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Evaluation of an organo-layered double hydroxide and two organic residues as amendments to immobilize metalaxyl enantiomers in soils: a comparative study

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ABSTRACT

Many pollutants released into the environment as a result of human activities are chiral. Pollution control strategies generally consider chiral compounds as if they were achiral and rarely consider enantiomers separately. We compared the performance of three different materials, an organically-modified anionic clay (HT-ELA) and two organic agro-food residues (ALP and ALPc), as amendments to immobilize the chiral fungicide metalaxyl in two soils with different textures, addressing the effects of the amendments on the sorption, persistence, and leaching of each of the two enantiomers of metalaxyl (*R*-metalaxyl and *S*-metalaxyl) separately. The effects of the amendments were both soil- and amendment-dependent, as well as enantiomer-selective. The organo-clay (HT-ELA) was much more efficient in increasing the sorption capacity of the soils for the two enantiomers of metalaxyl than the agro-food residues (ALP and ALPc), even when applied at a reduced application rate. The enhanced sorption in HT-ELA-amended soils reduced the bioavailability of metalaxyl enantiomers and their leaching in the soils, mitigating the particularly high leaching potential of the more persistent *S* enantiomer. The immobilizing capacity of the agro-food residues was more variable, mainly because their addition did not greatly ameliorate the sorption capacity of the soils and had variable effects on the enantiomers degradation rates. HT-ELA showed potential to reduce the bioavailability and mobility of metalaxyl enantiomers in soil and to mitigate the contamination problems particularly associated with the higher leaching potential of the more persistent enantiomer.

Keywords: Chiral pesticides; Groundwater pollution; Immobilization; Organic wastes; Organo-clays; Soil amendments

1. Introduction

Chiral pesticides have become a very important group of emerging pollutants. The use of this group of agrochemicals has spread in the last decades; currently, more than 25 % of the registered pesticides are chiral (Garrison, 2011; Ulrich et al., 2012). Chiral pesticides consist of one (or more) pair of enantiomers that present practically identical physico-chemical properties; however, enantiomers can display significant differences in their behavior in soil and in their toxicity towards target and non-target organisms. In the majority of cases, only one of the enantiomers is active against the target pest, and the fate of the non-active enantiomer is largely unknown. Sometimes, the inactive enantiomer can affect non-target species or act on the effectivity of the active enantiomer (Magrans et al., 2002). This is due to the fact that many reactions of pesticides in soil are enzymatic, and hence, enantioselective. For this reason, authorities have alarmed about the necessity to achieve a higher control of chiral agrochemicals and expressed the advantages of using active enantiomers separately (Magrans et al., 2002; Regulation (EC) N° 1107/2009). Nevertheless, because of economic reasons, chiral pesticides are yet often applied to soils as racemic mixtures rather than as pure active enantiomers, and this may pose serious environmental side-effects.

Metalaxyl is a phenylamide pesticide with an asymmetrical C in its structure, so that two different enantiomers can be identified: *S*-metalaxyl and *R*-metalaxyl (Fig. S1 of the Supplementary material). It is well known that the fungicidal activity of metalaxyl is due to *R*-metalaxyl mainly (Buerge et al., 2003; Chen and Liu, 2009). Previous studies on the sorption of racemic metalaxyl on soils and soil components have concluded that metalaxyl sorption is not enantioselective (Celis et al., 2013; Sukul et al., 2013), although recent findings have indicated that, when present in a non-racemic form, metalaxyl sorption can turn into enantioselective (Celis et al., 2015a).

The persistence of metalaxyl enantiomers in soil depends on several factors. One of the most important factors is the pH. Under aerobic conditions, the *R*-enantiomer degrades faster than the *S*-enantiomer in soils with pH > 5, whereas the *S*-enantiomer degrades faster than the *R*-

enantiomer in acid soils ($\text{pH} < 4$) (Buerge et al., 2003). In sewage sludge, *S*-metalaxyl was preferentially degraded over *R*-metalaxyl both in aerobic and anaerobic conditions (Chen and Liu, 2009; Müller and Buser, 1995). Other factors influencing the behavior of metalaxyl enantiomers in soil are the amount and nature of mineral constituents and soil porosity. Celis et al. (2013) observed that a soil with high clay content had a high sorption capacity for metalaxyl and this appeared to reduce the availability of metalaxyl enantiomers to be biodegraded compared to soils with lower clay contents. Similarly, entrapment in small-size pores can protect this chiral pesticide from biodegradation in soil and prolong its presence in a racemic form (Celis et al., 2013). Some agricultural practices may also alter the behavior of metalaxyl enantiomers. For example, Gámiz et al. (2013, 2016) reported how adding fresh, composted, or pyrolyzed olive-mill waste (OMW) to sandy loam soil samples affected the sorption, persistence, and leaching of metalaxyl enantiomers.

Recently, the performance of layered double hydroxides (LDHs) as sorbents of pesticides has received considerable attention (Celis et al., 2014; Cornejo et al., 2008; Lagaly, 2001). LDHs, also known as hydrotalcites (HTs) or anionic clays, consist of $\text{Mg}(\text{OH})_2$ brucite-type layers with isomorphic substitutions, which lead to positive charges in the layers that are balanced by exchangeable hydrated inorganic anions intercalated in the interlayer space (Cavani et al., 1991; Hou et al., 2003; Rives, 2001). The intercalated anion of LDHs can be exchanged by a specific anion with high affinity for a particular sorbate (Cornejo et al., 2008). Celis et al. (2014) prepared organo-LDHs consisting of nanohybrids of (3:1) Mg/Al layered double hydroxide (HT) with long-chain unsaturated fatty acid anions inserted in the interlayer space and evaluated them as pesticide sorbents. The study concluded that trans-unsaturated elaidate anion-modified hydrotalcite (HT-ELA) had a great sorption capacity for neutral pesticides.

As pesticide sorption can affect the dissipation of pesticides by reducing their availability to be biodegraded, as well as their transport to ground and surface water (Reichenberger et al., 2007; Rojas et al., 2013; Zipper et al., 1998), we hypothesized that increasing metalaxyl sorption in soil by using organo-LDHs as amendments could be used to control the enantioselective

behavior of this chiral pesticide in soils and as a pollution control strategy. Studies on the potential application of organo-LDHs as amendments for pollutant immobilization in soils are very scarce (Bruna et al., 2012), and to our knowledge, there are no published reports addressing their possible enantiomer-selective immobilizing effect for chiral pollutants.

Thus, the objective of this work was to assess under laboratory conditions whether the addition of HT-ELA could be used to increase the sorption of metalaxyl enantiomers in two soils with markedly different textures, a clay soil and a sandy clay loam soil. Subsequently, we evaluated the changes in the persistence and leaching of metalaxyl enantiomers due to the increase in sorption. For comparative purposes, the effects observed after the addition of two organic (OMW) residues to the tested soils are also reported.

2. Materials and methods

2.1. Fungicide

Metalaxyl [methyl-*N*-(2-methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate] is a phenylamide pesticide with a molecular mass of 279.3 g/mol, water solubility of 8.4 g/L (22 °C), and vapor pressure of 0.75 mPa (25°C) (Tomlin, 2006). Analytical standard grade, racemic-metalaxyl (chemical purity > 99.5 %) provided by Sigma-Aldrich (Spain) was used in the experiments.

2.2. Soils

Two agricultural soils with different textures and clay mineralogy were used in this study. The soils were sampled (0-20 cm), air-dried, sieved to pass a 2 mm mesh, and stored at 4 °C until their characterization and use in the experiments. The most important physicochemical properties of the soils are summarized in Table 1.

2.3. Amendments

The two organic residues, fresh and composted olive-mill waste or “*alperujo*” (ALP and ALPc), were supplied by Spanish olive-processing factories. Alperujo (ALP) is the solid residue

generated during the two-phase olive-oil extraction technology, which is currently widely used in Spain and other olive-oil producing countries. ALPc corresponded to the material resulting from composting fresh alperujo for about 5 months. Both ALP and ALPc were ground and sieved (2 mm), and stored at 4 °C until used. The main properties of the ALP and ALPc used in this work are summarized in Table S1 of the Supplementary material. The rate of ALP and ALPc applied in the soils (2% w/w) was equivalent to a typical agronomic dose of 50 t/ha, considering a soil bulk density of 1.3 g/cm³ and that the organic amendment is mixed with the top 0-20 cm soil layer. The third amendment used was a 3:1 Mg/Al LDH (hydrotalcite, HT) intercalated with elaidate anions (HT-ELA). It was prepared and characterized as described in Celis et al. (2014) and, on the basis of its high sorption capacity, was added to the soils at a rate of 1% (w/w). Its elemental composition was 12.2% Mg, 4.3% Al, and 39.1% C.

2.4. Sorption experiment

Sorption-desorption isotherms were measured by the batch equilibration technique. Triplicates of 1 g of unamended soils or soils amended with ALP (2% w/w), ALPc (2% w/w) or HT-ELA (1% w/w) were treated with 8 mL of aqueous solutions of *rac*-metalaxyl with concentrations (C_{ini}) ranging from 2 to 30 mg/L. After 24 h of shaking at 20 ± 2 °C, the suspensions were centrifuged (5000 rpm for 15 min) and 4 mL of the supernatant solutions were removed, filtered (0.45 µm pore size GHP membrane disk filters) and analyzed by chiral high-performance liquid chromatography (HPLC). The amount of *S*- and *R*-metalaxyl sorbed by the unamended and amended soil samples (C_s) was determined from the differences between the initial (C_{ini}) and equilibrium (C_e) solution concentrations of each enantiomer. Initial metalaxyl solutions without soil were also prepared and served as controls.

Desorption was measured immediately after sorption from the highest concentration point (C_{ini} = 30 mg/L) of the sorption isotherms. The 4 mL of supernatant used for the sorption analysis were replaced with 4 mL of distilled water and the soil suspensions were shaken again at 20 ± 2 °C for 24 h, centrifuged, and 4 mL of the supernatant solution removed and analyzed by

chiral HPLC as for the sorption experiment. This desorption procedure was repeated three times for each sample. Preliminary experiments had indicated that degradation of metalaxyl in the unamended and amended soils was insignificant in the course of the sorption-desorption test.

Sorption-desorption data were fitted to the Freundlich equation:

$$C_s = K_f \cdot C_e^{N_f} \quad (1)$$

where C_s (mg/kg) is the amount of enantiomer sorbed at the equilibrium concentration C_e (mg/L), and K_f ($\text{mg}^{1-N_f} \text{kg}^{-1} \text{L}^{N_f}$) and N_f (unitless) are the empirical Freundlich constants.

Thermodynamic index of irreversibility (TII) was calculated according to:

$$\text{TII} = 1 - (N_{fd}/N_f) \quad (2)$$

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

2.5. Dissipation experiment

Dissipation experiments were conducted by unamended and amended soil incubation under aerobic conditions. Samples of 100 g of each soil, either unamended or amended with 2% ALP, 2% ALPc or 1% HT-ELA, were moisturized with distilled water to a level close to the water holding capacity of the soils (40% for soil 1 and 30% for soil 2). Next, *rac*-metalaxyl was added in aqueous solution at a rate of 2 mg/kg and the spiked soil was thoroughly homogenized using a sterilized spatula. Periodically (0, 1, 7, 14, 21, 28, 40 and 50 days after treatment), triplicate 3 g of soil were sampled and immediately frozen until analyzed. Metalaxyl extraction was conducted by addition of 8 mL of methanol, 24 h of shaking, followed by centrifugation and chiral analysis

of the supernatant. A preliminary experiment revealed that this extraction procedure recovered >80% of the metalaxyl freshly applied to the unamended and amended soils (Table S2).

Metalaxyl dissipation data were fitted to first-order kinetics:

$$C = C_0 \cdot e^{-k \cdot t} \quad (3)$$

where C (mg/kg) is the enantiomer concentration in soil at time t (days), C_0 (mg/kg) is the concentration in soil at the time zero, and k (days⁻¹) is the single first-order dissipation rate. The half-life ($t_{1/2}$) of each enantiomer was calculated as $t_{1/2} = 0.693/k$.

For each sampling time, the enantiomer fraction in soil (EF) was calculated according to the equation (Harner et al., 2000):

$$EF = C_{S(+)} / (C_{S(+)} + C_{R(-)}) \quad (4)$$

where $C_{S(+)}$ and $C_{R(-)}$ are the soil concentrations of $S(+)$ - and $R(-)$ -enantiomers of metalaxyl, respectively.

In addition, an enantioselectivity factor (ES) was calculated for the whole experimental period according to the expression proposed by Müller and Buser (1995). These authors defined the excess of the rate of the faster over the slower degraded enantiomer in a particular medium as:

$$ES = (k_1 - k_2) / (k_1 + k_2) \quad (5)$$

where k_1 and k_2 are the rate constants of the faster and the slower degraded enantiomer, respectively. The ES value is set in the range from 0 (non-enantioselective process: $k_1 = k_2$) to 1 (enantioexclusive process: $k_2 = 0$ or $k_2 \ll k_1$).

2.6. Column leaching experiment

The leaching experiments were carried out in triplicate using glass columns of 30 cm length and 3.1 cm internal diameter. Glass wood plus 10 g of sea sand was placed on the bottom of the columns (5 cm) to prevent possible losses of soil and contamination of leachates with soil particles. Next, the columns were filled with unamended soil or with soil amended with ALP or ALPc (at a rate of 2% w/w along the entire column) or with HT-ELA (at the rate of 1% w/w for the top 0-2.5 cm of soil). In the case of the amended soil columns, the soils were separately mixed with the amendments and homogenized manually using a stainless steel spatula before being placed in the columns. Finally, 10 g of sea sand was added on the soil surface. The soil columns were saturated with 100 mL of distilled water and allowed to drain for 24 hours. The pore volume (V_p) of the columns was calculated from the amount of drained water. After saturating the columns, 0.15 mg of *rac*-metalaxyl dissolved in 1 mL of water was added to the top of the columns (amount equivalent to an agronomic application rate of 2 kg/ha). Daily, 15 mL of water was added to the columns and the leachates were collected after 24 hours of drainage. The concentration of each enantiomer in every filtered leachate was determined by chiral HPLC. To check if there was residual metalaxyl in the soil, when the leaching experiment was completed the soil columns were extracted, divided into four parts, and immediately frozen until analyzed. Metalaxyl extraction was conducted by addition of 100 mL of methanol, 24 h of shaking, followed by centrifugation and chiral analysis of the supernatant.

2.7. Enantioselective analysis of metalaxyl

The analysis of metalaxyl enantiomers was carried out by chiral HPLC using a Waters 600E chromatograph coupled to a Waters 996 diode-array detector (Celis et al., 2013). The conditions used were: Chiralpak IB column (150 mm length x 4.6 mm i.d., 5 μ m particle size), 60:40 water:acetonitrile eluent mixture at a flow rate of 1 mL/min, 50 μ L injection volume, and UV detection at 213 nm. External calibration curves with five standard solutions between 0.1 and 6 mg/L of *rac*-metalaxyl were used in the calculations. Instrumental LOQ calculated as the

concentration resulting in a signal to noise ratio 10:1 was 0.02 mg/L for both enantiomers. Under these conditions, the retention times for the *S*(+)- and *R*(-)-metalaxyl enantiomers were 4.9 and 6.1 min, respectively.

3. Results and discussion

3.1. Sorption of metalaxyl enantiomers by the unamended and amended soils

Figure 1 shows the individual sorption isotherms for *S*- and *R*-metalaxyl on the unamended soils and on the soils amended with ALP, ALPc and HT-ELA. Sorption of metalaxyl by both soils was found to be non-enantioselective, that is, the two enantiomers were sorbed to the same extent, and the addition of the amendments did not affect the non-enantioselective character of metalaxyl sorption in the soils tested. These results agree with previous sorption studies of *rac*-metalaxyl on soils and soil components (Celis et al., 2013; Gámiz et al., 2013; Sukul et al., 2013).

Sorption isotherms were fitted to the Freundlich equation. The sorption-desorption coefficients obtained are compiled in Table S3 of the Supplementary material. The N_f values for the untreated soils and for the soils treated with ALP and ALPc were all greater than 1, whereas those for the soils treated with HT-ELA were close to 1 (soil 1) or slightly less than 1 (soil 2). According to the Giles et al. (1960) classification, N_f values > 1 denote S-type isotherms, which are characteristic of systems where sorption becomes easier as the solute concentration increases (cooperative sorption). Giles et al. (1960) explained that these isotherms appear when three situations happen: the solute molecule is monofunctional, it has moderate intermolecular attraction, and there is a strong competition between solute molecules and molecules of the solvent or another sorbed species for substrate sites. Metalaxyl is a very polar pesticide that fulfills these three requirements. It may be sorbed on polar soil surfaces through its N- or O-containing moieties (Fig. S1) with both competition with water molecules for sorption sites and attractive interactions between the hydrophobic rings of adjacent molecules at the sorbed state (Gámiz et al., 2013). Addition of HT-ELA to both soils led to a decrease in the Freundlich N_f value, indicating a change in sorption mechanism. Metalaxyl sorption in soil amended with HT-ELA

may have become dominated by hydrophobic-type interactions between the fungicide and the organo-clay (Celis et al., 2014), thus explaining the noticeable change in the shape of the isotherms and associated N_f values.

The Freundlich K_f coefficients show much greater sorption of metalaxyl enantiomers on the clay soil (soil 1) than on the sandy clay loam soil (soil 2) (Table S3). In soils with low organic matter contents, such as the tested soils, sorption of metalaxyl has been proposed to be dominated by mineral clay constituents, because the fungicide, with a high polar character, has a high affinity for the internal surfaces of smectite-type clay minerals (Celis et al., 2013; Fernandes et al., 2003). Both unamended and ALP- and ALPc-amended soils presented similar K_f , i.e., the addition of these organic amendments did not affect significantly the sorption of metalaxyl. Previous studies have reported that the addition of organic amendments reduced the sorption of metalaxyl on soil by competition between the organic matter of the amendment and the fungicide for sorption sites on soil clay components (Fernandes et al., 2006). However, in this work, we did not observe significant changes in metalaxyl sorption upon addition of ALP or ALPc. These results agree with the effects observed by Gámiz et al. (2013, 2016) upon amending a sandy loam soil with similar amendments, and suggest that the new sorption sites provided by the addition of ALP and ALPc to our soils could have been counterbalanced by some reduction of the soil surfaces available for metalaxyl sorption.

In the case of soils amended with HT-ELA, the organo-clay greatly increased the sorption of metalaxyl in both soils (Fig. 1, Table S3), even though this sorbent had lower organic C content than ALP and was applied at reduced rate to the soils. This was because HT-ELA is known to present a high affinity for hydrophobic compounds, such as metalaxyl (Celis et al., 2014, 2015b). This great sorption can be attributed to hydrophobic interactions between the elaidate alkyl chain of HT-ELA and metalaxyl hydrophobic moieties. The increase in sorption was more significant in soil 2, in part because the original sorption capacity of this soil for metalaxyl was lower than that of soil 1.

The values of thermodynamic index of irreversibility (TII) for the assayed systems, calculated according to Sander et al. (2005), are included in Table S3. For soil 1, the TII value was lower (TII= 0.1) than for soil 2 (TII= 0.5), indicating that sorption of metalaxyl on soil 1 was more reversible than on soil 2. It was remarkable that for soil 2 amended with ALP or ALPc, the TII value was approximately the same (TII= 0.5-0.6) as for unamended soil. In contrast, when this soil was amended with HT-ELA, sorption became significantly more reversible (TII= 0.2).

3.2. Dissipation

3.2.1. Dissipation in unamended soils

Figures 2 and 3 show the *S*- and *R*-metalaxyl dissipation curves in the soils and the variation in the enantiomer fraction (EF) with time during the incubation experiment. The kinetic parameters obtained from the fit of the experimental data to Eq. (3) are summarized in Table 2. The dissipation of *rac*-metalaxyl was enantioselective in both soils. The *R*-enantiomer (active enantiomer) was degraded faster than the *S*-enantiomer (inactive enantiomer) (Fig. 2 and 3). This behavior is in accordance with the results reported by Buerge et al. (2003) indicating that in aerobic soils with pH > 5 there appeared to be a predominance of microorganisms or enzymatic activities that degraded *R*-metalaxyl preferably. Although the analytical methodology we used to determine metalaxyl did not allow us to identify metalaxyl degradation products, previous studies have shown that the degradation of metalaxyl in soils appears to proceed with accumulation of the metabolite metalaxyl acid, either in an extractable or non-extractable form, and little mineralization of the fungicide (Buser et al., 2002; Kalathoor et al., 2015; Sukul and Spiteller, 2001). Accordingly, metalaxyl was probably transformed to its acid metabolite during our incubation experiment. In studying the enantioselective dissipation of metalaxyl, it should also be noted that the enantiomerization of this fungicide in soil is unimportant compared to degradation, so that enantiomer interconversion can be ruled out when analyzing the dissipation data (Buser et al., 2002).

R-metalaxyl was degraded slower in soil 1 ($t_{1/2}$ = 72 days) than in soil 2 ($t_{1/2}$ = 18 days). This can be explained by the high sorption of metalaxyl enantiomers in soil 1 (Fig. 1, Table S3). The degradation of *S*-metalaxyl was not influenced by its higher sorption in soil 1, probably because its very slow degradation rate in both soils made the effect of sorption negligible. EF value in soil 1 at the end of the incubation experiment was 0.54; therefore, metalaxyl degradation in this soil was not highly enantioselective. In contrast, EF values in soil 2 increased with time reaching the value of 0.83 at the end of the incubation experiment. An EF= 0.83 denotes that 83% of metalaxyl residues are in the form of *S*-metalaxyl and only 17% are in the form of *R*-metalaxyl. The ES value for degradation of metalaxyl in soil 1, calculated according to eq. 5, was lower (ES= 0.23) than the value for degradation of metalaxyl in soil 2 (ES= 0.74), further reflecting how the enantioselectivity in soil 1 was less than in soil 2. The calculated ES values were thus consistent with the EF values. These results agree with those obtained by Celis et al. (2013) for similar low organic carbon content soils indicating that a high sorption of metalaxyl on soil can prolong the presence of the fungicide in a more racemic form. The explanation of this effect was that a high sorption of metalaxyl on soil led to a low bioavailability of the fungicide enantiomers.

3.2.2. Dissipation in ALP- and ALPc-amended soils

The effect of adding ALP or ALPc on the dissipation of metalaxyl enantiomers was soil-dependent. For soil 1, ALP and ALPc addition led to a faster degradation of both enantiomers (Fig. 2, Table 2). It is known that in soils with high clay contents, such as soil 1, the organic matter provided by organic amendments can compete with metalaxyl for sorption sites (Fernandes et al., 2006). Hence, metalaxyl molecules may have become more bioavailable to be degraded in soil 1 after amendment with ALP and ALPc. ES value indicated that the degradation of metalaxyl in soil 1 amended with ALP (ES= 0.19) and ALPc (ES= 0.21) was not highly enantioselective, as in the unamended soil (ES= 0.23). The EF value for soil 1 treated with ALP (EF= 0.63) and ALPc (EF= 0.59) at the end of the incubation experiment indicated also a moderate enantioselectivity of the dissipation process.

In soil 2 amended with ALP, *S*-metalaxyl was degraded faster while *R*-metalaxyl was degraded slower than in unamended soil (Fig. 3, Table 2). This result has previously been observed by Gámiz et al. (2013) in a soil very similar to our soil 2 amended with ALP, and was attributed to changes in microbial populations after amendment, the presence of a new C or energy source for microorganisms, and/or some toxic effects of ALP on metalaxyl degraders, as suggested for other pesticides after amending soil with fresh olive-mill wastes (Fernandes et al., 2006; Gámiz et al., 2012; Peña et al., 2015). In soil 2 amended with ALP, both the value of EF at the end of the incubation period (EF= 0.60) and the ES factor (ES= 0.27) confirmed that enantioselectivity in metalaxyl dissipation was less pronounced than in unamended soil (EF= 0.83, ES= 0.74), because the degradation patterns of the two enantiomers became more similar.

In soil 2 amended with ALPc, both enantiomers were degraded slightly slower than in unamended soil. This effect could result from the slightly higher sorption and resistance to desorption of metalaxyl in soil 2 amended with ALPc (Table S3), which may have protected metalaxyl from being degraded. It should be noted that composting of ALP reduces the amount of labile and soluble organic material and increases the pH of the residue (Table S1). These changes may have also accounted for the different effect of ALP and ALPc observed in soil 2.

3.2.3. Dissipation in HT-ELA-amended soils

Addition of HT-ELA increased the persistence of both enantiomers of metalaxyl in the two soils. The dissipation curves for soil 1 amended with HT-ELA (Fig. 2) show that the concentration of *S*-metalaxyl did not change along the experiment. The degradation of *R*-metalaxyl was faster compared to *S*-metalaxyl, but still slower than in unamended soil ($t_{1/2}$ =151 days) (Table 2). The EF value at the end of the experiment was 0.56 and the ES factor was 0.64. Soil 2 amended with HT-ELA showed the same trend as that observed for soil 1, i.e., slower degradation of both enantiomers compared to unamended soil (Fig. 3, Table 2). The EF value at the end of the experiment (EF= 0.64) and ES factor (ES= 0.74) indicated a moderate enantioselectivity of the dissipation process. The slow degradation of metalaxyl enantiomers in

both soils amended with HT-ELA can be attributed to the high sorption of the fungicide (Fig. 1, Table S3), that protected it from degradation. For soil 2, the effect of HT-ELA was more evident because the effect of the amendment on sorption was also more pronounced (Fig. 1, Table S3).

3.3. Leaching

3.3.1 Leaching in unamended soils

Figures 4 and 5 show the relative and cumulative breakthrough curves (BTCs) for *S*- and *R*-metalaxyl in the unamended soils and in the soils amended with ALP (2% w/w, 0-20 cm), ALPc (2% w/w, 0-20 cm) and HT-ELA (1% w/w, 0-2.5 cm). The enantiomer fractions (EFs) in the leachates collected during the experiment are represented in the relative BTCs. For the two soils tested, either unamended or amended, the maximum concentration of both enantiomers appeared in leachates after applying a similar amount of water. Therefore, both enantiomers had a similar retardation in the soil columns. This agrees with the batch sorption experiments, in which the sorption of the enantiomers by the unamended and amended soils was non-enantioselective.

The leaching of metalaxyl enantiomers in soil 1 was slower than in soil 2. In the case of soil 1, the maximum concentration (C_{\max}) of *S*- and *R*-metalaxyl appeared in the leachates after adding 255 mL of water, while in soil 2 C_{\max} appeared after adding 120 mL (Table S4). The oblate shape of the relative BTCs of soil 1 was also indicative of the slow leaching of metalaxyl enantiomers in columns of this soil. This was due to their high sorption on soil 1 (Fig. 1, Table S3), which retarded their movement throughout the column. The cumulative BTCs (Fig. 4 and 5) revealed that the overall leaching in soil 2 was more enantioselective than in soil 1. This result was confirmed by the EF values of soil leachates. In soil 1, EF was almost constant and close to 0.5 during all the leaching experiment, whereas in soil 2 the EF value increased over time to a value of 0.74. This behavior can be explained by the faster degradation of metalaxyl in soil 2, as dissipation experiments showed (Fig. 2 and 3) (Celis et al., 2013).

3.3.2. Leaching in ALP- and ALPc-amended soils

In soil 1 amended with ALP and ALPc, metalaxyl enantiomers leached slightly earlier than in unamended soil 1 (Fig. 4, Table S4). The form of the relative BTCs was less oblate than for the unamended soil, which is also typical of faster leaching. This may be the result of two related effects: i) the competence between metalaxyl and organic matter from ALP and ALPc for the sorption sites on the soil, and ii) the consequent increase in the bioavailability of metalaxyl enantiomers, which facilitated their leaching. Moreover, metalaxyl may have even become linked to the dissolver organic matter released by ALP and ALPc, making the fungicide leaching faster (Fenoll et al., 2015). The cumulative BTCs for soil 1 amended with ALP and ALPc show percentages of metalaxyl leached similar to those of unamended soil 1 for both enantiomers (Table S4).

In soil 2, ALP and ALPc did not result in significant changes in the position of C_{max} , but it is interesting to note that the enantioselectivity of the leaching process was less noticeable for soil 2 amended with ALP than for unamended or ALPc-amended soil 2 (Fig. 5). This effect can be explained by the differences in the degradation of metalaxyl during leaching (Gámiz et al., 2013), which should have been less enantioselective in ALP-amended soil than in unamended and ALPc-amended soil, primarily as a result of the slower degradation of the *R*-enantiomer (Fig. 3). These results were confirmed by the cumulative BTCs where the total amount of *R*-metalaxyl leached followed the order: ALP-amended (76%) > ALPc-amended (62%) > unamended soil 2 (58%). The amount of metalaxyl extracted with methanol from soil 2, unamended and amended with ALP and ALPc, were less than 1% (Table S4). This may be a consequence of the rapid leaching that metalaxyl showed in this soil.

3.3.3. Leaching in HT-ELA-amended soils

The relative BTCs for the soils amended with HT-ELA showed slower leaching of metalaxyl enantiomers compared to the respective unamended soils (Fig. 4 and 5). The C_{max} values for the amended soils were poorly defined, but they were noticeably lower and appeared at higher

volume of water added compared to the unamended soils (Fig. 4 and 5). The retarded leaching of metalaxyl in the soils treated with HT-ELA can be attributed to the increase in sorption of this fungicide after amending with the organo-LDH (Fig. 1, Table S3). The effect of HT-ELA on leaching was more evident in soil 2 because the original sorption capacity of this soil was less than that of soil 1. The cumulative BTC for soil 1 amended with HT-ELA revealed that only 58% of *S*-metalaxyl and 51% of *R*-metalaxyl was recovered in leachates. After extraction of the soil columns with methanol, we recovered an additional amount of 20% of *S*-metalaxyl and 25% of *R*-metalaxyl (Table S4). Considering the low degradation of metalaxyl in soil 1 amended with HT-ELA, the percentage of metalaxyl not-recovered (22% for *S*-metalaxyl and 24% for *R*-metalaxyl) can be attributed to the formation of residues strongly sorbed or entrapped by the soil or organo-LDH particles, which could not be extracted with methanol. The effect of the addition of HT-ELA to soil 2 was similar to that observed for soil 1. The total cumulative amounts of both enantiomers leached from the amended soil were lower than from the unamended soil. It was significant that the HT-ELA-treated soil was the only case where some fungicide appeared in the methanol-extractable fraction for soil 2 (Table S4). This was because HT-ELA helped reduce the leaching of metalaxyl enantiomers and maintain them within the soil column.

4. Conclusions

Exogenous materials can significantly affect the behavior of chiral pesticide enantiomers in soils. Our study conducted with three amendments (a synthetic organo-clay and two agro-food residues) and two soils with different textures revealed that the effects of the amendments on the behavior of metalaxyl enantiomers were both amendment- and soil-dependent, as well as enantiomer-selective. Addition of the agro-food residues did not greatly affect the sorption of *R*- or *S*-metalaxyl, and had variable effects on the degradation and leaching of the enantiomers depending on the residue and the soil to which this was added. In contrast, the high sorption capacity of the synthetic organo-clay for metalaxyl enantiomers increased their sorption and

reduced their bioavailability and mobility in the two soils. Hence, organically modified clays such as HT-ELA show potential as amendments to immobilize chiral pesticide enantiomers in soil and mitigate the contamination problems often associated with the higher leaching potential of the more persistent enantiomer.

Acknowledgments

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FIGURE CAPTIONS

Fig. 1. *S*- and *R*-metalaxyl sorption isotherms on unamended soils and ALP-, ALPc-, and HT-ELA-amended soils. Error bars correspond to standard errors of triplicate measurements.

Fig. 2. *S*- and *R*-metalaxyl dissipation curves and changes in the enantiomer fraction (EF) with time during the incubation experiment of *rac*-metalaxyl in unamended soil 1 and soil 1 amended with ALP, ALPc and HT-ELA. In the dissipation curves, symbols represent experimental data, whereas lines are the fittings to single first-order dissipation kinetics. Error bars correspond to standard errors of triplicate measurements.

Fig. 3. *S*- and *R*-metalaxyl dissipation curves and changes in the enantiomer fraction (EF) with time during the incubation experiment of *rac*-metalaxyl in unamended soil 2 and soil 2 amended with ALP, ALPc and HT-ELA. In the dissipation curves, symbols represent experimental data, whereas lines are the fittings to single first-order dissipation kinetics. Error bars correspond to standard errors of triplicate measurements.

Fig. 4. Relative and cumulative breakthrough curves (BTCs) of *S*- and *R*-metalaxyl in unamended and ALP-, ALPc-, and HT-ELA-amended columns of soil 1. Enantiomer fractions (EF) in the leachates are also indicated in the relative BTCs. Error bars correspond to standard errors of triplicate measurements.

Fig. 5. Relative and cumulative breakthrough curves (BTCs) of *S*- and *R*-metalaxyl in unamended and ALP-, ALPc-, and HT-ELA-amended columns of soil 2. Enantiomer fractions (EF) in the leachates are also indicated in the relative BTCs. Error bars correspond to standard errors of triplicate measurements.

Table 1

Physicochemical properties of the soils.

| Soil | Texture | Sand (%) | Silt (%) | Clay (M, I, K) ^a (%) | OC ^b (%) | pH ^c |
|--------|-----------------|-------------|-------------|------------------------------------|------------------------|-----------------|
| Soil 1 | Clay | 6 | 26 | 68 (20, 38, 10) | 0.49 | 8.6 |
| Soil 2 | Sandy clay loam | 74 | 4 | 22 (12, 4, 6) | 0.50 | 8.8 |

^a M: montmorillonite, I: illite/mica, K: kaolinite.^b OC: organic carbon content measured by dichromate oxidation.^c Measured in 1 g soil: 2.5 mL water suspension.

Table 2

First-order dissipation constants for *S*- *R*-, and (*S+R*)-metalaxyl in unamended soils and in soils amended with ALP, ALPc and HT-ELA.

| | | k (d ⁻¹) | $t_{1/2}$ (d) | R ² |
|--------------------|--------------------------|------------------------------|----------------|----------------|
| Soil 1 | <i>S</i> -metalaxyl | 0.0059 ± 0.0004 ^a | 117 | 0.974 |
| | <i>R</i> -metalaxyl | 0.0095 ± 0.0007 | 72 | 0.960 |
| | (<i>S+R</i>)-metalaxyl | 0.0077 ± 0.0005 | 90 | 0.972 |
| Soil 1 + 2% ALP | <i>S</i> -metalaxyl | 0.0193 ± 0.0019 | 34 | 0.945 |
| | <i>R</i> -metalaxyl | 0.0282 ± 0.0013 | 24 | 0.988 |
| | (<i>S+R</i>)-metalaxyl | 0.0231 ± 0.0011 | 30 | 0.987 |
| Soil 1 + 2% ALPc | <i>S</i> -metalaxyl | 0.0083 ± 0.0016 | 84 | 0.797 |
| | <i>R</i> -metalaxyl | 0.0127 ± 0.0016 | 55 | 0.902 |
| | (<i>S+R</i>)-metalaxyl | 0.0103 ± 0.0014 | 67 | 0.891 |
| Soil 1 + 1% HT-ELA | <i>S</i> -metalaxyl | 0.0010 ± 0.0006 | - ^b | 0.248 |
| | <i>R</i> -metalaxyl | 0.0046 ± 0.0007 | 151 | 0.875 |
| | (<i>S+R</i>)-metalaxyl | 0.0027 ± 0.0006 | 256 | 0.776 |
| Soil 2 | <i>S</i> -metalaxyl | 0.0055 ± 0.0006 | 126 | 0.938 |
| | <i>R</i> -metalaxyl | 0.0369 ± 0.0019 | 18 | 0.988 |
| | (<i>S+R</i>)-metalaxyl | 0.0164 ± 0.0005 | 42 | 0.995 |
| Soil 2 + 2% ALP | <i>S</i> -metalaxyl | 0.0106 ± 0.018 | 65 | 0.865 |
| | <i>R</i> -metalaxyl | 0.0184 ± 0.0016 | 38 | 0.963 |
| | (<i>S+R</i>)-metalaxyl | 0.0142 ± 0.0014 | 49 | 0.935 |
| Soil 2 + 2% ALPc | <i>S</i> -metalaxyl | 0.0048 ± 0.0005 | 144 | 0.936 |
| | <i>R</i> -metalaxyl | 0.0206 ± 0.0020 | 33 | 0.957 |
| | (<i>S+R</i>)-metalaxyl | 0.0114 ± 0.0009 | 61 | 0.969 |
| Soil 2 + 1% HT-ELA | <i>S</i> -metalaxyl | 0.0019 ± 0.0006 | - ^b | 0.610 |
| | <i>R</i> -metalaxyl | 0.0128 ± 0.0016 | 54 | 0.920 |
| | (<i>S+R</i>)-metalaxyl | 0.0067 ± 0.0009 | 103 | 0.902 |

^a Value ± standard error.

^b Not calculated because of negligible degradation during the experiment.

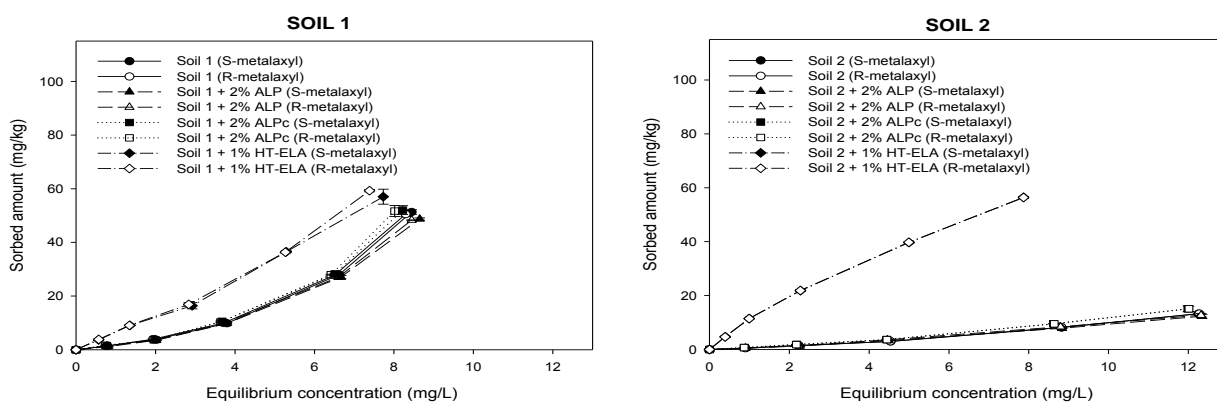


Fig. 1. *S*- and *R*-metalaxyl sorption isotherms on unamended soils and ALP-, ALPc-, and HT-ELA-amended soils. Error bars correspond to standard errors of triplicate measurements.

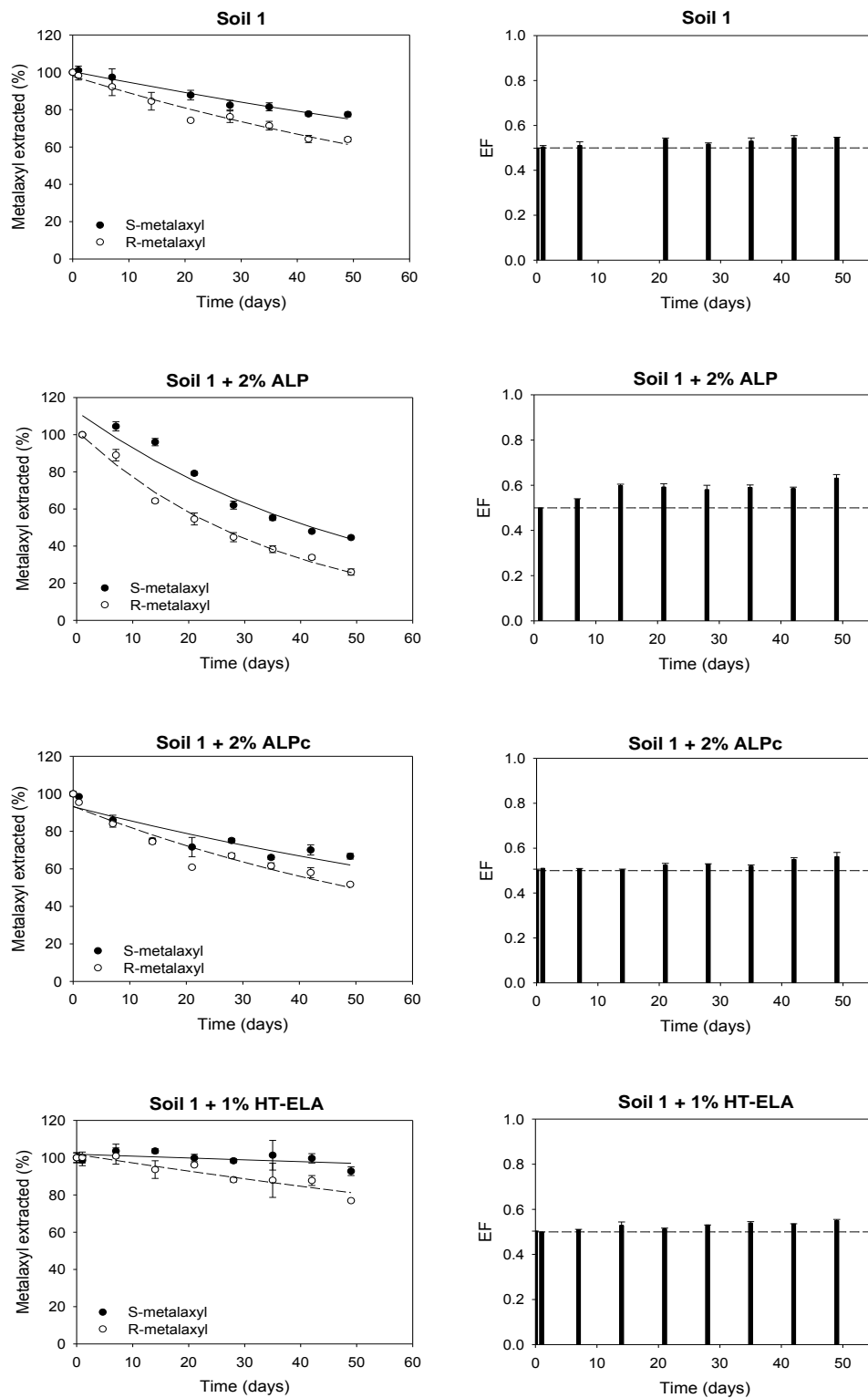


Fig. 2. *S*- and *R*-metalaxyl dissipation curves and changes in the enantiomer fraction (EF) with time during the incubation experiment of *rac*-metalaxyl in unamended soil 1 and soil 1 amended with ALP, ALPc and HT-ELA. In the dissipation curves, symbols represent experimental data, whereas lines are the fittings to single first-order dissipation kinetics. Error bars correspond to standard errors of triplicate measurements.

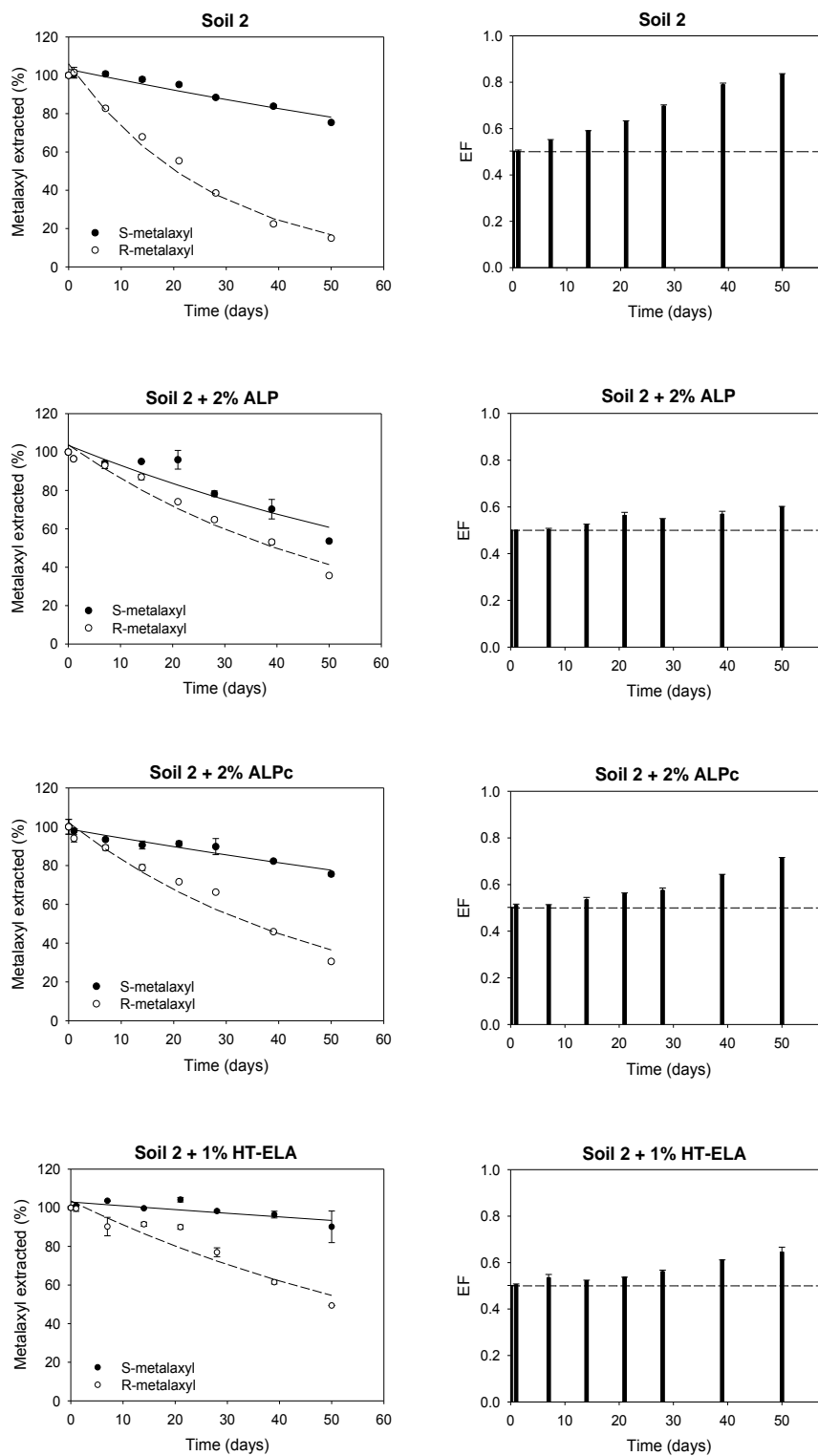


Fig. 3. S- and R-metalaxyl dissipation curves and changes in the enantiomer fraction (EF) with time during the incubation experiment of *rac*-metalaxyl in unamended soil 2 and soil 2 amended with ALP, ALPc and HT-ELA. In the dissipation curves, symbols represent experimental data, whereas lines are the fittings to single first-order dissipation kinetics. Error bars correspond to standard errors of triplicate measurements.

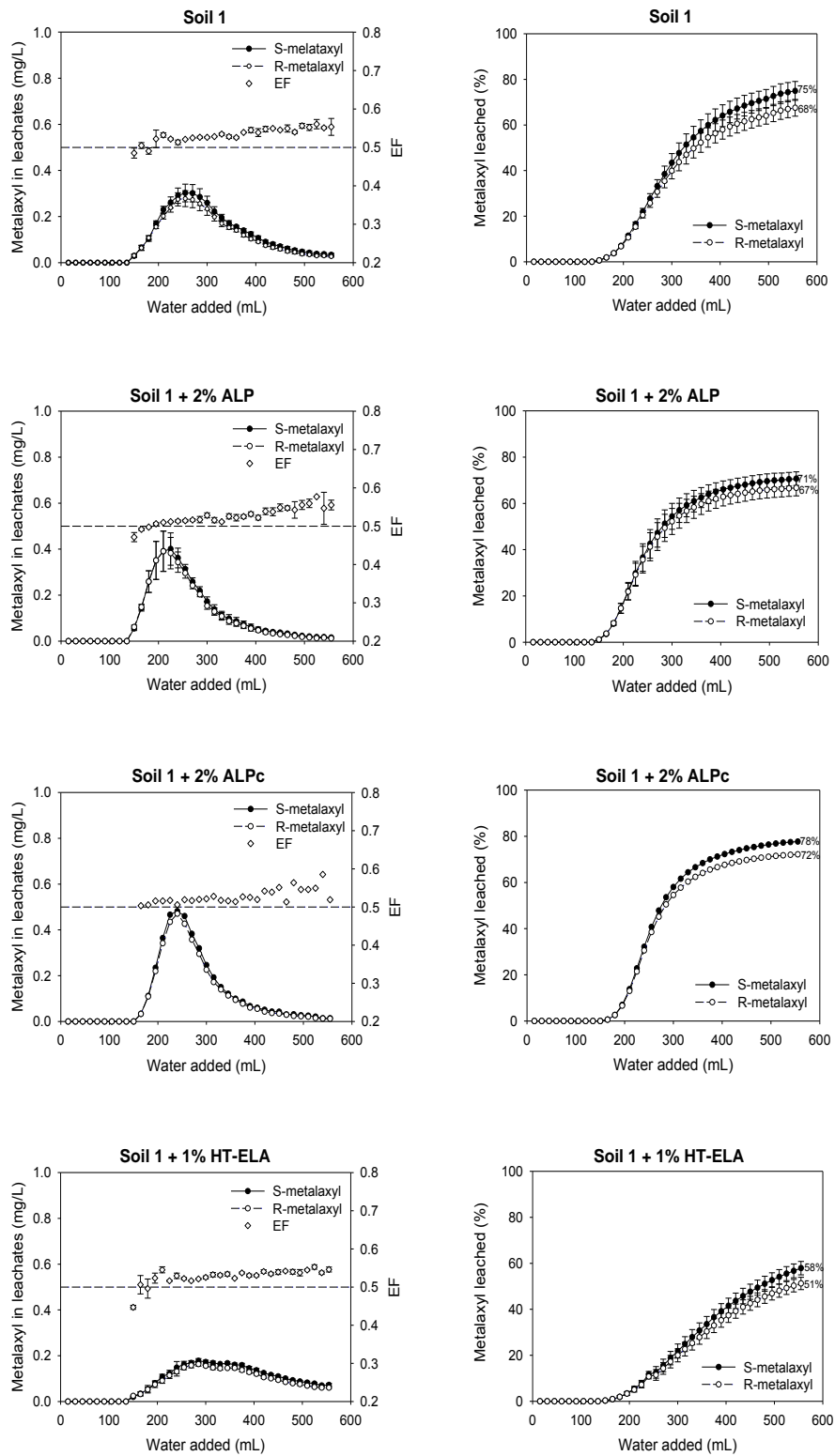


Fig. 4. Relative and cumulative breakthrough curves (BTCs) of *S*- and *R*-metaxyl in unamended and ALP-, ALPc-, and HT-ELA-amended columns of soil 1. Enantiomer fractions (EF) in the leachates are also indicated in the relative BTCs. Error bars correspond to standard errors of triplicate measurements.

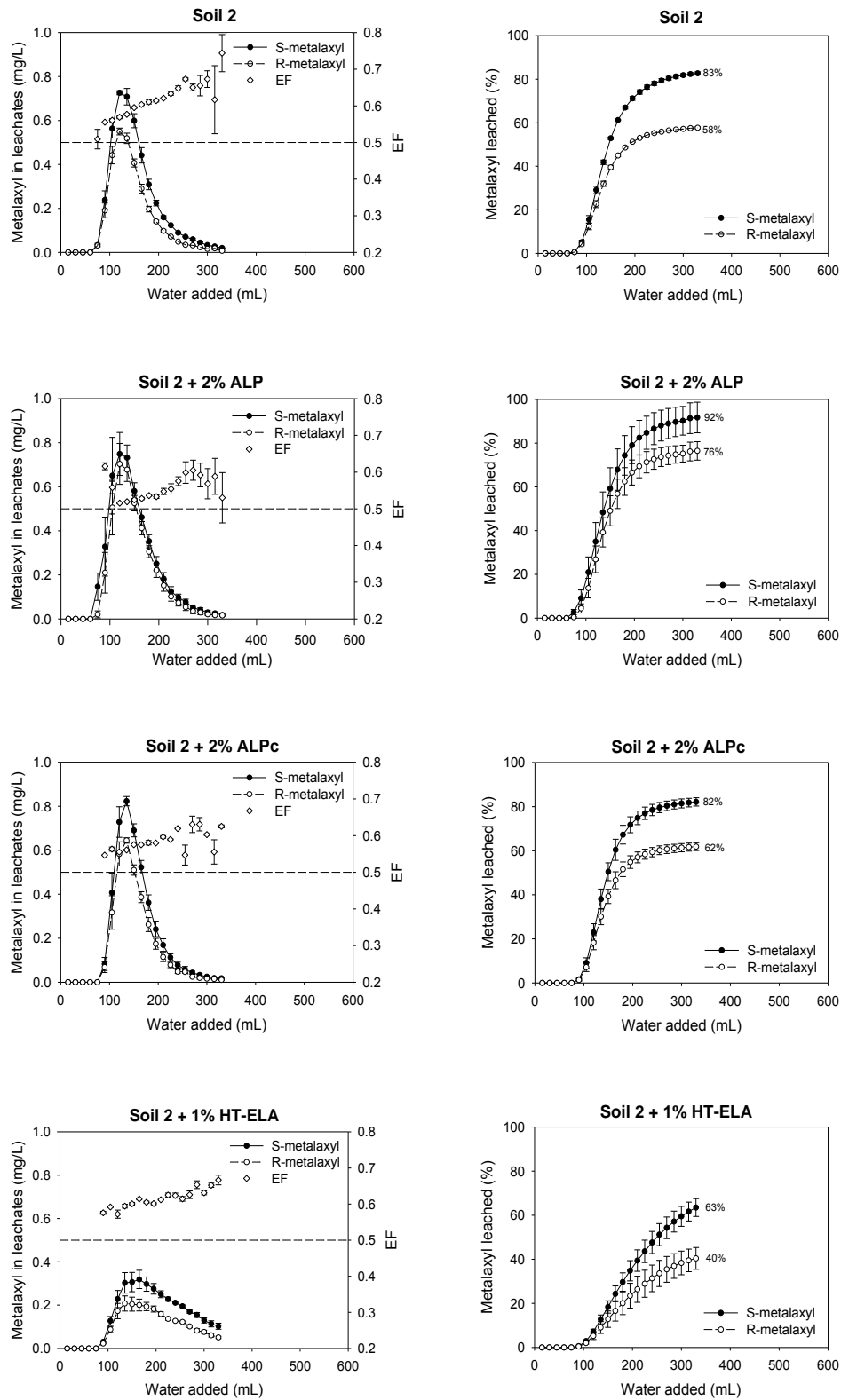


Fig. 5. Relative and cumulative breakthrough curves (BTCs) of *S*- and *R*-metolaxyl in unamended and ALP-, ALPc-, and HT-ELA-amended columns of soil 2. Enantiomer fractions (EF) in the leachates are also indicated in the relative BTCs. Error bars correspond to standard errors of triplicate measurements.

Supplementary data for

Evaluation of an organo-layered double hydroxide and two organic residues as amendments to immobilize metalaxyl enantiomers in soils: a comparative study

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Number of tables: 4

Number of figures: 1

Table S1

Physicochemical properties of the organic residues.

| Amendment | Total OC (%) | Soluble OC (g/l) ^a | pH ^a |
|-----------|--------------|-------------------------------|-----------------|
| ALP | 44 | 4.6 | 5.2 |
| ALPc | 20 | 1.2 | 8.6 |

^a Value measured in 1g ALP/ALPc : 20 mL CaCl₂ (0.01 M).

Table S2

Recovery in extraction procedure for *S*- and *R*-metalaxyl freshly applied to unamended soils and to soils amended with ALP, ALPc and HT-ELA.

| | Recovery (%) | |
|--------------------|-------------------------|---------------------|
| | <i>S</i> -metalaxyl | <i>R</i> -metalaxyl |
| Soil 1 | 84.1 ± 0.6 ^a | 85.2 ± 0.7 |
| Soil 1 + 2% ALP | 80.8 ± 0.7 | 81.1 ± 0.7 |
| Soil 1 + 2% ALPc | 91.2 ± 1.1 | 90.5 ± 0.6 |
| Soil 1 + 1% HT-ELA | 80.1 ± 2.2 | 81.1 ± 2.0 |
| Soil 2 | 96.7 ± 0.2 | 96.6 ± 1.2 |
| Soil 2 + 2% ALP | 99.1 ± 1.0 | 99.0 ± 0.9 |
| Soil 2 + 2% ALPc | 101.3 ± 3.8 | 101.1 ± 3.9 |
| Soil 2 + 1% HT-ELA | 106.5 ± 0.9 | 106.3 ± 0.4 |

^aValue ± standard error of triplicates.

Table S3

Freundlich coefficients and thermodynamic index of irreversibility for *S*-, *R*-, and (*S*+*R*)-metalaxyl sorption-desorption isotherms on unamended soils and on soils amended with ALP, ALPc and HT-ELA.

| | | K_f | N_f | R^2 | TII |
|--------------------|-----------------------------------|-------------------------------|-------------|-------|------|
| Soil 1 | <i>S</i> -metalaxyl | 1.69 (1.40-2.04) ^a | 1.49 ± 0.13 | 0.977 | 0.14 |
| | <i>R</i> -metalaxyl | 1.70 (1.45-2.00) | 1.50 ± 0.11 | 0.983 | 0.14 |
| | (<i>S</i> + <i>R</i>)-metalaxyl | 1.20 (0.94-1.54) | 1.49 ± 0.12 | 0.980 | 0.14 |
| Soil 1 + 2% ALP | <i>S</i> -metalaxyl | 1.38 (1.20-1.59) | 1.57 ± 0.10 | 0.900 | 0.34 |
| | <i>R</i> -metalaxyl | 1.67 (1.39-2.00) | 1.48 ± 0.13 | 0.978 | 0.31 |
| | (<i>S</i> + <i>R</i>)-metalaxyl | 1.06 (0.84-1.34) | 1.52 ± 0.11 | 0.984 | 0.32 |
| Soil 1 + 2% ALPc | <i>S</i> -metalaxyl | 1.72 (1.42-2.06) | 1.51 ± 0.13 | 0.989 | 0.20 |
| | <i>R</i> -metalaxyl | 1.96 (1.56-2.46) | 1.44 ± 0.16 | 0.963 | 0.16 |
| | (<i>S</i> + <i>R</i>)-metalaxyl | 1.22 (0.93-1.60) | 1.51 ± 0.13 | 0.977 | 0.19 |
| Soil 1 + 1% HT-ELA | <i>S</i> -metalaxyl | 6.35 (5.94-6.79) | 1.04 ± 0.05 | 0.993 | 0.00 |
| | <i>R</i> -metalaxyl | 6.60 (6.16-7.06) | 1.04 ± 0.05 | 0.998 | 0.00 |
| | (<i>S</i> + <i>R</i>)-metalaxyl | 6.30 (5.72-6.92) | 1.04 ± 0.05 | 0.993 | 0.00 |
| Soil 2 | <i>S</i> -metalaxyl | 0.56 (0.51-0.62) | 1.22 ± 0.06 | 0.993 | 0.47 |
| | <i>R</i> -metalaxyl | 0.51 (0.47-0.55) | 1.27 ± 0.05 | 0.996 | 0.53 |
| | (<i>S</i> + <i>R</i>)-metalaxyl | 0.45 (0.40-0.51) | 1.25 ± 0.05 | 0.997 | 0.50 |
| Soil 2 + 2% ALP | <i>S</i> -metalaxyl | 0.51 (0.48-0.55) | 1.25 ± 0.04 | 0.997 | 0.55 |
| | <i>R</i> -metalaxyl | 0.52 (0.51-0.53) | 1.28 ± 0.01 | 0.999 | 0.58 |
| | (<i>S</i> + <i>R</i>)-metalaxyl | 0.43 (0.45-0.41) | 1.26 ± 0.02 | 0.999 | 0.56 |
| Soil 2 + 2% ALPc | <i>S</i> -metalaxyl | 0.79 (0.72-0.87) | 1.15 ± 0.06 | 0.992 | 0.52 |
| | <i>R</i> -metalaxyl | 0.77 (0.70-0.85) | 1.16 ± 0.06 | 0.992 | 0.56 |
| | (<i>S</i> + <i>R</i>)-metalaxyl | 0.52 (0.47-0.57) | 1.26 ± 0.04 | 0.996 | 0.57 |
| Soil 2 + 1% HT-ELA | <i>S</i> -metalaxyl | 10.74 (10.41-11.09) | 0.82 ± 0.02 | 0.997 | 0.17 |
| | <i>R</i> -metalaxyl | 10.74 (10.40-11.09) | 0.82 ± 0.02 | 0.997 | 0.19 |
| | (<i>S</i> + <i>R</i>)-metalaxyl | 12.17 (11.67-12.72) | 0.82 ± 0.02 | 0.996 | 0.18 |

^a Values in parentheses correspond to the range in the values of the Freundlich coefficients.

Table S4

Summary of *S*-, *R*-, and (*S+R*)-metalaxyl column leaching data extracted from the relative and cumulative breakthrough curves (BTCs) in unamended soils and in soils with ALP, ALPc and HT-ELA.

| | | C_{\max}^a | Position of C_{\max}^b | | Total leached ^c (%) | Total extracted ^d (%) | Total non-recovered (%) |
|--------------------|--------------------------|--------------|--------------------------|------------------|-----------------------------------|-------------------------------------|----------------------------|
| | | (mg/L) | (mL) | ($\times V_p$) | | | |
| Soil 1 | <i>S</i> -metalaxyl | 0.30 | 255 | 4.3 | 75 | 6 | 19 |
| | <i>R</i> -metalaxyl | 0.28 | 255 | 4.3 | 68 | 17 | 15 |
| | (<i>S+R</i>)-metalaxyl | 0.58 | 255 | 4.3 | 71 | 12 | 18 |
| Soil 1 + 2% ALP | <i>S</i> -metalaxyl | 0.40 | 210 | 3.7 | 71 | 25 | 4 |
| | <i>R</i> -metalaxyl | 0.39 | 210 | 3.7 | 67 | 12 | 21 |
| | (<i>S+R</i>)-metalaxyl | 0.80 | 210 | 3.7 | 69 | 19 | 12 |
| Soil 1 + 2% ALPc | <i>S</i> -metalaxyl | 0.48 | 240 | 4.1 | 78 | 4 | 18 |
| | <i>R</i> -metalaxyl | 0.47 | 240 | 4.1 | 72 | 15 | 13 |
| | (<i>S+R</i>)-metalaxyl | 0.95 | 240 | 4.1 | 75 | 9 | 16 |
| Soil 1 + 1% HT-ELA | <i>S</i> -metalaxyl | 0.18 | p.d. ^e | p.d. | 58 | 20 | 22 |
| | <i>R</i> -metalaxyl | 0.16 | p.d. | p.d. | 51 | 25 | 24 |
| | (<i>S+R</i>)-metalaxyl | 0.34 | p.d. | p.d. | 55 | 23 | 22 |
| Soil 2 | <i>S</i> -metalaxyl | 0.73 | 120 | 1.9 | 83 | < 1 | 17 |
| | <i>R</i> -metalaxyl | 0.55 | 120 | 1.9 | 58 | < 1 | 42 |
| | (<i>S+R</i>)-metalaxyl | 1.28 | 120 | 1.9 | 70 | < 1 | 30 |
| Soil 2 + 2% ALP | <i>S</i> -metalaxyl | 0.75 | 120 | 1.9 | 92 | < 1 | 8 |
| | <i>R</i> -metalaxyl | 0.70 | 120 | 1.9 | 76 | < 1 | 24 |
| | (<i>S+R</i>)-metalaxyl | 1.47 | 120 | 1.9 | 84 | < 1 | 30 |
| Soil 2 + 2% ALPc | <i>S</i> -metalaxyl | 0.82 | 135 | 2.1 | 82 | < 1 | 18 |
| | <i>R</i> -metalaxyl | 0.64 | 135 | 2.1 | 62 | < 1 | 38 |
| | (<i>S+R</i>)-metalaxyl | 1.47 | 135 | 2.1 | 72 | < 1 | 28 |
| Soil 2 + 1% HT-ELA | <i>S</i> -metalaxyl | 0.32 | p.d. | p.d. | 63 | 12 | 25 |
| | <i>R</i> -metalaxyl | 0.21 | p.d. | p.d. | 40 | 12 | 48 |
| | (<i>S+R</i>)-metalaxyl | 0.52 | p.d. | p.d. | 52 | 12 | 36 |

^a C_{\max} : maximum concentration of *S*-, *R*- and (*S+R*)-metalaxyl in leachates.

^b Position of C_{\max} : mL or number of pore volumes (V_p) of water added at which C_{\max} appeared in leachates.

^c Total amount of *S*-, *R*- and (*S+R*)-metalaxyl recovered in leachates.

^d Total amount of *S*-, *R*- and (*S+R*)-metalaxyl extracted with methanol.

^e Poorly defined.

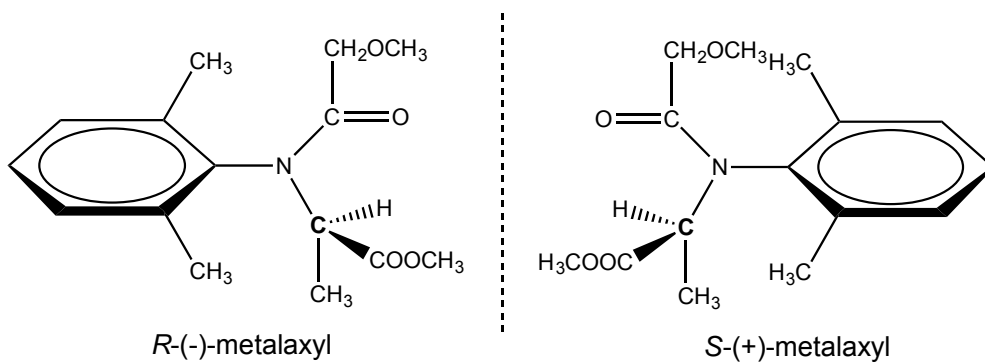


Fig. S1. Chemical structures of *R*-(-)-metalaxyl and *S*-(+)-metalaxyl.