

**EVIDENCE FOR THE EFFECT OF SORPTION ENANTIOSELECTIVITY ON THE  
AVAILABILITY OF CHIRAL PESTICIDE ENANTIOMERS IN SOIL**

Beatriz Gámiz, Gracia Facenda, Rafael Celis\*

*Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC, Avenida Reina  
Mercedes 10, 41012 Sevilla, Spain*

**\* Corresponding Author:** Rafael Celis

**Address:** Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC

Avenida Reina Mercedes 10

41012 Sevilla, Spain

**Phone:** +34 954624711

**Fax:** +34 954624002

**E-mail:** rcelis@irnase.csic.es

1 **ABSTRACT**

2 Although enantioselective sorption to soil particles has been proposed as a mechanism that  
3 can potentially influence the availability of individual chiral pesticide enantiomers in the  
4 environment, environmental fate studies generally overlook this possibility and assume that  
5 only biotic processes can be enantioselective, whereas abiotic processes, such as sorption, are  
6 non-enantioselective. In this work, we present direct evidence for the effect of the  
7 enantioselective sorption of a chiral pesticide in a natural soil on the availability of the single  
8 pesticide enantiomers for transport. Batch sorption experiments, with direct determination of  
9 the sorbed amounts, combined with column leaching tests confirmed previous observations  
10 that from non-racemic aqueous solutions the sorption of the chiral fungicide metalaxyl on the  
11 soil appeared to be enantioselective, and further demonstrated that the enantiomer that was  
12 sorbed to a greater extent (*R*-metalaxyl,  $K_d= 1.73$  L/kg) exhibited retarded leaching compared  
13 to its optical isomer (*S*-metalaxyl,  $K_d= 1.15$  L/kg). Interconversion and degradation of the  
14 pesticide enantiomers, which are potential experimental artifacts that can lead to erroneous  
15 estimates of sorption and its enantioselectivity, were discarded as possible causes of the  
16 observed enantioselective behavior. The results presented here may have very important  
17 implications for a correct assessment of the environmental fate of chiral pesticides that are  
18 incorporated into the environment as non-racemic mixtures, and also of aged chiral pesticide  
19 residues that have been transformed from racemic to non-racemic by biologically-mediated  
20 processes.

21

22 **Capsule abstract:** Enantiomer-selective sorption of non-racemic chiral pesticide residues in  
23 soil makes one of the enantiomers more available for transport than the other.

24 **Keywords:** Chiral pollutants; Leaching; Pesticides; Soil; Sorption

## 25 **1. Introduction**

26 Chiral pesticides comprise an important group among the chiral organic compounds that  
27 reach the environment as a result of anthropogenic activities. Although optical isomers or  
28 enantiomers of chiral pesticides have long been treated as a single compound both in standard  
29 chemical analyses and in toxicity and environmental fate studies, it is now widely accepted  
30 that studies on chiral pesticides should account for the behavior of individual enantiomers to  
31 avoid incorrect predictions of efficacy and environmental risks (Chen and Liu, 2008;  
32 Garrison, 2006; Li et al., 2013; Magrans et al., 2002; Williams, 1996; Wong, 2006). This is  
33 because enantiomers of a particular chiral pesticide can differ greatly in their toxicity to target  
34 and non-target organisms as well as in their environmental fate (Garrison, 2006; Lewis et al.,  
35 1999; Liu et al., 2005; Ramezani et al., 2010; Ye et al., 2010).

36 In achiral environments, chiral pesticide enantiomers have in general identical physical  
37 and chemical properties (water solubility, vapor pressure, dissociation constant, etc.). In  
38 biological systems, however, they usually show different activities because individual  
39 enantiomers often interact selectively with other substances that are also enantiomers, such as  
40 biological receptors (Garrison, 2006; Li et al., 2012; Liu et al., 2009). For this reason, changes  
41 in enantiomer composition or chiral signatures of a pesticide in environmental samples, e.g.  
42 changes from racemic to non-racemic enantiomer ratios, have been considered as an  
43 indication that the compound has been subject to biological degradation and to distinguish  
44 recent from aged pesticide residues, with the underlying assumption that abiotic processes  
45 such as dilution, transport, and chemical reactions are non-enantioselective (Bidleman et al.,  
46 1998, 2002; Buser et al., 1992; Hegeman and Laane, 2002; Kurt-Karakus et al., 2005;  
47 Williams et al., 2003; Wong, 2006; Zipper et al., 1998).

48 The question of whether enantioselective sorption to mineral and organic components can  
49 significantly contribute to the chiral signatures of pesticide residues in environmental samples

50 remains uncertain. Theoretically, chiral minerals and soil organic matter chiral regions  
51 provide soil particles with “chiral environments” with the potential to selectively sorb chiral  
52 pesticide enantiomers (Bidleman et al., 2002; Bonner and Kavasmaneck, 1976; Han and  
53 Sholl, 2010; Hazen et al., 2001), but evidence for the enantioselective sorption of chiral  
54 compounds by natural soils remains scarce. Wedyan and Preston (2005), Liu and Lee (2007),  
55 and Oravec et al. (2010), for example, reported results of batch sorption experiments  
56 indicating optical isomer selectivity of soil and sediment materials in their sorption of  
57 different chiral organic compounds. Other studies, however, failed to identify similar sorption  
58 enantioselectivities (Celis et al., 2013; Gámiz et al., 2013; Matallo et al., 1998; Qin et al.,  
59 2014; Shaner et al., 2006; Sukul et al., 2013). Since sorption is a key process determining  
60 pesticide availability for transport and degradation, enantioselective binding to soil particles  
61 would greatly affect the fate of chiral pesticides in the environment, and so their chiral  
62 signatures in soil, water, and air samples, by making one of the enantiomers more available  
63 than the other (Bidleman et al., 2002; Garrison, 2006). This relationship, however, has not yet  
64 been fully demonstrated.

65 A number of reasons may explain the apparent difficulty to obtain experimental evidence  
66 for the enantioselectivity of the soil sorption process of chiral compounds and its effect on  
67 pesticide enantiomer availability. Even though enantiomers may actually differ in their  
68 affinities for chiral soil constituents, differences can be too small to be measured, as it occurs  
69 with other enantiomer-selective properties (Smith and March, 2007). In fact, even working  
70 with pure chiral sorbents, such as those used as chiral stationary phases in liquid  
71 chromatography techniques, the enantioselective sorption of chiral compounds can be  
72 difficult to observe under batch conditions (Tobler et al., 2000). This difficulty is exacerbated  
73 in natural soils, where the “non-enantioselective” component of sorption (i.e., on achiral soil  
74 sorption sites) may predominate and obscure the enantioselective component that may be

75 occurring on chiral sorption sites. On the other hand, although several studies have shown the  
76 ability of certain chiral minerals and purified organic materials to selectively adsorb chiral  
77 molecules (Bonner and Kavasmaneck, 1976; Hazen et al., 2001; Oravec et al., 2010), the  
78 heterogeneity of natural soil colloids (Celis et al. 2013; Oravec et al., 2010) and the fact that  
79 the different enantiomeric morphologies of soil components can be equally abundant in nature  
80 (Frondel, 1978; Hazen, 2014; Pauzat et al., 2015) may lead to compensation of the individual  
81 sorption affinities and result in an apparent lack of enantioselectivity on the macroscopic scale  
82 at which sorption is measured by batch equilibration procedures.

83 In a previous study, we conducted batch sorption experiments to investigate the  
84 enantioselectivity of the soil sorption process for the chiral fungicide metalaxyl, not only from  
85 racemic, but also from non-racemic initial solutions containing different *R* to *S* enantiomer  
86 ratios (Celis et al., 2015). The fungicide sorbed on natural soil from solutions enriched with *R*  
87 enantiomer to a greater extent than from racemic solutions. On the basis of the shape of the  
88 sorption isotherms and the sorptive behavior of model sorbents, we proposed that the presence  
89 of the fungicide in a non-racemic form could have self-induced an enantioselective sorption,  
90 whereby the *R* enantiomer would be preferably sorbed over the *S* enantiomer within a surface  
91 layer already dominated by other *R*-metalaxyl molecules. This mechanism would be  
92 compatible with the lack of enantioselectivity previously observed for metalaxyl sorption on  
93 soil and soil components from racemic aqueous solutions, and would also explain previous  
94 findings indicating that the sorption of racemic metalaxyl on mineral samples differed from  
95 that of Metalaxyl-M, i.e. the *R*-enantiomer enriched form of metalaxyl (Hall, 2012). We also  
96 pointed out, however, that the indirect method commonly used to determine sorption by the  
97 batch equilibration procedure can lead to erroneous estimates of sorption and its  
98 enantioselectivity if enantiomer interconversion or degradation processes occurred during the  
99 experiment.

100 The objectives of the present study were: i) to employ a batch sorption methodology with  
101 direct determination of the sorbed amounts, to estimate the sorption coefficients for individual  
102 metalaxyl enantiomers on soil from racemic and non-racemic aqueous solutions, and ii) to  
103 design column leaching tests to provide experimental evidence that enantioselective sorption  
104 to soil can affect the availability of individual pesticide enantiomers for transport by making  
105 one of the isomers less available than the other.

106

## 107 **2. Materials and methods**

### 108 *2.1. Chemical and soil*

109 The racemic and non-racemic forms of metalaxyl used in this study, *Rac*-metalaxyl and  
110 Metalaxyl-M, were supplied by Sigma-Aldrich (Spain) with a chemical purity of 99.6 and  
111 99.2%, respectively. *Rac*-metalaxyl contained equal quantities of the *R*- and *S*-metalaxyl  
112 enantiomers, whereas Metalaxyl-M contained 96.8% of *R*-metalaxyl and 3.2% of *S*-metalaxyl  
113 (Celis et al., 2015), i.e., a composition close to that reported by Buser et al. (2002) and Hall  
114 (2012). Pure *S*-metalaxyl and *R*-metalaxyl aqueous solutions were obtained by semi-  
115 preparative high-performance liquid chromatography (HPLC), under the chromatographic  
116 conditions described in section 2.4. After injecting 100  $\mu$ L of a concentrated (5000 mg/L)  
117 *Rac*-metalaxyl aqueous solution into the HPLC system, the peaks eluting at 4.9 min (*S*-  
118 metalaxyl) and 6.1 min (*R*-metalaxyl) were collected, and then the organic solvent was  
119 evaporated from the collected fractions using a gentle stream of nitrogen. The resulting  
120 aqueous solutions contained the individual enantiomers at a concentration of ca. 250 mg/L  
121 with an enantiomeric purity > 99.9%.

122 The soil used was a sandy clay loam agricultural soil sampled from an olive orchard  
123 located in Seville (Spain, 37° 17' N, 6° 2' W). It had 74% sand, 4% silt, 22% clay (12%  
124 smectites, 4% illite/mica, 6% kaolinite), 0.50% organic C and 2.5% CaCO<sub>3</sub>. The pH of a 1:2.5

125 (w/v) soil:water suspension was 8.8. The soil was sampled from the 0-20 cm surface layer,  
126 and then air-dried and sieved to pass a 2 mm mesh.

127

## 128 2.2. Batch sorption experiment

129 The sorption of *Rac*-metalaxyl and Metalaxyl-M on the soil was determined by the batch  
130 equilibration procedure using glass centrifuge tubes lined with screw caps. Sorption  
131 conditions were optimized from those used in an earlier study (Celis et al., 2015) to: i)  
132 increase the precision of the measurements by keeping the solution concentration change in  
133 the range 30-40% (Green and Yamane, 1970) and ii) remain in the region of cooperative  
134 sorption of the isotherms according to the concentration-dependent study described in Celis et  
135 al. (2015). Thus, quadruplicate 3 g-samples of soil were equilibrated with 8 mL of a 90 mg/L  
136 initial aqueous solution of either *Rac*-metalaxyl or Metalaxyl-M, which was prepared from a  
137 2.5 g/L methanolic stock solution. The suspensions were shaken for 19 h at  $20 \pm 2$  °C in an  
138 end-over-end shaker (30 r.p.m.), centrifuged at 5000 r.p.m. for 15 min, and then 6 mL of the  
139 supernatant solutions were removed, filtered, and analyzed by chiral HPLC to determine the  
140 concentration of each enantiomer in the aqueous phase of the equilibrated suspensions,  $C_e$   
141 (mg/L). Next, the 6 mL of supernatant removed for the analysis of  $C_e$  were replaced with 6  
142 mL of methanol, and the tubes were resuspended and shaken for 8 h to promote the  
143 desorption of the sorbed metalaxyl enantiomers. The suspensions were again centrifuged, and  
144 the supernatants were filtered and analyzed by HPLC to determine the amount of *S*- and *R*-  
145 metalaxyl desorbed from the soil. The amount of *S*- and *R*-metalaxyl sorbed,  $C_s$  (mg/kg), was  
146 thus calculated by two independent methods: i) indirectly, from the difference between their  
147 initial ( $C_{ini}$ ) and equilibrium aqueous phase concentration ( $C_e$ ), and ii) directly, from their  
148 desorbed amounts directly determined during the desorption step. Tubes with the initial  
149 solutions without soil were also prepared and served as controls. Indirect and direct estimates

150 of the sorption distribution coefficients,  $K_d$  (L/kg), were obtained as  $K_d = C_s/C_e$ , where  $C_s$  was  
151 calculated by applying the indirect and direct methodologies, respectively. The fraction of *S*  
152 enantiomer (EF) in the solution and sorbed phases was calculated by dividing its aqueous or  
153 sorbed concentration by the respective total (*R+S*) concentration.

154

### 155 2.3. Column leaching test

156 Column leaching experiments were carried out in glass columns of dimensions 30 cm  
157 long and 3.1 cm internal diameter. The columns were hand-packed with 120 g (10 cm) of sea  
158 sand plus glass wool at the bottom and 80 g (10 cm) of air-dried soil plus 10 g (1 cm) of sea  
159 sand at the top. Afterward, the columns were saturated with 60 mL of distilled water and the  
160 column pore volume ( $V_{p-col}$ ) was calculated as the volume of water retained by the soil  
161 columns after saturation. To avoid differences in column efficiency between replicates (Wu et  
162 al., 2012), duplicate columns with pore volumes differing in less than 5% were selected for  
163 each fungicide treatment (see below). At the end of the leaching experiment, the columns  
164 were extracted and the water content of the soil segment was measured by determining the  
165 weight loss upon heating at 105 °C for 24 h. The amount of water retained by the 80 g of soil  
166 or soil pore volume ( $V_{p-soil}$ ) was found to vary in the small range of  $30.0 \pm 1.5$  mL. The “extra  
167 soil” pore volume of each column ( $V_{ext}$ ) was calculated from the difference between its total  
168 column pore volume ( $V_{p-col}$ ) and the soil pore volume ( $V_{p-soil}$ ).

169 Once saturated, the columns were immediately treated with *Rac*-metalaxyl or Metalaxyl-  
170 M at a rate of 100 kg/ha (7.5 mg). In addition, we also studied the leaching of Metalaxyl-M  
171 and of the pure *S*- and *R*-metalaxyl enantiomers at an application rate of 20 kg/ha (1.5 mg).  
172 *Rac*-metalaxyl and Metalaxyl-M were applied to the surface of the columns dissolved in a  
173 small amount of methanol ( $\leq 1$  mL) which was allowed to evaporate for 3 h, whereas the



174 purified *S*- and *R*-metalaxyl enantiomers were applied as 6 mL of the pure enantiomer  
175 aqueous solutions (250 mg/L).

176 The columns were eluted by conducting daily five additions of 12 mL of distilled water at  
177 2 h intervals for a period of 5-7 days. The leachate resulting from each water addition was  
178 collected, filtered, and analyzed by HPLC to determine the concentration of *S*- and *R*-  
179 metalaxyl. Elution curves were obtained by plotting the percentage of enantiomer leached  
180 versus the corrected elution volume ( $V_{\text{corr}}$ ), given as number of soil pore volumes, which was  
181 calculated as:

182

$$183 \quad V_{\text{corr}} = (V - V_{\text{ext}})/V_{\text{p-soil}} \quad [1]$$

184

185 where  $V$  (mL) is the elution volume,  $V_{\text{ext}}$  (mL) is the extra soil column volume and  $V_{\text{p-soil}}$  (mL)  
186 is the soil pore volume.

187

#### 188 *2.4. Enantioselective analysis*

189 The solution concentrations of the individual *S* and *R* metalaxyl enantiomers were  
190 determined by chiral HPLC using a Waters 600E chromatograph coupled to a Waters 996  
191 diode-array detector and a Waters 717 Autosampler injector. The chromatographic conditions  
192 used for the analysis were similar to those detailed in Celis et al. (2013). We used a Chiralpak  
193 IB column (150 mm length  $\times$  4.6 mm i.d., 5  $\mu\text{m}$  particle size) from Chiral Technologies  
194 Europe (France), a 60:40 water:acetonitrile eluent mixture at a flow rate of 1 mL/min, a 25  $\mu\text{L}$   
195 sample injection volume, and UV detection at 213 nm. Retention times for *S*- and *R*-metalaxyl  
196 were 4.9 and 6.1 min, respectively. External calibration curves with five standard solutions  
197 with concentrations ranging between 1 and 100 mg/L of *Rac*-metalaxyl were used for the  
198 calculations.

199

### 200 3. Results and discussion

#### 201 3.1. Batch sorption experiment

202 Compared to our preceding work (Celis et al., 2015), the experimental conditions used  
203 here resulted in an increase in the solution concentration change from less than 18% to 30-  
204 40%, while remaining in the region of cooperative sorption of the isotherm at  $C_e (R+S) = 50$ -  
205 60 mg/L (Tables 1 and 2). The overall ( $R+S$ ) distribution coefficients,  $K_d$ , obtained from the  
206 solution depletion (indirect) method were 1.43 L/kg for *Rac*-metalaxyl and 1.79 L/kg for  
207 Metalaxyl-M (Tables 1 and 2), in good agreement with the values of 1.41 and 1.91 L/kg  
208 predicted by the Freundlich coefficients reported by Celis et al. (2015) for their isotherm  
209 sorption data. When we applied the direct method to estimate the amounts sorbed ( $C_s$ ), we  
210 obtained slightly lower  $K_d$  values compared to the indirect method (Tables 1 and 2), but still  
211 the amounts extracted from the soil accounted for > 90% of the solution concentration  
212 depletion observed during the experiment. Incomplete extraction of the sorbed fungicide  
213 and/or the greater sample manipulation associated with the direct methodology would easily  
214 explain the small differences in  $C_s$  (and  $K_d$ ) determined by the indirect and direct methods.

215 Data in Table 1 evidence that for *Rac*-metalaxyl the *R* enantiomer was sorbed to the same  
216 extent as the *S* enantiomer. The distribution coefficients of both enantiomers were identical  
217 (Table 1) and the fungicide in solution remained racemic ( $EF = 0.500 \pm 0.001$ ) after  
218 equilibration with the soil (Table 3). The direct method confirmed the racemic nature of the  
219 fungicide sorbed and subsequently extracted from the soil ( $EF = 0.501 \pm 0.001$ ). As observed  
220 in earlier studies (Celis et al., 2013; Gámiz et al., 2013; Sukul et al., 2013), the process of  
221 *racemic* metalaxyl sorption on soil appeared to be non-enantioselective, at least on the  
222 macroscopic scale at which sorption is evaluated by the batch equilibration procedure.

223 Metalaxyl-M, i.e. the *R*-enantiomer-enriched form of metalaxyl, contains the two  
224 enantiomers at an *R* to *S* non-racemic ratio of ~ 30:1 (Celis et al., 2015; Hall, 2012). Data of  
225 indirect and direct sorption measurements both indicated that the sorption of Metalaxyl-M on  
226 the soil was enantioselective, with the *R* enantiomer being sorbed to a greater extent than the  
227 *S* enantiomer (Table 2). The overall (*R+S*) sorption of Metalaxyl-M was also greater than for  
228 *Rac*-Metalaxyl, as previously observed for soil and soil minerals using indirect batch  
229 methodologies (Celis et al., 2015; Hall, 2012). It should be noted here that the indirect  
230 procedure commonly used to determine  $C_s$  by the batch equilibration method can lead to  
231 erroneous estimates of sorption and/or its enantioselectivity if enantiomer interconversion or  
232 degradation processes occurred during equilibration and/or sample storage (Celis et al., 2015;  
233 Gámiz et al., 2016). A specific experiment was conducted to confirm that these processes did  
234 not occur during sample storage (Fig. 1). That they did not occur during equilibration either  
235 was confirmed by applying the direct method to estimate  $C_s$ . The direct procedure allowed us  
236 to perform a mass balance after the sorption experiments, which showed similar total  
237 recoveries of  $96.8 \pm 0.7\%$  for *S*-metalaxyl and  $98.3 \pm 0.7\%$  for *R*-metalaxyl. Consequently, *R*  
238 to *S* interconversion or preferential degradation of the *R* enantiomer during equilibration can  
239 be ruled out as a cause of the greater depletion in solution concentration observed for the *R*  
240 enantiomer compared to the *S* enantiomer (Table 2). The direct procedure also showed that,  
241 compared to the initial solution ( $EF= 0.032 \pm 0.001$ ), the equilibrated solution phase became  
242 enriched in *S* enantiomer ( $EF= 0.037 \pm 0.001$ ), whereas the soil solid phase became enriched  
243 in *R* enantiomer ( $EF= 0.025 \pm 0.001$ ) (Table 3). Thus, for Metalaxyl-M, enantioselective (*R*  
244 over *S*) sorption to the soil particles was strongly supported by the direct determination of the  
245 amounts of *S*- and *R*-metalaxyl sorbed on the soil. As a possible explanation, Celis et al.  
246 (2015) proposed that molecular interactions between metalaxyl enantiomer species at the  
247 sorbed state, with *R-R* interactions being energetically more favorable than *R-S* interactions,

248 could have promoted the enhanced sorption of the *R* enantiomer within a sorbed layer already  
249 dominated by other *R*-metalaxyl molecules. It is interesting to note that in preliminary  
250 experiments conducted with initial solutions enriched with *S*-enantiomer (~ 95% *S* and 5% *R*)  
251 we also observed enantioselective sorption of metalaxyl to soil particles, but in this case the *S*-  
252 enantiomer was sorbed to a greater extent than the *R* enantiomer (see Table S1 of the  
253 supplementary material). As expected, within a surface layer dominated by *S*-metalaxyl  
254 molecules the *S*-enantiomer was preferably sorbed over the *R*-enantiomer. This result supports  
255 the hypothesis that homochiral (*R-R* and *S-S*) interactions between metalaxyl enantiomers at  
256 the sorbed state are more favorable than heterochiral (*R-S*) interactions.

257

### 258 *3.2. Column leaching experiment*

259 To confirm the enantiomer-selective sorption for Metalaxyl-M on the soil, a column  
260 leaching experiment was designed. Our hypothesis was that if the *R*-enantiomer was actually  
261 preferentially sorbed over the *S*-enantiomer, then it should show enhanced retardation in a  
262 standard soil column leaching experiment, i.e. it should elute later than the *S*-enantiomer.  
263 Enhanced retardation of the *R* enantiomer would also constitute a direct evidence that sorption  
264 enantioselectivity can actually affect the availability of pesticide residues in soil for various  
265 environmental processes (leaching, volatilization, absorption by organisms, etc.) by making  
266 one of the enantiomers less available than the other. The fungicide application rate and the  
267 duration of the leaching experiment were optimized to: i) ensure that the leachate  
268 concentrations were high enough to fall in the region of cooperative (enantioselective)  
269 sorption of the isotherm (Celis et al., 2015), and ii) achieve complete leaching of the applied  
270 fungicide within 1 week, because preliminary data indicated that degradation of metalaxyl  
271 enantiomers during this period was insignificant in the tested soil.

272 The breakthrough curves (BTCs) for the *S* and *R* enantiomers of metalaxyl applied to the  
273 soil columns as Metalaxyl-M at rates of 20 and 100 kg/ha are shown in Fig. 2. The  
274 corresponding BTCs for *Rac*-Metalaxyl applied at 100 kg/ha are also given for comparative  
275 purposes. The results show that while the leaching of *Rac*-metalaxyl was non  
276 enantioselective, enantioselectivity was evident for Metalaxyl-M (Fig. 2). The BTCs of the *R*  
277 enantiomer were broader and the maximum concentration appeared at higher elution volumes  
278 compared to the *S* enantiomer, strongly indicating that the enhanced sorption of the *R*-  
279 enantiomer retarded its leaching through the soil column (Fig. 2 and Table 4). These effects  
280 were much more pronounced for the application rate of 100 kg/ha, reflecting that, in  
281 agreement with the shape of the sorption isotherms (Celis et al., 2015), enantioselectivity in  
282 leaching was also concentration-dependent. The total amount of *S*- and *R*-metalaxyl leached  
283 (> 95%) confirmed that degradation and enantiomer interconversion played a minor role, if  
284 any, during the experiment (Table 4). Interestingly, the BTCs of the purified *S*- and *R*-  
285 metalaxyl enantiomers applied separately to soil columns at 20 kg/ha were found to be nearly  
286 identical to each other and very similar to that of the *R*-enantiomer applied as Metalaxyl-M at  
287 the rate of 20 kg/ha (see Fig. S1 and Table S2 in the supplementary material). This indicated  
288 that the inherent affinity of the soil particles for metalaxyl was not enantiomer-selective and  
289 supported that enantioselectivity in the sorption and leaching of Metalaxyl-M was induced by  
290 the presence of the fungicide in a non-racemic form.

291 The soil-water distribution coefficients ( $K_d$ ) for the *S* and *R* enantiomers of *Rac*-metalaxyl  
292 and Metalaxyl-M experimentally determined by the direct batch equilibration method (Tables  
293 1 and 2) were used to calculate the predicted retardation factors (RF) for the soil section  
294 during leaching using the equation (Rao et al., 1985):

295

$$296 \quad \text{RF} = 1 + (\rho/\Theta) \cdot K_d \quad [2]$$

297

298 In Eq. 2 RF represents the number of soil pore volumes at which  $C_{\max}$  is expected to appear in  
299 leachates (after correcting for the extra soil column volume),  $\rho$  is the bulk density of the soil  
300 ( $\text{g}/\text{cm}^3$ ),  $\Theta$  is the volumetric water content of the soil, and  $K_d$  is the soil-water distribution  
301 coefficient for the analyte. Using eq. 2 is clearly an oversimplification for our system, since  
302 linear, instantaneous, and reversible sorption is assumed, and particularly the condition of  
303 linearity does not apply for metalaxyl sorption to the soil (Celis et al., 2015). Despite this, it is  
304 interesting to note that eq. 2 predicts an RF of 4.5 for the elution of both enantiomers when  
305 they are applied as *Rac*-metalaxyl and RF values of 4.1 and 5.6 for the elution of the *S* and *R*  
306 enantiomers, respectively, when applied as Metalaxyl-M. These predicted RF values are in  
307 reasonable agreement with the observed positions of  $C_{\max}$  (Table 4), particularly for the BTCs  
308 obtained at the application rate of 100 kg/ha (Table 4). For this application rate, the values of  
309  $C_{\max}$  monitored during the leaching experiment (Table 4) were very close to the equilibrium  
310 concentration ( $C_e$ ) of the batch sorption experiment (Tables 1 and 2), so that the batch  
311 equilibrium  $K_d$  value may have represented reasonably well the sorption of metalaxyl  
312 enantiomers during leaching.

313

### 314 *3.3. Environmental implications and concluding remarks*

315 The direct link between enantiomer-selective sorption and availability shown in this work  
316 has very important implications, as it demonstrates that not only biotic, but also abiotic  
317 processes can affect the chiral signatures of pesticides and related compounds in  
318 environmental samples. This is clearly illustrated in Fig. 3, where it can be seen that soil  
319 leachates from columns treated with Metalaxyl-M suffered from fluctuations in their  
320 enantiomer composition (EF values ranged between  $< 0.01$  and 0.08), as a result of the  
321 different leaching patterns of the *S* and *R* metalaxyl enantiomers (Fig. 2). On the assumption

322 that only biologically-mediated processes can be enantioselective, the enantiomer  
323 composition of the leachates should have remained constant with an EF= 0.032, since we  
324 demonstrated that biodegradation played a minor role during our experimental conditions.  
325 This was in fact the case of leachates from columns treated with *Rac*-metalaxyl, which  
326 presented a constant EF value equal to that of the added pesticide (EF= 0.500, Fig. 3).

327 The results of the present work, along with additional existing data suggesting that  
328 enantioselective sorption from non-racemic metalaxyl solutions can also occur on purified  
329 soil components (Hall, 2012; Celis et al., 2015) and on different soil types (see Table S1 in  
330 the supplementary material), support the conclusion that enantioselective sorption from non-  
331 racemic solutions should be considered as a possible mechanism that can contribute to the  
332 enantiomeric composition or chiral signatures of pesticides and related contaminants in soil,  
333 water, and air samples. Pesticide sorption enantioselectivity from non-racemic solutions  
334 should be particularly considered: i) when assessing the fate of chiral pesticides that are  
335 commercialized and applied to agricultural fields as formulations enriched with the  
336 biologically-active enantiomer, i.e. as non-racemic pesticide mixtures, and ii) when  
337 monitoring and assessing the fate of aged chiral pesticide residues in the environment, since  
338 these residues, even if originally racemic, may have become non-racemic as a result of  
339 enantioselective biologically-mediated processes. Metalaxyl, for example, is currently applied  
340 to agricultural fields both as a racemate and as *R*-enantiomer enriched product (Metalaxyl-M)  
341 at relatively high application rates of up to 10 kg/ha. It is also well known that the degradation  
342 of metalaxyl in soils is an enantioselective, biologically-mediated process that can yield non-  
343 racemic residues shortly after application of the fungicide in a racemic form (Buerge et al.,  
344 2003). These characteristics make metalaxyl a good candidate for displaying enantioselective  
345 sorption and availability under real agricultural application conditions. Moreover, the

346 mechanism reported here could also be applicable to other chiral pollutants reaching the soil  
347 as a result of anthropogenic activities.

348

### 349 **Acknowledgments**

350 This work was financed by the Spanish Ministry of Economy and Competitiveness (MINECO  
351 Project AGL2014-51897-R) and Junta de Andalucía (Research Group AGR-264), with  
352 European FEDER funds (Operative Program 2014-2020).

353

354

### 355 **References**

- 356 Bidleman, T.F., Jantunen, L.M., Harner, T., Wiberg, K., Wideman, J.L., Brice, K., Su, K.,  
357 Falconer, R.L., Aigner, E.J., Leone, A.D., Ridal, J.J., Kerman, B., Finizio, A., Alegria, H.,  
358 Parkhurst, W.J., Szeto, S.Y., 1998. Chiral pesticides as tracers of air-surface exchange.  
359 *Environ. Pollut.* 102, 43-49.
- 360 Bidleman, T.F., Leone, A.D., Falconer, R.L., Harner, T., Jantunen, L.M.M., Wiberg, K.,  
361 Helm, P.A., Diamond, M.L., Loo, B., 2002. Chiral pesticides in soil and water and  
362 exchange with the atmosphere. *Sci. World J.* 2, 357-373.
- 363 Bonner, W.A., Kavasmaneck, P.R., 1976. Asymmetric adsorption of DL-alanine  
364 hydrochloride by quartz. *J. Org. Chem.* 41, 2225-2226.
- 365 Buerge, I.J., Poiger, T., Müller, M.D., Buser, H.R., 2003. Enantioselective degradation of  
366 metalaxyl in soils: chiral preference changes with soil pH. *Environ. Sci. Technol.* 37, 2668-  
367 2674.
- 368 Buser, H.R., Müller, M.D., Poiger, T., Balmer, M.E., 2002. Environmental behavior of the  
369 chiral acetamide pesticide metalaxyl: enantioselective degradation and chiral stability in  
370 soil. *Environ. Sci. Technol.* 36, 221-226.



371 Buser, H.R., Müller, M.D., Rappe, C., 1992. Enantioselective determination of chlordane  
372 components using chiral high-resolution gas chromatography-mass spectrometry with  
373 application to environmental samples. *Environ. Sci. Technol.* 26, 1533-1540.

374 Celis, R., Gámiz, B., Adelino, M.A., Hermosín, M.C., Cornejo, J., 2013. Environmental  
375 behavior of the enantiomers of the chiral fungicide metalaxyl in Mediterranean agricultural  
376 soils. *Sci. Total Environ.* 444, 288-297.

377 Celis, R., Gámiz, B., Facenda, G., Hermosín, M.C., 2015. Enantioselective sorption of the  
378 chiral fungicide metalaxyl on soil from non-racemic aqueous solutions: environmental  
379 implications. *J. Hazard. Mater.* 300, 581-589.

380 Chen, S., Liu, W., 2008. Toxicity of chiral pesticide *Rac*-Metalaxyl and *R*-Metalaxyl to  
381 *Daphnia magna*. *Bull. Environ. Contam. Toxicol.* 81, 531-534.

382 Frondel, C., 1978. Characters of quartz fibers. *Am. Mineral.* 63, 17-27.

383 Gámiz, B., Celis, R., Hermosín, M.C., Cornejo, J., 2013. Effect of olive-mill waste addition to  
384 agricultural soil on the enantioselective behavior of the chiral fungicide metalaxyl. *J.*  
385 *Environ. Manag.* 128, 92-99.

386 Gámiz, B., Pignatello, J.J., Cox, L., Hermosín, M.C., Celis, R., 2016. Environmental fate of  
387 the fungicide metalaxyl in soil amended with composted olive-mill waste and its biochar:  
388 an enantioselective study. *Sci. Total Environ.* 541, 776-783.

389 Garrison, A.W., 2006. Probing the enantioselectivity of chiral pesticides. *Environ. Sci.*  
390 *Technol.* 40, 16-23.

391 Green, R.E., Yamane, V.K., 1970. Precision in pesticide adsorption measurements. *Soil Sci.*  
392 *Soc. Am. J.* 34, 353-355.

393 Hall, A., 2012. Sorption and Enantiomerization of Current Use Chiral Pesticides, All  
394 Dissertations, Paper 1046, Clemson University TigerPrints.

395 Han, J.W., Sholl, D.S., 2010. Enantiospecific adsorption of amino acids on hydroxylated  
396 quartz (10 $\bar{1}$ 0). *Phys. Chem. Chem. Phys.* 12, 8024-8032.

397 Hazen, R.M., Filley, T.R., Goodfriend, G.A., 2001. Selective adsorption of L- and D-amino  
398 acids on calcite: implication for biochemical homochirality. *Proc. Natl. Acad. Sci. USA* 98,  
399 5487-5490.

400 Hazen, R.M., 2014. Enantioselective adsorption on rock-forming minerals: a thought  
401 experiment. *Surf. Sci.* 629, 11-14.

402 Hegeman, W.J.M., Laane, W.P.M., 2002. Enantiomeric enrichment of chiral pesticides in the  
403 environment. *Rev. Environ. Contam. Toxicol.* 173, 85-116.

404 Kurt-Karakus, P.B., Bidleman, T.F., Jones, K.C., 2005. Chiral organochlorine pesticide  
405 signatures in global background soils. *Environ. Sci. Technol.* 39, 8671-8677.

406 Lewis, D.L., Garrison, A.W., Wommack, K.E., Whittemore, A., Steudler, P., Melillo, J.,  
407 1999. Influence of environmental changes on degradation of chiral pollutants in soils.  
408 *Nature* 401, 898-901.

409 Li, Y., Dong, F., Liu, X., Xu, J., Li, J., Kong, Z., Chen, X., Zheng, Y., 2012. Environmental  
410 behavior of the chiral triazole fungicide fenbuconazole and its chiral metabolites:  
411 enantioselective transformation and degradation in soils. *Environ. Sci. Technol.* 46, 2675-  
412 2683.

413 Li, J., Zhang, J., Li, C., Wang, W., Yang, Z., Wang, H., Gan, J., Ye, Q., Xu, X., Li, Z., 2013.  
414 Stereoisomeric isolation and stereoselective fate of insecticide paichongding in flooded  
415 paddy soils. *Environ. Sci. Technol.* 47, 12768-12774.

416 Liu, W., Gan, J., Schlenk, D., Jury, W.A., 2005. Enantioselectivity in environmental safety of  
417 current chiral insecticides. *Proc. Natl. Acad. Sci. USA* 102, 701-706.

418 Liu, W., Ye, J., Jin, M., 2009. Enantioselective phytoeffects of chiral pesticides. *J. Agric.*  
419 *Food Chem.* 57, 2087-2095.

420 Liu, Z., Lee, C., 2007. The role of organic matter in the sorption capacity of marine  
421 sediments. *Mar. Chem.* 105, 240-257.

422 Magrans, J.O., Alonso-Prados, J.L., García-Baudín, J.L., 2002. Importance of considering  
423 pesticide stereoisomerism – proposal of a scheme to apply directive 91/414/CEE  
424 framework to pesticide active substances manufactured as isomeric mixtures.  
425 *Chemosphere* 49, 461-469.

426 Matallo, M., Romero, E., Sánchez-Rasero, F., Peña, A., Dios, G., 1998. Adsorption of  
427 mecoprop and dichlorprop on calcareous and organic matter amended soils: comparative  
428 adsorption of racemic and pure enantiomeric forms. *J. Environ. Sci. Health B* 33, 61-66.

429 Oravec, M., Simek, Z., Holoubek, I., 2010. The effect of humic acid and ash on enantiomeric  
430 fraction change of chiral pollutants. *Colloids Surf. A* 359, 60-65.

431 Pauzat, F., Marloie, G., Markovits, A., Ellinger, Y., 2015. Global versus local adsorption  
432 selectivity. *Intern. J. Astrobiol.* 14, 563-570.

433 Qin, F., Gao, Y.X., Guo, B.Y., Xu, P., Li, J.Z., Wang, H.L., 2014. Environmental behavior of  
434 benalaxyl and furalaxyl enantiomers in agricultural soils. *J. Environ. Sci. Health B* 49, 738-  
435 746.

436 Ramezani, M.K., Oliver, D.P., Kookana, R.S., Lao, W., Gill, G., Preston, C., 2010. Faster  
437 degradation of herbicidally-active enantiomer of imidazolinones in soils. *Chemosphere* 79,  
438 1040-1045.

439 Rao, P.S.C., Hornsby, A.G., Jessup, R.E., 1985. Indices for ranking the potential for pesticide  
440 contamination of groundwater. *Proc. Soil Crop. Sci. Soc. Fla.* 44, 1-8.

441 Shaner, D.L., Brunk, G., Belles, D., Westra, P., Nissen, S., 2006. Soil dissipation and  
442 biological activity of metolachlor and *S*-metolachlor in five soils. *Pest Manag. Sci.* 62,  
443 617-623.

444 Smith, M.B., March, J., 2007. March's Advanced Organic Chemistry: Reactions,  
445 Mechanisms, and Structure, Sixth Edition, John Wiley & Sons.

446 Sukul, P., Lamshoft, M., Zuhlke, S., Spitteller, M., 2013. Evaluation of sorption-desorption  
447 processes for metalaxyl in natural and artificial soils. J. Environ. Sci. Health B 48, 431-  
448 441.

449 Tobler, E., Lämmerhofer, M., Oberleitner, W.R., Maier, N.M., Lindner, W., 2000.  
450 Enantioselective liquid-solid extraction for screening of structurally related chiral  
451 stationary phases. Chromatographia 51, 65-70.

452 Wedyan, M., Preston, M.R., 2005. Isomer-selective adsorption of amino acids by components  
453 of natural sediments. Environ. Sci. Technol. 39, 2115-2119.

454 Williams, A., 1996. Opportunities for chiral agrochemicals. Pestic. Sci. 46, 3-9.

455 Williams, G.M., Harrison, I., Carlick, C.A., Crowley, O., 2003. Changes in enantiomeric  
456 fraction as evidence of natural attenuation of mecoprop in a limestone aquifer. J. Contam.  
457 Hydrol. 64, 253-267.

458 Wong, C.S., 2006. Environmental fate processes and biochemical transformations of chiral  
459 emerging organic pollutants. Anal. Bioanal. Chem. 386, 544-558.

460 Wu, N., Bradley, A.C., Welch, C.J., Zhang, L., 2012. Effect of extra-column volume on  
461 practical chromatographic parameters of sub-2- $\mu$ m particle-packed columns in ultra-high  
462 pressure liquid chromatography. J. Sep. Sci. 35, 2018-2025.

463 Ye, J., Zhao, M., Liu, J., Liu, W., 2010. Enantioselectivity in environmental risk assessment  
464 of modern chiral pesticides. Environ. Pollut. 158, 2371-2383.

465 Zipper, C., Suter, M.J.F., Haderlein, S.B., Gruhl, M., Kohler, H.P.E., 1998. Changes in the  
466 enantiomeric ratio of (*R*)- to (*S*)-mecoprop indicate in situ biodegradation of this chiral  
467 herbicide in a polluted aquifer. Environ. Sci. Technol. 32, 2070-2076.

468

469

### Figure captions

470

471 **Fig. 1.** Concentrations of *S*- and *R*-metalaxyl (left) and enantiomer fractions (right) as a  
472 function of time of a Metalaxyl-M solution (90 mg/L) prepared in water and in aqueous soil  
473 extract. The soil extract was prepared by shaking 30 g of soil with 80 ml of water for 2 h,  
474 followed by centrifugation and collection of the supernatant.

475

476 **Fig. 2.** Relative (left) and cumulative (right) breakthrough curves (BTCs) for the *S* and *R*  
477 enantiomers of metalaxyl applied to soil columns as *Rac*-metalaxyl (100 kg/ha) or Metalaxyl-  
478 M (20 and 100 kg/ha).

479

480 **Fig. 3.** Evolution of the enantiomer fraction (EF) in soil leachates during the column leaching  
481 experiments with *Rac*-metalaxyl and Metalaxyl-M.

**Table 1**Overall and individual enantiomer batch sorption data for *Rac*-metalaxyl on soil.

| Enantiomer | Initial<br>concentration<br>(mg/L) | Equilibrium<br>concentration<br>(mg/L) | $C_s$<br>(indirect)<br>(mg/kg) | $C_s$<br>(direct)<br>(mg/kg) | $K_d$<br>(indirect)<br>(L/kg) | $K_d$<br>(direct)<br>(L/kg) |
|------------|------------------------------------|--|--------------------------------|------------------------------|-------------------------------|-----------------------------|
| <i>S</i>   | 44.8 (0.1) <sup>a</sup>            | 29.2 (0.1)                             | 41.8 (0.2)                     | 38.2 (0.1)                   | 1.43 (0.01)                   | 1.31 (0.01)                 |
| <i>R</i>   | 44.8 (0.1)                         | 29.1 (0.1)                             | 41.8 (0.2)                     | 38.1 (0.1)                   | 1.43 (0.01)                   | 1.31 (0.01)                 |
| <i>R+S</i> | 89.6 (0.1)                         | 58.3 (0.2)                             | 83.6 (0.4)                     | 76.4 (0.2)                   | 1.43 (0.01)                   | 1.31 (0.01)                 |

<sup>a</sup> Values in parentheses correspond to the standard deviations of quadruplicate measurements.

**Table 2**

Overall and individual enantiomer batch sorption data for Metalaxyl-M on soil.

| Enantiomer | Initial<br>concentration<br>(mg/L) | Equilibrium<br>concentration<br>(mg/L) | $C_s$<br>(indirect)<br>(mg/kg) | $C_s$<br>(direct)<br>(mg/kg) | $K_d$<br>(indirect)<br>(L/kg) | $K_d$<br>(direct)<br>(L/kg) |
|------------|------------------------------------|--|--------------------------------|------------------------------|-------------------------------|-----------------------------|
| <i>S</i>   | 2.90 (0.01) <sup>a</sup>           | 1.96 (0.02)                            | 2.50 (0.04)                    | 2.25 (0.06)                  | 1.27 (0.03)                   | 1.15 (0.03)                 |
| <i>R</i>   | 86.5 (0.1)                         | 51.6 (0.2)                             | 93.1 (0.5)                     | 89.1 (1.4)                   | 1.80 (0.02)                   | 1.73 (0.03)                 |
| <i>R+S</i> | 89.4 (0.1)                         | 53.6 (0.2)                             | 95.6 (0.5)                     | 91.4 (1.5)                   | 1.79 (0.02)                   | 1.71 (0.03)                 |

<sup>a</sup> Values in parentheses correspond to the standard deviations of quadruplicate measurements.

**Table 3**

Enantiomer fraction (EF) of the initial solution, equilibrated solution phase, and equilibrated soil-sorbed phase for the batch soil sorption experiment conducted with *Rac*-metalaxyl and Metalaxyl-M.

|                       | Initial solution           | Equilibrated<br>aqueous phase | Equilibrated<br>sorbed phase<br>(indirect) | Equilibrated<br>sorbed phase<br>(direct) |
|-----------------------|----------------------------|-------------------------------|--|--|
| <i>Rac</i> -metalaxyl | 0.500 (0.001) <sup>a</sup> | 0.500 (0.001)                 | 0.500 (0.001)                              | 0.501 (0.001)                            |
| Metalaxyl-M           | 0.032 (0.001)              | 0.037 (0.001)                 | 0.026 (0.001)                              | 0.025 (0.001)                            |

<sup>a</sup> Values in parentheses correspond to the standard deviations of quadruplicate measurements.



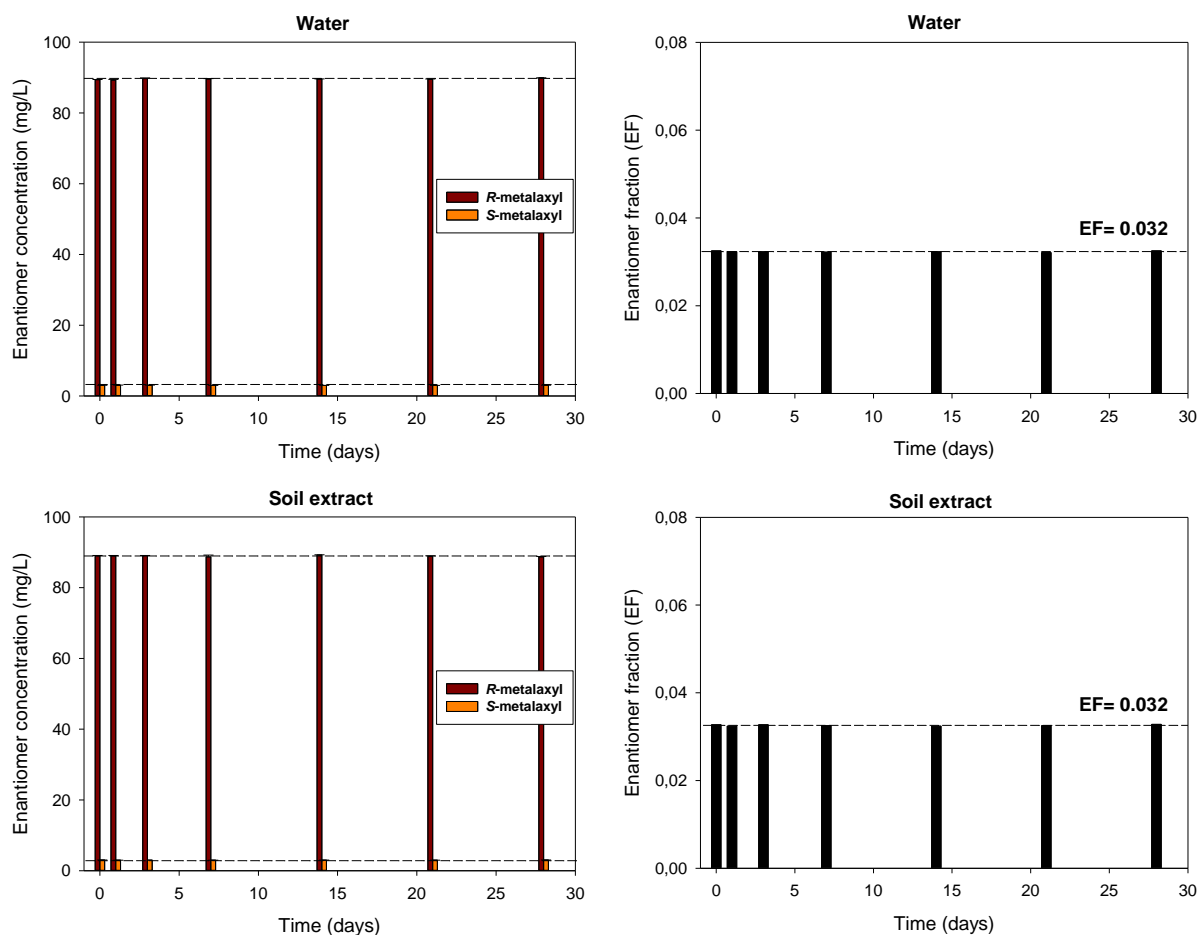
**Table 4**

Summary of *S*- and *R*-metalaxyl column leaching data extracted from the breakthrough curves (BTCs) of *Rac*-metalaxyl and Metalaxyl-M.<sup>a</sup>

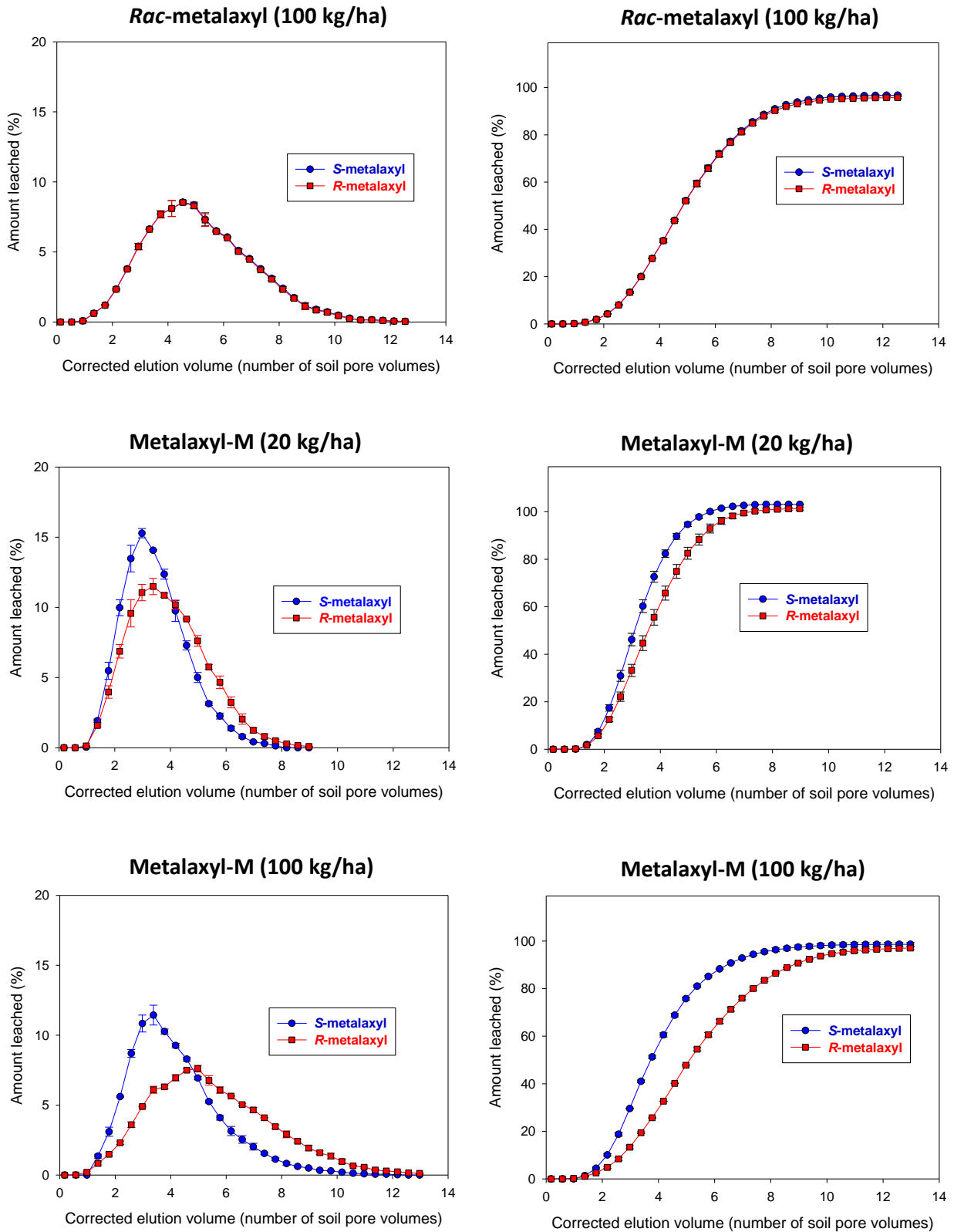
| Enantiomer                              | $C_{\max}$<br>(mg/L)    | Position of $C_{\max}$<br>( $\times V_{p\text{-soil}}$ ) | Total leached<br>(%) |
|---|-------------------------|--|----------------------|
| <b><i>Rac</i>-metalaxyl (100 kg/ha)</b> |                         |  |                      |
| <i>S</i> -metalaxyl                     | 28.4 (0.9) <sup>b</sup> | 4.53 (0.00)  | 96.8 (0.6)           |
| <i>R</i> -metalaxyl                     | 28.4 (0.9)              | 4.53 (0.00)  | 95.8 (0.6)           |
| <b>Metalaxyl-M (20 kg/ha)</b>           |                         |  |                      |
| <i>S</i> -metalaxyl                     | 0.64 (0.02)             | 2.97 (0.04)  | 103.0 (0.1)          |
| <i>R</i> -metalaxyl                     | 14.8 (0.6)              | 3.38 (0.04)  | 101.3 (0.8)          |
| <b>Metalaxyl-M (100 kg/ha)</b>          |                         |  |                      |
| <i>S</i> -metalaxyl                     | 2.16 (0.06)             | 3.37 (0.01)  | 98.6 (1.1)           |
| <i>R</i> -metalaxyl                     | 43.2 (2.2)              | 4.77 (0.01)  | 97.1 (0.9)           |

<sup>a</sup>  $C_{\max}$ : maximum concentration of *S*- and *R*-metalaxyl in leachates; position of  $C_{\max}$ : number of soil pore volumes ( $V_{p\text{-soil}}$ ) at which  $C_{\max}$  appeared in leachates (i.e., corrected elution volume given in number of soil pore volumes); total leached: total amount of *S* and *R* metalaxyl recovered in leachates.

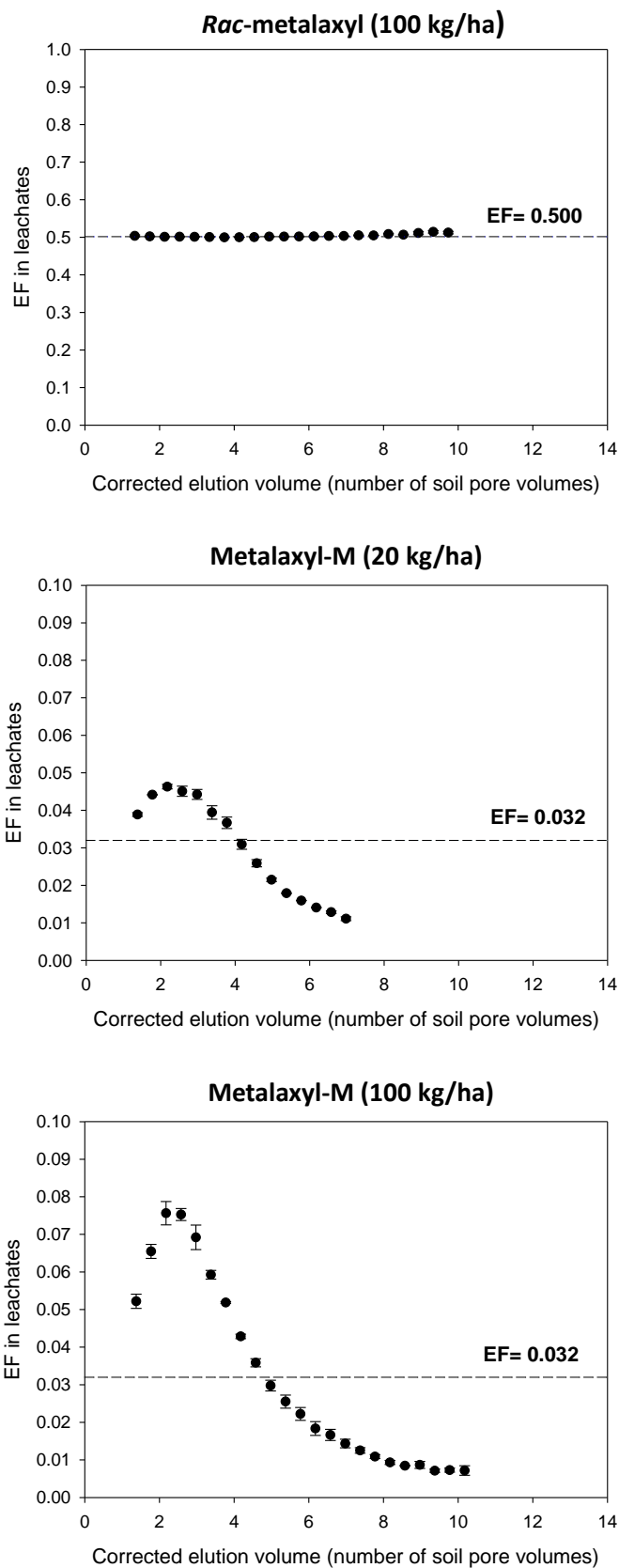
<sup>b</sup> Values in parentheses correspond to the standard deviations of duplicate soil columns.



**Figure 1.** Concentrations of *S*- and *R*-metalaxyl (left) and enantiomer fractions (right) as a function of time of a Metalaxyl-M solution (90 mg/L) prepared in water and in aqueous soil extract. The soil extract was prepared by shaking 30 g of soil with 80 ml of water for 2 h, followed by centrifugation and collection of the supernatant.



**Fig. 2.** Relative (left) and cumulative (right) breakthrough curves (BTCs) for the *S* and *R* enantiomers of metalaxyl applied to soil columns as *Rac*-metalaxyl (100 kg/ha) or Metalaxyl-M (20 and 100 kg/ha).



**Fig. 3.** Evolution of the enantiomer fraction (EF) in soil leachates during the column leaching experiments with *Rac*-metalaxyl and Metalaxyl-M.

## Supplementary data for

Evidence for the effect of sorption enantioselectivity on the availability of chiral pesticide  
enantiomers in soil

Beatriz Gámiz, Gracia Facenda, Rafael Celis\*

*Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC, Avenida Reina  
Mercedes 10, 41012 Sevilla, Spain*

Number of tables: 2

Number of figures: 1

**Table S1**

Overall and individual enantiomer batch sorption data for Metalaxyl-M and for a 95:5 *S*:*R* mixture of metalaxyl enantiomers on a clay soil.<sup>a</sup>

| Enantiomer   | Initial<br>concentration<br>(mg/L) | Equilibrium<br>concentration<br>(mg/L) | $C_s$<br>(indirect)<br>(mg/kg) | $C_s$<br>(direct)<br>(mg/kg) | $K_d$<br>(indirect)<br>(L/kg) | $K_d$<br>(direct)<br>(L/kg) |
|--|------------------------------------|--|--------------------------------|------------------------------|-------------------------------|-----------------------------|
| <b>Metalaxyl-M</b>   |                                    |  |                                |                              |                               |                             |
| <i>S</i>   | 0.85 (0.01) <sup>b</sup>           | 0.54 (0.01)                            | 2.55 (0.04)                    | 2.51 (0.06)                  | 4.8 (0.1)                     | 4.7 (0.1)                   |
| <i>R</i>   | 24.8 (0.1)                         | 12.5 (0.1)                             | 98.2 (0.6)                     | 89.2 (1.1)                   | 7.9 (0.1)                     | 7.1 (0.1)                   |
| <i>R+S</i>   | 25.6 (0.1)                         | 13.0 (0.1)                             | 100.8 (0.6)                    | 91.7 (1.1)                   | 7.7 (0.1)                     | 7.0 (0.1)                   |
| <b>95:5 <i>S</i>:<i>R</i> mixture of metalaxyl enantiomers</b> |                                    |  |                                |                              |                               |                             |
| <i>S</i>   | 24.8 (0.1)                         | 11.8 (0.1)                             | 101.9 (0.8)                    | nd <sup>c</sup>              | 8.6 (0.1)                     | nd                          |
| <i>R</i>   | 1.24 (0.01)                        | 0.73 (0.01)                            | 4.12 (0.07)                    | nd                           | 5.7 (0.2)                     | nd                          |
| <i>R+S</i>   | 26.1 (0.1)                         | 12.6 (0.1)                             | 106.1 (0.9)                    | nd                           | 8.4 (0.1)                     | nd                          |

<sup>a</sup> Sorption was measured at a soil to solution ratio of 1 g : 8 mL. The soil had 6% sand, 26% silt, 68% clay, 0.49% organic C, 1.6% CaCO<sub>3</sub> and a pH of 8.6.

<sup>b</sup> Values in parentheses correspond to the standard deviations of quadruplicate measurements.

<sup>c</sup> Not determined.

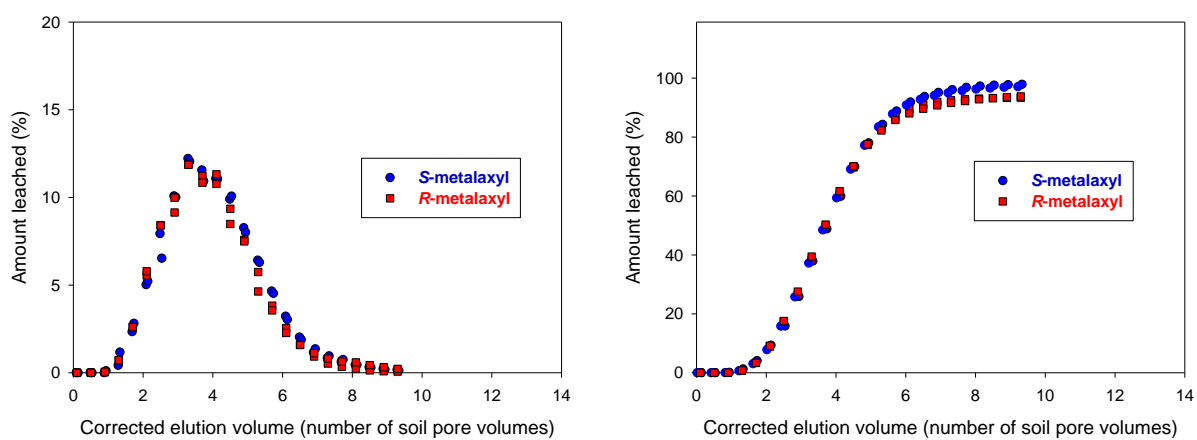
**Table S2**

Summary of *S*-metalaxyl and *R*-metalaxyl column leaching data extracted from the breakthrough curves (BTCs) obtained after their application to sandy clay loam soil columns as pure enantiomers.<sup>a</sup>

| Enantiomer          | $C_{\max}$<br>(mg/L)    | Position of $C_{\max}$<br>( $\times V_{p\text{-soil}}$ ) | Total leached<br>(%) |
|---------------------|-------------------------|--|----------------------|
| <i>S</i> -metalaxyl | 16.5 (0.1) <sup>b</sup> | 3.31 (0.03)  | 98.4 (0.6)           |
| <i>R</i> -metalaxyl | 16.7 (0.1)              | 3.30 (0.01)  | 93.6 (0.2)           |

<sup>a</sup>  $C_{\max}$ : maximum concentration in leachates; position of  $C_{\max}$ : number of soil pore volumes ( $V_{p\text{-soil}}$ ) at which  $C_{\max}$  appeared in leachates (i.e., corrected elution volume given in number of soil pore volumes); total leached: total amount recovered in leachates.

<sup>b</sup> Values in parentheses correspond to the standard deviations of duplicate soil columns.



**Fig. S1.** Relative (left) and cumulative (right) breakthrough curves (BTCs) for the *S* and *R* enantiomers of metalaxyl applied separately to sandy clay loam soil columns as pure enantiomers at a rate of 20 kg/ha.