

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

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1 ABSTRACT

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

19

20 *Keywords:* allelochemicals; biodegradation; chiral pesticides; soil biochemistry

21

22 **1. Introduction**

23 Modern agriculture needs to face the growing global demand for food due to the increasing
24 world population, which is expected to reach about 9 billion in 2050 (Gerwick and Sparks,
25 2014). To achieve a proper yield of crop production and pest control, the intensive use of
26 synthetic pesticides has been essential until now (Sparks et al., 2017). However, numerous
27 undesirable side effects are derived from pesticide use, mainly related to environmental
28 impacts resulting from water and soil contamination episodes (Cordeau et al., 2016) along
29 with gradual pest resistance (Gerwick and Sparks, 2014). For these reasons, the legislation is
30 becoming more restrictive and claims for new, alternative management strategies (and
31 products) in order to pursue an environmentally sustainable agriculture (Sparks et al., 2017).

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

43 Allelochemicals comprise a group of natural compounds which are responsible for the
44 stimulatory or inhibitory effects of one organism (plants, insects, microbes, etc.) upon the
45 growth, health, behavior, or population biology of neighboring organisms (Zeng et al., 2008).
46 Monoterpenes, foremost constituents of essential oils, are one of the most promising families

47 of allelochemicals to be used for pest management (van Roon et al., 2005), since insecticidal,
48 fungicidal, and herbicidal properties have been described for them (He et al., 2009; Inderjit et
49 al., 1997; Marei et al., 2012; Vokou et al., 2003; Zeng et al., 2008). As an example, the
50 monoterpenes eugenol, geraniol and thymol are currently approved in Europe as active
51 substances (a.s.) to control botrytis infection in grapes, at application rates up to 0.26 kg a.s.
52 ha⁻¹ per treatment (EU Pesticides Database, 2018).

53 Carvone is a monoterpene which occurs naturally as two enantiomers, S-carvone and R-
54 carvone (Fig. 1), and is a major constituent of the oils from caraway (S), gingergrass (both),
55 and spearmint (R) (De Carvalho and Da Fonseca, 2006; EFSA, 2016). Both enantiomers have
56 manifested striking pesticidal features, which potentially make carvone a target compound for
57 being included as a new active ingredient in pesticide formulations. For example, Vokou et al.
58 (2003) reported that both R- and S-carvone were extremely active in inhibiting seed
59 germination of *Lactuca sativa*. Similarly, De Martino et al. (2010) found that R-carvone
60 inhibited the radicle elongation for *Raphanus sativus* L. and *Lepidium sativum* L.
61 Additionally, R-carvone has been shown to be effective against insects, and S-carvone as a
62 fungistatic, bacteriostatic and potato sprout inhibiting compound (De Carvalho and Da
63 Fonseca, 2006; Oosterhaven et al., 1995). In fact, the active substance S-carvone has already
64 been authorized for use as a plant growth regulator (anti-sprouting agent) in Europe (EFSA,
65 2016), and R-carvone is registered as a biopesticidal active ingredient to be used in the
66 manufacture of insect repellents in the United States (USEPA, 2009).

67 Recently, chirality has received progressive attention in the field of synthetic pesticides.
68 The importance of enantiomers falls on the manifested selectivity in their responses, since one
69 of the enantiomers usually reacts preferentially with the (chiral) host system to which it is
70 exposed (Garrison, 2011). In addition, biodegradation of chiral pesticides is often an
71 enantioselective process which will be influenced by soil composition, pH, redox conditions,

72 and microbial populations (Buerge et al., 2003; Gámiz et al., 2017; Li et al., 2016; Matallo et
73 al., 1998). In spite that abiotic processes, such as chemical distribution or transport processes,
74 are often non-enantioselective, they can indirectly influence the relative abundance of one
75 enantiomer over the other in the different environmental compartments by affecting
76 biodegradation. It has been reported that sorption maintains enantiomer soil residues more
77 racemic by reducing their bioavailability (Celis et al., 2013; Gámiz et al., 2016a) and even
78 that sorption can turn into enantioselective when the starting initial pesticide solution is not
79 racemic (Celis et al., 2015; Gámiz et al., 2016b). Consequently, the assessment of the
80 enantioselective fate of chiral pesticides in various media has become an emerging area in
81 agricultural and environmental science (Celis et al., 2015; Gámiz et al., 2016a; Li et al.,
82 2016), which should be extrapolated to chiral allelochemicals such as carvone.

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

92 The objective of this work was to provide insight into the behavior of the two enantiomers
93 of the monoterpene carvone in agricultural soils. Correlations between sorption and soil
94 properties were established and several model sorbents were used to ascertain the role of
95 mineral and organic colloidal components in carvone sorption. The dissipation of carvone
96 enantiomers in selected soils was also investigated. The information provided should be

97 helpful to elucidate the behavior of this natural compound in soil and may also be valuable in
98 the design of new carvone-based pesticide formulations intended for soil applications.

99

100 **2. Materials and methods**

101 *2.1. Carvone, soils and model sorbents*

102 Analytical standard-grade S-carvone and R-carvone with chemical purities of 98.5%
103 and 99.9%, respectively, were purchased from Sigma-Aldrich (Spain). Carvone enantiomers
104 are classified as monoterpenes with a molecular weight of 150.2 g mol⁻¹, a vapor pressure of
105 21.3 Pa at 25 °C, and a water solubility of 27 mg L⁻¹ at 20 °C (BPDB, 2018). The racemic
106 aqueous solutions of carvone (RS-carvone) used in this work (0.1-2 mg L⁻¹) were prepared by
107 diluting a 200 mg L⁻¹ (R+S) methanolic stock solution, which was made by adding 10 mg of
108 each enantiomer to 100 mL of methanol.

109 Six agricultural soils (S1-S6) representative of Southern Spain were used in this study.
110 Soil samples were collected from a 0-20 cm depth, air-dried, sieved to pass a 2 mm mesh, and
111 stored at 4 °C. The soils were characterized by conventional methods described in the
112 literature. The hydrometer method was used to determine soil texture (Gee and Bauder, 1986).
113 The carbonate content was measured by the pressure calcimeter method and the organic
114 carbon content by dichromate oxidation (Nelson, 1982, Nelson and Sommers, 1982). The
115 amount of amorphous Fe-oxides was determined by extraction with oxalate (McKeague and
116 Day, 1966). The phyllosilicate mineralogy was determined by X-ray diffraction analysis of
117 oriented specimens of Mg²⁺- and K⁺-saturated soil clay (< 2 µm) samples, solvated with
118 ethylene glycol and calcined at 550°C, respectively (Brown, 1961). Soil pH values were
119 measured in 1:2.5 soil:water slurries. The main physico-chemical properties determined are
120 compiled in Table 1. It is remarkable that the soils had relatively low organic carbon (OC)
121 contents, as typical of Mediterranean soils (Gámiz et al., 2012), low amorphous Fe-oxide

122 contents (< 1%, not shown), and variable clay contents and mineralogies. Additional details
123 on the soils used are given in Supplementary Table S1.

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

135

136 2.2. Sorption of carvone enantiomers at a single initial concentration

137 The batch sorption technique was used as a conventional method to assess the sorption of
138 RS-carvone on the soils. Preliminary tests showed that carvone degraded rapidly in the tested
139 soils, so that, to avoid degradation losses, the soils were pre-treated once with steam in an
140 autoclave at 121 °C and 200 kPa for 20 min before using them in the sorption experiments.
141 Triplicate 4 g of the autoclaved soil samples (S1-S6) were weighed in Pyrex[®] glass screw-cap
142 centrifuge tubes and were shaken during 24 h at 20 ± 2 °C with 8 mL of an RS-carvone
143 aqueous solution of 2 mg L⁻¹. Next, the tubes were centrifuged at 5000 rpm for 10 min and 4
144 mL of the supernatant solutions were removed and stabilized with methanol (50:50 v/v), to
145 prevent the degradation of carvone before analysis. The solution concentration (C_e) of
146 carvone enantiomers was determined using reversed-phase chiral high-performance liquid

147 chromatography (HPLC), after filtering the samples with Acrodisc® syringe filters (GHP
148 membrane, pore size 0.45 µm, Pall Corp.). Analytical details are provided in section 2.5.
149 Triplicate RS-carvone initial solutions (2 mg L⁻¹) without soils were also shaken for 24 h and
150 revealed no losses of carvone enantiomers by volatilization or sorption to the tubes. Sorption
151 of RS-carvone by the model sorbents was determined using the same procedure, but reducing
152 the amount of sorbent used to 250 mg.

153

154 2.3. Sorption-desorption curves

155 Due to their different physico-chemical (organic C content and pH) and sorptive
156 properties, soils S2 and S5 were selected to perform a sorption-desorption curve study. To this
157 aim, RS-carvone aqueous solutions at concentrations of 0.1, 0.2, 1 and 2 mg L⁻¹ were shaken
158 for 24 h in Pyrex® glass screw-cap centrifuge tubes with the pre-autoclaved soils S2 and S5
159 (triplicate 4 g of soil/8 mL of solution) following the aforementioned procedure to determine
160 sorption. Additionally, in order to test the influence of pH on carvone sorption, an extra set of
161 S2 soil samples were acidified before the addition of the carvone solutions by adding 1 mL of
162 0.1 M HCl to each tube. In this way, a value of pH similar to that displayed by soil S5 (pH=
163 5.5 ± 0.5) was obtained in the course of the sorption curve. The acidified S2 sample was
164 denoted S2-H⁺.

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

171

172 2.4. Dissipation study

173 An incubation experiment was conducted to compare the dissipation of carvone
174 enantiomers in S2, S2 at low pH (S2-H⁺, pH= 5.5 ± 0.5), and S5, in all cases using non-
175 autoclaved soil as well as autoclaved soil samples. In this case, two different autoclaving
176 treatments were compared: i) a single autoclaving step (1×) at 121 °C and 200 kPa for 20 min,
177 and ii) three autoclaving steps (3×) on consecutive days with the soils being incubated at 25
178 °C for 24 h before conducting the second and third autoclaving processes. Initially, the water
179 content of 100 g of non-autoclaved or autoclaved soil samples was adjusted to 30% (S2) or
180 35% (S5), which were approximately the water holding capacity of each soil. For S2, an
181 additional treatment was prepared to reach acidic conditions (pH= 5.5 ± 0.5) by
182 supplementing with HCl (1 M) to give the S2-H⁺ sample. Then, the S2, S2-H⁺, and S5 soil
183 samples were spiked with 1 mL of a methanolic solution of RS-carvone (200 mg L⁻¹) to
184 obtain a final concentration of 2 mg kg⁻¹ dry soil. Assuming an average soil bulk density of
185 1.3 g cm⁻³ and a uniform distribution along a soil depth of 1 cm, this concentration would be
186 achieved by an agronomic dose of 0.26 kg ha⁻¹. Subsequently, the soil samples were
187 thoroughly mixed and incubated in glass jars in the dark at 20 ± 2 °C for 7 days. At selected
188 times, triplicate 3 g-soil aliquots were sampled and immediately frozen until analyzed.
189 Carvone residues in the soil samples were extracted by shaking for 24 h with 8 mL of
190 methanol followed by centrifugation and analysis of the supernatant by chiral HPLC.
191 Recoveries were > 95% of the RS-carvone freshly applied to the non-autoclaved and
192 autoclaved soils.

193 A parallel experiment was designed to determine the basal respirations of soils S2 and S5,
194 either non-autoclaved or autoclaved, and relate them to differences in carvone degradation
195 patterns in both soils. Soil respiration was measured following the alkali trapping–titrimetric
196 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
198 dissipation experiment.

199

200 *2.5. Enantioselective analysis of carvone*

201 The determination of R- and S-carvone was performed by reversed-phase chiral HPLC
202 using a Waters 2695 chromatograph coupled to a Waters 996 diode-array detector. The
203 enantiomers were resolved using a Chiralpak IG column packed with immobilized amylose
204 tris(3-chloro-5-methylphenylcarbamate) of 150 mm length × 4.6 mm i.d. and 3 μm of particle
205 size (Chiral Technologies Europe). The mobile phase consisted of 50:50 acetonitrile:water at
206 a flow rate of 1 mL min⁻¹, operating with an injection volume of 50 μL and UV detection at
207 236 nm. External calibration curves with four standard solutions ranging between 0.1 and 2
208 mg L⁻¹ of RS-carvone were used to construct individual external calibration curves for R-
209 carvone and S-carvone. The elution order of each enantiomer was determined by injecting
210 separately R- and S-carvone under the same conditions formerly detailed. The retention times
211 under these conditions were 8.0 and 8.9 min for R-carvone and S-carvone, respectively.
212 Instrumental limit of quantification (LOQ), calculated as the concentration resulting in a
213 signal to noise ratio of 10:1, was 0.01 mg L⁻¹. A representative chromatogram of RS-carvone
214 at 2 mg L⁻¹ concentration prepared in water is given in Fig. S1.

215

216 *2.6. Modeling and data analysis*

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

220

$$K_d = C_s/C_e$$

221 where C_e (mg L^{-1}) corresponded to the concentration of each enantiomer in the aqueous phase
 222 after 24 h-equilibration, and C_s (mg kg^{-1}) was the amount sorbed calculated from the
 223 difference between the initial and the final enantiomer concentration in the aqueous phase.

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

$$225 \quad \log C_s = \log K_f + N_f \log C_e$$

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

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

$$228 \quad \text{TII} = 1 - (N_{fd}/N_f)$$

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

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

$$233 \quad \ln C = \ln C_0 - kt$$

234 where C (mg kg^{-1}) and C_0 (mg kg^{-1}) are the concentration of R- or S-carvone in the soils at
 235 time t (days) and $t= 0$, respectively, and k (day^{-1}) is the first-order dissipation rate constant.

236 The half-lives ($t_{1/2}$) of R-carvone and S-carvone were calculated as $t_{1/2} = 0.693/k$.

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

$$239 \quad \text{EF} = [\text{S-carvone}]/([\text{S-carvone}] + [\text{R-carvone}])$$

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

243 Statistical analysis was performed using Sigmaplot 12.5. Standard error was used to
 244 specify variability among replicates. Soil respirations, carvone residues, enantiomer fractions
 245 (EF), and distribution coefficients (K_d) were compared pair-wise using the t-test to establish

246 differences between treatments. Correlations between K_d values and soil properties were
247 established using the Pearson correlation coefficient (r). Correlation coefficients and
248 differences between treatments were considered statistically significant at $P < 0.05$.

249

250 **3. Results and discussion**

251 *3.1. Sorption on soils and model sorbents*

252 Figure 2 shows the distribution coefficients, K_d , obtained for carvone in the six pre-
253 autoclaved agricultural soils and the model sorbents. Results indicated that the sorption
254 processes were non-enantioselective, always with solution EF values of 0.50 ± 0.01 .
255 Consequently, the K_d coefficients calculated for the total (R+S) carvone concentrations
256 coincided with those calculated for the individual enantiomers. Non-enantioselective sorption
257 is a common observation when soil sorption studies are performed using racemic initial
258 solutions of chiral compounds (Gámiz et al., 2017; Liang et al., 2016; López-Cabeza et al.,
259 2017).

260 Overall, the sorption of carvone on the six agricultural soils was relatively low (Fig. 2A).
261 K_d values ranged between 0.24 and 1.26 L kg⁻¹ (Fig. 2A), with S5 and S6 sorbing carvone to a
262 greater extent than the rest of soils ($P < 0.05$). Correlations were performed to identify
263 physicochemical soil properties that could have been relevant in the sorption of carvone. A
264 strong positive correlation was found between the K_d values and the OC content of the soils (r
265 = 0.917, $P < 0.05$), whereas the correlation coefficients between K_d and the clay, silt, sand, or
266 carbonate content and between K_d and soil pH were not statistically significant ($P > 0.05$)
267 (Supplementary Table S2). This indicated that organic matter may have been the most
268 important soil component determining the sorption of carvone in the tested soils.

269 Sorption of carvone on various model sorbents was determined to confirm the correlation
270 outcomes. Sorption followed the order: humic acid >> montmorillonite ~ ferrihydrite > illite ~

271 kaolinite. Thus, higher sorption of carvone ($P < 0.05$) was measured on the organic HA ($K_d =$
272 28 L kg^{-1}) compared to the mineral model sorbents ($K_d < 6.3 \text{ L kg}^{-1}$) (Fig. 2B). Among the
273 mineral soil constituents, the affinity of carvone for montmorillonite ($K_d = 6.3 \text{ L kg}^{-1}$) and
274 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
275 illite ($K_d = 1.8 \text{ L kg}^{-1}$). Although a direct relationship appeared to exist between the sorption of
276 carvone on the layer silicates and the CEC of the minerals (Fig. 2B), the K_d values measured
277 for kaolinite and illite were very low and not significantly different ($P > 0.05$), which made
278 the correlation meaningless. Furthermore, carvone is a neutral (non-ionizable) compound and
279 its sorption on the layer silicates was not expected to occur by a cation exchange mechanism.
280 The K_d values on model sorbents confirmed the importance of organic matter in the sorption
281 of carvone enantiomers, but also indicated that the role of soil minerals might become
282 relevant in low organic carbon content soils rich in expandable clay minerals and/or metal
283 oxides. This latter finding can be related to the polarity of monoterpenes (Vokou et al., 2003)
284 and, in particular, to the oxygen-containing functionality present in the structure of carvone
285 (Fig. 1). The role of the soil mineral fraction in the sorption of organic compounds containing
286 polar functional groups has previously been reported, particularly in soils with low organic
287 carbon contents (Celis et al., 2006, 1997; Laird et al., 1992).

288

289 3.2. Sorption-desorption curves

290 Sorption-desorption curves of R- and S-carvone on (pre-autoclaved) S2, S2-H⁺ (pH= 5.5
291 ± 0.5), and S5 soil samples are shown in Fig. 3, and Freundlich sorption parameters are listed
292 in Supplementary Table S3. Firstly, the sorption of racemic carvone by the soils was non-
293 enantioselective, as revealed by the overlapped sorption curves of the individual enantiomers
294 (Fig. 3) and the carvone EF values of 0.5 in the supernatant solutions at different points of the
295 sorption curves (data not shown). All sorption curves were close to linear with N_f values close

296 to 1 (Supplementary Table S3), which indicated that sorption was minimally concentration-
297 dependent (Giles et al., 1960) and that there was no limited number of available sorption sites,
298 interpreting soil as an homogeneous matrix at macroscopic scale. The K_f values followed the
299 order: $S2 \sim S2-H^+ < S5$ (Supplementary Table S3), which was consistent with the K_d sorption
300 data obtained at a single initial concentration, where S5 also sorbed carvone to a greater
301 extent than S2 (Fig. 2). When K_f values were normalized to the OC content of the soils, much
302 less variability in K_{foc} among soils was obtained (Supplementary Table S3). In fact, similar
303 K_{foc} calculated for S5 and S2 supported that the OC content could have been a major factor
304 determining the sorption of carvone by the soils, as previously discussed. On the other hand,
305 bearing in mind the non-ionizable character of carvone, it was expected that the pH had little
306 influence in the sorption of this chemical. Accordingly, differences in sorption between S2
307 and S2- H^+ were insignificant (Supplementary Table S3). It also follows that lowering the pH
308 of S2 did not trigger conformational changes in the soil organic matter that could affect the
309 sorption of carvone by the soil (Alonso et al., 2011).

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

321 enantioselectivity of the degradation of carvone in S2 compared to S2-H⁺ or S5 (see section
322 3.3) (Gámiz et al., 2013, 2016a).

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

331

332 *3.3. Incubation experiment*

333 Dissipation curves of carvone enantiomers in non-autoclaved and autoclaved (1× and 3×)
334 soil samples and the evolution of the enantiomer fraction during the incubation experiment are
335 shown in Fig. 4. The first-order dissipation rate constants, k , and half-lives, $t_{1/2}$, for R- and S-
336 carvone in the non-autoclaved soils and in the soils subjected to one autoclaving process (1×)
337 are listed in Table 2. To prevent reactivation of the soil microbial activity, the number of
338 samples taken for the 3-autoclaving treatment (3×) was reduced from nine to three, so that the
339 fitting was not viable. For all other cases, the curves fitted well to first-order kinetics ($R^2 >$
340 0.881), with the exception of those for autoclaved (1×) S2-H⁺ due to the very low degradation
341 rate. Given that racemization can influence the individual enantiomer dissipation rates, a
342 preliminary experiment was performed to monitor the dissipation of the isolated carvone
343 enantiomers, and showed no interconversion of R- to S-enantiomer or viceversa (data not
344 shown).

345 Dissipation of carvone in the alkaline soil S2 under non-autoclaved conditions was
346 enantioselective; S-carvone was degraded faster compared to R-carvone. Between day 1 and 3
347 of the experiment, differences in the enantiomer concentrations were statistically significant
348 ($P < 0.05$). Nevertheless, there was a fast dissipation of both enantiomers, with 100% of the
349 spiked R- and S-carvone being completely depleted 7 days after treatment (DAT) (Fig. 4 and
350 Table 2). This rendered short half-lives of 0.8 and 1.2 days for S- and R-carvone, respectively.
351 Additionally, the faster degradation of S-carvone resulted in EF of 0.20 ± 0.03 at 3 DAT, a
352 value significantly lower ($P < 0.05$) than the initial EF of 0.49 ± 0.01 at the beginning of the
353 experiment (Fig. 4). Variations in EF are considered an indication that the compound has been
354 subjected to microbial degradation, since other attenuation processes such as diffusion,
355 transport, and chemical reactions in achiral environments are supposed to be non-
356 enantioselective (Buerge et al., 2003; Gámiz et al., 2013; Kurt-Karakus et al., 2005).

357 The role of biodegradation in the dissipation of carvone enantiomers in soil S2 was
358 confirmed when the soil was autoclaved. As seen in Fig. 4, significantly greater amounts of
359 each enantiomer ($P < 0.05$) were recovered from the autoclaved soil samples compared to the
360 non-autoclaved soil at most sampling times. When S2 was autoclaved once (1×), the
361 dissipation rates of R- and S-carvone greatly decreased, and the (extrapolated) half-lives
362 increased to 17-19 days (Table 2). For the 3-autoclaving treatment (3×), 85% of the initially
363 added carvone enantiomers remained at 7 DAT. Furthermore, EF remained unaltered at a
364 value of 0.50 ± 0.01 ($P > 0.05$) at the end of the experiment, either for the 1× or the 3×
365 autoclaving treatment (Fig. 4). These results supported that the dissipation of carvone in non-
366 autoclaved S2 soil was essentially biological, with little contribution of abiotic processes,
367 such as chemical degradation or volatilization (Gámiz et al., 2013; Kurt-Karakus et al., 2005;
368 Li et al., 2009). Accordingly, lower soil respiration was measured for autoclaved S2 as
369 compared to non-autoclaved S2 ($P < 0.05$), especially for the soil subjected to three

370 consecutive autoclaving treatments, case in which the respiration drastically decreased (Table
371 2). The fact that soil respiration was not fully suppressed by the single autoclaving treatment
372 may indicate that some re-establishment of the microbial population could have occurred in
373 the course of the respiration experiment (Carter et al., 2007). This phenomenon would explain
374 the enantioselectivity observed in the desorption experiment with autoclaved S2 (Fig. S2).

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

392 In non-autoclaved S5 soil, the enantiomers of carvone dissipated at a slower rate than in
393 non-autoclaved S2 and only a slight enantioselectivity was developed, with an EF of 0.44 by
394 the end of the experiment. As in S2, S-carvone was degraded faster than R-carvone. Half-lives

395 were 6.5 days for S-carvone and 9.1 days for R-carvone (Table 2). One possible explanation
396 for the longer persistence of carvone enantiomers in S5 compared to S2 is their higher
397 sorption in S5 (Fig. 2 and 3). In general, sorption reduces the bioavailability of organic
398 compounds in soils, prolonging the presence of chiral pesticides and natural compounds in
399 their (initially) racemic form (Celis et al., 2013; Gámiz et al., 2016a,c, 2017). Furthermore,
400 the low soil respiration measured for this soil could also explain the longer persistence of
401 carvone enantiomers (Table 2). The low pH of S5 (5.4) did not appear to completely prevent
402 the preferential degradation of S- over R-carvone, as observed in S2-H⁺ under non-autoclaved
403 conditions (Fig. 4), but the acidity of S5 could have also contributed to delay the enantiomers
404 degradation rate and to reduce the enantioselectivity in degradation. Autoclaving also reduced
405 the degradation rates in S5, most clearly when three consecutive autoclaving steps were
406 conducted. In this case, only 5% of the initially added carvone enantiomers dissipated after 7
407 days (Fig. 4). This confirmed the biological character of the dissipation losses also in S5.

408

409 **4. Summary and conclusions**

410 The sorption of the chiral monoterpene carvone by soils and model sorbents from racemic
411 initial solutions was a non-enantioselective process. Organic carbon was indicated to play a
412 major role in the sorption of carvone enantiomers, but the mineral fraction, namely the
413 presence of high amounts of (expandable) smectites and/or Fe-oxides, could also play a role
414 in the sorption of this organic compound in soils with very low organic carbon contents. The
415 pH did not affect the extent of sorption of carvone enantiomers in one soil sample. Carvone
416 desorption curves showed hysteretic behavior, but reactivation of the soil microbial activity in
417 the course of the desorption experiment probably contributed to the observed sorption-
418 desorption hysteresis. The dissipation of racemic carvone in soils was attributed to
419 biodegradation, and differences in biodegradation rates between enantiomers were soil-

420 dependent, presumably due to specific soil microbial activities and to the extent of sorption
421 process. S-carvone was degraded faster than R-carvone both in natural alkaline and acidic
422 soils, but low pH levels provoked changes in the endogenous microbial populations or their
423 activities that decreased the rate and enantioselectivity of carvone degradation. Finally, our
424 results indicate that, even though the low persistence of carvone enantiomers in soil could
425 represent a limitation to their use as soil-applied agrochemicals, selecting the application
426 conditions, such as soil type or soil pH, or even increasing the soil organic matter content by
427 soil amendment, may be good strategies to achieve prolonged persistence of this monoterpene
428 in soil. On the basis of the affinity of carvone towards smectitic clay minerals, the possibility
429 also exists to assay these materials as carriers for the preparation of carvone formulations
430 specifically designed to control the release and increase the persistence of this compound in
431 soils.

432

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Figure captions

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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 ($1\times$) 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 ($1\times$ and $3\times$) 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 ($1\times$) (dashed) soils. Errors bars denote standard errors of triplicate measurements.

Table 1 Selected properties of the soils used in this work.

Soil	Texture	Sand (%)	Silt (%)	Clay (%) (M, I, K) ^a	CaCO ₃ (%)	OC (%)	pH
S1	loamy sand	89	5	5 (2, 2, 1)	5.9	0.27	8.3
S2	sandy loam	75	9	17 (9, 3, 5)	1.9	0.63	7.3
S3	clay	26	32	41 (0, 36, 5)	1.0	1.06	8.6
S4	loam	50	29	21 (11, 4, 6)	32.5	1.17	8.3
S5	sandy loam	68	23	8 (0, 4, 4)	0.8	1.23	5.4
S6	clay loam	22	51	27 (17, 9, 1)	24.0	1.37	8.3

^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.

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

^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.

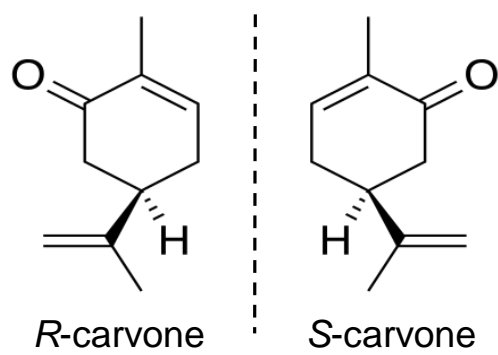


Fig. 1. Structures of *R*-carvone and *S*-carvone.

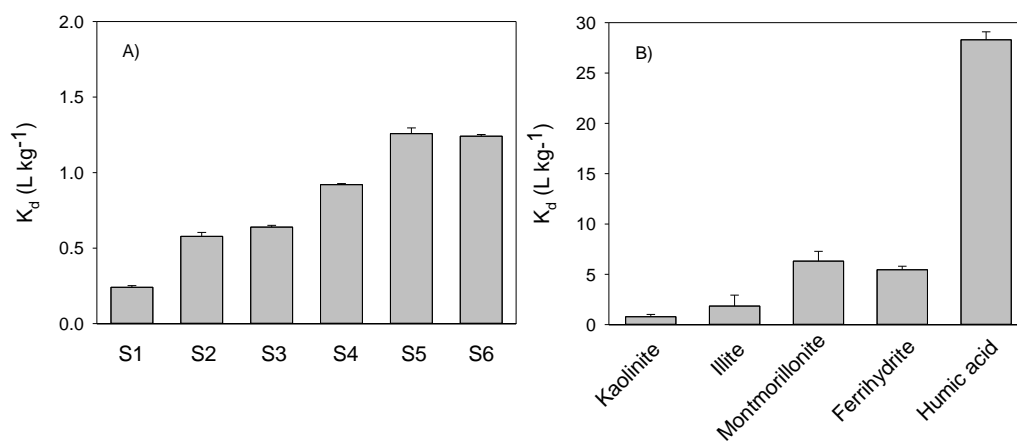


Fig. 2. Distribution coefficient, K_d , for RS-carvone on soils (A) and on model sorbents (B) measured at a single initial RS-carvone concentration of 2 mg L^{-1} and 24 h equilibration time.

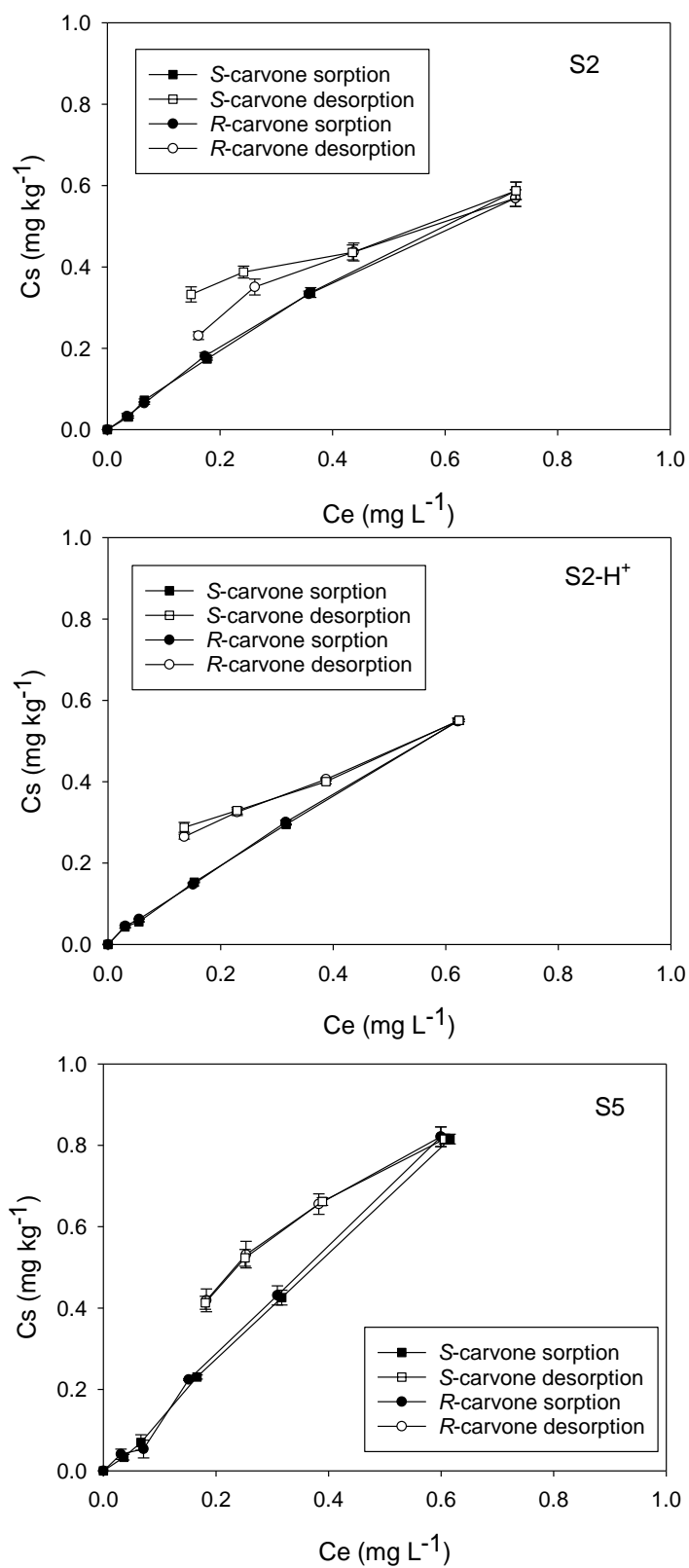


Fig. 3. Sorption-desorption curves of R- and S-carvone on autoclaved (1×) samples of S2, S2-H⁺ and S5.

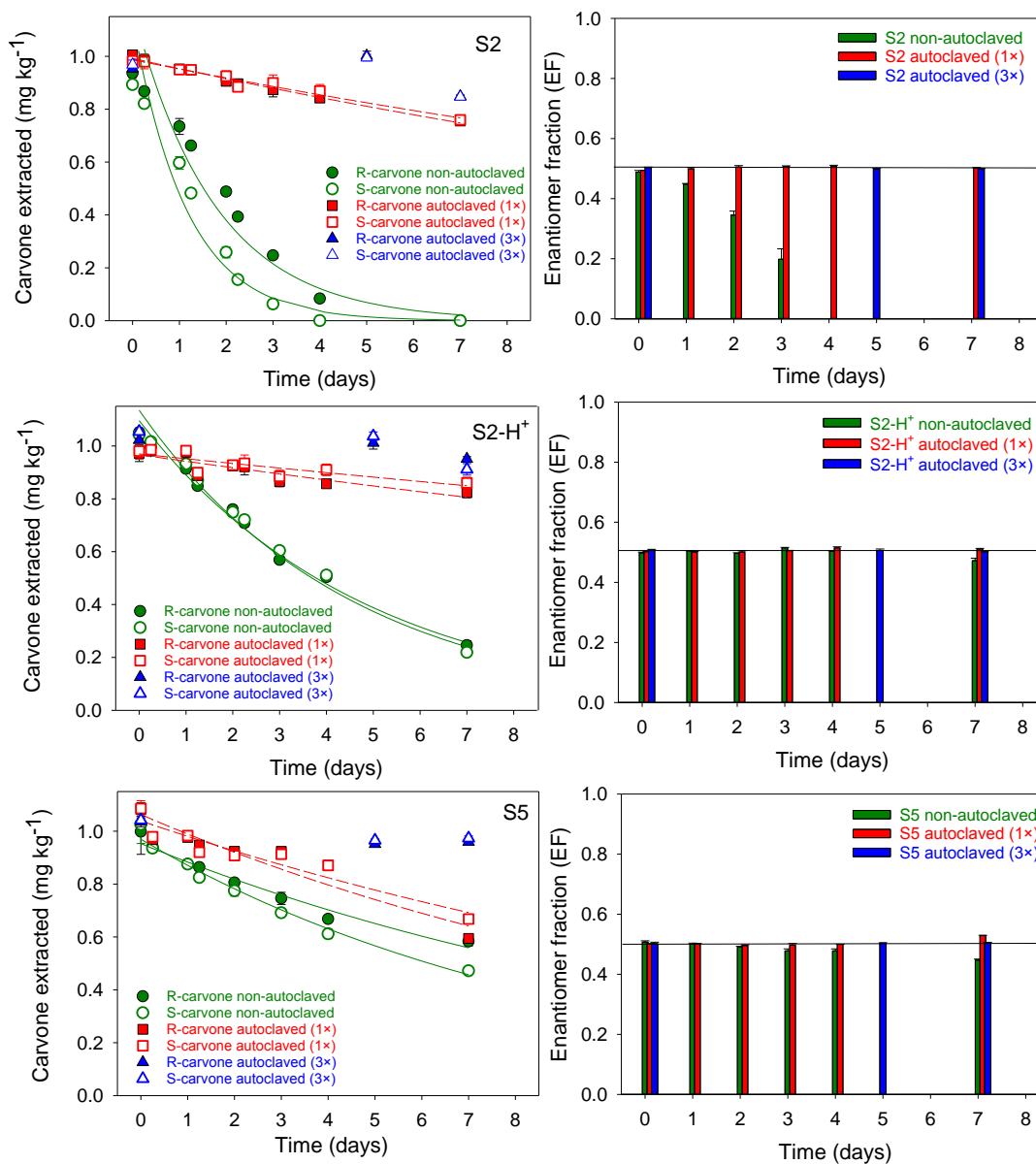


Fig. 4. Dissipation curves for R- and S-carvone (left) and enantiomer fraction evolution (right) during the incubation experiment with non-autoclaved and autoclaved (1× and 3×) soils. In the dissipation curves, symbols indicate experimental data points whereas lines correspond to the fits to first-order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