

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

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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 μ 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% CaCO₃. 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 ± 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 ± 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 (≤ 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_{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_{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 μm 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 μL
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

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

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), ρ is the bulk density of the soil
300 (g/cm^3), Θ 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

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353

354

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

Enantiomer	Initial concentration (mg/L)	Equilibrium concentration (mg/L)	C_s (indirect) (mg/kg)	C_s (direct) (mg/kg)	K_d (indirect) (L/kg)	K_d (direct) (L/kg)
<i>S</i>	44.8 (0.1) ^a	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)

^a 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 concentration (mg/L)	Equilibrium concentration (mg/L)	C_s (indirect) (mg/kg)	C_s (direct) (mg/kg)	K_d (indirect) (L/kg)	K_d (direct) (L/kg)
<i>S</i>	2.90 (0.01) ^a	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)

^a 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 aqueous phase	Equilibrated sorbed phase (indirect)	Equilibrated sorbed phase (direct)
<i>Rac</i> -metalaxyl	0.500 (0.001) ^a	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)

^a 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.^a

Enantiomer	C_{\max} (mg/L)	Position of C_{\max} ($\times V_{p\text{-soil}}$)	Total leached (%)
<i>Rac</i>-metalaxyl (100 kg/ha)			
<i>S</i> -metalaxyl	28.4 (0.9) ^b	4.53 (0.00)	96.8 (0.6)
<i>R</i> -metalaxyl	28.4 (0.9)	4.53 (0.00)	95.8 (0.6)
Metalaxyl-M (20 kg/ha)			
<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)
Metalaxyl-M (100 kg/ha)			
<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)

^a 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.

^b Values in parentheses correspond to the standard deviations of duplicate soil columns.

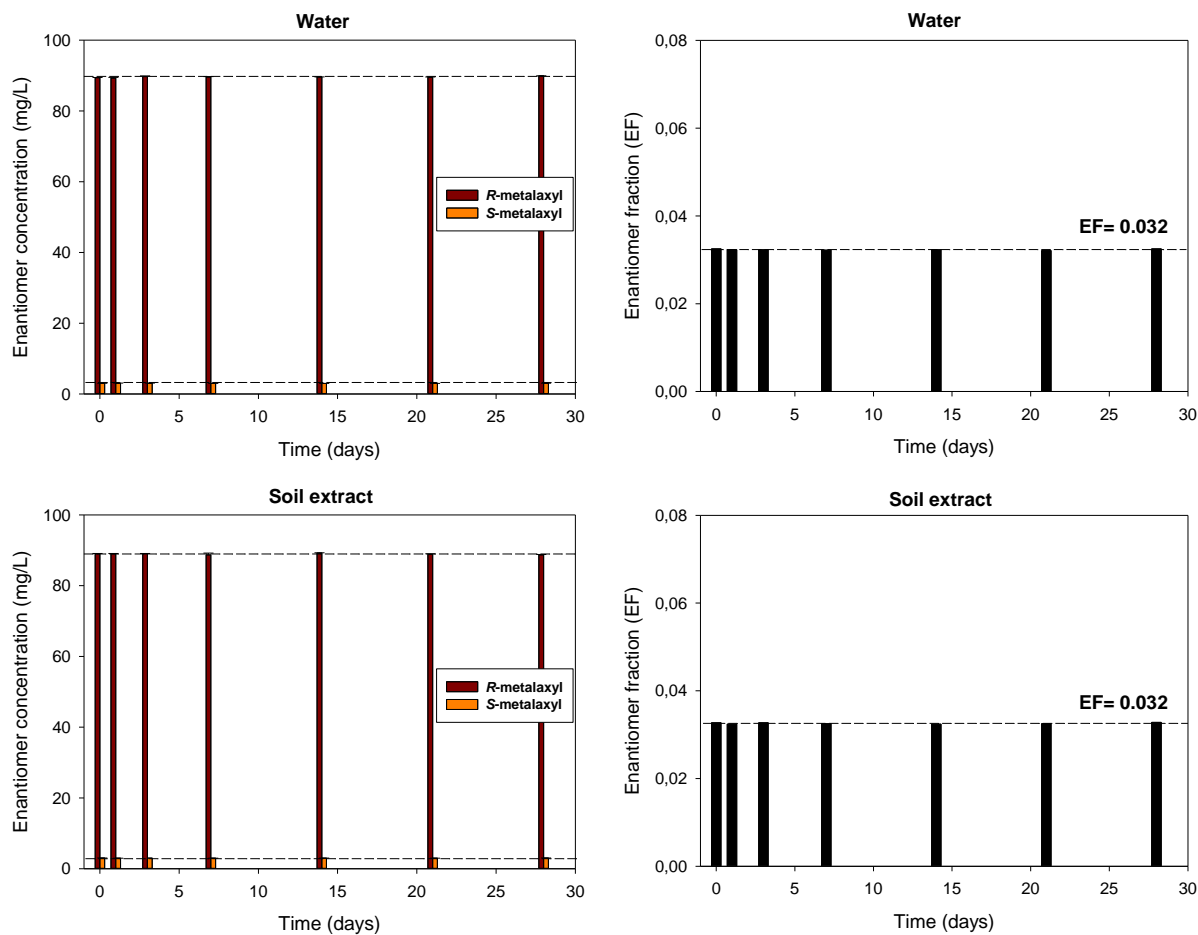


Figure 1. Concentrations of *S*- and *R*-metalaxyl (left) and enantiomer fractions (right) as a function of time of a Metalaxyl-M solution (90 mg/L) prepared in water and in aqueous soil extract. The soil extract was prepared by shaking 30 g of soil with 80 ml of water for 2 h, followed by centrifugation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

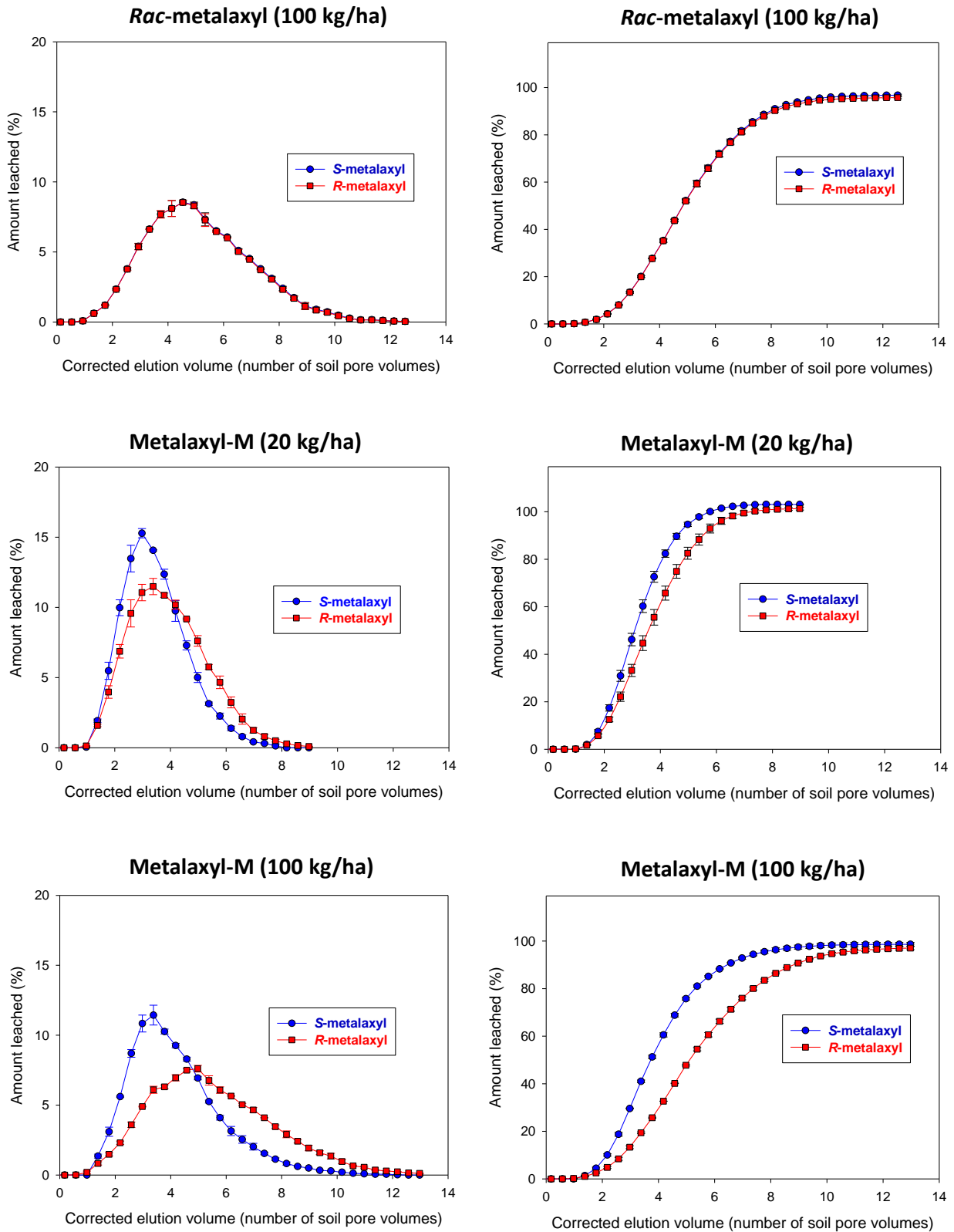


Fig. 2. Relative (left) and cumulative (right) breakthrough curves (BTCs) for the *S* and *R* enantiomers of metalaxyl applied to soil columns as *Rac*-metalaxyl (100 kg/ha) or Metalaxyl-M (20 and 100 kg/ha).

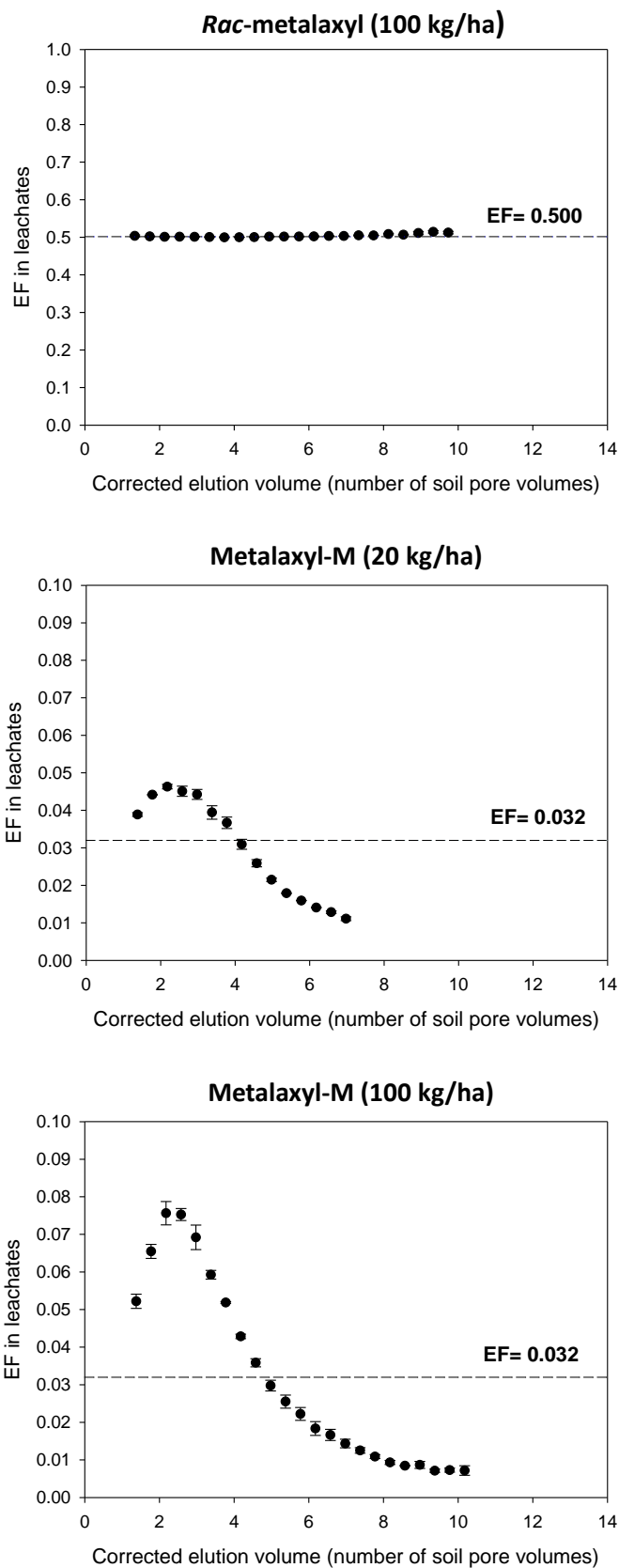


Fig. 3. Evolution of the enantiomer fraction (EF) in soil leachates during the column leaching experiments with *Rac*-metalaxyl and Metalaxyl-M.

Supplementary data for

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

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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.^a

Enantiomer	Initial concentration (mg/L)	Equilibrium concentration (mg/L)	C_s (indirect) (mg/kg)	C_s (direct) (mg/kg)	K_d (indirect) (L/kg)	K_d (direct) (L/kg)
Metalaxyl-M						
<i>S</i>	0.85 (0.01) ^b	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)
95:5 <i>S</i>:<i>R</i> mixture of metalaxyl enantiomers						
<i>S</i>	24.8 (0.1)	11.8 (0.1)	101.9 (0.8)	nd ^c	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

^a 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% CaCO₃ and a pH of 8.6.

^b Values in parentheses correspond to the standard deviations of quadruplicate measurements.

^c 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.^a

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

^a 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.

^b Values in parentheses correspond to the standard deviations of duplicate soil columns.

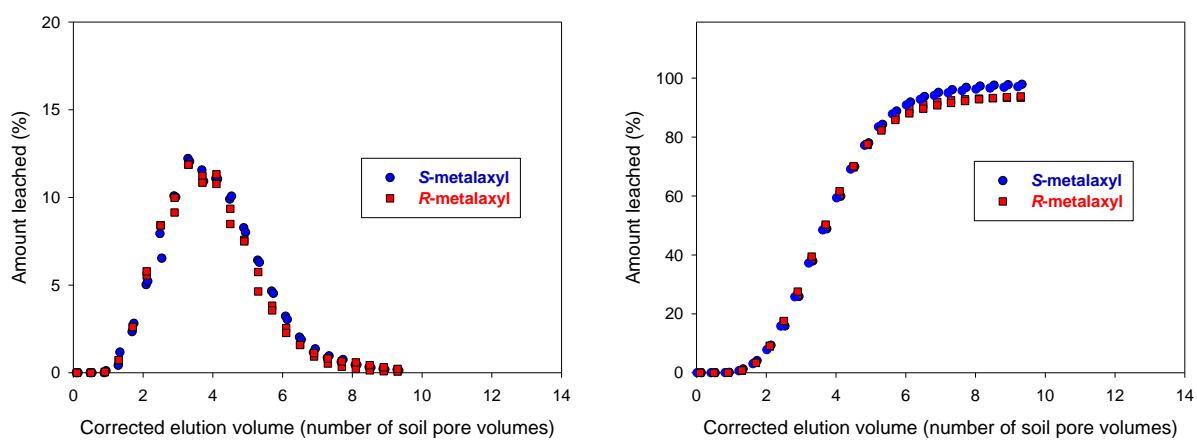


Fig. S1. Relative (left) and cumulative (right) breakthrough curves (BTCs) for the *S* and *R* enantiomers of metalaxyl applied separately to sandy clay loam soil columns as pure enantiomers at a rate of 20 kg/ha.