Contribution by different organic fractions to triazines sorption in Calcaric Regosol amended with raw and biotransformed olive cake

L. Delgado-Moreno [a,*], A. Peña [a], G. Almenbdros [b]

[a] *Estación Experimental del Zaidín, CSIC, c/Profesor Albareda, 1. 18008 Granada, Spain*
[b] *Centro de Ciencias Medioambientales, CSIC, c/Serrano, 115, duplicado. 28006 Madrid, Spain*

ABSTRACT

A soil, olive cake, compost and vermicompost of olive cake, were subjected to sequential laboratory extraction to progressively remove water-soluble, lipid and alkali-soluble (humic-type) fractions. Sorption experiments with triazines were carried out with non-amended and amended soil and with soil residues in the intermediate stages during the laboratory removal of the different organic fractions. Herbicide sorption in soil amended with olive cake was between two and three times higher than sorption in composted substrates. In non-amended soil, the removal of humic and fulvic acids led to a decrease of triazines sorption indicating the importance of these fractions in the sorption of these pesticides. The greater triazines sorption in soil amended with olive cake could be associated with the high concentration of water-soluble substances. In contrast, olive cake lipids did not favour the sorption of the more hydrophobic herbicides as indicated by the fact that the sorbed amount increased 30–40% when this fraction was removed. No significant ($P > 0.05$) differences in K_{oc} values were found in soils amended with compost and vermicompost in the course of the progressive removal of the different organic fractions, indicating triazine sorption was related more with the total amount of organic matter than with its chemical composition.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Triazine herbicides have been largely used in agriculture for the control of different weeds in crops, such as maize or vineyards. However, due to their physico-chemical properties (in particular, their relatively long persistence) there have been numerous reports of their presence in surface or ground waters [1,2]. Since organic components have been considered to play a key role on the sorption of pesticides [3], in last years, the use of organic amendments has been proposed to modify surfaces of soils and subsurface materials to promote adsorption and reduce triazines water pollution [4,5]. In that sense, raw olive cake (*alperujo* in Spanish) generated in large amounts by olive industries in Mediterranean areas, could be used to bioremediate pesticide contaminated soil. Natural olive cake has, however, some drawbacks due to its content in polyphenols and organic acids with phytotoxic and antimicrobial effects. The transformation of olive cake into more stabilized amendments has become an attractive option for agricultural residues as long as negative effects on soil and ground water can be eliminated, because degradation and transformation reactions during these processes affect both organic matter and organic contaminants present in the original substrate. Among the different biotransfor-

mation processes of agricultural wastes, composting, an aerobic process during which microorganisms decompose the organic matter into a humus-like product [6,7] or vermicomposting, in which biological degradation and stabilisation of organic matter occurs, apart from microorganisms, as earthworms feed on organic waste materials [6], can be considered.

Although the effect on triazines sorption of different organic amendments has been subject of a number of studies, e.g. [4,5,8], relatively little attention has been devoted to raw olive cake [9] and its biotransformed products, i.e., compost and vermicompost [10]. On the other hand, the studies about the role of different organic matter fraction of these amendments on triazines sorption are scarce and focused on dissolved organic matter [11].

In the present work we assess the importance of different organic fractions from a soil and from olive cake on the sorption of four triazines. In order to compare the effect of several organic constituents differing in their molecular complexity and transformation degree on triazines sorption, we have also employed compost and vermicompost of olive cake, as soil amendments.

2. Materials and methods

2.1. Herbicides

The herbicides selected were terbuthylazine, cyanazine, simazine and prometryn, all with purity >99% (Dr. Ehrenstorfer,

---

* Corresponding author. Tel.: +34 1 95182722692; fax: +34 1 8273993.
  E-mail address: lmoreno@ucr.edu (L. Delgado-Moreno).

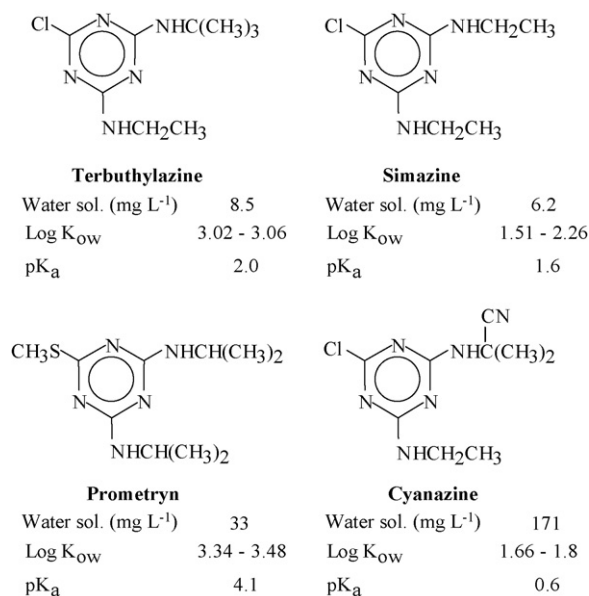**Fig. 1.** Chemical structure and some properties of the triazine herbicides studied.

Substrate h: soil and amendments without the water-soluble fraction

Substrate hlip: soil and amendments without the water-soluble and lipid fractions

Substrate THE: soil and amendments without water-soluble, lipid, and total humic extract

Fig. 2. Extraction diagram of the several organic fractions from soil and amendments.

Augsburg, Germany). They are pre- or post-emergence herbicides to control weeds in many crops such as vines, fruit trees, olive trees, etc. [12]. Some properties of these herbicides, which are weakly basic compounds, are presented in Fig. 1. For the range of soil pH these triazines will always be in a neutral form. Although simazine exhibits the lowest water solubility, it is considered, together with cyanazine, more polar than prometryn and terbuthylazine according to the partition coefficient, K_{ow}.

2.2. Soil and organic amendments

The soil material (S), Calcaric Regosol from an olive orchard located near Iznalloz (Granada, Southern Spain), was collected with a spade from the upper layer (0–25 cm), air-dried and passed through a 2-mm sieve. This soil had a silty clay loam texture, 34.0% clay, 56.0% silt and 10.0% sand, and contained 44.1% $CaCO_3$. Total organic carbon (OC) content determined by wet oxidation with $K_2Cr_2O_7$ [14] was 2.18% and pH (5/20 soil/water ratio) was 7.9 ± 0.1.

The amendments used were prepared from olive wastes by-products and consisted of: raw olive cake from a two-stage centrifugation process (*alperujo*, A), 6-month matured compost from olive cake (C) and vermicompost from olive cake (V). The olive cake was supplied by an olive oil industry (Romeroliva, Deifontes, Granada, Spain). The mature compost and vermicompost were prepared as described elsewhere [10]. The main chemical properties of raw olive cake, compost and vermicompost are summarized in [13]. Among them, OC content and pH are shown in Table 1.

Each amendment was air-dried, ground, homogenized to <.2 mm and added to the soil at a rate equivalent to 200 Mg ha^{-1}. The soil and amendments were mixed and kept at room temperature.

2.3. Sequential extraction of organic matter fractions

Different organic fractions of soil and amendments were isolated as described in Fig. 2. Firstly, the soil and amendments were extracted with distilled water and centrifuged at $5000 \times g$ for 10 min several times to remove the supernatant liquid with the water-soluble fraction (Fig. 2A, substrate h). From the previous residue, the lipid fraction was removed by Soxhlet's extraction using 40–60 °C petroleum ether. The organic extract was dehydrated with anhydrous Na_2SO_4, the solvent was distilled and the lipid was weighed (Fig. 2B, substrate hlip) (Table 1). From the above residue, after the complete elimination of the petroleum ether by evaporation at room temperature (substrate hlip), the total humic extract (THE) was removed by successive extractions with
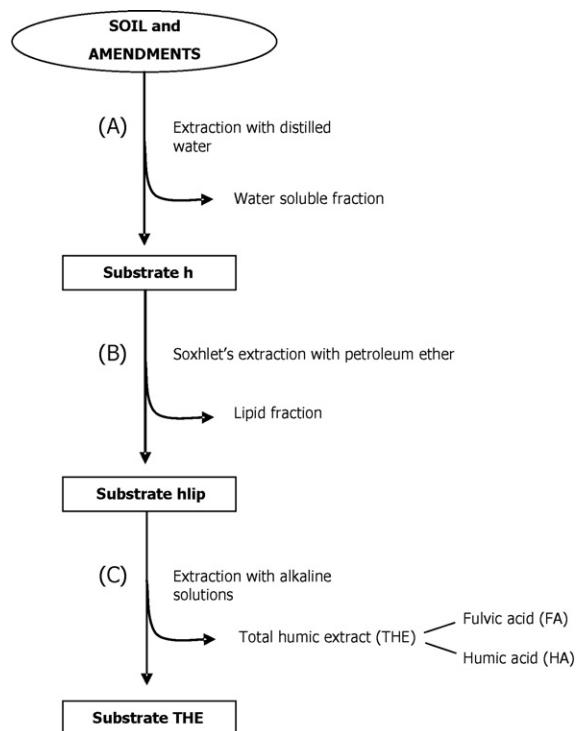
Table 1
Total organic carbon content and pH of soil and amendments, as well as distribution of organic carbon in different organic fractions (S, soil; A, olive cake; C, compost of olive cake; V, vermicompost of olive cake).

	OC content (g C/100 g)	pH	H	LIP	HA	FA	Humin[a]	HA (%)[b]	HA/FA
			g OC (100 g OC)$^{-1}$						
S	2.1	7.9	0	0.2	26.0	11.2	62.6	69.8	2.3
A	53.0	5.3	9.6	10.4	13.7	14.5	51.8	48.6	1.0
V	35.3	8.0	3.2	0.3	27.6	9.6	59.3	74.1	2.9
C	22.7	8.4	6.0	0.04	14.7	7.7	71.6	65.7	1.9

pH for 5/20 soil/water ratio. H: water-soluble fraction; LIP: lipid fraction; HA: humic acid; FA: fulvic acid.

[a] The OC content in this fraction was calculated by difference between the OC total content and the OC content of the rest of organic fractions.
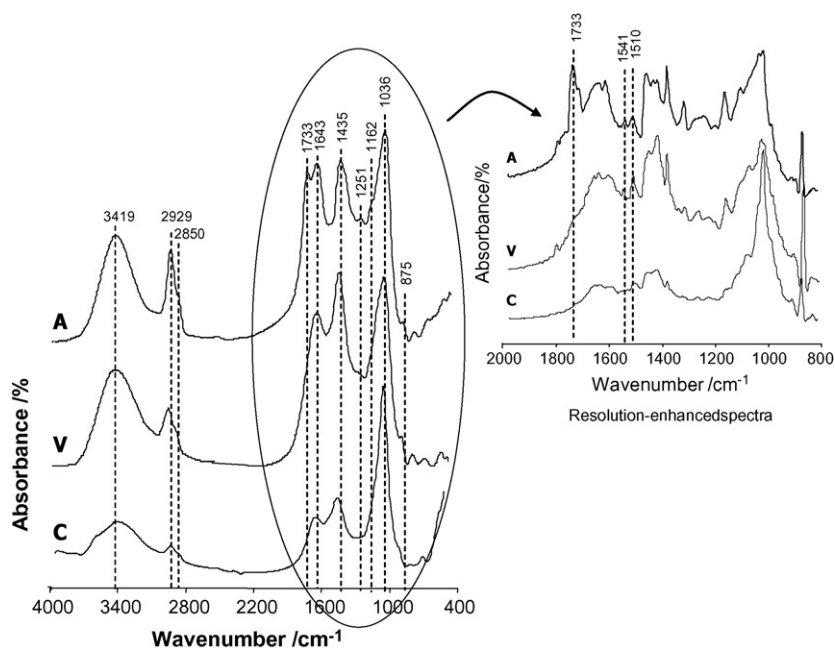
[b] Calculated as (HA/THE) × 100.

Fig. 3. Infrared spectra and resolution-enhanced spectra for olive cake (A), vermicompost (V) and compost (C).

0.1 M $Na_4P_2O_7$. After shaking the samples for 1 h with the alkaline solution, the suspension was centrifuged at $900 \times g$ for 10 min. The residue after centrifugation was subjected to four successive extractions with 0.1 M NaOH. The soil residue was exhaustively washed with distilled water to remove traces of the alkaline extraction reagents (Fig. 2C, substrate THE). In order to quantify the organic carbon amount of the HA-like fractions, a 100 ml aliquot of the THE obtained by mixing the alkaline supernatant solutions after centrifugation was acidified to pH 1 with H_2SO_4 to precipitate the HA-like fraction. The supernatant solution with the acid-soluble organic matter (i.e., fulvic acid-like, FA) was removed by siphoning and the residue (HA) was centrifuged at 900 g for 10 min and then redissolved in 0.5 M NaOH, centrifuged at $40,000 \times g$ to sediment particulate fractions which were discarded, acidified with HCl (pH = 1), dialysed and desiccated.

Total OC content of the organic fractions extracted from soil and amendments was determined by wet oxidation with $K_2Cr_2O_7$ [14] (Table 1). The concentration of FA (acid-soluble fraction) was calculated by difference between THE and HA.

The substrates, referred to with the subscripts h, hlip and THE (Fig. 2), indicating the different organic fractions that were removed, were dried at room temperature and later used as sorbates in the sorption studies.

2.4. Spectroscopic analyses

Soil and amendments were analyzed by Fourier transform infrared spectroscopy, by mixing 1.0 mg of each sample with 100 mg KBr, dried at 110 °C for 24 h. The spectra were acquired in the range between 4000 and 400 cm^{-1} with a PerkinElmer Spectrum One apparatus (PerkinElmer, MA, USA). For resolution enhancement, an algorithm based on the digital subtraction from the original spectrum of a positive multiple of its second derivative was applied [15]. Infrared bands of spectra from the amendments were interpreted according to [16,17].

2.5. Herbicides sorption

The herbicides sorption was determined using the batch equilibration method, with non-amended and amended soils. Soil

samples (5 g) were suspended in aliquots of 20 ml of herbicide solutions at four concentrations (5, 10, 15 and 20 mg l^{-1}) prepared in Milli-Q® water (Millipore, Bedford, MA). To facilitate herbicide solubility, the solutions were prepared by dilution from standards in acetone at 1 g l^{-1}, keeping the organic solvent amount always below 0.5–0.8% in volume, so that it could not have effect on adsorption [18]. The soil-solution samples were mechanically shaken end-over-end in a thermostatic chamber at 15 ± 1 °C for 12 h. In the preliminary kinetic study, the above contact time was found to be sufficient to achieve equilibrium. The soil material was separated by centrifugation at $1720 \times g$ at 15 °C for 15 min. A 1-ml portion was taken from the supernatant solution and analyzed as described below. The experiments were run in duplicate with a control of the herbicides solution without soil, to account for possible herbicide degradation or sorption onto the glass material during the process. No variation in concentrations for the control was found during the batch experiments. The amount of herbicide sorbed by the soil at equilibrium was determined as the difference between the initial and equilibrium herbicide concentrations in solution. Sorption parameters were calculated using the Freundlich equation, $X = K_f \times C_e^{1/n}$, where X (μg g^{-1}) is the amount of herbicide sorbed at the equilibrium concentration C_e (μg ml^{-1}) and K_f and $1/n$ are the Freundlich constants that describe pesticide sorption capacity and intensity, respectively. The sorption linear equation, $X = K_d \times C_e$, was employed to calculate the distribution coefficient (K_d). The OC soil sorption coefficient (K_{oc}) was calculated from the K_d values as $K_{OC} = (K_d \times 100)/OC\%$.

Sorption experiments were also carried out with non-amended and amended soil at the different stages of removal of the corresponding organic fractions (substrates h, hlip and THE), keeping the same proportion substrate:herbicide solution.

2.6. Pesticide analysis

In order to extract the pesticide from the aqueous solution, a 1-ml aliquot of the supernatant solution resulting from the sorption experiments was vortexed in a test tube with 2 ml of hexane/toluene (1/1, v/v). Phase separation was achieved by sample freezing in a conventional freezer (−18 °C) for 2 h. The organic

Table 2
Organic carbon content (g C/100 g) of non-amended soil (S) and soil amended with olive cake (SA), compost (SC) and vermicompost (SV) and of the corresponding organic fractions.

	Substrate	h[a]	hlip	THE
S	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	1.3 ± 0.2
SA	4.9 ± 0.2	4.6 ± 0.3	4.4 ± 0.3	3.2 ± 0.4
SC	3.6 ± 0.2	3.5 ± 0.2	3.5 ± 0.2	2.8 ± 0.2
SV	3.8 ± 0.2	3.7 ± 0.2	3.7 ± 0.1	2.8 ± 0.2

[a] h: substrate without water-soluble fraction; hlip: substrate without water-soluble and lipid fractions; THE: substrate without water-soluble, lipid and HA and FA fractions.

phase was transferred into a vial and analyzed. Herbicides recoveries ranged from 73.2 to 92.1%.

Samples were analyzed by gas chromatography in a Varian Star 3400 CX, equipped with a thermoionic specific detector and an 8200 automatic injector (all from Varian, Madrid, Spain). One microliter of the sample was injected in the splitless mode, using He as carrier gas, on a Hewlett-Packard Ultra 2 column (cross-linked 5% phenylmethylsilicone) (25 m, 0.32 mm i.d., 0.17 mm film thickness). Injector and detector temperatures were 280 and 300 °C, respectively. Oven temperature was programmed starting at 45 °C (1 min), then at 25 °C min^{-1} until 160 °C and at 7 °C min^{-1} until 210 °C (2 min). Under these experimental conditions the retention times were 11.6, 12.1, 13.9 and 15.0 min, for simazine, terbuthylazine, prometryn and cyanazine, respectively. Limits of detection for a herbicide standard mixture in organic solvent were 0.11 mg l^{-1} for simazine and terbuthylazine and 0.10 mg l^{-1} for prometryn and cyanazine.

3. Results and discussion

3.1. Organic matter fractions

Table 1 shows the distribution in soil and amendments of the different organic fractions. In the case of soil, there were only traces of OC in the water-soluble fraction and the lipid content was low. The OC concentration of the water-soluble fraction in olive cake was approximately two and three times higher than in compost and vermicompost, respectively. The lipid content of compost and vermicompost was negligible in comparison with that of the olive

cake (Table 1). These results agree with earlier observations [19] which indicated that the water-soluble OC sources of low molecular weight, such as monosaccharides and some lipids, are used by microorganisms in the early stages of organic matter degradation. In fact, the lipid concentration was inversely related to the compost stability and degree of maturity [20]. In the present study the decrease in lipid concentration of compost and vermicompost, with regard to olive cake, was 99.8 and 98.3%, respectively. Similar results were found by [20] after one year of olive cake composting.

To compare the effect of the humification process on soil and amendments, the HA to FA ratio (HA/FA) and the HA percentage (HA%), calculated as (HA/THE) × 100, were used. It is important to emphasize the high soil HA/FA ratio, which is pointing to a high condensation degree of its organic matter [16].

Olive cake has a larger content of FA-like substances than compost and vermicompost because it is mainly composed by acid-soluble compounds readily degradable during the transformation processes [21,22]. This decrease in FA concentration during composting and vermicomposting processes in part could be attributed to the FA transformation into complex and condensed aromatic structures more similar to the HA-like fraction. This would explain the greater values of HA/FA ratio and HA% in the stabilized amendments (compost and vermicompost) than in olive cake and suggests increase in the humification level [22]. For this study, it is worthy to point out that both HA/FA and HA% values of compost and vermicompost are similar to those of soil.

The comparison between the infrared spectra of the amendments (Fig. 3) confirms the humification pattern suggested in the previous paragraph. The infrared spectra of compost and vermicompost showed comparable patterns to those of olive cake, only differing in terms of absorption intensities. Similar results were reported by [17] when studying the organic matter transformation during composting of olive wastes. These authors concluded that extended composting time did not qualitatively modify compost composition. In our case, when the infrared absorption bands of biotransformed products are compared with those in the olive cake spectra (Fig. 3) the following observations can be made: (i) the band at 2929 cm^{-1} and the shoulder at 2092 cm^{-1}, which correspond to the symmetric and asymmetric stretching vibrations of aliphatic C–H bonds, decrease in the order A > V > C indicating the preferential degradation of alkyl structures in the biotrans-

Table 3
Sorption constants, K_f (μg$^{1-1/n}$ ml$^{1/n}$ g^{-1}) and $1/n$, for simazine, cyanazine, terbuthylazine and prometryn in non-amended soil (S) and soil amended with olive cake (SA), compost (SC) and vermicompost (SV) and these substrates without the corresponding organic fractions.

	Simazine		Cyanazine		Terbuthylazine		Prometryn	
	K_f ± sd[a]	$1/n$ ± sd[a]	K_f ± sd[a]	$1/n$ ± sd[a]	K_f ± sd[a]	$1/n$ ± sd[a]	K_f ± sd[a]	$1/n$ ± sd[a]
S	0.8 ± 0.1	0.9 ± 0.0	0.6 ± 0.1	1.0 ± 0.1	2.6 ± 0.3	0.9 ± 0.1	3.6 ± 0.0	0.7 ± 0.1
S$_h$	0.7 ± 0.1	1.0 ± 0.1	0.6 ± 0.3	1.1 ± 0.1	2.6 ± 0.5	0.9 ± 0.1	3.6 ± 0.6	0.7 ± 0.1
S$_{hlip}$	0.7 ± 0.1	0.9 ± 0.1	0.8 ± 0.2	1.0 ± 0.1	2.8 ± 0.3	0.8 ± 0.1	3.8 ± 0.3	0.7 ± 0.0
S$_{THE}$	0.5 ± 0.1	0.9 ± 0.1	0.5 ± 0.2	1.1 ± 0.1	1.9 ± 0.3	0.9 ± 0.1	1.6 ± 0.3	0.8 ± 0.1
SA	1.8 ± 0.3	0.9 ± 0.1	1.8 ± 0.3	0.9 ± 0.1	7.0 ± 1.0	0.9 ± 0.1	9.4 ± 1.7	0.8 ± 0.1
SA$_h$	1.7 ± 0.3	0.9 ± 0.1	2.1 ± 0.2	0.9 ± 0.0	10.6 ± 1.2	0.6 ± 0.1	13.5 ± 1.0	0.5 ± 0.0
SA$_{hlip}$	1.7 ± 0.3	0.9 ± 0.1	1.5 ± 0.2	1.0 ± 0.0	9.2 ± 0.4	0.8 ± 0.0	10.8 ± 0.4	0.7 ± 0.0
SA$_{THE}$	1.0 ± 0.1	1.0 ± 0.1	1.5 ± 0.2	0.9 ± 0.0	3.9 ± 0.3	0.9 ± 0.0	4.7 ± 0.3	0.8 ± 0.0
SC	1.4 ± 0.2	0.9 ± 0.1	1.7 ± 0.4	0.9 ± 0.1	5.4 ± 0.7	0.6 ± 0.1	5.8 ± 0.5	0.6 ± 0.0
SC$_h$	1.4 ± 0.3	0.9 ± 0.1	1.3 ± 0.2	0.9 ± 0.1	6.8 ± 0.5	0.5 ± 0.0	6.7 ± 0.3	0.5 ± 0.0
SC$_{hlip}$	1.8 ± 0.3	0.8 ± 0.1	1.9 ± 0.2	0.8 ± 0.0	6.9 ± 0.6	0.5 ± 0.0	7.3 ± 0.5	0.5 ± 0.0
SC$_{THE}$	1.1 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.0	3.6 ± 0.5	0.7 ± 0.1	3.1 ± 0.3	0.8 ± 0.0
SV	2.0 ± 0.5	0.8 ± 0.1	1.7 ± 0.3	0.9 ± 0.1	5.2 ± 0.6	0.7 ± 0.0	6.5 ± 0.3	0.6 ± 0.0
SV$_h$	1.4 ± 0.2	0.9 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	7.2 ± 0.7	0.6 ± 0.0	8.3 ± 0.7	0.4 ± 0.0
SV$_{hlip}$	1.0 ± 0.2	1.1 ± 0.1	1.4 ± 0.3	1.0 ± 0.1	6.4 ± 0.7	0.6 ± 0.0	7.3 ± 0.7	0.5 ± 0.0
SV$_{THE}$	1.4 ± 0.1	0.8 ± 0.1	1.0 ± 0.3	0.9 ± 0.1	3.8 ± 0.4	0.7 ± 0.0	4.9 ± 0.6	0.5 ± 0.1

h: soil without water-soluble fraction; hlip: soil without water-soluble and lipid fractions; THE: soil without water-soluble, lipid and HA and FA fractions.

[a] Standard deviation for two replicates.

Table 4
Linear sorption constant, K_d (ml g^{-1}) and organic carbon-normalised linear sorption constant, K_{oc} (ml g^{-1} C), for simazine, cyanazine, terbuthylazine and prometryn in non-amended soil (S) and soil amended with olive cake (SA), compost (SC) and vermicompost (SV) and these substrates without the corresponding organic fractions.

	Simazine		Cyanazine		Terbuthylazine		Prometryn	
	$K_d \pm sd$[a]	$K_{oc} \pm sd$[a]	$K_d \pm sd$[a]	$K_{oc} \pm sd$[a]	$K_d \pm sd$[a]	$K_{oc} \pm sd$[a]	$K_d \pm sd$[a]	$K_{oc} \pm sd$[a]
S	0.7 ± 0.0	32.3 ± 1.3	0.7 ± 0.0	31.8 ± 1.5	1.9 ± 0.0	90.8 ± 3.9	1.7 ± 0.1	77.6 ± 4.7
S$_h$	0.6 ± 0.0	29.1 ± 1.6	0.7 ± 0.0	33.2 ± 1.7	2.0 ± 0.1	93.1 ± 4.6	1.7 ± 0.1	79.6 ± 4.7
S$_{hlip}$	0.6 ± 0.0	28.7 ± 1.6	0.7 ± 0.0	31.4 ± 1.6	1.9 ± 0.0	90.5 ± 4.3	1.6 ± 0.1	75.3 ± 4.5
S$_{THE}$	0.4 ± 0.0	30.4 ± 4.3	0.6 ± 0.0	41.2 ± 5.7	1.3 ± 0.0	100.5 ± 13.7	1.1 ± 0.0	83.3 ± 11.4
SA	1.5 ± 0.0	31.3 ± 1.5	1.6 ± 0.0	32.5 ± 1.5	6.6 ± 0.1	136.4 ± 6.4	6.8 ± 0.2	141.1 ± 7.5
SA$_h$	1.5 ± 0.0	31.4 ± 1.9	1.5 ± 0.0	32.7 ± 1.9	4.6 ± 0.3	99.7 ± 7.8	5.1 ± 0.4	110.3 ± 9.8
SA$_{hlip}$	1.4 ± 0.0	31.7 ± 2.1	1.5 ± 0.0	33.7 ± 2.1	5.9 ± 0.2	133.7 ± 8.8	5.9 ± 0.2	133.9 ± 9.3
SA$_{THE}$	1.1 ± 0.0	33.8 ± 3.9	1.0 ± 0.0	31.9 ± 3.7	3.0 ± 0.1	92.4 ± 11.0	2.9 ± 0.9	90.2 ± 10.7
SC	1.2 ± 0.0	32.3 ± 1.6	1.2 ± 0.0	33.6 ± 1.8	2.5 ± 0.1	70.4 ± 4.1	2.3 ± 0.1	64.8 ± 4.1
SC$_h$	1.0 ± 0.0	29.3 ± 1.6	1.1 ± 0.0	31.0 ± 1.5	2.4 ± 0.1	67.8 ± 4.8	2.3 ± 0.1	65.8 ± 4.7
SC$_{hlip}$	1.1 ± 0.0	30.1 ± 1.6	1.2 ± 0.0	34.0 ± 1.7	2.4 ± 0.1	67.7 ± 4.8	2.4 ± 0.1	67.0 ± 4.7
SC$_{THE}$	0.7 ± 0.0	23.8 ± 2.0	0.6 ± 0.0	21.1 ± 1.8	1.8 ± 0.1	63.1 ± 5.6	1.7 ± 0.1	60.9 ± 5.2
SV	1.1 ± 0.0	30.7 ± 1.6	1.2 ± 0.0	31.5 ± 1.6	2.4 ± 0.1	64.4 ± 3.7	2.5 ± 0.1	65.6 ± 4.2
SV$_h$	1.2 ± 0.0	32.3 ± 1.1	1.4 ± 0.0	36.9 ± 1.3	2.6 ± 0.1	69.1 ± 4.3	2.5 ± 0.2	67.5 ± 5.0
SV$_{hlip}$	1.2 ± 0.0	33.1 ± 1.3	1.2 ± 0.0	34.8 ± 1.5	2.6 ± 0.1	71.0 ± 4.0	2.4 ± 0.1	65.2 ± 4.1
SV$_{THE}$	0.9 ± 0.0	32.7 ± 2.1	0.8 ± 0.0	29.1 ± 2.1	1.8 ± 0.1	66.5 ± 4.7	1.6 ± 0.1	56.6 ± 5.0

h: soil without water-soluble fraction; hlip: soil without water-soluble and lipid fractions; THE: soil without water-soluble, lipid and HA and FA fractions.

[a] Standard deviation for two replicates. $K_{oc} = K_d$/OC content × 100.

formed products [17]: (ii) the band at 1733 cm^{-1}, which decreases following the same trend, could correspond to the breakdown of esters resulting in the release of alkyl material and the persistence of free carboxyl groups [17]. The intensity of the band close to 1541 cm^{-1} (resolution-enhanced spectra), attributed to amide II, N–H bending vibration, decreases in the composted and vermicomposted materials suggesting degradation of peptide structures during the transformation and maturation processes [17]. To evaluate the aromaticity of the different amendments, the intensity ratio between the bands with maximum at 1510 cm^{-1} (resolution-enhanced spectra) (stretching vibrations of aromatic C=C bonds) and the band between 2933 and 2929 cm^{-1}, for the different amendments (stretching vibrations of aliphatic C–H bonds) can be used. This ratio was 1.09, 1.33 and 1.38 for olive cake, vermicompost and compost, respectively which confirms the increased aromaticity of the biotransformed products.

3.2. Sorption study

It is important to point out that during the extraction processes of the different organic fractions, some modifications in the substrate properties may occur which could lead to changes in herbicide sorption. For instance, the Soxhlet extraction of the lipid fraction could cause some decrease in the specific soil surface as has been previously reported in soil autoclaving as a consequence of temperature increase [23]. Besides, the extraction with alkaline solutions also removes soil polyvalent cations that could act as bridges between organic and mineral fractions, affecting competition for the active sorption sites in soil. Under alkaline conditions, autooxidation of some labile organic constituents is also possible [16]. Other chemical changes which could occur in alkaline solution, include condensation between aminoacids and the C=O group of aromatic aldehydes or quinones to form humic-type compounds through browning reactions [16]. Therefore, the study of sorption on the different organic fractions has to be considered carefully but as a valuable tool for understanding the mechanisms affecting the sorption of herbicides in soil, illustrating the role of individual constituents of the heterogeneous substrates.

Table 2 shows the OC content and Table 3 the Freundlich sorption parameters (K_f, and $1/n$) ($R^2 \geq 0.89$) for both non-amended and amended soils, as well as all the substrates without the different organic fractions. The K_f con-

stant in all the substrates studied increases in the order cyanazine $\leq$ simazine < terbuthylazine < prometryn, following the same trend as log K_{ow} values (Fig. 1). So, the most hydrophobic triazines, terbuthylazine and prometryn, were more sorbed than the less hydrophobic ones, simazine and cyanazine, a fact that agrees with previous triazine sorption results [24,25].

In general, $1/n$ values showed certain lack of linearity corresponding to both, the soil and amendments heterogeneity, the diversity of sorption mechanisms [26] and the possible occurrence of competition mechanisms between the different pesticides used [24]. The non-linearity grade was less pronounced for simazine and cyanazine, the less sorbed pesticides. For these triazines, experimental sorption data was well explained ($R^2 \geq 0.89$ for all the substrates) by linear sorption equation. By contrast, terbuthylazine and prometryn sorption isotherms presented higher non-linearity grade as indicated by the lower $1/n$ values, e.g., in the case of soil amended with compost (SC) and vermicompost (SV), as well as these substrates without the different organic fractions (SC$_h$, SC$_{hlip}$, SC$_{THE}$, SV$_h$, SV$_{hlip}$ and SV$_{THE}$). The sorption capacity of these substrates for terbuthylazine and prometryn decreases when the triazine concentration increases.

No relationship between the non-linearity grade and the nature of the substrate or herbicide was found. However, $1/n$ value is an important factor to take into account since the sorption phenomenon is strongly influenced by this parameter and the solute concentration. So, when $1/n$ values are significantly different, as observed in this study, the use of the Freundlich constant, K_f, as a measure of the sorption extent for different substrates and pesticides could lead to wrong conclusions. Some authors avoid the $1/n$ effect using, for comparative purpose, the sorbed pesticide amount at an equilibrium concentration, calculated from the Freundlich equation [27]. Alternatively, the linear sorption constant, K_d is also considered a good approach and will be used from now on to compare sorption isotherms with different $1/n$ values (Table 4).

3.2.1. Non-amended soil

Values of K_d for all herbicides decreased with the OC content (Table 4). No significant ($P > 0.05$) differences were observed in K_d for all pesticides in S, S$_h$ and S$_{hlip}$ since the concentration of both fractions, water-soluble and lipid fractions, in non-amended soil was very low. Thus, only the extraction of HA and FA fractions (S$_{THE}$), which removed around 37% of the total soil OC (Table 2), led

to a slight decrease in herbicide sorption. In general the removal of the different fractions did not significantly modify the sorption values normalised to the OC content (K_{oc} values) as can be seen in Table 4. These results confirm the outstanding role of OC in the sorption of triazine herbicides [10].

3.2.2. Soil amended with raw olive cake

The raw olive cake was the amendment that caused the highest increase in triazines sorption (Table 3). In soil amended with this product it is necessary to distinguish between two sorption patterns related with the hydrophobicity of pesticides. The more polar pesticides, simazine and cyanazine, showed the same behaviour as described for the soil, i.e., the K_d values decreased only when the HA and FA were removed (SA$_{THE}$) (Table 4). The removal of the water-soluble and lipid fractions did not modify the sorption values for both herbicides in spite of the considerable concentration of these two fractions in olive cake (Table 1). Besides, the removal of the different extractable organic fractions did not cause significant differences ($P > 0.05$) between the K_{oc} values of simazine and cyanazine in the different substrates (SA, SA$_h$, SA$_{hlip}$, SA$_{THE}$) (Table 4). For both herbicides the correlation coefficients between OC content and sorbed herbicide amount was higher than 0.98.

In contrast, K_d values for terbuthylazine and prometryn were poorly correlated with OC content of the substrates. The sorption capacity of SA for the comparatively more hydrophobic triazines, terbuthylazine and prometryn, were significantly ($P < 0.05$) reduced with the removal of the water-soluble fraction (30% less when compared with SA) (Table 4) indicating that this fraction from olive cake conferred greater sorption ability to the substrates. This assumption is supported by the decrease in $1/n$ values when soluble OC is removed (SA and SA$_h$ in Table 3) which indicates less available active sites for triazines sorption [28]. However, the removal of lipids (SA$_{hlip}$) induced an increase in the K_d values of terbuthylazine and prometryn with regard to SA$_h$ (Table 4). This suggests that the olive cake lipid could hamper the sorption of these triazine herbicides and its removal would probably lead to increased availability of sorption sites in the remaining organic and/or mineral fractions [29]. Finally, the extraction of HA and FA fractions (SA$_{THE}$) significantly decreased the K_d values of terbuthylazine and prometryn (Table 4).

When K_{oc} values for terbuthylazine and prometryn in soil amended with olive cake were analyzed, no significant differences ($P > 0.05$) were found between SA and SA$_{hlip}$, which were significantly higher than SA$_h$ and SA$_{THE}$ (Table 4). The removal of the water-soluble fraction was responsible for a decrease in K_{oc} values of 20% for terbuthylazine and 28% for prometryn, with regard to SA.

The above results suggest preference of all the triazines for macromolecular structures like those in HA and FA fractions, where diffusion phenomena [10] and macromolecular encapsulation processes could occur to a large extent. From this viewpoint, the low molecular weight fractions, even assuming their possible high reactivity and mobility, do not have a similar bearing on the sorption of the triazines of different hydrophobicity even in the case of the raw olive cake where extractive compounds (soluble organic matter and lipids) amount to more than 20% of the total OC.

The water-soluble fraction from raw olive cake favoured the sorption of terbuthylazine and prometryn. This fraction in solution could form stable solution-phase complexes with the pesticide molecules or compete with pesticides for sorption sites inducing a herbicide sorption reduction [8,30]. But the water-soluble fraction could also sorb on soil particles thus enhancing pesticides sorption by providing additional sorption sites as reported for triazines for dissolved organic matter [4,24]. In that instance, sorption of part of water-soluble compounds of olive cake on soil could modify the hydrophobic–hydrophilic characteristics of the soil surfaces, increasing the number of sorption sites for the more hydrophobic

herbicides like terbuthylazine and prometryn and decreasing [8] or not modifying [31] the number of sorption sites available for the hydrophilic ones, like simazine and cyanazine. Moreover the water-soluble molecules could also act as bridges between the organic matter and the mineral fraction (mainly in the presence of Ca^{2+}, Fe^{3+}, Al^{3+} and Mg^{2+}) or could be involved in active chemisorption processes (i.e., those promoted by charge-transfer or free-radical condensation with aminoacids or phenols).

On the other hand, the increase of K_{oc} values for terbuthylazine and prometryn when the HA-like and FA-like fractions were extracted could be explained because the removal of these organic components would increase the number of sorption sites in the sorbate surface from the remaining humin fraction and/or from inorganic components as the increase in $1/n$ values indicates (Table 3). Linear regression analysis showed that HA and humin were the most important organic fractions explaining the sorption of atrazine [32]. On the contrary, other soil components, such as clay, silt and Al, Mg and Fe oxides, which usually play a minor role [32], may be of increasing importance in soils with high clay/OC content proportion [33,34].

3.2.3. Soil amended with compost and vermicompost

Tables 3 and 4 show that the addition of amendments to soil increased its sorption capacity for the triazines.

In soil amended with compost and vermicompost the sorption of triazines showed similar patterns to that observed in non-amended soil, i.e., a decrease of K_d values after the removal of OC (Table 4), with correlation coefficients between the OC content and K_d ranging from 0.90 to 0.99 ($0.008 < P < 0.07$) for all herbicides. The maturity of compost and vermicompost seems to be comparable to that of soil organic matter (Table 1). Nevertheless, the similar results could also be due to the fact that triazine sorption did not take advantage from the characteristic features associated to highly stabilized soil organic matter (i.e., aromaticity and high carboxyl content). If the sorptive processes were favoured by a low-condensed, flexible macromolecular structure in the case of triazines [35] this fact could be pointing out to prevalence of encapsulation and diffusion processes not necessarily requiring specific surface properties.

On the other hand, no significant differences were observed ($P > 0.05$) in K_{oc} values between soil amended with compost and vermicompost and the corresponding substrates without the different organic fractions (Table 4). This fact indicates that, in the soil amended with compost and vermicompost, the amount of OC was the major factor in triazine sorption, so the presumably efficient interactions between humic substances of compost and vermicompost and the mineral and/or humic soil fractions could also limit the active sites available for further interactions with herbicides.

These results should be interpreted with caution since they show that the whole sorption process depends on complex interactions between different organic fractions leading to relevant emergent properties probably related to active organo–mineral interactions. Additional studies should be carried out to establish the interactions between the exogenous organic fractions and the organic and mineral soil components, as well as to identify the physico-chemical mechanisms with a bearing on the sorption processes.

4. Conclusion

In non-amended soil, HA and FA are the main fractions involved in triazine sorption. On the other hand, the addition of olive-cake-derived amendments to soil increased its sorption capacity for all triazine herbicides studied. However, in soil amended with compost and vermicompost, this increase was only related with their

greater OC content whereas no differences in terms of the organic matter quality were observed. In contrast, in soil amended with raw olive cake, the high concentration of water-soluble fraction was associated to enhanced sorption of terbuthylazine and prometryn. Therefore, the highest content of this fraction just in olive cake in comparison with the soil and the other two amendments could be the reason of the greater sorption of both triazine compounds in soil amended with olive cake as regards the other substrates. On the contrary, the lipid fraction of olive cake to some extent hampers the sorption of the herbicides terbuthylazine and prometryn.

Acknowledgements

References

[1] G.M. Clark, D.A. Goolsby, Occurrence and load of selected herbicides and metabolites in the lower Mississippi river, Sci. Total Environ. 248 (2000) 101–113.
[2] H. Blanchoud, E. Moreau-Guigon, F. Farrugia, M. Chevreuil, J.M. Mouchel, Contribution by urban and agricultural pesticide uses to water contamination at the scale of the Marne watershed, Sci. Total Environ. 375 (2007) 168–179.
[3] R. Ahmad, R.S. Kookana, A.M. Alston, J.O. Skjemstad, The nature of soil organic matter affects sorption of pesticides. 1. Relationships with carbon chemistry as determined by C CPMAS NMR spectroscopy, Environ. Sci. Technol. 35 (2001) 878–884.
[4] R. Celis, E. Barriuso, S. Houot, Sorption and desorption of atrazine by sludge-amended soil: dissolved organic matter effects, J. Environ. Qual. 27 (1998) 1348–1356.
[5] G. Briceño, R. Demanet, M. de la Luz Mora, G. Palma, Effect of liquid cow manure on andisol properties and atrazine adsorption, J. Environ. Qual. 37 (2008) 1519–1526.
[6] N. Senesi, Composted material as organic fertilizers, Sci. Total Environ. 81/82 (1989) 521–542.
[7] S.M. Tiquia, Microbiological parameters as indicators of compost maturity, J. Appl. Microbiol. 99 (2005) 816–828.
[8] E. Barriuso, U. Baer, R. Calvet, Dissolved organic matter and adsorption-desorption of dimefuron, atrazine and carbetamide by soils, J. Environ. Qual. 21 (1992) 359–367.
[9] A. Albarrán, R. Celis, M.C. Hermosín, A. López-Piñeiro, J. Cornejo, Behaviour of simazine in soil amended with the final residue of the olive-oil extraction process, Chemosphere 54 (2004) 717–724.
[10] L. Delgado-Moreno, G. Almendros, A. Peña, Fresh or incubated by-products of the olive culture as soil amendments: effect on triazine herbicides sorption–desorption, J. Agr. Food Chem. 55 (2007) 836–843.
[11] L. Cox, R. Celis, M.C. Hermosín, J. Cornejo, A. Zsolnay, K. Zeller, Effect of organic amendments on herbicide sorption as related to the nature of the dissolved organic matter, Environ. Sci. Technol. 34 (2000) 4600–4605.
[12] C.D.S. Tomlin, The Pesticide Manual, 13th ed., British Crop Protection Council, Alton, Hampshire, UK, 2003.
[13] R. Nogales, E. Benítez, Effect of olive-derived organic amendments on lead, zinc and biochemical parameters of an artificially contaminated soil, Commun. Soil Sci. Plant Anal. 38 (2007) 795–811.
[14] MAPA, Métodos Oficiales de Análisis (Tomo III), Ministerio de Agricultura Pesca y Alimentación, Dirección General de Política Alimentaria, Madrid, 1986.
[15] G. Almendros, J. Sanz, A structural study of alkyl polymers in soil after perborate degradation of humin, Geoderma 53 (1992) 79–95.
[16] F.J. Stevenson, Humus Chemistry: Genesis, Composition, Reactions, John Wiley, New York, 1982.
[17] G. Ait Baddi, J.A. Alburquerque, J. Gonzálvez, J. Cegarra, M. Hafidi, Chemical and spectroscopic analyses of organic matter transformations during composting of olive mill wastes, Int. Biodeter. Biodegr. 54 (2004) 39–44.
[18] S.Y. Lee, S.J. Kim, S.Y. Chung, C.H. Jeong, Sorption of hydrophobic organic compounds onto organoclays, Chemosphere 55 (2004) 781–785.
[19] A. Saviozzi, R. Cardelli, R. Levi-Minzi, R. Riffaldi, Evolution of biochemical parameters during composting of urban wastes, Compost Sci. Util. 12 (2004) 153–160.
[20] H. Dinel, M. Schnitzer, S. Dumontet, Compost maturity: extractable lipids as indicators of organic matter stability, Compost Sci. Util. 4 (1996) 6–12.
[21] C. Paredes, A. Roig, M.P. Bernal, M.A. Sánchez-Monedero, J. Cegarra, Evolution of organic matter and nitrogen during co-composting of olive mill wastewater with solid organic wastes, Biol. Fertil. Soils 32 (2000) 222–227.
[22] A. García-Gómez, M.P. Bernal, A. Roig, Organic matter fractions involved in degradation and humification processes during composting, Compost Sci. Util. 13 (2005) 127–135.
[23] D.C. Wolf, T.H. Dao, H.D. Scott, T.L. Lavy, Influence of sterilization methods on soil microbiological, physical, and chemical properties, J. Environ. Qual. 18 (1989) 39–44.
[24] D. Businelli, Pig slurry amendment and herbicide coapplication effects on s-triazine mobility in soil: an adsorption–desorption study, J. Environ. Qual. 26 (1997) 102–108.
[25] C. Sluszny, E.R. Graber, Z. Gerstl, Sorption of s-triazine herbicides in organic matter amended soils: fresh and incubated systems, Water Air Soil Pollut. 115 (1999) 395–410.
[26] L. Cox, A. Cecchi, R. Celis, M.C. Hermosín, W.C. Koskinen, J. Cornejo, Effect of exogenous carbon on movement of simazine and 2,4-D in soils, Soil Sci. Soc. Am. J. 65 (2001) 1688–1695.
[27] L. Sánchez, E. Romero, F. Sánchez-Rasero, G. Dios, A. Peña, Enhanced soil sorption of methidathion using sewage sludge and surfactants, Pest. Manage. Sci. 59 (2003) 857–864.
[28] C.H. Giles, T.H. McEwan, S.N. Nakhwa, D. Smith, Studies in adsorption. Part XI. A system of classification of solution adsorption isotherms, and its use in diagnosis of adsorption mechanisms and in measurement of specific surface areas of solids, J. Chem. Soc. 3 (1960) 3973–3993.
[29] L. Cox, P. Velarde, A. Cabrera, M.C. Hermosín, J. Cornejo, Dissolved organic carbon interactions with sorption and leaching of diuron in organic-amended soils, Eur. J. Soil Sci. 58 (2007) 714–721.
[30] E.R. Graber, I. Dror, F.C. Bercovich, M. Rosner, Enhanced transport of pesticides in a field trial with treated sewage sludge, Chemosphere 44 (2001) 805–811.
[31] T. Ilani, E. Schulz, B. Chefetz, Interactions of organic compounds with wastewater dissolved organic matter: role of hydrophobic fractions, J. Environ. Qual. 34 (2005) 552–562.
[32] A.B. Payá-Pérez, A. Cortés, M.N. Sala, B. Larsen, Organic matter fractions controlling the sorption of atrazine in sandy soils, Chemosphere 25 (1992) 887–898.
[33] T. Grundl, G. Small, Mineral contributions to atrazine and alachlor sorption in soil mixtures of variable organic carbon and clay content, J. Contam. Hydrol. 14 (1993) 117–128.
[34] G. Sheng, C.T. Johnston, B.J. Teppen, S.A. Boyd, Potential contributions of smectite clays and organic matter to pesticide retention in soils, J. Agr. Food Chem. 49 (2001) 2899–2907.
[35] G. Almendros, Sorptive interactions of pesticides in soils treated with modified humic acids, Eur. J. Soil Sci. 46 (1995) 287–301.