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Organic Amendments from Olive Cake as a Strategy To Modify the Degradation of Sulfonylurea Herbicides in Soil

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Amending soil with products rich in organic matter, such as raw olive cake or alperujo and its compost and vermicompost, could be a simple bioremediation strategy for soil pollutants such as pesticides. To investigate this hypothesis in relation to sulfonylurea herbicides, these amendments were applied to a Mediterranean agricultural soil at rates 4 times higher than agronomical dosage to stimulate biodegradation of chlorsulfuron, prosulfuron, and bensulfuron, added in a mixture to the soils. Degradation studies were conducted in microbially active and sterile soils to check the importance of biological and chemical degradation of sulfonylurea herbicides in nonamended and amended soil. The addition of alperujo stimulated soil microbial activity, as determined by dehydrogenase activity measurements, but it did not enhance the degradation of the sulfonylurea herbicides. In contrast, compost and vermicompost slightly favored the biological degradation of bensulfuron during the first week of incubation. Chlorsulfuron and prosulfuron were mainly degraded by chemical pathways in all substrates, which is probably due to a competitive or inhibitory phenomenon observed between chlorsulfuron and bensulfuron. The first-order kinetic equation satisfactorily explained the experimental data for chlorsulfuron and prosulfuron; however, a biphasic model, such as that proposed by Hoerl, better predicted the results obtained for bensulfuron.

KEYWORDS: Compost; vermicompost; bioremediation; chlorsulfuron; bensulfuron-methyl; prosulfuron

INTRODUCTION

The application of herbicides is an integral and economically essential method of modern agricultural practice. Despite the beneficial impact of herbicides on agricultural productivity, the soil exposure to potential contaminants, such as herbicides, represents a considerable side effect of agricultural practice. Chlorsulfuron, prosulfuron, and bensulfuron are sulfonylurea herbicides. These compounds are weak acids ($pK_a = 3.6–5.2$), and their solubilities in water, which vary with pH, range from 120 to 31800 mg L^{-1} (*1*). These herbicides are applied at very low application rates ranging from 9 to 100 g ha^{-1}. They possess unprecedented herbicidal activity, and susceptible crops, such as legumes and pastures, are highly sensitive to trace level residues of these herbicides in the soil (*2*). Therefore, the persistence of these herbicides could affect the performance of subsequent crops. Moreover, residues have been found in surface water and groundwater (*3*).

The need to protect the environment and to preserve the water quality, especially in semiarid and arid regions, has increased the search for new methods to prevent or remediate the pollution by pesticides. In that instance, increased interest has recently been given to the consequences of application of exogenous organic matter on the behavior of pesticides in soil. Although some effort has been recently devoted to the modification of

soil sorption/desorption or leaching processes of contaminants by the addition of organic amendments (*4, 5*), less information can be found on how these amendments affect their degradation. Some research has indicated that the addition of exogenous amendments would increase pesticide degradation rates due to the introduction of non-indigenous microbial population that stimulate biodegradation (*6*). Additional research finds that pesticides are less available for degradation because the addition of exogenous organic matter promotes their sorption (*7, 8*). Finally, it has also been indicated that the microbial population tends to degrade more rapidly the more labile organic fractions from the amendments than the target pesticides (*4, 8*). Also, the type and origin of the amendment have a great influence on pesticide degradation (*7, 9, 10*).

Olive oil production is an important industry in the Mediterranean countries and generates huge amounts of waste, the disposal of which is regarded as a serious environmental issue (*11*). The main waste from the olive oil extraction in the "continuous centrifugation two-phase process" is an olive cake (or alperujo) (*12*). This waste can be reused without treatment or after its biostabilization and maturation, via composting or vermicomposting, by addition to soils in remediation techniques or for agricultural purposes. In that instance, Semple et al. (*13*) consider the addition of organic matter to soil as a strategy of bioremediation dependent on the catabolic activity of soil microbial population. These authors distinguish two processes of bioremediation: (i) the transformation and humification of

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fresh organic matter in soil as occurs with alperujo and (ii) the addition of composted or vermicomposted organic matter to soil. The bioremediation strategies are, however, affected by different factors, such as chemical properties of pesticides, soil pH, temperature, water content, and composition of the organic and mineral fractions (*14–16*), but few studies have focused on the interactive effects of joint herbicide applications.

The aim of this work was to examine the influence of alperujo and its bioproducts, compost and vermicompost, on the degradation of chlorsulfuron, prosulfuron, and bensulfuron added together to a typical soil of the Mediterranean region. These results will be related with microbial activity assessed by measurements of dehydrogenase activity (DHA). The degradation of chlorsulfuron when applied either individually or in conjunction with prosulfuron and bensulfuron was also studied.

MATERIALS AND METHODS

Soil and Organic Amendments. A soil from the plain of Granada (southeastern Spain) (S) was sampled from the upper layer (0–25 cm) and passed through a 2 mm sieve. It is a calcareous silt loam soil (31% sand; 58% silt; 11% clay) (Typical xerofluvent), with 12.1 g kg^{-1} organic carbon (OC) content, pH 8.1 (5:20 soil/water ratio) and 21% water-holding capacity (WHC) (0.33 bar). The particle-size distribution was determined by sieving and sedimentation, applying Robinson's pipet method (*17*) after organic matter had been removed with H$_2$O$_2$ using sodium hexametaphosphate as a dispersing agent. OC content was determined by oxidation with K$_2$Cr$_2$O$_7$ (*18*). Amendments were also air-dried and passed through a 2 mm sieve. The amendments used were obtained from olive residues and consisted of alperujo (A) from a two-stage centrifugation process and compost (C) and vermicompost (V) of alperujo. The alperujo was obtained from an olive oil industry (Romeroliva, Deifontes, Granada). The mature compost, supplied by Sociedad Cooperativa Sierra de Génave (Jaén, Spain), was prepared from a mixture of raw alperujo, olive leaves, and manure at a ratio of 7:4:1 (dw), to form a compost pile of 40 m³. The compost pile was mechanically turned once a month during 6 months. The material was then left undisturbed for a further 3 month maturation period. Finally, the vermicompost was produced at the Estación Experimental del Zaidín. In the latter case, a mixture of alperujo and residual biosolids (8:1 w/w) was vermicomposted during 6 months (*19*), using clitellated and non-clitellated worms of *Eisenia andrei*.

An amendment dosage equivalent to 200 t ha^{-1} was applied to the soil. This dosage, 4 times higher than the usual field dosage, was applied with bioremediation purposes to stimulate the sulfonylurea degradation. The OC contents of soil amended with alperujo, compost, and vermicompost (*18*) were 48.5, 35.9, and 37.5 g kg^{-1}, respectively and the corresponding pH (5:20 ratio) values were 7.3, 8.2, and 8.0.

Autoclaving was used to sterilize soil and amendments by heating under pressure for 1 h each day for three consecutive days. To verify the effectiveness of this sterilization method, 100 μL of soil and amendment suspensions was pipetted into Petri dishes containing 15 mL of agar–water (10%). The substrate suspension was spread over the surface, and the dishes were incubated at 22–25 °C for 2 months. During this incubation time no bacterial colonies or fungi were observed.

Herbicides. The selected sulfonylurea herbicides, chlorsulfuron (100% pure, a gift from DuPont, Wilmington, DE), bensulfuron-methyl, and prosulfuron (Dr. Ehrenstofer, Germany, 98.5 and 99% purities, respectively), are weak acids with pK_a values of 3.6, 5.2, and 3.76, respectively (*1*). Their physicochemical properties are markedly affected by pH. Their water solubilities at pH 7 for bensulfuron, chlorsulfuron, and prosulfuron are 0.1, 31.8, and 4 g L^{-1}, and the log partition coefficients octanol/water (log K_{ow}) at the same pH are 0.62, −0.99, and −0.21, respectively (*1*).

Persistence Studies. Nonamended and amended soil (1 kg) was preincubated for 3 days with a moisture level equivalent to 40% of soil water-holding capacity at 15 °C to stimulate the microbial activity. Subsequently, a solution containing a mixture of the herbicides in Milli-Q water (Millipore, Bedford, MA) was incorporated into nona-

mended and amended soil to give a nominal initial concentration of 1.0 μg g^{-1} (dry weight) for each sulfonylurea herbicide. Further water was added to adjust the soil moisture content to 70% of water-holding capacity. Each sample was thoroughly mixed by passing it several times through a 2 mm sieve. The soil samples were split into two aliquots of 500 mg each, which were transferred to loosely capped glass containers. All of the soil–herbicide mixtures were incubated at 15 °C, and soil moisture contents were maintained by periodic additions of Milli-Q water followed by vigorous shaking. All treatments were sampled immediately after preparation and then at intervals during the subsequent 98 days. The degradation studies were also carried out in sterile nonamended and amended soils. For nonamended and compost-amended soil, independent persistence studies were carried out with chlorsulfuron, as described above. In that case, the incubation time was 60 days.

The analysis of sulfonylurea residues was carried out by ultrasound-assisted extraction. Soil samples (10 g) were extracted, per duplicate, with 30 mL of residue analysis grade acetonitrile (Merck, Darmstadt, Germany) for 15 min. Then the samples were centrifuged at 3000 rpm and 15 °C for 15 min, and the supernatant was filtered. Finally, the extract was concentrated in a rotary evaporator at 40 °C. The dry residue was dissolved with 2 mL of acetonitrile/Milli-Q water (40:60). Recoveries ranged between 73 and 95%, depending on the herbicide and the substrate.

Analytical Procedure. The samples were filtered by 0.45 μm HV filters (Millipore, Bedford, MA) before analysis. Sulfonylurea herbicides were analyzed by HPLC-DAD (series 1100, Agilent) on a Zorbax C8 column (5 μm, 150 × 2.1 cm) connected to an Eclipse XOB-C8 (2.1 × 12.5 mm) precolumn (Agilent). The mobile phase was a 1:1 mixture of acetonitrile and 0.1% trifluoroacetic acid brought to pH 3.0 with triethylamine. The flow was set at 0.2 mL min^{-1}, the injection volume to 20 μL, the oven temperature to 40 °C, and the detector wavelengths to 237 nm for bensulfuron and to 225 nm for chlorsulfuron and prosulfuron. Retention times were 6.2, 9.7, and 14.5 min for chlorsulfuron, bensulfuron, and prosulfuron, respectively.

Dehydrogenase Activity. For the determination of the DHA, aliquots of nonamended or amended microbially active soil samples (1 g) were removed at different incubation times and incubated, in triplicate, during 20 h at 25 °C with 0.2 mL of 0.4% 2-p-iodophenyl-3 p-nitrophenyl-5 tetrazolium chloride (INT) as a substrate. The iodonitrotetrazolium formazan (INTF) produced in the reduction of INT was extracted with a mixture of acetone/tetrachloroethylene (2:3) by shaking and measured in a spectrophotometer at 490 nm.

Mathematical Equations. The simple first-order equation ($C = C_0 \times e^{-kt}$) and two biphasic models, the Hoerl function ($C = C_0 \times e^{bt} \times t^c$) and a biexponential equation [$C = F(C_0 \times e^{-k_1 t}) + (1 - F)(C_0 \times e^{-k_2 t})$], were fitted by simple and multiple regression, respectively. In the different equations, C corresponds to the herbicide concentration at time t (days), C_0 represents the initial herbicide concentration, and k_i (day^{-1}) refers to the degradation constants. For ease of data treatment, C is expressed as percent of applied pesticide, such that $C_0 = 100$. The Hoerl function includes the parameters b (similar to k in the first-order equation) and c, which is a measurement of the deviation from the exponential behavior (*20*). Finally, in the biexponential equation, F is lower than one and represents the partitioning coefficients between pools 1 and 2. k_1 and k_2 are the rate constants for the two pools.

Statistical Analysis. The degradation curves were compared among them with the method of regression lines comparison using the Statgraphics Plus 5.1 statistical software (Statistical Graphics Corp., Princeton, NJ).

RESULTS AND DISCUSSION

Remaining concentration values of sulfonylurea herbicides in microbially active and sterile substrates were fitted to first-order kinetics (**Table 1**), with regression coefficients in general >0.89 and highly significant ($P < 0.01$), thus indicating that the assumption of first-order kinetics was acceptable.

Degradation in Microbially Active and Sterile Nonamended Soil. In microbially active nonamended soil the

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Table 1. Kinetic Parameters for the Degradation of Bensulfuron, Chlorsulfuron, and Prosulfuron in Nonamended Soil (S) and Soil Amended with Alperujo (SA), Compost (SC), and Vermicompost (SV)

| | simple first order equation | | | | |
	$C_0 \pm SD^a$ (%)	$k^b \times 10^2 \pm SD$ (day^{-1})	$t_{1/2}$ (days)	R^2	DT_{50}^c
bensulfuron					
S	85.91 ± 7.30	1.96 ± 0.08a	35.40	0.95	18.53
SA	85.71 ± 8.93	2.23 ± 0.08a	31.05	0.96	24.71
SC	71.73 ± 12.43	2.57 ± 0.15b	26.90	0.90	16.19
SV	76.96 ± 10.22	2.72 ± 0.11b	25.44	0.95	13.63
S$_{sterile}$	90.74 ± 7.33	1.42 ± 0.07	48.62	0.95	37.55
SA$_{sterile}$	90.18 ± 12.75	1.23 ± 0.13	54.34	0.85	46.18
SC$_{sterile}$	94.05 ± 7.35	1.15 ± 0.06	60.01	0.95	58.95
SV$_{sterile}$	87.86 ± 7.40	1.37 ± 0.07	50.67	0.95	41.12
chlorsulfuron					
S	106.79 ± 5.81	0.76 ± 0.05a	91.14	0.94	97.18
SA	89.42 ± 3.19	0.83 ± 0.03a	83.10	0.96	83.72
SC	95.11 ± 5.18	0.74 ± 0.05a	93.71	0.90	92.94
SV	100.25 ± 5.28	0.84 ± 0.05a	82.63	0.93	79.81
S$_{sterile}$	90.74 ± 7.33	0.89 ± 0.08^d	77.99	0.89	72.81
SA$_{sterile}$	95.48 ± 7.01	0.98 ± 0.06^d	70.64	0.92	61.65
SC$_{sterile}$	100.40 ± 5.02	0.73 ± 0.03^d	94.62	0.95	95.48
SV$_{sterile}$	98.10 ± 5.56	0.92 ± 0.05^d	74.90	0.95	80.27
prosulfuron					
S	101.88 ± 5.07	0.82 ± 0.05a	84.38	0.94	83.61
SA	98.25 ± 5.64	0.80 ± 0.05a	86.24	0.93	90.46
SC	97.55 ± 2.43	0.72 ± 0.02a	95.56	0.98	91.19
SV	109.57 ± 6.32	0.95 ± 0.06a	72.67	0.96	87.60
S$_{sterile}$	97.35 ± 4.44	0.93 ± 0.05^d	74.21	0.96	65.32
SA$_{sterile}$	100.33 ± 5.82	0.91 ± 0.06^d	76.20	0.93	76.64
SC$_{sterile}$	97.74 ± 6.08	0.66 ± 0.05^d	105.02	0.90	92.21
SV$_{sterile}$	93.19 ± 5.15	0.80 ± 0.04^d	86.13	0.94	>98

^{a} Standard deviation ($n = 2$). ^{b} k values followed by the same letter show no significant differences ($P > 0.05$) among the microbially active substrates. ^{c} Graphically derived by interpolating the values between successive measurements. ^{d} No significant differences ($P > 0.05$) were found between the sterile and the corresponding microbially active substrates.

degradation rate of bensulfuron was greater than those of chlorsulfuron and prosulfuron (**Table 1**). At the end of the incubation time (98 days) the herbicide concentrations in S were 49, 45, and 18% of the initial concentration for chlorsulfuron, prosulfuron, and bensulfuron, respectively.

Sulfonylurea degradation occurs via microbial breakdown or chemical hydrolysis or a combination of the two and is pH and temperature sensitive. Therefore, the different degradation behaviors observed for the three sulfonylurea herbicides could be explained because (a) the biological degradation for bensulfuron was faster, (b) the biological degradation was not taking place for some herbicides, or (c) the chemical degradation for bensulfuron was faster than that for chlorsulfuron and prosulfuron.

The degradation pathways of chlorsulfuron and prosulfuron could be mainly attributed to chemical processes as shown by the absence of significant differences ($P > 0.05$) in degradation rates between microbially active and sterile soils (**Table 1**). In the case of bensulfuron, however, the biological degradation process plays an important role, the degradation rate in sterile soil being significantly ($P < 0.05$) lower than that in microbially active nonamended soil (**Table 1**).

These results are in disagreement with the ones observed in other studies. In these reports, the microbiological degradation was the main pathway of degradation for chlorsulfuron and prosulfuron in alkaline soils (*15, 21, 22*), although chlorsulfuron can also be biologically degraded in acid soils (*14*).

On the other hand, the contribution of biotic processes on bensulfuron soil degradation found in the present study is not

in accordance with the postulations of other authors (*23−25*), who considered that this herbicide was preferably degraded by chemical processes.

The greater biological degradation of bensulfuron than of chlorsulfuron and prosulfuron in our case could be explained by a phenomenon of competition among the herbicides studied or of inhibition of the chlorsulfuron and prosulfuron degradation, such as those described previously for other herbicides such as triazines (*4*) or nitroaromatic compounds (*26*). Therefore, the presence of bensulfuron as a carbon source for microorganisms could limit chlorsulfuron and prosulfuron biological degradation. The higher degradation rate for bensulfuron could be the result of an easier degradation pathway for this herbicide in comparison with chlorsulfuron and prosulfuron or its effect on the microorganisms degrading chlorsulfuron and prosulfuron specifically. The inhibition of the degradation would then depend on the herbicide itself and on the microbial communities involved in the degradation process. A previous paper on other sulfonylurea herbicides indicated that their addition as a mixture to soil did not have any effect on their degradation rate (*10*). Our results show that the degradation rate of chlorsulfuron in microbially active nonamended soil was greater when it was added to soil alone than in the presence of prosulfuron and bensulfuron (**Figure 1a**). This confirms the existence of mechanisms of competition or inhibition between bensulfuron and chlorsulfuron in this case and the preferred biological degradation of bensulfuron when applied together with chlorsulfuron and prosulfuron. Additional studies could be necessary for determining the causes of this behavior because many pesticide formulations mix several active ingredients from the same or different chemical groups. As a consequence of this phenomenon an organic compound with *a priori* low polluting characteristics could become a harmful compound in the presence of other more easily degradable compounds.

On the other hand, the main chemical reaction affecting sulfonylurea degradation in soil is the pH-dependent hydrolysis of the sulfonylurea linkage (*27*). The neutral form of sulfonylurea herbicides is at least 250−1000 times more susceptible to hydrolysis than the anionic form (*2*). In aqueous solution the hydrolysis rate of many weak acid sulfonylurea herbicides is slowed with the increase of pH (*28*) because the proportion of the anionic form of the molecule increases. A similar pH dependence of sulfonylurea degradation observed in aqueous solution has been found in soil (*16, 29*). Due to this, the greater pK_a value of bensulfuron (5.2) in relation with chlorsulfuron and prosulfuron (3.6 and 3.76, respectively) determines that a higher proportion of neutral bensulfuron will be found at the soil pH, and so this herbicide will be more easily hydrolyzed.

The hydrolysis rate of sulfonylurea herbicides in sterile soil can be compared to the hydrolysis rate in aqueous solution at the same pH. At soil pH (8.1), no relevant hydrolysis of the studied sulfonylureas was found in water (data not shown), the transformation rate being lower in solution than in soil. Similar results were found for other sulfonylureas such as metsulfuron-methyl (*30*), chlorimuron-ethyl (*2, 31*), and trisulfuron-methyl (*32*) with higher transformation rates in sterilized soils than in solution, under the same conditions. This could indicate that the soil constituents, such as mineral and organic fractions, play a catalytic role in sulfonylureas degradation (*22, 33*).

Comparison between herbicides half-lives ($t_{1/2}$) obtained in earlier studies is difficult because there were differences in the experimental setup, such as initial concentration, water content, pH, temperature, content and composition of mineral and organic matter fractions, and analytical techniques (*15, 34*). However,
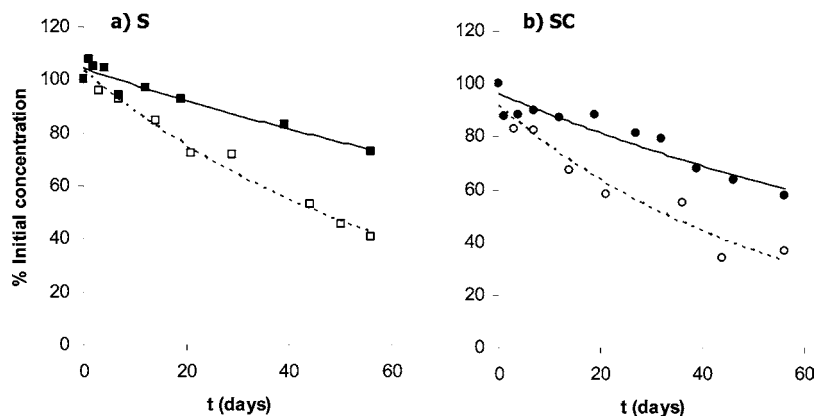
Figure 1. Degradation of chlorsulfuron in (**a**) nonamended soil (S) and (**b**) in soil amended with compost (SC), added alone (open symbol and discontinuous line) or in a mixture with bensulfuron and prosulfuron (solid symbol and continuous line).
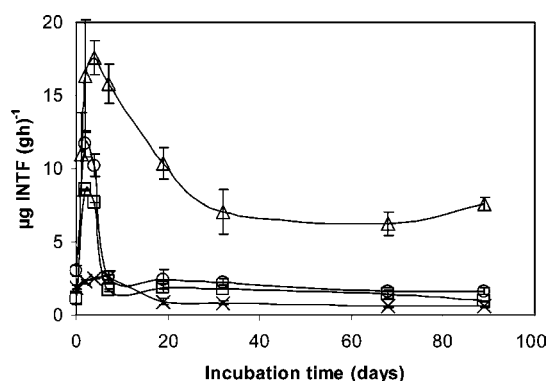

Figure 2. Dehydrogenase activity (DHA) in nonamended soil ($\times$) and in soil amended with alperujo ($\triangle$), compost ($\bigcirc$), and vermicompost ($\square$) during the incubation time. The vertical lines represent the standard deviation in each sample ($n = 3$).

Table 2. Kinetic Parameters ($\pm$ Standard Error) for the Degradation of Bensulfuron in Nonamended Soil (S) and Soil Amended with Alperujo (SA), Compost (SC), and Vermicompost (SV)

	substrate			
equation	S	SA	SC	SV
first order				
C_0	85.91 ± 7.30	85.71 ± 8.93	71.73 ± 12.43	76.96 ± 10.22
$K \times 10^2$	1.96 ± 0.08	2.23 ± 0.08	2.57 ± 0.15	2.72 ± 0.15
RSE^a	49.47	35.06	65.82	93.64
Hoerl				
C_0	88.28 ± 6.22	85.08 ± 11.31	83.24 ± 17.53	84.55 ± 14.06
$-b \times 10^2$	1.58 ± 0.08	2.25 ± 0.15	2.04 ± 0.26	2.38 ± 0.20
$-c \times 10^2$	6.64 ± 1.60	−6.15 ± 2.94	12.08 ± 4.83	7.69 ± 3.83
RSE^a	29.20	16.54	31.109	68.70
biexponential				
F	0.67 ± 0.055	0.80 ± 0.02	0.78 ± 0.03	0.81 ± 0.041
$K_1 \times 10^2$	1.60 ± 0.20	2.01 ± 0.99	3.00 ± 0.21	2.99 ± 0.31
$K_2 \times 10^2$	18.4 ± 5.01	293.40 ± 318.30	231.50 ± 213.21	476.20 ± 4169.21
RSE^a	17.86	15.09	30.06	69.81

^{a} Residual standard error.

half-lives for the three sulfonylureas considered in the present study are intermediate with respect to reported values in the literature under similar conditions, taking into account that soil pH is the major environmental determinant of both abiotic and biological processes affecting their fate (*16*). In the case of chlorsulfuron in soil $t_{1/2}$ (91 days, **Table 1**) for this herbicide oscillated between 70 and 229 days at incubation temperatures between 10 and 25 °C (*15, 21, 29, 35, 36*) for alkaline soils ranging in pH from 7.5 to 9.4. For prosulfuron, for which a half-life of 84 days was calculated, Menniti et al. (*22*) found $t_{1/2}$ values of 24 days in a soil with pH 7.9, but Hultgren et al. (*16*) reported higher $t_{1/2}$ values, 73 and 110 days, with pH soil conditions, 8.0 and 7.9, respectively, similar to the ones used in this study. The higher soil acidity and incubation temperature (30 °C) of the former authors may explain the disagreement in the data. Finally, for bensulfuron half-lives in the literature range from 1 to 3 weeks, depending on the conditions (*23−25*).

Effect of Amendments on Sulfonylurea Degradation. Sulfonylurea herbicide degradation in soil was not affected by the addition of the untreated amendment, alperujo, despite the fact that soil amended with alperujo showed the highest DHA during the incubation period (**Figure 2**). DHA is thought to reflect the total range of oxidative activity of soil microflora, and consequently it can be considered as a good indicator of microbiological activity. Alperujo stimulated microbial activity, but it did not enhance the degradation of herbicides. The microorganisms may have preferred the more easily degradable organic compounds of alperujo (*4, 8*) as a carbon and/or nitrogen source instead of the sulfonylurea herbicides.

The addition of compost and vermicompost to soil did not modify the degradation rate for chlorsulfuron and prosulfuron, because no significant differences ($P > 0.05$) were found between the k values for these herbicides in nonamended and amended soil (**Table 1**). As occurred with nonamended soil, sterile and microbially active soils amended with compost and vermicompost were not significantly different ($P > 0.05$) with regard to the k values (**Table 1**). Thus, the dominant degradation pathway of these herbicides in the amended soil corresponds to abiotic processes.

On the contrary, the k values for bensulfuron were slightly but significantly ($P < 0.05$) higher in soil amended with the biotransformed products than in nonamended soil (**Table 1**). The microbial activity of compost- and vermicompost-amended soils seems to coincide with the enhanced degradation of bensulfuron observed in these substrates. The higher DHA in soil amended with compost and vermicompost than in nonamended soil during the first week of incubation is attributed to the greater organic matter content of amended soils and to the presence of new active microbial populations introduced with the amendments (**Figure 2**), indicating their bioremediation capacity. These introduced microorganisms would be able to degrade bensulfuron rather than chlorsulfuron and prosulfuron as indicated by the comparison of k values between sterile and microbially active amended soils.

The competitive or inhibitory process was also observed in amended soil as can be seen in **Figure 1b**. Compost stimulated chlorsulfuron degradation in the microbially active soil treated
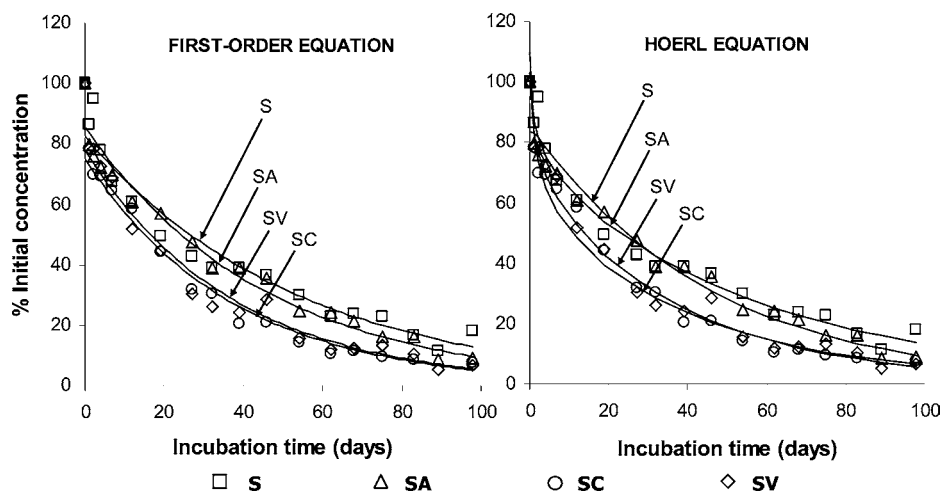
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Figure 3. Bensulfuron degradation in nonamended soil (S) and in soil amended with alperujo (SA), compost (SC), and vermicompost (SV). Experimental data were fitted to (**a**) a first-order equation and (b) the Hoerl equation.

with the herbicide alone to a greater extent than in the soil treated with the sulfonylurea mixture.

Previous studies have shown that some organic amendments promoted pesticide degradation in soil by stimulating microbial activity as has occurred with compost and vermicompost (*37*), but they can also reduce pesticide degradation (*7*). The latter has been attributed to reduced pesticide availability to microorganisms as a result of sorption to the organic amendment (*9*) or to a toxic effect of the organic amendment to the soil microbial population (*4*). Previous studies with these sulfonylurea herbicides on these substrates (*5*) have shown that bensulfuron sorption was low or moderate (K_f between 1.26 and 2.48), whereas that of chlorsulfuron and prosulfuron was negligible even in amended soil. In these instances, sorption had no important influence on herbicide degradation.

Although the first order approach produced high linear correlation coefficients, a difference between the $t_{1/2}$ and DT_{50} values was observed in all soils, especially for bensulfuron (**Table 1**). Therefore, the degradation rate was faster than predicted from the first-order kinetic fit. To explain this pattern, two more complex models, the Hoerl and biexponential equations, were applied for bensulfuron in the microbially active substrates (**Table 2**). Both models explain better the very rapid bensulfuron decay during the first 1−3 days of incubation, because they provide residual standard errors lower than the first-order kinetic equation (**Table 2**). From both models, the biexponential equation provides k_2 values with too large standard errors, making this approach inappropriate. Therefore, the Hoerl equation better predicts the bensulfuron behavior, in agreement with previous results (**Figure 3**) (*8*).

None of the equations improved any of the parameters for chlorsulfuron and prosulfuron, which confirms the simple first-order assumption as a good estimation, with good agreement between $t_{1/2}$ and DT_{50} values (**Table 1**). These results are in disagreement with other studies in which degradation kinetics for chlorsulfuron (*15*) and prosulfuron (*16*) were better fitted to a two-stage kinetic equation, which stresses the importance of microbial degradation of both herbicides when assayed alone, opposite to what happens in the present study.

Conclusion. Chlorsulfuron and prosulfuron were preferably degraded by chemical means. Thus, the addition of amendments to soil did not significantly affect their degradation. Bensulfuron was the sulfonylurea herbicide more rapidly degraded due to the importance of the microbial degradation and its higher pK_a value, which favors the chemical degradation at soil pH. The

degradation of this compound in all substrates followed a two-stage degradation process. Compost and vermicompost were the amendments that were most consistent in the stimulation of microorganisms, which enhanced bensulfuron degradation, whereas alperujo stimulated general microbial population and activity without concurrent increases in herbicide degradation, suggesting that the specific microbial populations responsible for degrading the herbicides were not stimulated. Moreover, the results of this study show the complexity of bioremediating herbicide mixtures, as well as the influence of the source and type of amendment added. Probably the presence in soil of bensulfuron limits the biological degradation of chlorsulfuron and prosulfuron.

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