

## Environmental Fate of Terbutylazine in Soils Amended with Fresh and Aged Final Residue of the Olive-Oil Extraction Process

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**ABSTRACT:** The impact of de-oiled and dried two-phase olive mill waste (DW) amendment on the sorption, degradation, leaching, and persistence of the herbicide terbutylazine in a Mediterranean agricultural soil were studied under laboratory and long-term field conditions. The results are compared with those of a previous study, where the intermediary by-product of the olive-oil processing technology (OW) was applied to the same soil. The adsorption of terbutylazine by the soil significantly increased with the increase of the amount of DW in the laboratory and field-amended soils. The amount of terbutylazine leached was significantly decreased or even not detected in the field-amended soil columns, but leaching losses of the herbicide increased by up to 52.7% in the laboratory amended-soils. The DW soil application significantly increased the half-life of terbutylazine from 6.7 days for the original soil to 21 and 104 days in the field and laboratory amended soils, respectively. In the field study, DW addition decreased the vertical movement of terbutylazine through the amended soils and increased the persistence of the herbicide in the upper 5 cm of the soils. This study suggests that DW amendment can play a positive role in preventing contamination of groundwater by terbutylazine, especially after the transformation of the organic matter by aging processes. The results were quantitatively different from those previously obtained with the intermediary by-product OW, showing the importance of the specific characteristics of the amendment in determining its effect on pesticide environmental fate and behaviour.

**Key words:** Adsorption, Leaching, Degradation, Terbutylazine, Fresh and aged olive mill waste

### INTRODUCTION

Olive oil extraction is one of the most traditional agricultural industries, with great economic importance, in most Mediterranean countries (Owen *et al.*, 2000). The new technology for olive-oil extraction is a continuous centrifuge two-phase process that generates a liquid phase (olive oil) and organic slurry (two-phase olive mill waste, OW) that only in Spain amounts to more than 4 000 000 Mg per year. After drying the OW, the remaining oil still present in this waste is usually extracted with hexane, leaving a final solid residue — de-oiled two-phase olive mill waste (DW) with lower organic matter content than the first residue (OW). Traditionally, DW has mainly been used as fuel for small boilers. However, the enactment of international regulations limiting the emission of CO<sub>2</sub> has lately been restricting this practice, so that new solutions are required for appropriate recycling.

Soil characteristics enhancement by the use of different pesticides, fertilizers and conditioners has caused environmental concerns (Ahsan and DelValls, 2011; Assassi *et al.*, 2011, Nasrabadi *et al.*, 2011; Mhadhbi and Boumaiza, 2012; Ghaderi *et al.*, 2012). Low organic matter content is a common feature of Mediterranean soils. It causes deterioration of workability, and contributes to limiting fertility and productivity. Therefore, agricultural practices based on periodic inputs of organic amendments are strongly recommended for Mediterranean agro-ecosystems. Because OW and DW contain an important level of organic matter content, their recycling as organic amendment may be an alternative for its disposal either raw or processed by composting (Delgado-Moreno and Peña, 2008; López-Piñero *et al.*, 2008), although the addition of composted products is considered an expensive option (Dolaptsoglou *et al.*, 2007). This strategy could be of especial importance for the maintenance of Mediterranean agricultural

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ecosystems, particularly olive grove ecosystems. Indeed, López-Piñeiro *et al.*, (2008) found that the repeated application of fresh DW to an olive grove soil led to a positive effect on soil quality and productivity.

The development of agriculture activities has led to an increase in pesticide use as a complement or alternative to tillage, and hence an increase in the contamination of water bodies (Dabrowski and Schulz, 2003). The presence of pesticides in drinking water supplies has a socio-economic impact in causing social alarm and increasing water purification costs. Terbutylazine, a chloro-s-triazine, is one of the commonest herbicides used to control weeds in olive groves, as in many other crops. However, due to its moderate sorption by soil and relatively long persistence, it is frequently responsible for the contamination of ground and surface water at concentrations above the European threshold for drinking water, 0.1 µg/L (Blanchoud *et al.*, 2007). These environmental problems are particularly to be expected in a Mediterranean climate characterized by short but heavy rainfall events, and in olive grove soils which are very often poor in organic matter content and with steep slopes, hence the importance of practices designed to avoid or reduce water contamination by this pesticide.

One way of reducing the direct contamination of water bodies by pesticide is by favouring sorption processes in the soil. Soil amendment with organic wastes is a common management practice in Mediterranean countries, but it can cause changes in soil structure and transport characteristics such as porosity, bulk density and water retention (Tester, 1990). Organic amendments also can favour the pesticide sorption by a soil, which can affect pesticide transport and distribution in the amended soils (Si *et al.*, 2011). The impact of the organic amendment on the behaviour of a pesticide depends on the type of organic material and the dosage, as well as on the pesticide's molecular configuration, polarity, and size (Albarrán *et al.*, 2004; Si *et al.*, 2006; Ahangar *et al.*, 2008). Nonetheless, a limitation of many previous studies is that the impact of organic amendments on a pesticide's behaviour is only examined after a relatively short residence time in the soil. The use of DW as soil amendment could be an effective management practice to enhance the retention of pesticides by soils, and therefore to reduce the risk of groundwater contamination (Albarrán *et al.*, 2004). However, the extent of impact of DW amendment on fate of pesticides has so far received limited attention. Moreover, the potential effect of any water-soluble organic carbon that may result from the organic

amendment is poorly understood and results have frequently been contradictory (Celis *et al.*, 1998; Said-Pullicino *et al.*, 2004). Several studies conducted on amendment of soils with different wastes have found that it may result in excessive levels of WSOC, which could hence increase pesticide mobility to lower soil layers (Graber *et al.*, 2001; Cox *et al.*, 2007). Moreover, the transformation of organic matter after an incubation period may also modify the further interactions of pesticides with the amended soils (Morillo *et al.*, 2002), so that it would be of great interest to know the effect of aging, preferably under long-term field conditions, on the transformation of the organic amendments added to soils, and on pesticide behaviour and fate.

Few studies have investigated the effects of OW on a pesticide's behaviour (e.g., Cabrera *et al.*, 2007; Cabrera *et al.*, 2010; López-Piñeiro *et al.*, 2011), and even fewer have been carried out on DW-amended soils. Although Mediterranean soils would appear to be suitable for DW application (López-Piñeiro *et al.*, 2008), and terbutylazine is widely used to control weeds, to the best of our knowledge, there have been no published studies evaluating its behaviour and environmental fate in DW-amended soils, although such information would be useful to help optimize the combined use of DW with pesticides in agriculture as well as determining the appropriate doses of the pesticide applied to these soils to avoid the risk of water contamination.

The aims of the present study were (1) to investigate the effects of seven years of repeated DW addition when compared to laboratory amendments on the sorption-desorption, degradation, and leaching of the herbicide terbutylazine applied to a typical Mediterranean agricultural soil in the field; (2) to determine the influence of DW amendment on the persistence and distribution of terbutylazine down these field amended soil profiles; (3) to evaluate the influence of the transformation of the organic matter in the processes of aging on terbutylazine's behaviour. The results are compared with those of a previous study, where the intermediary by-product OW was applied to the same soil.

## MATERIALS & METHODS

For the laboratory experiments, terbutylazine (N2-ter-butyl-6-chloro-N4-ethyl-1,3,5-triazine-2,4-diamine) (purity 98.6%), purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany), was used to prepare the herbicide solutions. For the field experiment, Tyllanex 80 (80% concentrated suspension, Kenogard, S.A., Spain), commonly used in olive groves, was used as a reference standard formulation. The study was

conducted in Elvas, Portugal (38°53' N; 7°9' W; 290 m above sea level) on a representative olive grove (*Olea europaea* L.) soil amended or unamended with DW for seven successive years. The soil, classified as a Cutanic Luvisol (ISSS-ISRIC-FAO 1994), consisted of 19.7% clay, 19.7% silt, and 60.6% sand. The climate is semi-arid Mediterranean with an average annual rainfall of 500 mm occurring mostly in autumn and spring and a mean annual temperature of 16.7 °C. The DW was obtained from the UCASUL oil industry located in Beja (Portugal), which employs chemical and heat treatment to obtain a second-extraction olive-oil. Its main properties were as follows: pH 5.30, 516 g/kg organic carbon, 24.0 g/kg total N, 1.94 g/kg total P, 12.5 g/kg total K, 14.6 g/kg water soluble phenols, 5.30 dS/m electrical conductivity, 23.7, 16.7, and 21.5 g/kg lignin, hemicellulose, and cellulose, respectively, and 5.40% moisture content.

The experimental design consisted of 9 14 x 15-m plots in the olive grove, with amendments made in a complete randomized design with three replicates per treatment. The three amendment treatments consisted of 27 (DW27) and 54 (DW54) Mg DW/ha, dry weight equivalent, and a control (DW) (unamended soil). Amendments were applied annually in February (from 1999 to 2005), manually spreading the waste on the soil surface, followed by arable-level homogenization.

For the laboratory experiments, soil samples from the unamended and both amended plots were collected in October 2005. To assess the effects of weathered and aged DW application on terbuthylazine (residual effect), soil samples were also collected in October 2007, two years after the last DW application. Four soil subsamples from each olive grove plot were taken randomly at a 25-cm depth. Samples were air dried, and the fraction that passed through a 2-mm sieve was stored at 4 °C until use. Since no DW amendments were added after 2005, the measurements made in 2005 and 2007 represented the “cumulative” (DWC, DW27C, and DW54C samples) and “aging or residual” (DWR, DW27R, and DW54R samples) effects, respectively. Unamended soil collected in 2005 (DWC) was also amended in the laboratory at a rate of 5% (DW5) and 10% (DW10) by weight of the same fresh OW as was applied in the field experiments.

Total organic carbon content (TOC) was determined by dichromate oxidation (Nelson and Sommers, 1996). Water-soluble organic carbon (WSOC) was extracted with de-ionized water at a 3:1 (water to soil) and 100:1 (water to OW) ratio. Humic and fulvic acids (HA and FA, respectively) were extracted by a solution of 0.1 M  $\text{Na}_4\text{P}_2\text{O}_7$  + NaOH using a ratio of extractant to sample of 10:1, and to precipitate humic

acid the supernatant was acidified to pH 2 with  $\text{H}_2\text{SO}_4$ . The WSOC and the TOC associated with each fraction of HA (CHA) and FA (CFA) were determined by dichromate oxidation and measurement of the absorbance at 590 nm (Sims and Haby, 1971). The polymerization grade (PG) was calculated as (CHA/CFA), and the humification index (HI) as (CHA/TOC). Texture was determined by sedimentation using the pipette method. Water content of the DW was calculated from weight loss after oven drying to constant weight at 105 °C. Electrical conductivity (EC) was measured in a saturation extract (US Salinity Laboratory Staff, 1954). The pH was measured in 1:1 (w/v) soil/water and 1:5 (w/v) DW water mixtures using a combination electrode.

Terbuthylazine sorption isotherms on unamended and amended soils were obtained using the batch equilibration procedure by equilibrating (24 h) triplicate soil samples (5 g) with 10 mL of initial terbuthylazine solutions (5 to 50  $\mu\text{M}$  in 0.01 M  $\text{CaCl}_2$ ). Adsorption isotherms were set up using the batch equilibration method. After equilibration, the suspensions were centrifuged and the equilibrium concentration ( $C_e$ ) was determined in the supernatant by high performance liquid chromatography (HPLC). The amount of terbuthylazine sorbed ( $C_s$ ) was calculated from the difference between the initial ( $C_i$ ) and equilibrium ( $C_e$ ) solution concentrations. The measurements of desorption of terbuthylazine from the soils were made immediately after the adsorption experiments, by successive dilution from the 5, 20, and 50  $\mu\text{M}$  initial concentration points. The 5 mL of supernatant removed for sorption analysis was replaced with the same amount of terbuthylazine-free 0.01 M  $\text{CaCl}_2$ . The samples were resuspended, shaken for 24 hours, and centrifuged, and the equilibrium concentration in the supernatant was determined. All treatments had three replicates. The herbicide sorption and desorption results were fitted by the Freundlich model,  $C_s = K_f C_e^{1/n_f}$ , where  $C_s$  ( $\mu\text{M}/\text{kg}$ ) is the amount of herbicide sorbed at the equilibrium concentration  $C_e$  ( $\mu\text{M}/\text{L}$ ), and  $K_f$  ( $\mu\text{M}^{1-1/n_f} / \text{kg}/\text{L}^{1/n_f}$ ) and  $n_f$  are the empirical Freundlich constants. 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.

Unamended, laboratory, and field amended soils (500 g) were spiked with 8 mL of an ethanol solution of terbuthylazine to give a concentration of 3 mg terbuthylazine/kg 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 at least five times.

Triplicate treatments were transferred to 1 L glass jars where they were incubated at  $20 \pm 2$  °C for 58 days in the dark. The moisture content was maintained at a constant level throughout the experiment by adding distilled water as necessary. The soils were sampled periodically and frozen until analysed. For analysis, 5 g of soil samples in duplicate were extracted with 10 mL of methanol for 24 h, and the terbuthylazine concentration in the extracts was determined by HPLC. Terbuthylazine dissipation curves in soils were fitted to first-order kinetics, and the half-lives ( $t_{1/2}$ ) were calculated.

Leaching was studied in disturbed-soil columns. Soil columns were constructed of PVC tubes (30 cm length  $\times$  5 cm i.d.). The top 5 cm of the columns was filled with sea sand and the bottom 5 cm with sea sand plus glass wool, to minimize losses of soil and contamination of leachates with soil particles. The remaining 20 cm was hand-packed with unamended or amended air-dried soil. The soil columns were saturated with 0.01 M  $\text{CaCl}_2$ , allowed to drain for 24 h, and then the amount of terbuthylazine corresponding to an application rate of 3 kg/ha dissolved in methanol was applied to the top of the columns. Each day the columns were leached with 0.01 M  $\text{CaCl}_2$  at a rate of 50 mL/day 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 then sectioned into 5-cm deep portions. Soil samples (10 g) from different depths were extracted once with 10 mL of methanol by shaking mechanically at  $20 \pm 2$  °C for 24 h. The suspensions were centrifuged, filtered, and assayed by HPLC in order to determine the residual amount of terbuthylazine at the different depths of the soil column. The leaching experiment was conducted in triplicate.

Once the leaching experiment was concluded, an undisturbed soil core (1 g) was sampled from the bottom 5-cm soil ring of the columns, dried at 80 °C for 24 h, and its pore size distribution (from  $1 \times 10^5$  to 1 nm) was determined by mercury intrusion porosimetry using Quantachrome Poremaster 33GT mercury intrusion porosimeter. A value for the surface tension of mercury of  $\gamma = 0.48$  N/m and a mercury solid contact angle of  $\theta = 140^\circ$  were assumed to use the Washburn (1921) equation for the calculation of the pore size distributions.

Terbuthylazine was applied at a rate of 3 kg/ha, equivalent to the recommended olive grove dosage, to the unamended and DW-amended plots, as a standard commercial formulation (80% concentrated suspension, Kenogard, S.A., Spain) in November (2005 and 2007). At selected times after the herbicide application, each

year four soil subsamples were taken randomly from different soil depths (0-5, 5-10, 10-30, and 30-60 cm) using a gouge auger. Samples were extracted twice with methanol at a 1:1 soil solution ratio, and the extracts were assayed by HPLC in order to determine the persistence and distribution of terbuthylazine down the soil profiles. Since no DW amendments were added after 2005, the measurements made in 2005 (DWC, DW27C, and DW54C samples) and 2007 (DWR, DW27R, and DW54R samples) represented the cumulative and residual effects, respectively. The two years had very similar rainfall over the complete period of the field study, with total amounts of 166.8 and 172.9 mm in the study area for 2005 and 2007, respectively.

Terbuthylazine assay was performed 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  $\div$  3.9 mm i.d.), 50:50 water:acetonitrile eluent mixture at a flow rate of 1 mL/min, 25  $\mu\text{L}$  injection volume, and UV detection at 223 nm. External calibration curves with standard terbuthylazine solutions between 0.05 and 20  $\mu\text{M}$  were used in the calculations.

#### *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.

## **RESULTS & DISCUSSION**

As a consequence of repeated DW amendment, the TOC significantly ( $p < 0.05$ ) increased by factors of 3.2 and 5.0 at 27 and 54 Mg/ha of DW application, respectively (Table 1). The increase in TOC provided by DW was more pronounced than those reported by López-Piñeiro *et al.* (2011) for the intermediary by-product OW. The greater TOC increase measured in the DW-amended soils suggests the occurrence of less extensive mineralization processes, i.e., a smaller priming effect, in these soils than in OW-amended ones (López-Piñeiro *et al.*, 2008).

Repeated DW applications also led to significant positive effects on CHA, CFA, and WSOC contents, with higher percentages attained at the 54 Mg/ha application rate (91%, 132%, and 333% greater for CHA, CFA, and WSOC, respectively, Table 1). As would be expected, two years after the last DW application the CHA content significantly increased, while CFA decreased (Table 1). Thus, compared with the control, the CHA increased by factors of 1.9 and 3.4 for the cumulative and residual years, respectively, at the higher rate of DW application. On the contrary, the

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

Properties	Unit	DWC	DW27C	DW54D	DWR	DW27R	DW54R	DW5	DW10
Organic carbon	(g/kg)	11.07	35.70*	55.90*	10.26	33.50*	48.10*	39.32*	57.30*
WSOC	(mg/kg)	142	466*	615*	122	236*	296*	3293*	6054*
Humic acid	(g/kg)	1.348	1.702*	2.569*	1.268	3.002*	4.320*	3.166*	4.396*
Fulvic acid	(g/kg)	0.873	1.439*	2.032*	0.830	0.916	1.201*	4.928*	8.756*
HI		12.1	4.76*	4.44*	12.3	8.96*	9.64	8.10*	7.70*
PG		1.54	1.18*	1.26*	1.52	3.27*	3.59*	0.64*	0.50*
pH		8.00	7.94	7.5*	8.03	7.77*	7.55*	6.07*	5.56*

WSOC=water soluble organic carbon. HI=humification index. PG=polymerization grade. DWC=unamended soil; DW27C=amended soil at rate of 27 Mg/ha/yr; DW54C=amended soil at rate of 54 Mg/ha/yr; all in the last year of DW application. DWR=unamended soil; DW27R=amended soil at rate of 27 Mg/ha/yr; DW54R= amended soil at rate of 54 Mg/ha/yr; all two years after the last DW application. DW5 and DW10 are laboratory amended soils at rates of 5% and 10% by weight of the DW, respectively. Values in the same row followed by \* indicate that there is a significant difference between the control and the respective treatment at a  $p<0.05$  level of probability

CFA content was much lower in the residual year at both rates of DW. Consequently, in the residual year the polymerization grade (PG) of DW-amended soils also increased significantly. The residual year had a much lower WSOC content (236 and 296 mg/kg) than the cumulative year (466 and 615 mg/kg) at the 27 and 54 Mg/ha DW application rates, respectively (Table 1). These results suggest that repeated raw DW amendment can lead to an increase in the native soil organic matter stability by increasing the humified organic matter fraction, which represents a positive effect in the context of the beneficial recycling of DW.

In the laboratory amended soils, the DW amendments also led to a significant increase of TOC, WSOC, CHA, and CFA. Compared to the control, these increases were by factors of 5.2, 42, 3.3, and 10, respectively, at the higher DW application rate (Table 1).

The Freundlich equation satisfactorily fit the sorption isotherms for terbuthylazine in unamended and DW-amended soils ( $R^2>0.990$ , Table 2). The  $k_f$  values increased by factors of 5.7 and 7.5 for DW5 and DW10, respectively (Table 2), showing the affinity of this herbicide for the organic amendment. The increase in herbicide adsorption by organic amendment to soil has previously been reported by several workers (Sanchez-Camazano *et al.*, 2000; Albarrán *et al.* 2004; López-Piñeiro *et al.* 2010; Si *et al.* 2011). Similarly, Cabrera *et al.* (2007) found that terbuthylazine sorption increased by a factor of 4.6 in a soil amended in the laboratory with fresh OW at a rate of 10%. López-Piñeiro *et al.* (2011) found that the sorption of terbuthylazine increased by factors of 4.8 and 6.5 in the same soil amended at rates of 5 and 10% with fresh OW,

respectively. The increase in terbuthylazine sorption provided by OW was less pronounced than that found in the present study for DW. This can be attributed to the higher TOC content observed in DW-amended (Table 2) than in OW-amended soils (López-Piñeiro *et al.*, 2011). However, in a study using a soil with very high initial organic matter content, atrazine sorption decreased after manure addition (Briceño *et al.*, 2008). This is consistent with previous reports indicating that the addition of organic amendments increases pesticides sorption in soils, especially in those with low organic matter content (Briceño *et al.*, 2007).

The repeated DW application also significantly increased the adsorption of terbuthylazine in the field amended soils. Although the increase in terbuthylazine sorption has previously been related to the TOC content of soils (e.g. Karanasios *et al.*, 2010), the highest  $K_f$  coefficients were obtained in the residual year, despite its lower TOC content compared with those observed in the cumulative year. Thus, the average increases over the unamended plots were by factor of 2.2 and 3.3 for the DW54C and DW54R treatments, respectively. This seems to indicate that the quality rather than quantity of the organic matter influenced the soil's ability to adsorb terbuthylazine. In fact,  $K_f$  was correlated positively and highly significantly ( $p<0.001$ ) with CHA ( $r=0.966$ ), and significantly ( $p<0.05$ ) with TOC ( $r=0.778$ ), also confirming that most of the sorptive performance of the DW-amended soils depends on the organic matter transformed into humic substances. Similar results have previously been reported indicating that aromatic C has the highest affinity for different pesticides such as diuron (Ahangar *et al.*, 2008) and ethametsulfuron-

**Table 2. Effects of dried and de-oiled olive mill waste addition on the Freundlich sorption coefficients and half-life ( $t_{1/2}$ ) in dissipation studies for terbuthylazine**

Soils	$K_f$	$1/n_f$	$R^2$ sorption	H	$t_{1/2}$ (days)	$R^2$ ( $t_{1/2}$ )
DWC	4.35±0.29	0.804±0.037	0.996	2.94	6.66±0.19	0.931
DW27C	7.31*±0.17	0.918±0.016	0.999	3.25	13.8*±0.67	0.857
DW54C	9.48*±1.23	0.833±0.101	0.971	4.12*	21.1*±1.06	0.801
DWR	4.80±0.27	0.881±0.034	0.990	2.85	6.78±0.16	0.806
DW27R	9.77*±0.58	0.965*±0.046	0.993	5.66*	12.6*±0.48	0.989
DW54R	14.5*±1.03	0.956*±0.056	0.991	8.97*	15.7*±0.71	0.984
DW5	24.8*±2.9	0.970*±0.040	0.999	2.46*	84.0*±2.85	0.810
DW10	32.6*±1.3	1.03*±0.060	0.999	2.35*	104*±4.50	0.835

DWC=unamended soil; DW27C=amended soil at rate of 27 Mg/ha/yr;

DW54C=amended soil at rate of 54 Mg/ha/yr; all in the last year of DW application.

DWR=unamended soil; DW27R=amended soil at rate of 27 Mg/ha/yr;

DW54R=amended soil at rate of 54 Mgt/ha/yr; all two years after the last DW

application. DW5 and DW10 are laboratory amended soils at rates of 5% and 10% by

weight of the DW, respectively. Values of  $K_f$ ,  $n_f$ , and  $t_{1/2}$  are presented as mean±standard deviation. Values in the same column followed by \* indicate that there is a significant difference

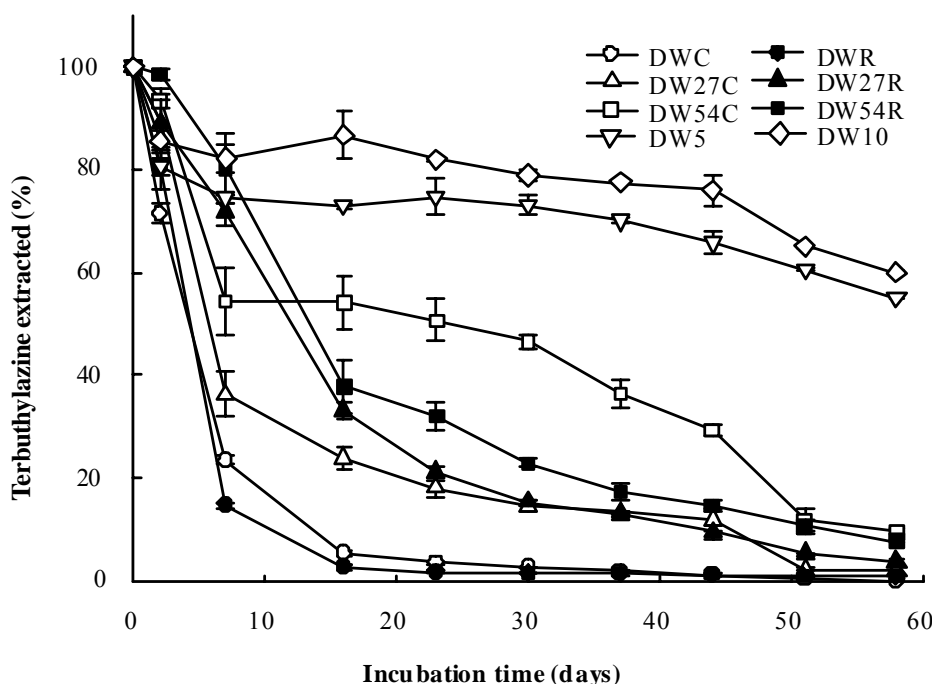
between the control and the respective treatment at a  $p<0.05$  level of probability

methyl (Si *et al.*, 2006). The results reported here for the effects of DW addition on the sorption of terbuthylazine in field-amended soils are also similar to those found for OW (López-Piñero *et al.*, 2011). However, the increase in terbuthylazine sorption provided by DW (Table 2) were significantly more pronounced than those obtained for OW (López-Piñero *et al.*, 2011) because of the much higher TOC and CHA of the waste applied in the present study. This suggests, therefore, that DW has a higher affinity for terbuthylazine than OW, resulting in higher sorption.

In relation to the control, greater hysteresis coefficients (lower reversibility) were observed in the field amended soils, especially in the residual year (Table 2) in which DW organic matter underwent a humification process. However, in the laboratory amended soils these coefficients decreased (Table 2), suggesting that adsorbed terbuthylazine was more easily desorbed in the laboratory than in the field amended soils. This may have been caused by the greater WSOC content observed in the laboratory than in the field amended soils (Table 1), that could promote pesticide desorption and enhance its apparent water solubility through stable interactions in solution between pesticide and soluble organic matter (Graber *et al.*, 2001; Navarro *et al.*, 2003). Similar observations have previously been reported in which it was found that the high WSOC level of fresh olive mill wastes may increase the reversibility of pesticides such as simazine (Albarrán *et al.*, 2004), diuron (López-Piñero *et al.* 2010), and terbuthylazine (López-Piñero *et al.* 2011).

The dissipation rates of terbuthylazine in the presence and absence of DW are shown in Fig. 1. The DW amendment had a significant influence on the degradation. The recovery of applied terbuthylazine significantly ( $p<0.05$ ) increased with increasing amounts of DW addition. Indeed, in the case of the soils amended in the laboratory, the DW addition increased the half-life ( $t_{1/2}$ ) of terbuthylazine from 6.7 to 84 and 104 days for DWC, DW5, and DW10, respectively (Table 2). This finding is consistent with the literature reports that degradation of pesticides in amended soils is limited by increasing pesticide sorption, which in particular can reduce terbuthylazine availability for microbial decomposition (Cabrera *et al.*, 2007; Delgado-Moreno and Peña, 2009; López-Piñero *et al.*, 2011) or lead to a toxic effect of the organic amendment on the soil microbial population (Albarrán *et al.*, 2004). As was to be expected, the dissipation increases were significantly greater than those obtained by López-Piñero *et al.* (2011) for OW (from 6.7 days for OWC to 60 and 89 days for OW5 and OW10, respectively), because of the higher  $k_f$  values observed in the present study.

At very short incubation times (<10 days) (Fig. 1), there was little difference observed in the terbuthylazine extracted between the unamended and DW5 and DW10 treatments. However, at longer incubation times, there was a pronounced decrease in terbuthylazine extractability from the unamended soil, but little change in DW5 and DW10 (Fig. 1) even towards the end of the incubation experiment. Indeed, at the end of the experiment (58 days), 58% and 63% of



**Fig. 1. Cumulative and residual effects of dried and de-oiled olive mill waste addition on terbuthylazine dissipation (error bars represent one standard error of the mean)**

the herbicide applied to the DW5 and DW10 soils, respectively, remained extractable, while a negligible amount of terbuthylazine was extractable from the unamended soil (Fig. 1). Similar results have previously been found with this pesticide but using OW (López-Piñero *et al.*, 2011), and with the same organic amendment used in this study but with simazine (Albarrán *et al.*, 2004) and diuron (López-Piñero, *et al.*, 2010), with the prolonged availability of the herbicides in the fresh OW and DW-amended soils being a result of herbicide sorption to the organic amendment, or a toxic effect of the organic amendment on the soil's microbial population.

The effect of DW additions on the half-life of terbuthylazine in the field amended soils was not as pronounced as in the laboratory amended soils, which have greater sorption coefficients (Table 2). Thus, the DW additions increased the  $t_{1/2}$  of terbuthylazine from 6.7 to 21.1 and 15.7 days for the unamended, DW54C, and DW54R treatments, respectively. These results are in agreement with those found by López-Piñero *et al.* (2011) who reported that a toxic effect of the organic amendment (OW) on the microbial population of the fresh laboratory amended soils would explain why the  $t_{1/2}$  was shorter in field amended soils.

The cumulative breakthrough curves (BTCs) of the terbuthylazine applied in unamended and DW-amended soil columns are shown in Fig. 2. In the unamended soil, terbuthylazine breakthrough occurred after

passing 3.5 and 2.5 pore volumes of water (DWC and DWR, respectively). However, in the field amended soils breakthrough was delayed, occurring after passing 6.8, 8.7, and 9.5 pore volumes of water for DW27C, DW54C, and DW27R, respectively. On the other hand, it is interesting to note that the effectiveness of DW in raising soil porosity was significantly greater in the cumulative than in the residual year (Table 3). Thus, the average increases relative to the unamended plots were 137% and 55% for the DW54C and DW54R treatments, respectively. This fact was due to a significant reduction of pores  $> 10 \mu\text{m}$  observed in the DW-amended soils in the residual year (Table 3). The observed decrease in the volume of pores with radii  $> 10 \mu\text{m}$  (Table 3) coupled with the higher  $k_f$  values (Table 2) could therefore well explain the slower breakthrough of terbuthylazine in the field amended soil columns in the residual year compared to those in the cumulative year. This slower herbicide breakthrough agrees with earlier observations with other organic amendments and pesticides (e.g., Cox *et al.*, 1997; Majumdar and Singh, 2007; López-Piñero *et al.*, 2010; Si *et al.*, 2011). This retardation as a result of DW addition to the soil was, however, more pronounced than that reported for OW, probably as a result of the higher sorption capacity of the DW-amended soils (López-Piñero *et al.*, 2011).

In the case of columns filled with laboratory DW-amended soils, the breakthrough time of terbuthylazine

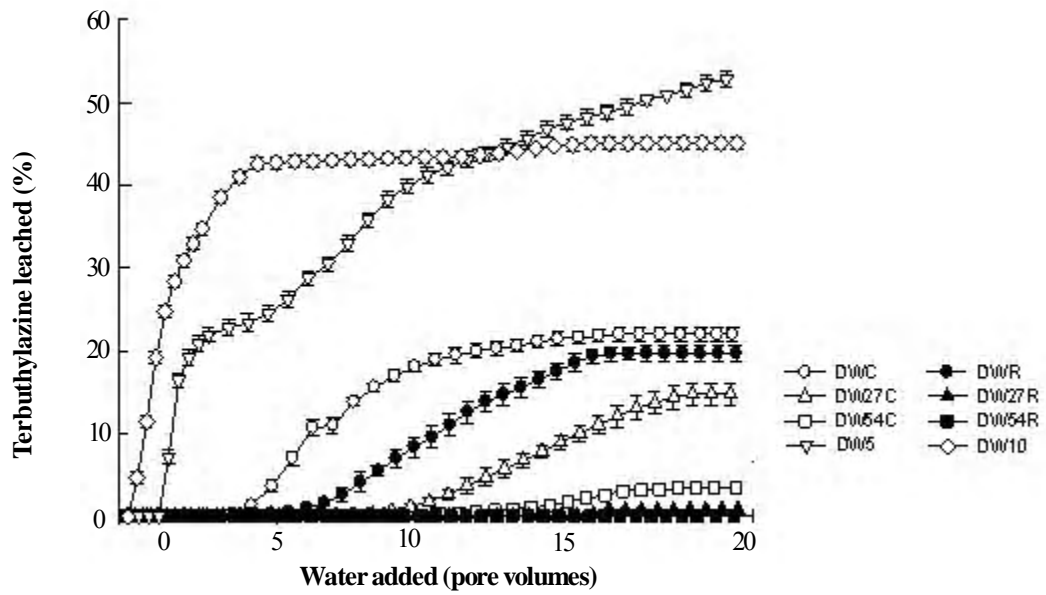


Fig. 2. Cumulative breakthrough curves of terbuthylazine in unamended and dried and de-oiled olive mill waste amended soils (error bars represent one standard error of the mean)

Table 3. Pore volumes ( $\text{mm}^3 \text{g}^{-1}$ ) of the unamended and dried and de-oiled olive mill waste amended soils

Range	DWC	DW27C	DW54C	DWR	DW27R	DW54R	DW5	DW10
0.001 – 0.10 $\mu\text{m}$	22.7	43.1*	60.3*	20.5	59.0*	62.6*	44.0*	38.4*
0.10 – 1.0 $\mu\text{m}$	26.5	52.6*	73.0*	27.3	70.6*	84.3*	31.5	34.2*
1.0 – 10 $\mu\text{m}$	45.4	69.7*	64.7*	41.5	67.5*	73.8*	63.5*	76.9*
10 – 100 $\mu\text{m}$	127.1	293.1*	322.5*	120.9	96.2*	104.4*	72.7*	85.9*
Cumulative volume	221.7	458.5*	523.5*	210.2	293.3*	325.1*	211.7	235.4*

DWC=unamended soil; DW27C=amended soil at rate of 27 Mg/ha/yr;

DW54C=amended soil at rate of 54 Mg/ha/yr; all in the last year of DW application.

DWR=unamended soil; DW27R=amended soil at rate of 27 Mg/ha/yr;

DW54R=amended soil at rate of 54 Mg/ha/yr; all two years after the last DW application.

DW5 and DW10 are laboratory amended soils at rates of 5% and 10% by weight of the OW, respectively.

Values in the same row followed by \* indicate that there is a significant difference between the control and the respective treatment at a  $p < 0.05$  level of probability

was advanced, occurring after only passing 1.6 and 0.6 pore volumes of water. Similarly, López-Piñero et al. (2011) reported that the addition of fresh OW also advanced the breakthrough time of terbuthylazine, indicating that the greater reversibility of the pesticide sorption in the laboratory than in the field-amended soils could account for its faster breakthrough.

Leaching losses of terbuthylazine were increased by up to 52.7% for the laboratory amended soil columns. However, applications of DW to soil significantly reduced the amount of terbuthylazine leached in the field amended soil columns, especially in the residual year (Table 4). Thus, whereas nearly 20% of the applied herbicide was recovered in leachates from unamended soils, only 3.3% and no residues of

herbicide were detected in the leachates of the cumulative and residual-amended columns, respectively, at the greater application rate of DW. According to López-Piñero et al. (2011), the transformation of the organic matter of the fresh amendment due to humification and maturation processes could be responsible for this behaviour. Indeed, the lower WSOC content of DW27R and DW54R (236 and 296 mg/kg, respectively) and their higher PG (3.27 and 3.59) in comparison with the WSOC content of DW27C and DW54C (466 and 615 mg/kg, respectively) and the PG values of these last two soils (1.18 and 1.26) (Table 1) can well explain why the terbuthylazine content was far lower or not detected in the residual year (Table 4).



**Table 4. Terbutylazine leached and extracted from the soil columns at the end of the leaching experiment**

DWC	DW27C	DW54C	DWR	DW27R	DW54R	DW5	DW10	
Leached	21.9±2.75	14.7*±2.56	3.35*±0.20	19.7±1.21	0.30*±0.01	0.00*	52.8*±0.24	45.1*±0.22
Total extracted	2.38±0.01	5.44*±0.22	7.71*±0.11	5.61±0.61	21.2*±1.89	22.7*±1.66	23.8*±0.70	33.7*±1.06
Extracted 0-5 cm	0.08±0.00	0.41*±0.03	0.47*±0.04	1.26±0.05	6.87*±1.06	10.1*±1.19	1.77*±0.22	19.9*±0.45
Extracted 5-10 cm	0.34±0.00	1.23*±0.03	3.26*±0.00	1.23±0.04	7.87*±1.21	9.82*±0.36	7.38*±0.12	13.4*±0.53
Extracted 10-15 cm	0.99±0.01	1.32±0.09	0.59*±0.04	1.49±0.13	4.61*±1.02	1.68±0.15	7.51*±.21	0.37*±0.07
Extracted 15-20 cm	0.98±0.01	2.48*±0.07	3.38*±0.01	1.62±0.33	1.85±0.49	1.07*±0.04	7.17*±0.14	0.00*

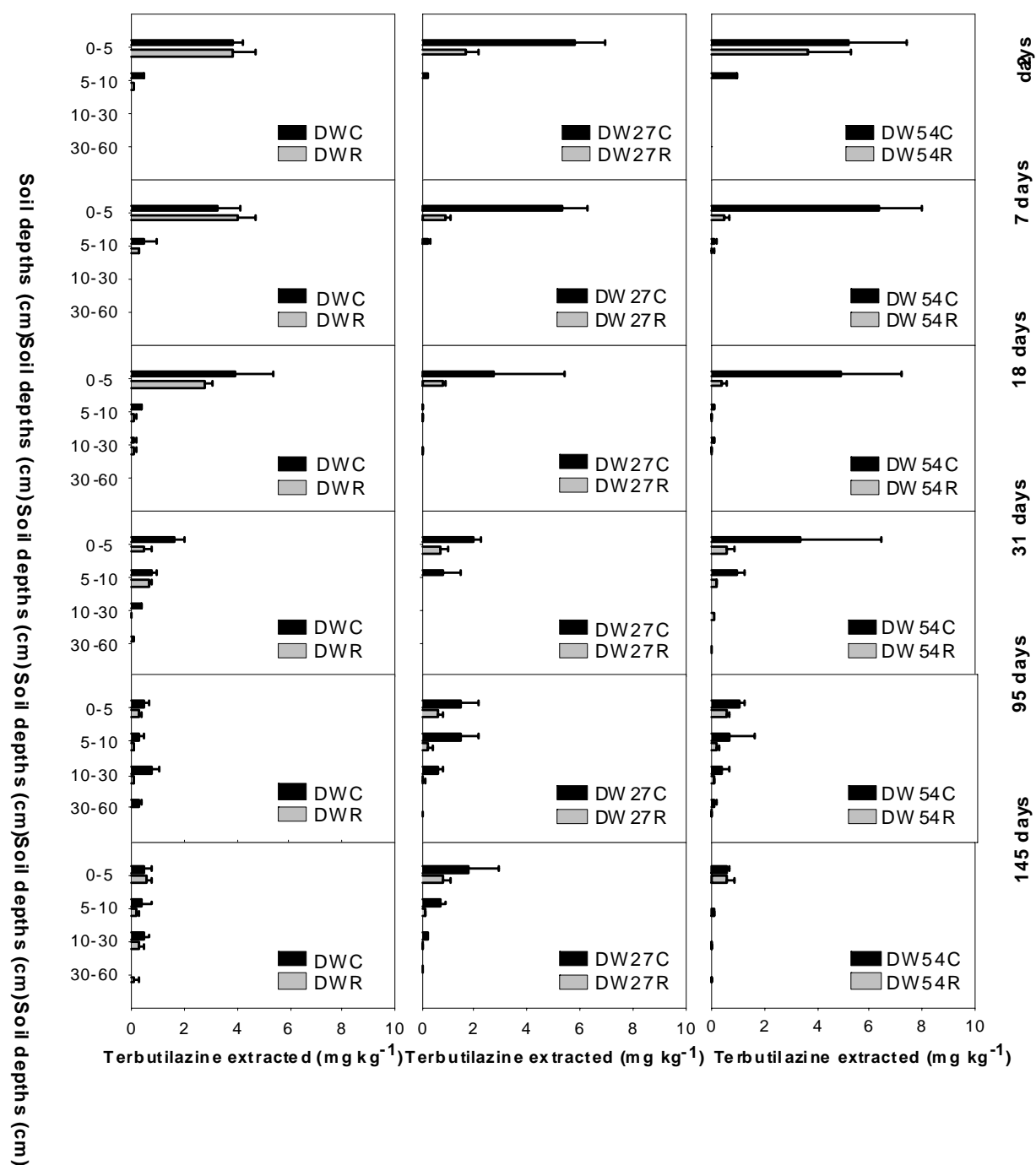
DWC=unamended soil; DW27C=amended soil at rate of 27 Mg/ha/yr; DW54C=amended soil at rate of 54 Mg/ha/yr; all in the last year of DW application. DWR=unamended soil; DW27R=amended soil at rate of 27 Mg/ha/yr; DW54R=amended soil at rate of 54 Mg/ha/yr; all two years after the last DW application. DW5 and DW10 are laboratory amended soils at rates of 5% and 10% by weight of the OW, respectively. Values in the same row followed by \* indicate that there is a significant difference between the control and the respective treatment at a  $p < 0.05$  level of probability

Although terbutylazine leached down to 20 cm depth in all soil columns (Table 4), its distribution pattern in DW-amended soils was quite different from that in the unamended soil column. Moreover, the addition of DW increased the total amounts of terbutylazine recovered, especially in the laboratory amended soils and in the residual year of the field amended soils (Table 4). Thus, whereas only 0.50% of terbutylazine retention was observed within the first 10 cm depth in the unamended soil column, 3.7%, 19.9%, and 33.3% of the applied herbicide was recovered from the DW54C, DW54R and DW10 soil columns, respectively, which agrees with the results observed previously in the sorption experiment of this study (Table 2). Similar results using OW as organic amendment were reported by López-Piñero et al. (2011), indicating that the greater persistence of terbutylazine in the laboratory-amended soils compared to the field-amended soils could also explain the observed difference in the leaching behaviour.

Fig. 3 shows the amounts of terbutylazine extracted from different depths of field plots in selected sampling dates after herbicide application. Although a difference in terbutylazine distribution between the unamended and amended plots is readily apparent, for all treatments the greatest amounts of pesticide were extracted from the 5 cm depth, thus indicating a limited mobility of this herbicide in the selected soil, even in the DW27C and DW54C plots which had greater WSOC values (Table 1). According to López-Piñero et al. (2011), the differences between disturbed and undisturbed systems, together with the large total amount of 0.01 M CaCl<sub>2</sub> added in the laboratory columns (equivalent to 1476 mm) compared with the weak rainfall observed over the complete period of the

field study (total amounts of 166.8 and 172.9 mm for 2005 and 2007, respectively) could well explain the observed differences in the leaching behaviour of the pesticide under the controlled (Table 4) and field conditions (Fig. 3).

In all treatments, terbutylazine was detected in decreasing concentrations up to the end of the study. However, we observed that incorporation of DW into the soil profile can be expected to significantly decrease the downward movement of pesticide, leading to an accumulation of pesticide at the soil surface, especially in the cumulative year (Fig. 3). Thus, 2 days after herbicide application, whereas only 3.8 mg/kg of pesticide was observed within the first 5 cm depth in DWC and DWR, 5.2 and 3.6 mg/kg were recovered for DW54C and DW54R, respectively. Similarly, 95 days after herbicide application, the amounts of terbutylazine recovered within that same depth were 0.4, 1.1, and 0.5 mg/kg for unamended, DW54C, and DW54R plots, respectively, thus corroborating that availability of terbutylazine in the amended soils is reduced by increasing the humified organic matter fraction. These results are in agreement with those obtained in López-Piñero et al. (2010) in which diuron herbicide decreased in field DW-amended soils due to an increase in the humified organic matter fraction. These results are, however, quantitatively different to those reported upon OW addition to the same soils where the amounts of terbutylazine recovered were greater than in DW-amended soils (López-Piñero *et al.*, 2011). This illustrates how the specific characteristics of the organic amendment is a critical parameter determining pesticide sorption and persistence in amended soils.



**Fig. 3.** Cumulative and residual effects of dried and de-oiled olive mill waste addition on terbutylazine extracted from different depths of field plots after herbicide application: (A) unamended soils, (B) soils amended with 27 Mg ha<sup>-1</sup> yr<sup>-1</sup> of DW, and (C) soils amended with 54 Mg ha<sup>-1</sup> yr<sup>-1</sup> of DW; error bars represent one standard error of the mean

## CONCLUSION

The agronomic application of DW, either fresh or aged, has the potential to greatly affect the environmental fate of the herbicide terbuthylazine. The effect depends on the application rate, but even more so on the degree of organic matter maturity. The DW amendment may be an effective management practice to reduce the risk of groundwater contamination by terbuthylazine in Mediterranean soils, which frequently are very poor in organic matter. However, leaching loss of this pesticide could potentially increase in fresh DW amended soils. In addition, the use of DW could increase the persistence of terbuthylazine in the topsoil, helping the farmer reduce the rate and frequency of herbicide application, but also increasing the risk of surface water contamination, especially if application rates are greater than 27 Mg/ha.

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