Appraising factors governing sorption and dissipation of the monoterpenic carvone in agricultural soils

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ABSTRACT

The use of monoterpenes as agrochemicals has been proposed, but the behavior of this family of compounds once they reach the soil environment has not been completely examined. In this work, we investigated the sorption of the two optical isomers of the monoterpene carvone, R-carvone and S-carvone, on different soils and model sorbents, as well as their dissipation in selected soils. Sorption was a non-enantioselective process; from racemic initial solutions, R-carvone was sorbed to the same extent as S-carvone. Correlations with soil properties indicated that organic matter was the most important component determining the sorption of carvone on the soils. Accordingly, higher sorption of carvone enantiomers was measured on organic model sorbent (humic acid, \( K_d = 28 \text{ L kg}^{-1} \)) compared to mineral model sorbents (kaolinite, illite, montmorillonite, ferrihydrite, \( K_d < 6.3 \text{ L kg}^{-1} \)). Desorption from the soils was hysteretic, most likely because of the rapid degradation of the enantiomers in the soils. Dissipation of carvone in soils was microbial mediated and enantioselective, with S-carvone being degraded faster than R-carvone. The individual enantiomer dissipation rates and enantioselectivity depended on soil characteristics, such as pH. The findings of this study constitute a base for the understanding of the fate of monoterpenes in soils and for further investigations on their applicability as environmentally friendly agrochemicals.

Keywords: allelochemicals; biodegradation; chiral pesticides; soil biochemistry
1. Introduction

Modern agriculture needs to face the growing global demand for food due to the increasing world population, which is expected to reach about 9 billion in 2050 (Gerwick and Sparks, 2014). To achieve a proper yield of crop production and pest control, the intensive use of synthetic pesticides has been essential until now (Sparks et al., 2017). However, numerous undesirable side effects are derived from pesticide use, mainly related to environmental impacts resulting from water and soil contamination episodes (Cordeau et al., 2016) along with gradual pest resistance (Gerwick and Sparks, 2014). For these reasons, the legislation is becoming more restrictive and claims for new, alternative management strategies (and products) in order to pursue an environmentally sustainable agriculture (Sparks et al., 2017).

Primary and secondary metabolites produced by living cells have inspired the development of natural product-based pesticides, and have been proposed as a source of new pest control agents (Duke et al., 2000; Sparks et al., 2017). Two examples of this are the herbicide mesotrione and the fungicide azoxystrobin, based on triketone and strobilurin compounds, respectively (Gerwick and Sparks, 2014). Nevertheless, the direct use of natural products for crop protection still requires more investigation and has not been fully explored. Natural products are usually very unstable to exert their action in the environment and their structures are usually more complex than those of synthetic pesticides, factors that should be overcome to commercialize them as agrochemicals (Duke et al., 2000). Hence, a major challenge in agricultural science is to search for the ideal pesticides, that would be economically affordable and have low environmental impact while ensuring consumer safety (Cordeau et al., 2016).

Allelochemicals comprise a group of natural compounds which are responsible for the stimulatory or inhibitory effects of one organism (plants, insects, microbes, etc.) upon the growth, health, behavior, or population biology of neighboring organisms (Zeng et al., 2008). Monoterpenes, foremost constituents of essential oils, are one of the most promising families
of allelochemicals to be used for pest management (van Roon et al., 2005), since insecticidal, fungicidal, and herbicidal properties have been described for them (He et al., 2009; Inderjit et al., 1997; Marei et al., 2012; Vokou et al., 2003; Zeng et al., 2008). As an example, the monoterpenes eugenol, geraniol and thymol are currently approved in Europe as active substances (a.s.) to control botrytis infection in grapes, at application rates up to 0.26 kg a.s. ha\(^{-1}\) per treatment (EU Pesticides Database, 2018).

Carvone is a monoterpane which occurs naturally as two enantiomers, S-carvone and R-carvone (Fig. 1), and is a major constituent of the oils from caraway (S), gingergrass (both), and spearmint (R) (De Carvalho and Da Fonseca, 2006; EFSA, 2016). Both enantiomers have manifested striking pesticidal features, which potentially make carvone a target compound for being included as a new active ingredient in pesticide formulations. For example, Vokou et al. (2003) reported that both R- and S-carvone were extremely active in inhibiting seed germination of Lactuca sativa. Similarly, De Martino et al. (2010) found that R-carvone inhibited the radicle elongation for Raphanus sativus L. and Lepidium sativum L. Additionally, R-carvone has been shown to be effective against insects, and S-carvone as a fungistatic, bacteriostatic and potato sprout inhibiting compound (De Carvalho and Da Fonseca, 2006; Oosterhaven et al., 1995). In fact, the active substance S-carvone has already been authorized for use as a plant growth regulator (anti-sprouting agent) in Europe (EFSA, 2016), and R-carvone is registered as a biopesticidal active ingredient to be used in the manufacture of insect repellents in the United States (USEPA, 2009).

Recently, chirality has received progressive attention in the field of synthetic pesticides. The importance of enantiomers falls on the manifested selectivity in their responses, since one of the enantiomers usually reacts preferentially with the (chiral) host system to which it is exposed (Garrison, 2011). In addition, biodegradation of chiral pesticides is often an enantioselective process which will be influenced by soil composition, pH, redox conditions,
and microbial populations (Buerge et al., 2003; Gámiz et al., 2017; Li et al., 2016; Matallo et al., 1998). In spite that abiotic processes, such as chemical distribution or transport processes, are often non-enantioselective, they can indirectly influence the relative abundance of one enantiomer over the other in the different environmental compartments by affecting biodegradation. It has been reported that sorption maintains enantiomer soil residues more racemic by reducing their bioavailability (Celis et al., 2013; Gámiz et al., 2016a) and even that sorption can turn into enantioselective when the starting initial pesticide solution is not racemic (Celis et al., 2015; Gámiz et al., 2016b). Consequently, the assessment of the enantioselective fate of chiral pesticides in various media has become an emerging area in agricultural and environmental science (Celis et al., 2015; Gámiz et al., 2016a; Li et al., 2016), which should be extrapolated to chiral allelochemicals such as carvone.

There is very little information regarding the fate of carvone in soils and even less concerning its possible enantiomer-selective behavior. Given that carvone can exit in nature as two enantiomers, both of them with pesticidal properties, its enantioselective behavior merits attention. In fact, few studies have addressed the enantiomer-selective behavior of natural compounds in soils. Gámiz et al. (2016c) found that the sorption of racemic abscisic acid (ABA) in soils was non-enantioselective and that ABA dissipation occurred with the natural S-enantiomer being degraded faster than the unnatural R-enantiomer. Likewise, the enantiomer dissipation rates were affected by amending the soil with organoclays and biochar (Gámiz et al., 2017).

The objective of this work was to provide insight into the behavior of the two enantiomers of the monoterpene carvone in agricultural soils. Correlations between sorption and soil properties were established and several model sorbents were used to ascertain the role of mineral and organic colloidal components in carvone sorption. The dissipation of carvone enantiomers in selected soils was also investigated. The information provided should be
helpful to elucidate the behavior of this natural compound in soil and may also be valuable in the design of new carvone-based pesticide formulations intended for soil applications.

2. Materials and methods
2.1. Carvone, soils and model sorbents

Analytical standard-grade S-carvone and R-carvone with chemical purities of 98.5% and 99.9%, respectively, were purchased from Sigma-Aldrich (Spain). Carvone enantiomers are classified as monoterpenes with a molecular weight of 150.2 g mol⁻¹, a vapor pressure of 21.3 Pa at 25 °C, and a water solubility of 27 mg L⁻¹ at 20 °C (BPDB, 2018). The racemic aqueous solutions of carvone (RS-carvone) used in this work (0.1-2 mg L⁻¹) were prepared by diluting a 200 mg L⁻¹ (R+S) methanolic stock solution, which was made by adding 10 mg of each enantiomer to 100 mL of methanol.

Six agricultural soils (S1-S6) representative of Southern Spain were used in this study. Soil samples were collected from a 0-20 cm depth, air-dried, sieved to pass a 2 mm mesh, and stored at 4 °C. The soils were characterized by conventional methods described in the literature. The hydrometer method was used to determine soil texture (Gee and Bauder, 1986). The carbonate content was measured by the pressure calcimeter method and the organic carbon content by dichromate oxidation (Nelson, 1982, Nelson and Sommers, 1982). The amount of amorphous Fe-oxides was determined by extraction with oxalate (McKeague and Day, 1966). The phyllosilicate mineralogy was determined by X-ray diffraction analysis of oriented specimens of Mg²⁺- and K⁺-saturated soil clay (< 2 µm) samples, solvated with ethylene glycol and calcined at 550°C, respectively (Brown, 1961). Soil pH values were measured in 1:2.5 soil:water slurries. The main physico-chemical properties determined are compiled in Table 1. It is remarkable that the soils had relatively low organic carbon (OC) contents, as typical of Mediterranean soils (Gámiz et al., 2012), low amorphous Fe-oxide
contents (< 1%, not shown), and variable clay contents and mineralogies. Additional details on the soils used are given in Supplementary Table S1.

Several model sorbents were chosen to assess the importance of soil constituents in the sorption of carvone: three reference phyllosilicates (kaolinite, illite and montmorillonite), a poorly crystallized Fe-oxyhydroxide (ferrihydrite), and a commercial humic acid (HA). The three phyllosilicates, used as supplied by The Clay Minerals Society (Purdue University), were KGa-2 kaolinite (> 95% kaolinite, CEC = 3.3 cmol kg\(^{-1}\)), IMt-1 illite (85-90% illite, CEC = 26.6 cmol kg\(^{-1}\)) and SWy-2 montmorillonite (> 90% montmorillonite, CEC = 76.4 cmol kg\(^{-1}\)). Detailed physical and chemical data of these clays can be found in The Clay Minerals Society webpage (CMS, 2018). The Fe-oxyhydroxide (ferrihydrite) was prepared following the procedure described in Celis et al. (1997). The humic acid, used as a representative of natural organic matter, was supplied by Sigma-Aldrich (HA sodium salt, technical grade) and had 39% C and 0.68% N.

2.2. Sorption of carvone enantiomers at a single initial concentration

The batch sorption technique was used as a conventional method to assess the sorption of RS-carvone on the soils. Preliminary tests showed that carvone degraded rapidly in the tested soils, so that, to avoid degradation losses, the soils were pre-treated once with steam in an autoclave at 121 °C and 200 kPa for 20 min before using them in the sorption experiments. Triplicate 4 g of the autoclaved soil samples (S1-S6) were weighed in Pyrex\textsuperscript{®} glass screw-cap centrifuge tubes and were shaken during 24 h at 20 ± 2 °C with 8 mL of an RS-carvone aqueous solution of 2 mg L\(^{-1}\). Next, the tubes were centrifuged at 5000 rpm for 10 min and 4 mL of the supernatant solutions were removed and stabilized with methanol (50:50 v/v), to prevent the degradation of carvone before analysis. The solution concentration (C\(_e\)) of carvone enantiomers was determined using reversed-phase chiral high-performance liquid
chromatography (HPLC), after filtering the samples with Acrodisc® syringe filters (GHP membrane, pore size 0.45 µm, Pall Corp.). Analytical details are provided in section 2.5. Triplicate RS-carvone initial solutions (2 mg L\(^{-1}\)) without soils were also shaken for 24 h and revealed no losses of carvone enantiomers by volatilization or sorption to the tubes. Sorption of RS-carvone by the model sorbents was determined using the same procedure, but reducing the amount of sorbent used to 250 mg.

2.3. Sorption-desorption curves

Due to their different physico-chemical (organic C content and pH) and sorptive properties, soils S2 and S5 were selected to perform a sorption-desorption curve study. To this aim, RS-carvone aqueous solutions at concentrations of 0.1, 0.2, 1 and 2 mg L\(^{-1}\) were shaken for 24 h in Pyrex\(^{®}\) glass screw-cap centrifuge tubes with the pre-autoclaved soils S2 and S5 (triplicate 4 g of soil/8 mL of solution) following the aforementioned procedure to determine sorption. Additionally, in order to test the influence of pH on carvone sorption, an extra set of S2 soil samples were acidified before the addition of the carvone solutions by adding 1 mL of 0.1 M HCl to each tube. In this way, a value of pH similar to that displayed by soil S5 (pH= 5.5 ± 0.5) was obtained in the course of the sorption curve. The acidified S2 sample was denoted S2-H\(^{+}\).

Desorption was measured immediately after sorption from the highest concentration point of the sorption curve. The 4 mL of supernatant solution removed for the sorption analysis were replaced with 4 mL of distilled water. The tubes were re-suspended, shaken at 20 ± 2 °C for 24 h, centrifuged, and then 4 mL aliquots of the supernatant solutions were removed, stabilized with methanol, filtered, and analyzed by chiral HPLC. Reiterated desorption cycles were carried out up to three times.
2.4. Dissipation study

An incubation experiment was conducted to compare the dissipation of carvone enantiomers in S2, S2 at low pH (S2-H+, pH = 5.5 ± 0.5), and S5, in all cases using non-autoclaved soil as well as autoclaved soil samples. In this case, two different autoclaving treatments were compared: i) a single autoclaving step (1×) at 121 °C and 200 kPa for 20 min, and ii) three autoclaving steps (3×) on consecutive days with the soils being incubated at 25°C for 24 h before conducting the second and third autoclaving processes. Initially, the water content of 100 g of non-autoclaved or autoclaved soil samples was adjusted to 30% (S2) or 35% (S5), which were approximately the water holding capacity of each soil. For S2, an additional treatment was prepared to reach acidic conditions (pH = 5.5 ± 0.5) by supplementing with HCl (1 M) to give the S2-H+ sample. Then, the S2, S2-H+, and S5 soil samples were spiked with 1 mL of a methanolic solution of RS-carvone (200 mg L⁻¹) to obtain a final concentration of 2 mg kg⁻¹ dry soil. Assuming an average soil bulk density of 1.3 g cm⁻³ and a uniform distribution along a soil depth of 1 cm, this concentration would be achieved by an agronomic dose of 0.26 kg ha⁻¹. Subsequently, the soil samples were thoroughly mixed and incubated in glass jars in the dark at 20 ± 2 °C for 7 days. At selected times, triplicate 3 g-soil aliquots were sampled and immediately frozen until analyzed. Carvone residues in the soil samples were extracted by shaking for 24 h with 8 mL of methanol followed by centrifugation and analysis of the supernatant by chiral HPLC. Recoveries were > 95% of the RS-carvone freshly applied to the non-autoclaved and autoclaved soils.

A parallel experiment was designed to determine the basal respirations of soils S2 an S5, either non-autoclaved or autoclaved, and relate them to differences in carvone degradation patterns in both soils. Soil respiration was measured following the alkali trapping–titrimetric procedure described by Anderson (1982). The method consisted of quantifying the amount of
197 CO2 released by soil samples during 7 days, under the same conditions as those used in the
dissipation experiment.

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2.5. *Enantioselective analysis of carvone*

The determination of R- and S-carvone was performed by reversed-phase chiral HPLC
using a Waters 2695 chromatograph coupled to a Waters 996 diode-array detector. The
enantiomers were resolved using a Chiralpak IG column packed with immobilized amylose
tris(3-chloro-5-methylphenylcarbamate) of 150 mm length × 4.6 mm i.d. and 3 µm of particle
size (Chiral Technologies Europe). The mobile phase consisted of 50:50 acetonitrile:water at
a flow rate of 1 mL min⁻¹, operating with an injection volume of 50 µL and UV detection at
236 nm. External calibration curves with four standard solutions ranging between 0.1 and 2
mg L⁻¹ of RS-carvone were used to construct individual external calibration curves for R-
carbene and S-carvone. The elution order of each enantiomer was determined by injecting
separately R- and S-carvone under the same conditions formerly detailed. The retention times
under these conditions were 8.0 and 8.9 min for R-carvone and S-carvone, respectively.
Instrumental limit of quantification (LOQ), calculated as the concentration resulting in a
signal to noise ratio of 10:1, was 0.01 mg L⁻¹. A representative chromatogram of RS-carvone
at 2 mg L⁻¹ concentration prepared in water is given in Fig. S1.

2.6. *Modeling and data analysis*

Distribution coefficients (Kd) were used to express the sorption of the enantiomers on the
soils and model sorbents from a 2 mg L⁻¹ initial racemic solution of carvone, and were
calculated as:

\[ K_d = C_s/C_e \]
where $C_e$ (mg L$^{-1}$) corresponded to the concentration of each enantiomer in the aqueous phase after 24 h-equilibration, and $C_s$ (mg kg$^{-1}$) was the amount sorbed calculated from the difference between the initial and the final enantiomer concentration in the aqueous phase.

Sorption-desorption data were fitted to the Freundlich equation using a log-log linear fit:

$$\log C_s = \log K_f + N_f \log C_e$$

where $K_f$ (mg$^{1-Nf}$ kg$^{-1}$L$^{Nf}$) and $N_f$ (unitless) are the empirical Freundlich constants.

The thermodynamic index of irreversibility, TII, was calculated as:

$$TII = 1 - \frac{N_{fd}}{N_f}$$

where $N_f$ and $N_{fd}$ are the Freundlich constants obtained from the sorption and desorption curve, respectively (Sander et al., 2005). TII ranges from 0 to 1, where TII= 0 denotes completely reversible sorption and TII= 1 indicates irreversible sorption.

Dissipation data were modelled using the linearized form of a first-order kinetic rate law:

$$\ln C = \ln C_0 - kt$$

where $C$ (mg kg$^{-1}$) and $C_0$ (mg kg$^{-1}$) are the concentration of R- or S-carvone in the soils at time $t$ (days) and $t= 0$, respectively, and $k$ (day$^{-1}$) is the first-order dissipation rate constant. The half-lives ($t_{1/2}$) of R-carvone and S-carvone were calculated as $t_{1/2} = 0.693/k$.

The enantiomer fraction (EF) was used to estimate the enantioselectivity in sorption and dissipation of carvone and was calculated according to Harner et al. (2000) criteria as:

$$EF = \frac{[S-carvone]}{([S-carvone]+[R-carvone])}$$

where [S- carvone] and [R- carvone] are the individual concentration of each enantiomer. EF equal to 0.5 denoted racemic carvone residues and EF higher or lower than 0.5 indicated non-racemic carvone residues.

Statistical analysis was performed using Sigmaplot 12.5. Standard error was used to specify variability among replicates. Soil respirations, carvone residues, enantiomer fractions (EF), and distribution coefficients ($K_d$) were compared pair-wise using the t-test to establish
differences between treatments. Correlations between $K_d$ values and soil properties were established using the Pearson correlation coefficient ($r$). Correlation coefficients and differences between treatments were considered statistically significant at $P < 0.05$.

3. Results and discussion

3.1. Sorption on soils and model sorbents

Figure 2 shows the distribution coefficients, $K_d$, obtained for carvone in the six pre-autoclaved agricultural soils and the model sorbents. Results indicated that the sorption processes were non-enantioselective, always with solution EF values of $0.50 \pm 0.01$. Consequently, the $K_d$ coefficients calculated for the total (R+S) carvone concentrations coincided with those calculated for the individual enantiomers. Non-enantioselective sorption is a common observation when soil sorption studies are performed using racemic initial solutions of chiral compounds (Gámiz et al., 2017; Liang et al., 2016; López-Cabeza et al., 2017).

Overall, the sorption of carvone on the six agricultural soils was relatively low (Fig. 2A). $K_d$ values ranged between $0.24$ and $1.26$ L kg$^{-1}$ (Fig. 2A), with S5 and S6 sorbing carvone to a greater extent than the rest of soils ($P < 0.05$). Correlations were performed to identify physicochemical soil properties that could have been relevant in the sorption of carvone. A strong positive correlation was found between the $K_d$ values and the OC content of the soils ($r = 0.917$, $P < 0.05$), whereas the correlation coefficients between $K_d$ and the clay, silt, sand, or carbonate content and between $K_d$ and soil pH were not statistically significant ($P > 0.05$) (Supplementary Table S2). This indicated that organic matter may have been the most important soil component determining the sorption of carvone in the tested soils.

Sorption of carvone on various model sorbents was determined to confirm the correlation outcomes. Sorption followed the order: humic acid $>>$ montmorillonite $\sim$ ferrihydrite $>$ illite $\sim$
kaolinite. Thus, higher sorption of carvone ($P < 0.05$) was measured on the organic HA ($K_d = 28 \text{ L kg}^{-1}$) compared to the mineral model sorbents ($K_d < 6.3 \text{ L kg}^{-1}$) (Fig. 2B). Among the mineral soil constituents, the affinity of carvone for montmorillonite ($K_d = 6.3 \text{ L kg}^{-1}$) and ferrihydrite ($K_d = 5.5 \text{ L kg}^{-1}$) was greater ($P < 0.05$) than that for kaolinite ($K_d = 0.8 \text{ L kg}^{-1}$) or illite ($K_d = 1.8 \text{ L kg}^{-1}$). Although a direct relationship appeared to exist between the sorption of carvone on the layer silicates and the CEC of the minerals (Fig. 2B), the $K_d$ values measured for kaolinite and illite were very low and not significantly different ($P > 0.05$), which made the correlation meaningless. Furthermore, carvone is a neutral (non-ionizable) compound and its sorption on the layer silicates was not expected to occur by a cation exchange mechanism. The $K_d$ values on model sorbents confirmed the importance of organic matter in the sorption of carvone enantiomers, but also indicated that the role of soil minerals might become relevant in low organic carbon content soils rich in expandable clay minerals and/or metal oxides. This latter finding can be related to the polarity of monoterpenes (Vokou et al., 2003) and, in particular, to the oxygen-containing functionality present in the structure of carvone (Fig. 1). The role of the soil mineral fraction in the sorption of organic compounds containing polar functional groups has previously been reported, particularly in soils with low organic carbon contents (Celis et al., 2006, 1997; Laird et al., 1992).

3.2. Sorption-desorption curves

Sorption-desorption curves of R- and S-carvone on (pre-autoclaved) S2, S2-H+ (pH= 5.5 ± 0.5), and S5 soil samples are shown in Fig. 3, and Freundlich sorption parameters are listed in Supplementary Table S3. Firstly, the sorption of racemic carvone by the soils was non-enantioselective, as revealed by the overlapped sorption curves of the individual enantiomers (Fig. 3) and the carvone EF values of 0.5 in the supernatant solutions at different points of the sorption curves (data not shown). All sorption curves were close to linear with $N_r$ values close
to 1 (Supplementary Table S3), which indicated that sorption was minimally concentration-dependent (Giles et al., 1960) and that there was no limited number of available sorption sites, interpreting soil as an homogeneous matrix at macroscopic scale. The $K_f$ values followed the order: $S2 \sim S2-H^+ < S5$ (Supplementary Table S3), which was consistent with the $K_d$ sorption data obtained at a single initial concentration, where $S5$ also sorbed carvone to a greater extent than $S2$ (Fig. 2). When $K_f$ values were normalized to the OC content of the soils, much less variability in $K_{foc}$ among soils was obtained (Supplementary Table S3). In fact, similar $K_{foc}$ calculated for $S5$ and $S2$ supported that the OC content could have been a major factor determining the sorption of carvone by the soils, as previously discussed. On the other hand, bearing in mind the non-ionizable character of carvone, it was expected that the pH had little influence in the sorption of this chemical. Accordingly, differences in sorption between $S2$ and $S2-H^+$ were insignificant (Supplementary Table S3). It also follows that lowering the pH of $S2$ did not trigger conformational changes in the soil organic matter that could affect the sorption of carvone by the soil (Alonso et al., 2011).

Desorption of carvone enantiomers from the soils showed hysteresis, that is, desorption curves followed different pathways than sorption curves (Fig. 3). The intensity of this phenomenon was estimated by the thermodynamic index of irreversibility ($TII$), which ranged between 0.38 and 0.65 (Supplementary Table S3). Carvone resistance to desorption can be ascribed to irreversible/strong binding to the soil particles or slow kinetics of sorption or desorption (Alonso et al., 2011; Celis and Koskinen, 1999). Nevertheless, degradation of the enantiomers could have also contributed to the observed hysteresis (Gámiz et al., 2016c; Koskinen et al., 1979), since some reactivation of the soil microbial population during desorption from the soils subjected to a single soil autoclaving treatment was expected, as will be shown in the next section. In fact, the desorption of carvone enantiomers from $S2$ exhibited greater enantioselectivity than that from $S2-H^+$ or $S5$, and this was consistent with the greater
enantioselectivity of the degradation of carvone in S2 compared to S2-H⁺ or S5 (see section 3.3) (Gámiz et al., 2013, 2016a).

In order to illustrate to what extent biodegradation could have been responsible of the hysteresis detected during desorption, an estimation of the R- and S-carvone desorption curves was made after assuming reversible sorption but first order kinetics for the degradation of the enantiomers, with a total degradation of 10% during the desorption experiment. This value was close to the dissipation registered for the enantiomers at 3-4 days after carvone application in the incubation study with the soils autoclaved once. The results are shown in Fig. S2 and illustrate how an extent of degradation as low as 10% during the desorption is sufficient to explain the observed hysteresis for most of the systems studied.

3.3. Incubation experiment

Dissipation curves of carvone enantiomers in non-autoclaved and autoclaved (1× and 3×) soil samples and the evolution of the enantiomer fraction during the incubation experiment are shown in Fig. 4. The first-order dissipation rate constants, k, and half-lives, t₁/₂, for R- and S-carvone in the non-autoclaved soils and in the soils subjected to one autoclaving process (1×) are listed in Table 2. To prevent reactivation of the soil microbial activity, the number of samples taken for the 3-autoclaving treatment (3×) was reduced from nine to three, so that the fitting was not viable. For all other cases, the curves fitted well to first-order kinetics (R² > 0.881), with the exception of those for autoclaved (1×) S2-H⁺ due to the very low degradation rate. Given that racemization can influence the individual enantiomer dissipation rates, a preliminary experiment was performed to monitor the dissipation of the isolated carvone enantiomers, and showed no interconversion of R- to S-enantiomer or viceversa (data not shown).
Dissipation of carvone in the alkaline soil S2 under non-autoclaved conditions was enantioselective; S-carvone was degraded faster compared to R-carvone. Between day 1 and 3 of the experiment, differences in the enantiomer concentrations were statistically significant ($P < 0.05$). Nevertheless, there was a fast dissipation of both enantiomers, with 100% of the spiked R- and S-carvone being completely depleted 7 days after treatment (DAT) (Fig. 4 and Table 2). This rendered short half-lives of 0.8 and 1.2 days for S- and R-carvone, respectively. Additionally, the faster degradation of S-carvone resulted in EF of $0.20 \pm 0.03$ at 3 DAT, a value significantly lower ($P < 0.05$) than the initial EF of $0.49 \pm 0.01$ at the beginning of the experiment (Fig. 4). Variations in EF are considered an indication that the compound has been subjected to microbial degradation, since other attenuation processes such as diffusion, transport, and chemical reactions in achiral environments are supposed to be non-enantioselective (Buerge et al., 2003; Gámiz et al., 2013; Kurt-Karakus et al., 2005).

The role of biodegradation in the dissipation of carvone enantiomers in soil S2 was confirmed when the soil was autoclaved. As seen in Fig. 4, significantly greater amounts of each enantiomer ($P < 0.05$) were recovered from the autoclaved soil samples compared to the non-autoclaved soil at most sampling times. When S2 was autoclaved once (1×), the dissipation rates of R- and S-carvone greatly decreased, and the (extrapolated) half-lives increased to 17-19 days (Table 2). For the 3-autoclaving treatment (3×), 85% of the initially added carvone enantiomers remained at 7 DAT. Furthermore, EF remained unaltered at a value of $0.50 \pm 0.01$ ($P > 0.05$) at the end of the experiment, either for the 1× or the 3× autoclaving treatment (Fig. 4). These results supported that the dissipation of carvone in non-autoclaved S2 soil was essentially biological, with little contribution of abiotic processes, such as chemical degradation or volatilization (Gámiz et al., 2013; Kurt-Karakus et al., 2005; Li et al., 2009). Accordingly, lower soil respiration was measured for autoclaved S2 as compared to non-autoclaved S2 ($P < 0.05$), especially for the soil subjected to three
17 consecutive autoclaving treatments, case in which the respiration drastically decreased (Table 2). The fact that soil respiration was not fully suppressed by the single autoclaving treatment may indicate that some re-establishment of the microbial population could have occurred in the course of the respiration experiment (Carter et al., 2007). This phenomenon would explain the enantioselectivity observed in the desorption experiment with autoclaved S2 (Fig. S2).

In acidified S2 (S2-H\(^+\)) under non-autoclaved conditions, an increase in the persistence of both enantiomers occurred compared to the original non-autoclaved S2. Thus, R- and S-carvone residues in S2-H\(^+\) were greater \((P < 0.05)\) than those in untreated (alkaline) S2 (Fig. 4). In contrast to the alkaline medium, the dissipation rates of the enantiomers were almost identical (Fig. 4, Table 2). This revealed that the low pH, apart from extending the persistence of carvone, also altered the enantioselectivity of its dissipation in S2. Using EF as an indicator of the enantioselectivity, it was noticeable that EF scarcely changed along the experiment, ranging from 0.49 at 0 DAT to 0.47 at 7 DAT, thus differing \((P < 0.05)\) from the behavior observed for untreated S2 (Fig. 4). Changes in the pH have been reported to provoke alterations in the preferential degradation of one enantiomer over the other for chiral compounds (Buerge et al., 2003), as reported here for carvone. In autoclaved S2-H\(^+\), longer persistence of the enantiomers was observed, as happened in S2 (Fig. 4, Table 2), and R- and S-carvone residues by the end of the experiment derived in EF values that were not significantly different \((P > 0.05)\) respect to the initial value of 0.50 (Fig. 4). Consequently, these results supported the idea that, independent of pH, the main carvone degradation pathway in S2 was microbially mediated, although the biodegradation rate and enantioselectivity were influenced by pH.

In non-autoclaved S5 soil, the enantiomers of carvone dissipated at a slower rate than in non-autoclaved S2 and only a slight enantioselectivity was developed, with an EF of 0.44 by the end of the experiment. As in S2, S-carvone was degraded faster than R-carvone. Half-lives
were 6.5 days for S-carvone and 9.1 days for R-carvone (Table 2). One possible explanation for the longer persistence of carvone enantiomers in S5 compared to S2 is their higher sorption in S5 (Fig. 2 and 3). In general, sorption reduces the bioavailability of organic compounds in soils, prolonging the presence of chiral pesticides and natural compounds in their (initially) racemic form (Celis et al., 2013; Gámiz et al., 2016a,c, 2017). Furthermore, the low soil respiration measured for this soil could also explain the longer persistence of carvone enantiomers (Table 2). The low pH of S5 (5.4) did not appear to completely prevent the preferential degradation of S- over R-carvone, as observed in S2-H+ under non-autoclaved conditions (Fig. 4), but the acidity of S5 could have also contributed to delay the enantiomers degradation rate and to reduce the enantioselectivity in degradation. Autoclaving also reduced the degradation rates in S5, most clearly when three consecutive autoclaving steps were conducted. In this case, only 5% of the initially added carvone enantiomers dissipated after 7 days (Fig. 4). This confirmed the biological character of the dissipation losses also in S5.

4. Summary and conclusions

The sorption of the chiral monoterpenic carvone by soils and model sorbents from racemic initial solutions was a non-enantioselective process. Organic carbon was indicated to play a major role in the sorption of carvone enantiomers, but the mineral fraction, namely the presence of high amounts of (expandable) smectites and/or Fe-oxides, could also play a role in the sorption of this organic compound in soils with very low organic carbon contents. The pH did not affect the extent of sorption of carvone enantiomers in one soil sample. Carvone desorption curves showed hysteretic behavior, but reactivation of the soil microbial activity in the course of the desorption experiment probably contributed to the observed sorption-desorption hysteresis. The dissipation of racemic carvone in soils was attributed to biodegradation, and differences in biodegradation rates between enantiomers were soil-
dependent, presumably due to specific soil microbial activities and to the extent of sorption
process. S-carvone was degraded faster than R-carvone both in natural alkaline and acidic
soils, but low pH levels provoked changes in the endogenous microbial populations or their
activities that decreased the rate and enantioselectivity of carvone degradation. Finally, our
results indicate that, even though the low persistence of carvone enantiomers in soil could
represent a limitation to their use as soil-applied agrochemicals, selecting the application
conditions, such as soil type or soil pH, or even increasing the soil organic matter content by
soil amendment, may be good strategies to achieve prolonged persistence of this monoterpene
in soil. On the basis of the affinity of carvone towards smectitic clay minerals, the possibility
also exists to assay these materials as carriers for the preparation of carvone formulations
specifically designed to control the release and increase the persistence of this compound in
soils.

Acknowledgments

This work has been financed by the Spanish Ministry of Economy, Industry and
Competitiveness (MINEICO Project AGL2017-82141-R) and Junta de Andalucía (JA Project
P11-AGR-7400), co-financed with European FEDER-FSE funds. The authors thank I.
Pavlovic for supplying the commercial humic acid sample and G. Facenda for her technical
assistance. B.G. also thanks MINEICO for her Juan de la Cierva-Incorporación postdoctoral
contract (IJCI-2015-23309).
References


Figure captions

Fig. 1. Structures of R-carvone and S-carvone.

Fig. 2. Distribution coefficient, $K_d$, for RS-carvone on soils (A) and on model sorbents (B) measured at a single initial RS-carvone concentration of 2 mg L$^{-1}$ and 24 h equilibration time.

Fig. 3. Sorption-desorption curves of R- and S-carvone on autoclaved (1×) samples of S2, S2-H$^+$ and S5.

Fig. 4. Dissipation curves for R- and S-carvone (left) and enantiomer fraction evolution (right) during the incubation experiment with non-autoclaved and autoclaved (1× and 3×) soils. In the dissipation curves, symbols indicate experimental data points whereas lines correspond to the fits to first-order dissipation kinetics for non-autoclaved (solid) and autoclaved (1×) (dashed) soils. Errors bars denote standard errors of triplicate measurements.
Table 1 Selected properties of the soils used in this work.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Texture</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
<th>CaCO₃ (%)</th>
<th>OC (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>loamy sand</td>
<td>89</td>
<td>5</td>
<td>5</td>
<td>5.9</td>
<td>0.27</td>
<td>8.3</td>
</tr>
<tr>
<td>S2</td>
<td>sandy loam</td>
<td>75</td>
<td>9</td>
<td>17</td>
<td>1.9</td>
<td>0.63</td>
<td>7.3</td>
</tr>
<tr>
<td>S3</td>
<td>clay</td>
<td>26</td>
<td>32</td>
<td>41</td>
<td>1.0</td>
<td>1.06</td>
<td>8.6</td>
</tr>
<tr>
<td>S4</td>
<td>loam</td>
<td>50</td>
<td>29</td>
<td>21</td>
<td>32.5</td>
<td>1.17</td>
<td>8.3</td>
</tr>
<tr>
<td>S5</td>
<td>sandy loam</td>
<td>68</td>
<td>23</td>
<td>8</td>
<td>0.8</td>
<td>1.23</td>
<td>5.4</td>
</tr>
<tr>
<td>S6</td>
<td>clay loam</td>
<td>22</td>
<td>51</td>
<td>27</td>
<td>24.0</td>
<td>1.37</td>
<td>8.3</td>
</tr>
</tbody>
</table>

(M, I, K)\(^a\)

\(^a\) Percentage corresponding to montmorillonite (M), illite (I), and kaolinite (K).
Table 2 Dissipation rate constants, k, half-lives, t$_{1/2}$, and R$^2$ for the fits of R- and S-carvone dissipation data to first order kinetics and soil respiration values in non-autoclaved or autoclaved soils.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Treatment</th>
<th>k</th>
<th>t$_{1/2}$</th>
<th>$R^2$</th>
<th>k</th>
<th>t$_{1/2}$</th>
<th>$R^2$</th>
<th>Soil respiration$^a$ (mg CO$_2$ kg$^{-1}$ soil week$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2</td>
<td>non-autoclaved</td>
<td>0.568 ± 0.068$^b$</td>
<td>1.2</td>
<td>0.920</td>
<td>0.867 ± 0.095</td>
<td>0.8</td>
<td>0.943</td>
<td>979 ± 1</td>
</tr>
<tr>
<td></td>
<td>autoclaved (1×)</td>
<td>0.040 ± 0.001</td>
<td>17</td>
<td>0.991</td>
<td>0.036 ± 0.002</td>
<td>19</td>
<td>0.969</td>
<td>732 ± 39</td>
</tr>
<tr>
<td></td>
<td>autoclaved (3×)</td>
<td>n.f.$^c$</td>
<td></td>
<td></td>
<td>n.f.</td>
<td></td>
<td></td>
<td>127 ± 93</td>
</tr>
<tr>
<td>S2-H$^+$</td>
<td>non-autoclaved</td>
<td>0.208 ± 0.007</td>
<td>3.3</td>
<td>0.992</td>
<td>0.222 ± 0.011</td>
<td>3.1</td>
<td>0.983</td>
<td>n.d.$^d$</td>
</tr>
<tr>
<td></td>
<td>autoclaved (1×)</td>
<td>0.026 ± 0.005</td>
<td>27</td>
<td>0.797</td>
<td>0.019 ± 0.005</td>
<td>37</td>
<td>0.698</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>autoclaved (3×)</td>
<td>n.f.</td>
<td></td>
<td></td>
<td>n.f.</td>
<td></td>
<td></td>
<td>n.d.</td>
</tr>
<tr>
<td>S5</td>
<td>non-autoclaved</td>
<td>0.076 ± 0.006</td>
<td>9.1</td>
<td>0.969</td>
<td>0.107 ± 0.005</td>
<td>6.5</td>
<td>0.986</td>
<td>325 ± 6</td>
</tr>
<tr>
<td></td>
<td>autoclaved (1×)</td>
<td>0.072 ± 0.011</td>
<td>10</td>
<td>0.881</td>
<td>0.058 ± 0.008</td>
<td>12</td>
<td>0.907</td>
<td>143 ± 33</td>
</tr>
<tr>
<td></td>
<td>autoclaved (3×)</td>
<td>n.f.</td>
<td></td>
<td></td>
<td>n.f.</td>
<td></td>
<td></td>
<td>50 ± 17</td>
</tr>
</tbody>
</table>

$^a$ Measured under incubation experiment conditions.

$^b$ Value ± standard error.

$^c$ Not fitted due to the low number of samples.

$^d$ Not determined due to interferences by HCl-induced inorganic C dissolution.
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