Enantioselective sorption of the chiral fungicide Metalaxyl on soil from non-racemic aqueous solutions: environmental implications

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ABSTRACT

Mechanisms governing the enantioselectivity of the processes that determine the behavior of chiral pollutants in the environment need to be better understood. Understanding these mechanisms should help improve predictions of the hazards and risks chiral compounds can pose to people and the environment. We report the results of batch sorption experiments indicating that the sorption of the chiral fungicide metalaxyl on soil from non-racemic initial solutions was enantioselective. While from a racemic initial solution the two enantiomers of metalaxyl were sorbed on the soil to the same extent, increasing the fraction of *R*-enantiomer in the initial solution led to enhanced sorption of this enantiomer and to reduced sorption of the *S*-enantiomer. Considering the shape of the sorption isotherms (S-type) and the sorption behavior of model sorbents, we attributed this effect to molecular interactions between metalaxyl enantiomer species at the sorbed state, where *R-R* metalaxyl interactions appeared to be more favorable than *R-S* metalaxyl interactions. We discuss important environmental implications of the proposed mechanism, such as those related to the fact that the biological degradation of metalaxyl is known to be an enantioselective process that can yield non-racemic residues in soils shortly after application of the fungicide as a racemic mixture.

1. Introduction

Chiral pesticides have become an important group of currently used agrochemicals. One reason is that the likelihood of introducing pesticides containing chiral centers into the market has increased as natural products and more complex structures have become the source of inspiration for designing new pesticides [1-4]. Of a list of 1,693 pesticides compiled by Ulrich et al. [4], 482 (28%) were found to be chiral. Since it is well known that chiral pesticide enantiomers can differ greatly in their biological efficacy, toxicity, and environmental fate, improving the existing knowledge on the enantioselectivity of processes determining the fate of chiral pesticides in the environment is considered a current research opportunity [5-8]. Despite this, many studies on chiral pesticides do not yet explicitly account for individual stereoisomers, implicitly and incorrectly assuming that enantiomers have identical environmental behavior [9,10].

Optical isomers or enantiomers have practically identical chemical and physical properties; they show different reactivity mainly in the presence of other substances that are also enantiomers, i.e. in chiral environments. For example, chiral pesticide enantiomers can differ in their toxicity and biological degradation due to the fact that the molecular receptor responsible for the toxicity or biological degradation of a pesticide is often an enzyme, the active center of which is also chiral and in turn enantioselective [2,11-15]. Abiotic processes such as chemical, distribution, or transport processes are supposed to affect both enantiomers equally and are generally assumed to be non-enantioselective [10,16,17].

Despite being commonly considered as non-enantioselective, little research has been conducted to ascertain the non-enantioselectivity of the chiral pesticide sorption process in soils [18]. Even working with pure chiral sorbents, the enantioselective sorption of chiral compounds can be difficult to observe under batch conditions [19], and the heterogeneity and complexity of organomineral soil colloids make the identification of sorption enantioselectivity

even more complicated [20,21]. Thus, questions remain as to whether soil components

assemblages are net "isomer-blind" at the macro-scale in their sorption of chiral compounds or

whether chiral constituents, e.g. L-amino acids forming proteins, D-ribose and D-deoxyribose

units forming ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), or even chiral

minerals [20,22], could lead to the enantioselectivity that is evident in biological systems [23].

In this regard, although several authors have reported results of batch sorption experiments

where different chiral compounds seemed to exhibit strong enantioselectivity in their sorption

on natural soil and sediment materials [23-25], others have failed to identify sorption

enantioselectivity [21,26-28]. Since sorption is a key process determining the amount of

pesticide available for other transport and degradation processes, enantioselective sorption to

chiral soil, sediment, or aquatic components would greatly affect the fate of chiral pesticides in

the environment by making one of the enantiomers more available than the other [2,9,17].

Metalaxyl [methyl-*N*-(2-methoxyacetyl)-*N*-(2,6-xylyl)-*DL*-alaninate] is a chiral phenylamide

fungicide widely used in the control of plant diseases caused by pathogens of the *Oomycota*

division in several crops. It can exist as two enantiomers, *R*-(-) and *S*-(+) (Fig. S1), but the

fungicidal activity mostly originates from the *R*-(-) enantiomer [29,30]. Currently, metalaxyl is

marketed and used both as a racemic formulation and as *R*-(-)-single enantiomer-rich product,

called Metalaxyl-M. Previous studies have indicated that the degradation of metalaxyl in soil is

an enantioselective, microbiologically-mediated process, with *S*-metalaxyl being degraded more

rapidly than *R*-metalaxyl in anaerobic soils and in aerobic soils with pH < 4, and *R*-metalaxyl

being degraded more rapidly than *S*-metalaxyl in aerobic soils with pH > 5 [9,21,29-31].

Previous work on the sorption of racemic metalaxyl by soils and soil components either i)

considered metalaxyl as an achiral compound, indirectly assuming that the *R*- and *S*-metalaxyl

enantiomers had identical sorption behavior [32-35], or ii) failed to identify enantiomer

selectivity in its sorption to soils and purified soil components [21,27,28]. Recently, Hall [18]

reported data indicating that sorption of racemic metalaxyl on montmorillonite and bentonite samples was significantly lower than sorption of Metalaxyl-M. She hypothesized that selective sorption of *R*-(-)-metalaxyl could have taken place, and stressed the need for further research to determine whether enantioselective sorption of metalaxyl could actually occur in the environment. On the basis that the biological degradation of metalaxyl can lead to non-racemic fungicide residues in soil, we considered it was relevant to characterize the possible enantioselectivity of metalaxyl sorption on soil and model sorbents not only from racemic metalaxyl aqueous solutions, but also from different non-racemic solutions. The results reported here may have very important implications in predicting the enantiomer availability of aged chiral pesticide residues in soils.

2. Materials and methods

2.1. Fungicide

Analytical standard grade racemic-Metalaxyl (*rac*-Metalaxyl) and Metalaxyl-M, supplied by Sigma-Aldrich (Spain) with chemical purities higher than 99%, were used in the experiments. The HPLC peak areas of standard solutions (see HPLC method below) confirmed the racemic nature of *rac*-Metalaxyl ($50.0 \pm 0.1\%$ of each enantiomer), and revealed that Metalaxyl-M consisted of $96.8 \pm 0.1\%$ of *R*-metalaxyl and $3.2 \pm 0.1\%$ of *S*-metalaxyl.

2.2. Soil and model sorbents

The soil used in the experiments was a sandy clay loam agricultural soil sampled from an olive orchard located in Seville (SW Spain, 37° 17´ N, 6° 2´ W). It had 74% sand, 4% silt, 22% clay (12% smectites, 4% illite/mica, 6% kaolinite), 0.50% organic C and 2.5% $CaCO_3$, and a pH of 8.8 in a 1:2.5 soil:water suspension. The soil was sampled from the 0-20 cm surface layer, and then air-dried and sieved to pass a 2 mm mesh before used.

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The model sorbents used were SWy-2 Wyoming montmorillonite from the Source Clays Repository of the Clay Minerals Society (Purdue university, IN), and a 3:1 Mg:Al layered double hydroxide, also known as hydrotalcite (HT) or anionic clay, modified with elaidate anions (HT-ELA), which was synthesized in the laboratory as described in Celis et al. [36]. Relevant physical/chemical data of the samples HT-ELA and SWy-2 can be found elsewhere [36,37].

2.3. Sorption experiments

The sorption of metalaxyl enantiomers on the soil and model sorbents was determined by the batch equilibration procedure using glass centrifuge tubes closed with Teflon®-lined screw caps. Three different batches of initial metalaxyl aqueous solutions were prepared from: i) *rac*-Metalaxyl, ii) Metalaxyl-M, and iii) a mixture of equal quantities of *rac*-Metalaxyl and Metalaxyl-M. The purpose was that the three batches of initial metalaxyl solutions contained identical total ($R+S$) enantiomer concentrations (2, 5, 10, 20, 30, 40, or 50 mg/L), but differed in their relative contents in R and S enantiomers (R to S enantiomer ratio of 1:1 for the initial solutions prepared from *rac*-Metalaxyl, ~ 30:1 for the initial solutions prepared from Metalaxyl-M, and ~ 3:1 for the initial solutions prepared from the mixture of equal quantities of *rac*-Metalaxyl and Metalaxyl-M). Aliquots of 1 g of soil, 50 mg of SWy-2 or 10 mg of HT-ELA were equilibrated with 8 mL of the initial metalaxyl solutions by shaking in an end-over-end shaker (30 r.p.m.) at 20 ± 2 ºC for 24 h. After equilibration, the suspensions were centrifuged at 5000 × *g* for 15 min, and 4 mL of the supernatant solution (pH= 7.5-8.2) was removed, filtered using GHP membrane disk filters (pore diameter= 0.45 µm), and analyzed by chiral high-performance liquid chromatography (HPLC) to determine the solution concentration of each enantiomer in the equilibrated suspensions. The amount of *S*-metalaxyl and *R*-metalaxyl sorbed on the solids, C_s (mg/kg), was calculated from the difference between

the initial and equilibrium (C_e) solution concentration of each enantiomer. Tubes with the initial solutions without sorbent were also shaken for 24 h, centrifuged, and filtered, and revealed no loss of the enantiomers. It was also previously checked that no degradation of metalaxyl occurred within 24 h in the soil tested. All sorption measurements were conducted in triplicate.

2.4. FTIR and XRD study

Duplicate aliquots of 20 mg of SWy-2 montmorillonite were subjected to 5 successive treatments with 8 mL of *rac*-Metalaxyl or Metalaxyl-M aqueous solutions containing total ($R+S$) enantiomer concentrations of 120 mg/L. After each treatment, the clay suspension was shaken for 24 h, centrifuged, and the supernatant was removed and replaced with 8 mL of the original 120 mg/L solution of *rac*-Metalaxyl or Metalaxyl-M. Control and treated clay samples were air-dried, and then analyzed by X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FT-IR). XRD patterns were obtained on oriented clay specimens, both air-dried and heated at 200 ºC, using a Siemens D-5000 diffractometer (Siemens, Stuttgart) with CuKα radiation. FT-IR spectra were obtained using a Jasco FT/IR 6300 spectrometer (Jasco Europe s.r.l.) provided with a diffuse reflectance accessory by directly placing the clay samples in a horizontal trough, attenuated total reflectance (ATR) cell.

2.5. Enantioselective analysis of metalaxyl

Metalaxyl enantiomers were determined by reverse-phase chiral HPLC using a Waters 600E chromatograph coupled to a Water 996 diode-array detector [21]. For the analysis, we used a Chiralpak IB column (150 mm length × 4.6 mm i.d., 5 µm particle size) purchased from Chiral Technologies Europe (France), a 60:40 water:acetonitrile eluent mixture at a flow rate of 1 mL/min, a 50 µL sample injection volume, and UV detection at 213 nm. External calibration curves with five standard solutions between 0.1 and 50 mg/L of *rac*-metalaxyl were

used in the calculations. Under the analytical conditions used, the retention times for the $S(+)$- and $R(-)$-enantiomers of metalaxyl were 4.9 and 6.0 min, respectively. The limit of quantification (LOQ), calculated as the concentration resulting in a signal to noise ratio of 10:1, was 0.02 mg/L for both enantiomers.

2.6. Data treatment

Sorption isotherms were fitted to the logarithmic form of the Freundlich equation: $\log C_s = \log K_f + 1/n_f \log C_e$, where C_s (mg/kg) was the amount of metalaxyl sorbed at the equilibrium concentration C_e (mg/L), and K_f and $1/n_f$ are the empirical Freundlich coefficients, which were calculated from the linear plot of $\log C_s$ versus $\log C_e$. At each single initial concentration used, a sorption distribution coefficient, K_d (L/kg), was also calculated by dividing the amount of each enantiomer sorbed (C_s) by its equilibrium solution concentration (C_e). The statistical comparison between sorption coefficients (K_d) was performed using the t-test in Sigma Plot 12.5 software. Sorption coefficients were considered to be statistically different for p values < 0.05.

3. Results and discussion

3.1. Sorption of metalaxyl on soil from racemic and non-racemic initial solutions

Figure 1a shows metalaxyl sorption isotherms on soil from the three batches of initial solutions prepared with the same total ($R+S$) metalaxyl concentrations but differing in their R to S enantiomer ratios. The isotherms were first analyzed as if the fungicide was an achiral compound, i.e. plotting the total ($R+S$) amount of metalaxyl sorbed (mg/kg) against the total ($R+S$) amount of metalaxyl in solution (mg/L).

Sorption isotherms were well described by the Freundlich equation ($R^2 > 0.995$, Table S1) and were all S-type ($1/n_f > 1$) according to the Giles et al. [38] classification (Fig. 1; Table S1).

S-type isotherms are commonly observed for the sorption of polar pesticides on polar surfaces from water, particularly when the pesticide also contains a hydrophobic moiety that may result in hydrophobic intermolecular attraction between adjacent pesticide molecules in the sorbed state [27,38-40]. Such intermolecular attractions are favored at higher surface coverages, thus explaining the enhanced sorption at higher fungicide concentrations (cooperative sorption) characteristic of S-type isotherms [38]. Considering the structure of metalaxyl and that (polar) clay constituents are known to play an important role in the sorption of this fungicide in low organic carbon content soils [21,32,34], it is likely that sorption of the fungicide in the studied soil involved polar interactions through its oxygen- and nitrogen-containing moieties (Fig. S1) allowing intermolecular attraction between the (hydrophobic) phenyl rings of adjacent sorbed metalaxyl molecules [27].

Figure 1a clearly shows that increasing the R to S enantiomer ratio in the initial metalaxyl solutions led to an increase in the total (R+S) amount of metalaxyl sorbed, particularly at higher fungicide concentrations. This result is noteworthy because it indicates that the R and S enantiomers of metalaxyl are not indistinguishable in their sorption on the soil, specifically in the region of cooperative sorption of the isotherms (Fig. 1a). Matallo et al. [26] compared the sorption isotherms of the racemic and enantiopure forms of the herbicides mecoprop and dichlorprop on different soils, and found they were identical; the R- and S-enantiomers of the herbicides were thus indistinguishable in their sorption to the soils. However, data in Figure 1a are in accordance with the results of sorption experiments conducted by Hall [18], who found that Metalaxyl-M was sorbed by montmorillonite and bentonite samples to a greater extent than racemic metalaxyl. Our data further indicate that this effect is reproduced in natural soil and for initial solutions of metalaxyl containing an intermediate R to S enantiomer ratio of 3:1.

One straightforward explanation for the enhancement in metalaxyl sorption on the soil as the initial solutions were enriched with R-enantiomer (Fig. 1a) would be the existence of

sorption enantioselectivity, whereby the affinity of soil surfaces for the R enantiomer was greater than that for the S enantiomer. However, data from the enantioselective analysis of the sorption isotherms (Fig. 2) are in contradiction with this possibility, since greater affinity of the soil surfaces for the R-enantiomer should have resulted in different sorption of the individual enantiomers also from racemic initial solutions. For example, after equilibrating racemic amino acid solutions with mineral sorbents and sediment samples, Wedyan and Preston [23] found solution D/L ratios significantly different from 1 and proposed the existence of natural chirally selective components in sediment. This behavior, however, is not seen in Figure 2a, because, as observed in earlier studies [18,21,27,28], the differences in sorption of the R- and S-enantiomers from initial racemic metalaxyl solutions were not statistically significant ($p > 0.05$). Only from high-concentration non-racemic initial solutions, the R-enantiomer was sorbed on soil to greater extents ($p < 0.05$) than the S-enantiomer (Fig. 2b and 2c).

A potential experimental artifact that should be considered when analyzing the individual distribution coefficients of the S and R metalaxyl enantiomers on soil (Fig. 2) is enantiomerization. Hall [18] reported that mineral-mediated R to S conversion of metalaxyl enantiomers seemed to occur in aqueous solutions of metalaxyl-M in the presence of calcite and kaolinite. The amount of each enantiomer sorbed during our 24 h-sorption experiment was calculated by subtracting the equilibrium concentration of each enantiomer from its initial (total) concentration. If R-metalaxyl converted to S-metalaxyl during the 24 h equilibration period, the total amount of S-metalaxyl at the end of the equilibration period would have been underestimated and so its sorbed amount, whereas the total amount of R-metalaxyl (and its sorbed amount) would have been overestimated. Assuming rapid redistribution of the enantiomers after interconversion, we calculated that an increase of 0-10% in the total concentration of S-metalaxyl as a result of enantiomerization of R-metalaxyl would account for the differences in distribution coefficients between the enantiomers observed in Figure 2.

Nevertheless, it should be noted that previous works have indicated that metalaxyl enantiomers are configurationally stable in soil, with no interconversion of *R* to *S*-enantiomers, and vice-versa [31,41]. Furthermore, enantiomerization would not explain the greater total (*R*+*S*) sorption of metalaxyl from initial solutions enriched with *R* enantiomer (Fig. 1a) without also considering that the *R* and *S* enantiomers of metalaxyl were not indistinguishable in their sorption to the soil.

The greater sorption of Metalaxyl-M on soil compared to *rac*-Metalaxyl (Fig. 1a) and the preferential sorption of *R*-metalaxyl over *S*-metalaxyl from solutions enriched with the *R*-enantiomer (Fig. 2) could be explained by considering that, from non-racemic solutions, the two enantiomers became different in their sorption to the soil. For example, interactions between metalaxyl enantiomers at the sorbed state where *R*-*R* attraction was more favorable than *R*-*S* interactions would explain: i) that the solutions enriched with *R*-metalaxyl displayed enhanced total sorption than racemic metalaxyl solutions in the region of cooperative sorption (Fig. 1) and ii) that the *R*-enantiomer became preferentially sorbed over the *S*-enantiomer from the solutions enriched with *R* enantiomer (Fig. 2). This is because the *R*-enantiomer would be preferentially sorbed over the *S*-enantiomer within a sorbed layer already dominated by other *R*-metalaxyl molecules. In other words, the enantioselectivity of the soil surfaces for *R*-metalaxyl would have been self-induced by the sorbate [42]. A similar behavior was reported by Kühnle et al. [43] for the adsorption of cysteine on a (110) gold surface, where adsorbed DL dimers were calculated to be energetically less stable than DD or LL homochiral dimers, which suppressed the formation of DL dimers at the gold surface.

3.2. Sorption on model sorbents

The sorption isotherms of metalaxyl on SWy-2 Wyoming montmorillonite and the individual sorption coefficients for *S*- and *R*-metalaxyl from racemic and non-racemic metalaxyl

initial solutions are included in Figures 1 and 3, respectively. SWy-2 sorbed considerably greater amounts of metalaxyl than the soil, but the shape of the isotherms (S-type) and the effects of enriching the initial metalaxyl solutions with *R*-enantiomer on metalaxyl sorption on SWy-2 were noticeably similar to those observed for soil. These results indicate that, as suggested in earlier studies [21,32,34], clay minerals (e.g., soil smectites) could have dominated the metalaxyl sorption process in our low organic carbon content soil.

Fourier-transform infrared (FT-IR) analysis of untreated and metalaxyl-saturated SWy-2 samples (see saturation curves in Fig. S2 of the supporting information) revealed the presence of bands corresponding to functional groups of metalaxyl in the FT-IR spectra of the saturated clay samples (Fig. 4). The shift of bands corresponding to C-H (2816-3020 cm^{-1}), amide (1667 cm^{-1}), and ester (1757 cm^{-1}) vibrations upon sorption of *rac*-Metalaxyl and Metalaxyl-M to SWy-2 suggested involvement of these groups in the interaction of metalaxyl enantiomers with the clay. X-ray diffractograms showed an increase in the basal spacing value of the clay mineral after saturation with *rac*-Metalaxyl and Metalaxyl-M, both at room temperature and after dehydrating at 200ºC, clearly showing that the sorption of metalaxyl enantiomers occurred in the clay mineral interlayer space (Fig. 5). Differences between *rac*-Metalaxyl and Metalaxyl-M were not evident in the FT-IR spectra and XRD diffractograms of the corresponding saturated SWy-2 samples.

For comparison purposes, we also measured the sorption of metalaxyl enantiomers on HT-ELA, an organo-clay prepared by intercalation of the *trans*-monounsaturated carboxylate anion elaidate (ELA) in the interlayer space of synthetic hydrotalcite or anionic clay [36]. It was previously shown that the presence of ELA provided the anionic clay mineral surfaces with hydrophobic properties, increasing their affinity for neutral pesticides, which sorbed on the organo-clay mainly through hydrophobic-type interactions [36]. Figure 1c shows that, similar to SWy-2, HT-ELA displayed very high sorption of metalaxyl, but the isotherms were L-type,

suggesting lack of cooperative sorption for this sorbent [38]. Interestingly, the total ($R+S$) amounts of metalaxyl sorbed from non-racemic initial solutions were identical to the amounts sorbed from the corresponding racemic solutions. Thus, the R and S enantiomers of metalaxyl appeared to be indistinguishable in their sorption to HT-ELA, which may be related to the fact that cooperative sorption should have played a minor role for this sorbent and the R and S enantiomers could have equally competed for the same sorption sites at the HT-ELA surfaces.

The results of the enantioselective analysis of the sorption isotherms on HT-ELA are included in Figure 3. The two enantiomers were found to sorb on HT-ELA to similar extents from racemic solutions and from the non-racemic solutions with an R to S enantiomer ratio of 3:1. For an R to S enantiomer ratio of 30:1, the K_d values for R-metalaxyl were similar to those measured for the 1:1 and 3:1 solutions, but the S-enantiomer was found to be sorbed to a slightly lesser extent than expected. The cause for such a decrease is uncertain, although it may also indicate a small self-induced enantioselectivity as a result of some cooperative sorption of metalaxyl on HT-ELA and/or organoclay-induced enantiomerization, that became visible only at very high R to S enantiomer ratio (Fig. 3) and had little impact on the overall ($R+S$) sorption on the organo-clay (Fig. 1c) .

3.3. Data modeling

Table S2 shows the Freundlich coefficients resulting from fitting the Freundlich equation to the individual sorption data of R-metalaxyl on soil, SWy-2, and HT-ELA from initial solutions prepared with an R to S enantiomer ratio of 30:1 (Metalaxyl-M). Due to the small amount of S-metalaxyl in solution (3.2%), we assumed that the presence of the S-enantiomer had little impact on the sorption of the R-enantiomer and that the above-mentioned Freundlich coefficients represented well the individual sorption behavior of isolated R-metalaxyl. As mentioned above, if the R and S enantiomers of metalaxyl were *indistinguishable* in their

sorption to soil, SWy-2, or HT-ELA, the isotherms of *rac*-metalaxyl were expected to be well described by the individual sorption isotherms of isolated *R*-metalaxyl (Table S2). In contrast, if *R*- and *S*-metalaxyl behaved *independently* in their sorption to soil, SWy-2 or HT-ELA, then the isotherms of *rac*-metalaxyl will be given by:

$$C_s(R+S) = C_s(R) + C_s(S) = K_f(R) \cdot C_e(R)^{1/nf(R)} + K_f(S) \cdot C_e(S)^{1/nf(S)} \qquad (1)$$

where $C_s(R+S)$ is the total $(R+S)$ sorbed amount (mg/kg) of metalaxyl, $C_s(R)$ and $C_s(S)$ are the individual sorbed amounts (mg/kg) of *R*- and *S*-metalaxyl, $C_e(R)$ and $C_e(S)$ are the individual equilibrium concentrations (mg/L) of *R*- and *S*-metalaxyl, and K_f and $1/n_f$ are the Freundlich coefficients describing the sorption of the individual *R*- and *S*-metalaxyl enantiomers.

On the basis that from racemic initial solutions, the two enantiomers of metalaxyl were sorbed to the same extents on the assayed sorbents (Fig. 2 and 3), we can assume that the individual Freundlich isotherm of *R*-metalaxyl would also describe the individual sorption of *S*-metalaxyl:

$$C_s(R+S) = 2 \cdot K_f(R) \cdot C_e(R)^{1/nf(R)} \qquad (2)$$

and that the equilibrium concentration of the *R* enantiomer, $C_e(R)$, will be half of the total $(R+S)$ metalaxyl equilibrium concentration, $C_e(R+S)$:

$$C_s(R+S) = 2 \cdot K_f(R) \cdot [C_e(R+S)/2]^{1/nf(R)} \qquad (3)$$

or

$$C_s(R+S) = 2^{[1-1/nf(R)]} \cdot K_f(R) \cdot C_e(R+S)^{1/nf(R)} \qquad (4)$$

Thus, assuming an equal but independent sorption behavior for the R and S enantiomers of metalaxyl, the sorption isotherm of rac-metalaxyl will follow a Freundlich isotherm with the same slope, $1/n_{f(R)}$, as that describing the sorption of isolated R-metalaxyl (Table S2), but with an intercept, $2^{[1-1/nf(R)]} \cdot K_f(R)$, equal to that describing the sorption of R-metalaxyl multiplied by a factor of $2^{[1-1/nf(R)]}$.

According to eq. 4, if $1/n_{f(R)} > 1$ (S-type isotherm), then $2^{[1-1/nf(R)]}$ will be < 1 and an independent sorption behavior of R and S metalaxyl will imply that the sorption isotherm for rac-metalaxyl will lie below that of R-metalaxyl. In terms of sorption mechanism, this can be interpreted by considering that R-metalaxyl does not contribute to the cooperative sorption of S-metalaxyl, and vice-versa. If $1/n_{f(R)} < 1$ (L-type isotherm), then $2^{[1-1/nf(R)]}$ will be > 1 and an independent sorption behavior of R and S metalaxyl will imply that the sorption isotherm for rac-metalaxyl will lie above that of R-metalaxyl, which can be interpreted by considering that the R-enantiomer does not compete with the S-enantiomer for the same sorption sites at the sorbent surface.

Figure 6 shows the experimental data obtained for the sorption of (R+S) rac-metalaxyl on soil, SWy-2, and HT-ELA together with the isotherms calculated from the individual sorption isotherms of R-metalaxyl (Table S2) assuming identical but either *indistinguishable* or *independent* sorption behavior of the S and R-metalaxyl enantiomers. Interestingly, the sorption of rac-metalaxyl on soil was close to that expected assuming an independent sorption behavior of the R-and S-metalaxyl enantiomers, which may indicate little contribution of R-S interactions to the cooperative sorption of metalaxyl. In contrast, the sorption of rac-metalaxyl on HT-ELA was well described by assuming an indistinguishable behavior of the S and R enantiomers of metalaxyl, which may indicate that both enantiomers would compete for the same sorption sites at the HT-ELA surfaces. SWy-2 showed an intermediate behavior

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attributable to *R-S* interactions existing, but contributing less to the cooperative sorption than *R-R* interactions.

4. Conclusions

The results of this work strongly indicated that the sorption of metalaxyl on soil from non-racemic initial solutions, enriched with *R* enantiomer, was an enantioselective process whereby the *R*-enantiomer was sorbed to a greater extent than the *S*-enantiomer and the overall (*R+S*) sorption was greater than from racemic solutions containing the same total (*R+S*) metalaxyl concentrations. We suggest that molecular interactions between metalaxyl enantiomers in the sorbed state, probably at soil clay mineral interlayers and with *R-R* interactions being more favorable than *R-S* interactions, may have led to an enantioselective sorption behavior self-induced by the presence of the sorbate in a non-racemic form. The existence of the proposed mechanism would have important implications considering that metalaxyl is applied to soil both as a racemic mixture and as non-racemic *R*-(-)-enantiomer enriched product, and also that the biological degradation of metalaxyl is known to be an enantioselective process that can yield non-racemic metalaxyl residues in soils shortly after application of the fungicide in a racemic form. It follows that accurate sorption estimates for modeling should consider the changes in metalaxyl enantiomers sorption with time resulting from the enantioselective biodegradation of the fungicide in the environment, a mechanism which could also be applicable to other chiral pollutants. Since enantiomerization, even if occurring to a small extent, can represent an experimental artifact for individual enantiomer sorption measurements conducted by the batch indirect methodology, its occurrence should be considered to avoid misinterpretation of individual enantiomer sorption data.

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Fig. 1. Metalaxyl sorption isotherms on soil, SWy-2 and HT-ELA from initial solutions containing different R to S enantiomer ratios. Symbols correspond to experimental data points, lines are the Freundlich-fit sorption isotherms, and error bars correspond to the standard errors of triplicate measurements.
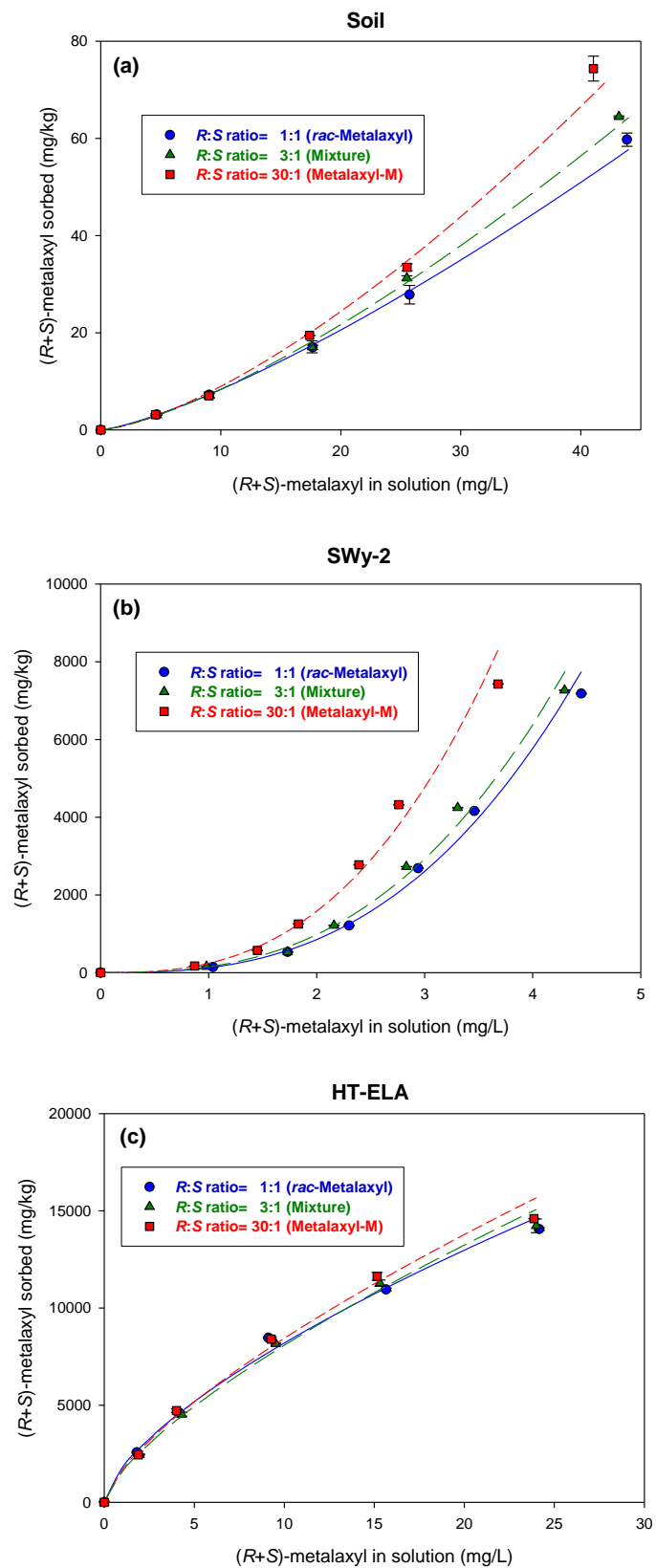
Fig. 2. Individual distribution coefficients for the R and S enantiomers of metalaxyl after sorption on soil from initial solutions containing identical total ($R+S$) concentrations but differing in their R to S enantiomer ratios. Error bars correspond to standard errors of triplicates. The asterisk indicates that the difference is statistically significant at the $p < 0.05$ level.

Fig. 3. Individual distribution coefficients for the R and S enantiomers of metalaxyl after sorption on SWy-2 and HT-ELA from initial solutions containing identical total ($R+S$) concentrations but differing in their R to S enantiomer ratios. Error bars correspond to standard errors of triplicates. The asterisk indicates that the difference is statistically significant at the $p < 0.05$ level.

Fig. 4. FT-IR spectra of a) *rac*-Metalaxyl, b) SWy-2, c) SWy-2 saturated with *rac*-Metalaxyl, and d) SWy-2 saturated with Metalaxyl-M.
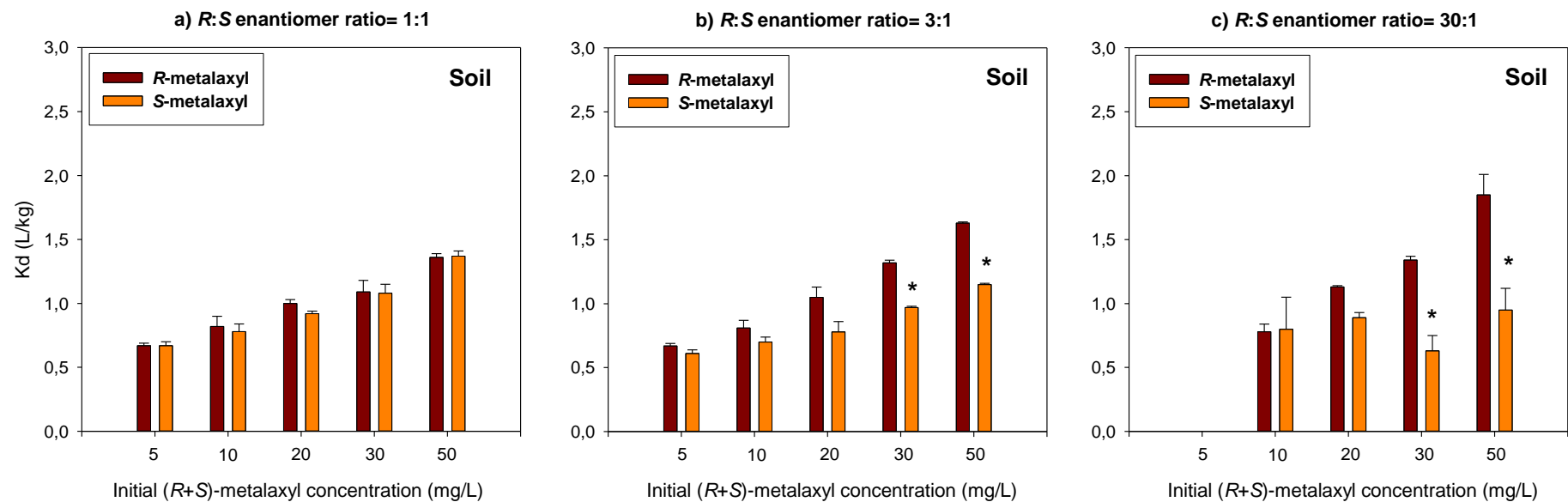
Fig. 5. X-ray diffractograms of oriented specimens of a) SWy-2, b) SWy-2 saturated with *rac*-Metalaxyl, and c) SWy-2 saturated with Metalaxyl-M.

Fig. 6. Experimental and calculated sorption isotherms for *rac*-Metalaxyl on soil, SWy-2, and HT-ELA. Symbols correspond to experimental sorption data, solid lines correspond to the isotherms expected assuming that *R*- and *S*-metalaxyl were indistinguishable in their sorption to the sorbents, and dashed lines correspond to the isotherms expected assuming an independent sorption behavior for *R*- and *S*-metalaxyl.
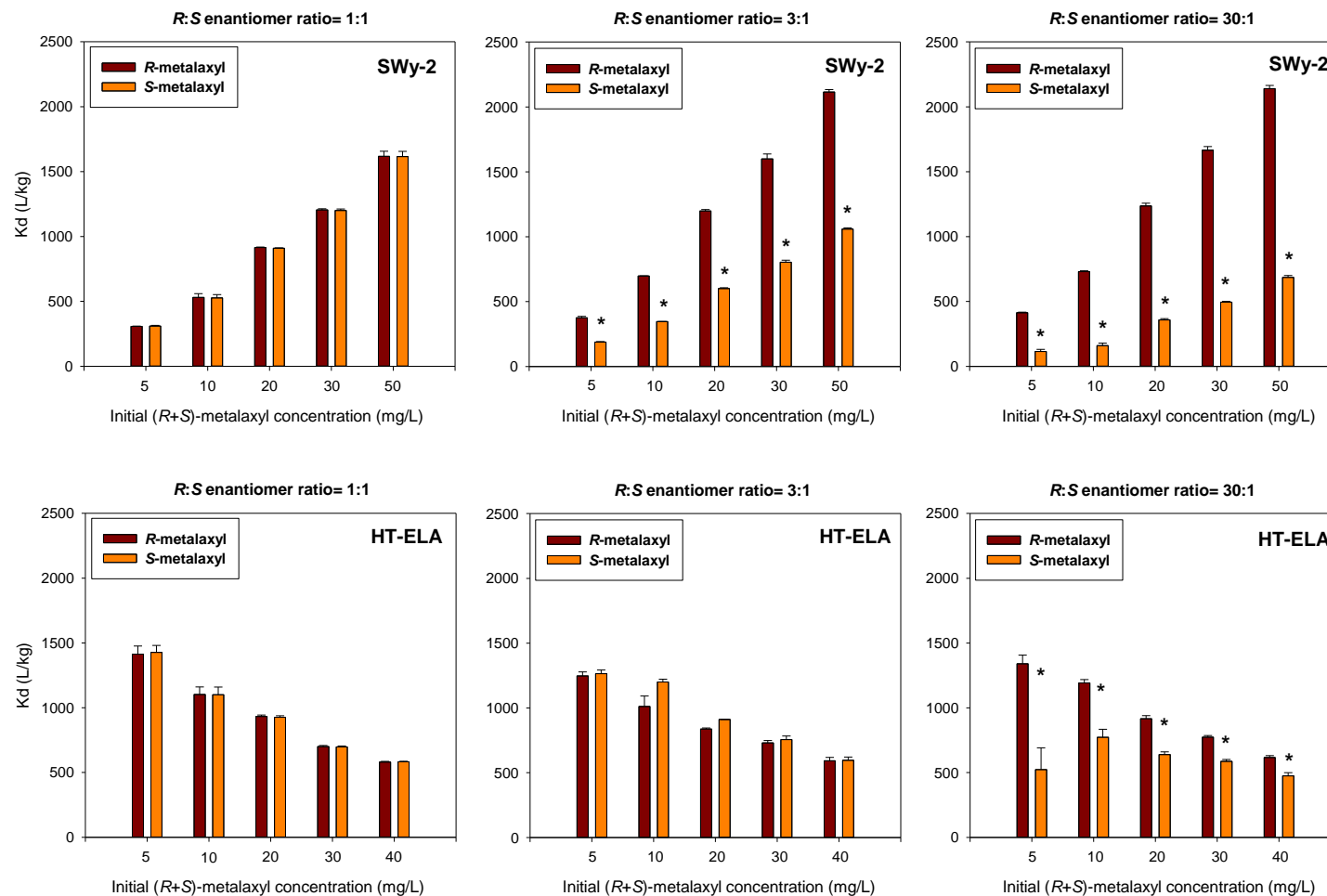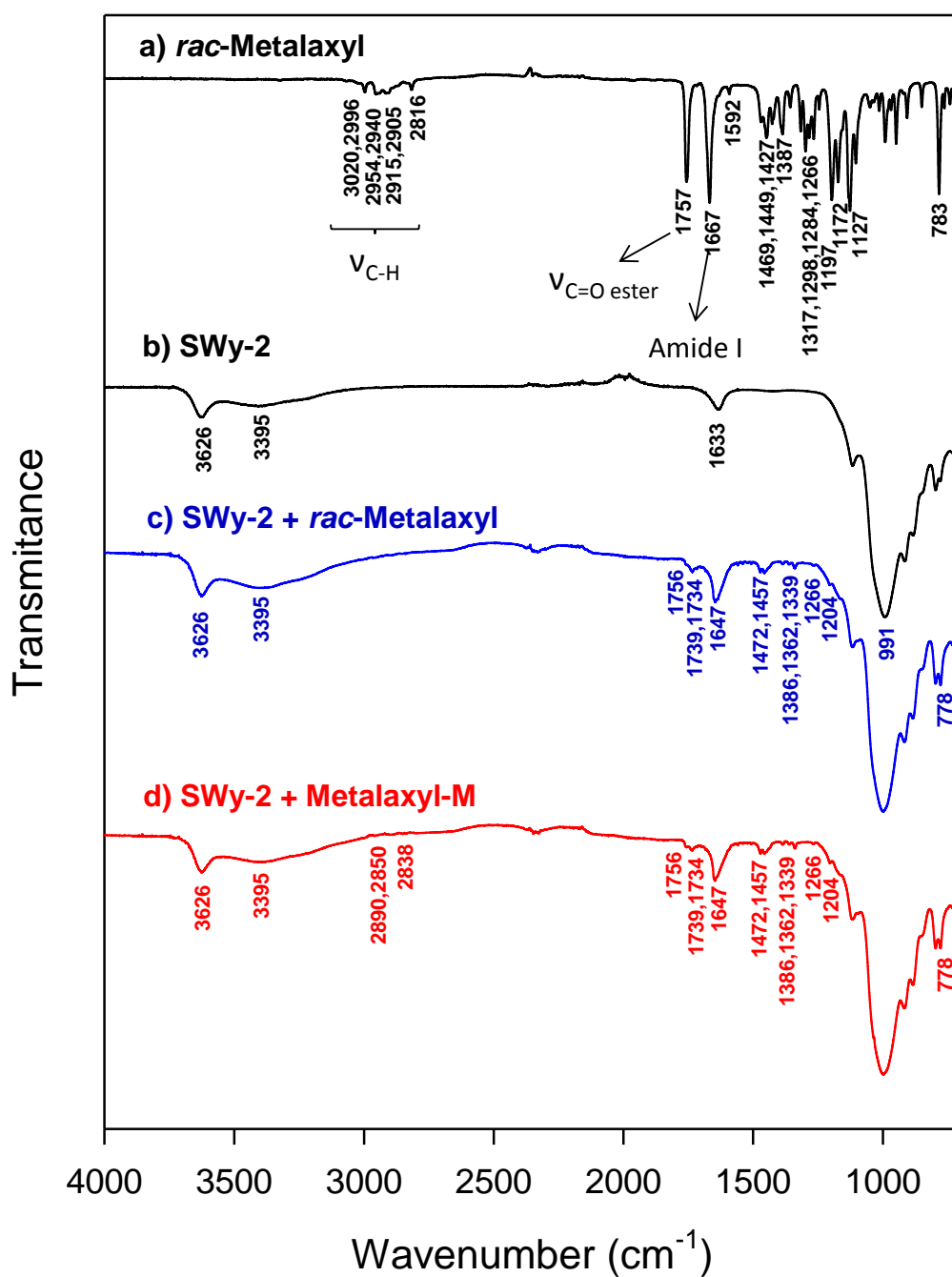
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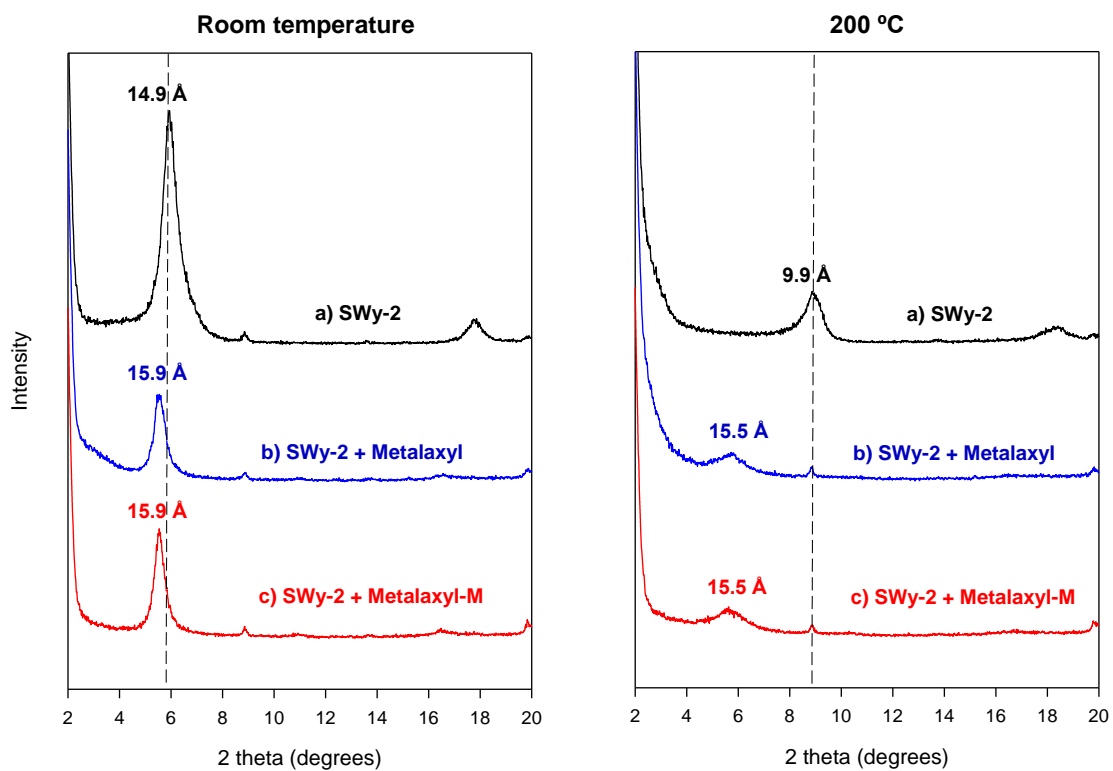
Fig. 2. Individual distribution coefficients for the *R* and *S* enantiomers of metalaxyl after sorption on soil from initial solutions containing identical total (*R*+*S*) concentrations but differing in their *R* to *S* enantiomer ratios. Error bars correspond to standard errors of triplicates. The asterisk indicates that the difference is statistically significant at the $p < 0.05$ level.
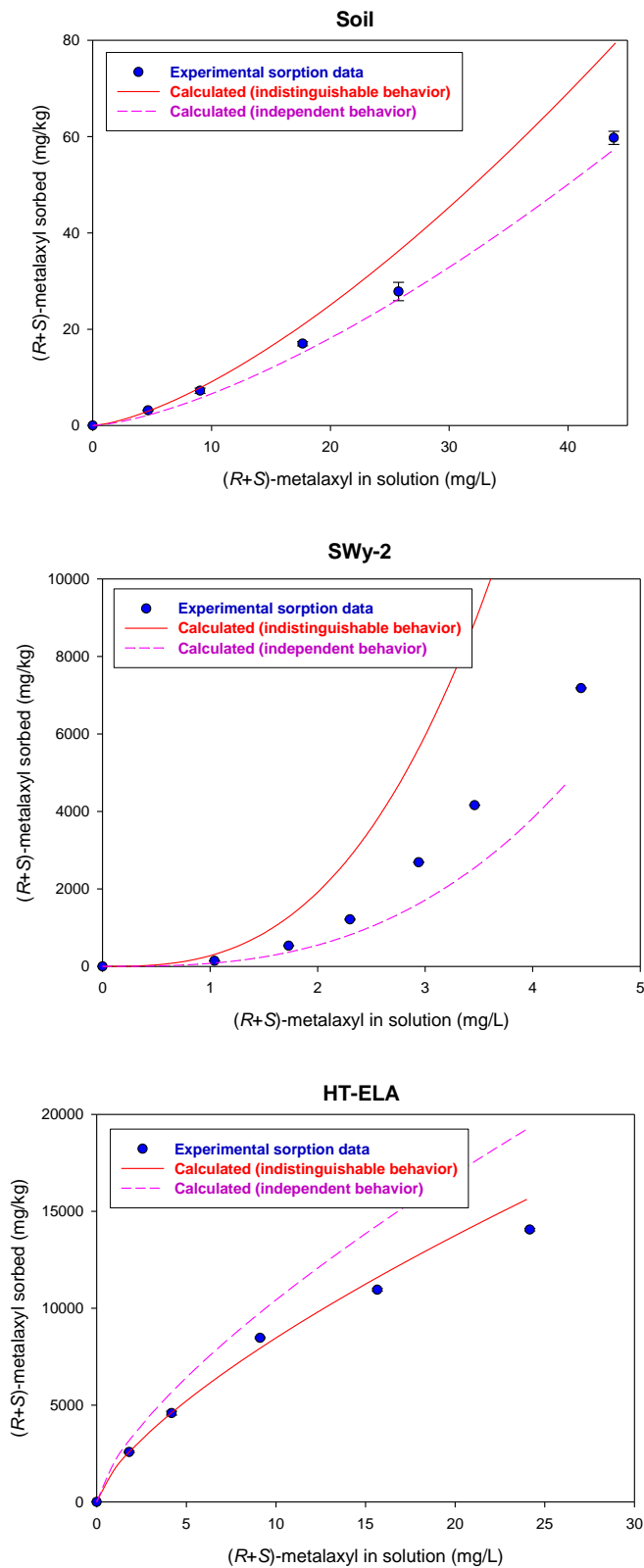
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Supplementary data for

Enantioselective sorption of the chiral fungicide Metalaxyl on soil from non-

racemic aqueous solutions: environmental implications

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Number of tables: 2

Number of figures: 2

Table S1

Freundlich coefficients for (R+S)-metalaxyl sorption on soil, SWy-2, and HT-ELA from racemic and non-racemic initial solutions.

| R to S ratio	K_f $mg^{1-1/nf}$ kg^{-1} $L^{1/nf}$	$1/n_f$	R^2
Soil			
1:1	0.41 (0.39-0.43)[a]	1.31 (1.29-1.33)[a]	0.999
3:1	0.35 (0.32-0.38)	1.38 (1.35-1.41)	0.998
30:1	0.32 (0.28-0.37)	1.45 (1.40-1.50)	0.996
SWy-2			
1:1	126 (117-134)	2.76 (2.69-2.83)	0.998
3:1	150 (134-169)	2.71 (2.82-2.82)	0.992
30:1	240 (220-261)	2.72 (2.62-2.82)	0.994
HT-ELA			
1:1	1786 (1670-1911)	0.66 (0.63-0.69)	0.994
3:1	1574 (1487-1666)	0.71 (0.69-0.73)	0.996
30:1	1663 (1538-1799)	0.71 (0.67-0.74)	0.993

[a] Standard error.

Table S2

Freundlich coefficients for *R*-metalaxyl sorption on soil, SWy-2, and HT-ELA from initial solutions with an *R* to *S* enantiomer ratio of 30:1 (Metalaxyl-M).

Sorbent	K_f	$1/n_f$	R^2
	$mg^{1-1/nf}\ kg^{-1}\ L^{1/nf}$		
Soil	0.31 (0.27-0.36)[a]	1.46 (1.41-1.51)[a]	0.997
SWy-2	277 (253-302)	2.80 (2.68-2.91)	0.993
HT-ELA	1694 (1577-1820)	0.70 (0.67-0.73)	0.993

[a] Standard error.
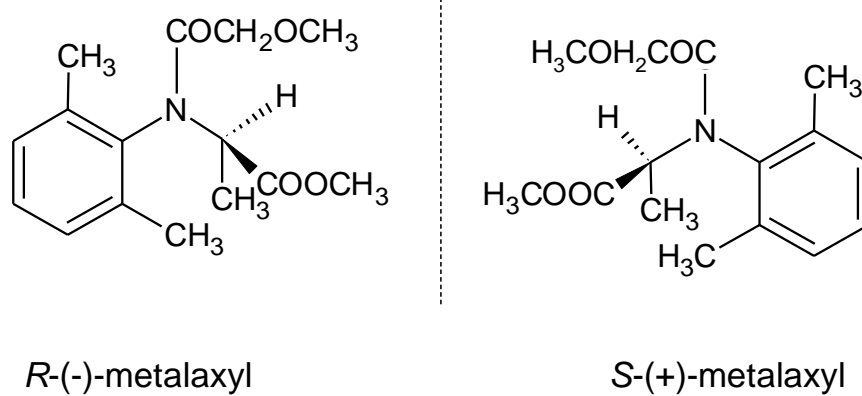
Fig. S1. Structure of the *R* and *S* metalaxyl enantiomers.
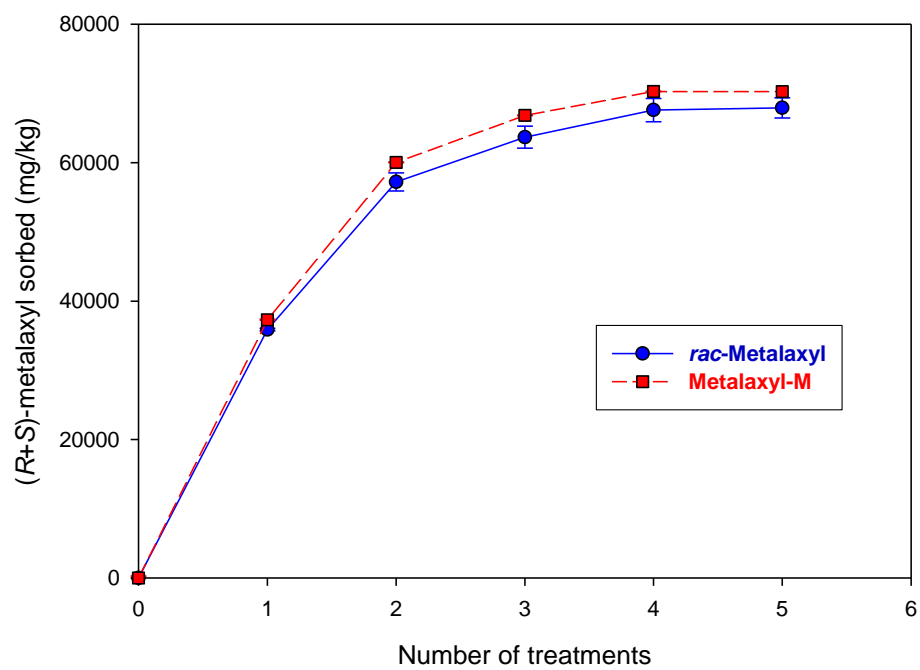
Fig. S2. *Rac*-Metalaxyl and Metalaxyl-M saturation curves on SWy-2.