**Environmental behavior of the enantiomers of the chiral fungicide metalaxyl in
Mediterranean agricultural soils**

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ABSTRACT

Improving the existing knowledge on the enantioselectivity of processes affecting chiral pesticide enantiomers in the environment is necessary to maximize the efficacy and minimize the environmental impact caused by the use of pesticides with chiral properties. In this work, the enantioselectivity of the sorption, degradation, and leaching processes of the chiral fungicide metalaxyl in three slightly alkaline, agricultural soils from southern Spain was studied. Batch sorption experiments indicated that the sorption of racemic-metalaxyl on soils, their clay ($< 2 \mu\text{m}$) fractions, and a number of model sorbents simulating naturally-occurring soil colloidal particles was non-enantioselective; the *S*-enantiomer was sorbed to the same extent as the *R*-enantiomer on all soil materials. Soil incubation experiments revealed that the *R*-enantiomer of metalaxyl was degraded faster than the *S*-enantiomer in all three soils, but the extent and enantioselectivity of metalaxyl degradation was soil-dependent, occurring more slowly and with less enantioselectivity in the fine-textured soil (soil 1) than in the coarse-textured soils (soils 2 and 3). For soils 2 and 3, *S*- and *R*-metalaxyl dissipation data were very well described by single first-order kinetics, whereas for soil 1 dissipation data were better fitted by two coupled first-order equations. It is suggested that sorption and entrapment of metalaxyl enantiomers in the abundant small-size pores of soil 1 (i.e., pore radius $< 100 \text{ nm}$) could have resulted in a fraction of the fungicide of reduced bioavailability, and consequently, protected from enantioselective degradation. Metalaxyl leaching through soil columns was also enantioselective; the concentration of *S*-metalaxyl in all leachates collected was greater than that of *R*-metalaxyl. Despite being non-enantioselective, sorption influenced the enantioselectivity of metalaxyl leaching, as it determined the residence time of the fungicide within the soil column, and consequently, the extent and enantioselectivity of its degradation during leaching.

Keywords: Chiral pesticides; Degradation; Enantioselectivity; Leaching; Sorption; Soil porosity

1. Introduction

Factors influencing the enantioselective environmental behavior of chiral pesticides have been pointed out as a current research opportunity in the field of environmental and agricultural chemistry, because some enantioselective environmental processes are not fully understood (Garrison, 2006; Pérez and Barceló, 2008). Although studies on the enantioselective environmental behavior of chiral pesticides first appeared in the early 1990s, they have only recently become a growing area of research. This is because it has become clear that improving the current knowledge about the different efficacy, toxicity, and environmental behavior of chiral pesticide enantiomers is necessary to comply with legislations directed to avoid unacceptable risks to human health and the environment, as well as to address the suitability of replacing pesticide racemic mixtures with single biologically-active enantiomers for a more sustainable pesticide use (Garrison, 2006; Magrans et al., 2002; Ye et al., 2010).

It is well known that the degradation of chiral pesticides in soil is usually enantioselective, primarily due to the enantioselectivity of soil biological degradation processes (Buerge et al., 2006; Garrison et al., 1996; Kurt-Karakus et al., 2005; Li et al., 2006; Ramezani et al., 2010); however, information on factors influencing such enantioselective degradation is still scarce (Buerge et al., 2003; Garrison, 2006; Lewis et al., 1999; Romero et al., 2001; Tan et al., 2008). Enantioselectivity of soil degradation processes can be affected by environmental conditions, such as soil type, pH, and microbial populations, so that agricultural practices affecting those parameters can influence the enantioselective degradation of chiral pesticides (Buerge et al., 2003, 2006; Lewis et al., 1999; Monkiedje et al., 2003; Romero et al., 2001).

The enantioselectivity of the soil sorption process for chiral pesticides is particularly poorly understood. Enantioselective sorption to chiral soil, sediment, or aquatic components could greatly affect the fate of chiral pesticides in the environment (Garrison, 2006), because sorption is a key process determining pesticide bioavailability and hence can affect other transport and

transformation processes such as leaching, volatilization or biodegradation (Celis and Koskinen, 1999; Koskinen and Harper, 1990; Oliveira et al., 2001). Even though the ability of certain soil components with chiral crystalline structures (quartz) or chiral surface properties (calcite) to enantioselectively adsorb different chiral compounds was reported long ago (Bonner and Kavasmaneck, 1976; Hazen et al., 2001), the enantioselectivity of pesticide sorption in soil is difficult to identify and predict due to the heterogeneity and complexity of organomineral soil colloids and also to the fact that the different enantiomeric morphologies of soil components can be equally abundant (Frondel, 1978, Matallo et al., 1998). Thus, questions remain as to whether soil components assemblages are net "isomer-blind" at the macro-scale or whether inherent selectivity could lead to the homochirality that is evident in biological systems (Wedyan and Preston, 2005). In this regard, many biological structures are chiral and, consequently, are good candidates to display a chiral preference. For example, L-aminoacids forming proteins or D-ribose and D-deoxyribose units forming ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) make these soil-occurring structures potential enantioselective adsorbents for chiral pesticides. In accordance with this hypothesis, Wedyan and Preston (2005) presented evidence that an ordinary estuarine sediment exhibited strong optical isomer selectivity in its sorption of a number of aminoacids. Similar results were reported by Liu and Lee (2007) and Oravec et al. (2010) for the sorption of different chiral compounds on soil and sediment materials. Enantioselective sorption of chiral pesticides to soil components would influence the enantiomer bioavailability (Wen et al., 2010), as well as their potential for transport to ground and surface waters (Zipper et al., 1998).

Metalaxyl [methyl-*N*-(2-methoxyacetyl)-*N*-(2,6-xylyl)-*DL*-alaninate] is a chiral acylanilide fungicide widely used in the control of plant diseases caused by pathogens of the *Oomycora* division in several crops. Its fungicidal activity mostly originates from the *R*(-)-enantiomer (Buerge et al., 2003; Chen and Liu, 2009). Previous studies have demonstrated 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 $\text{pH} < 4$, and *R*-metalaxyl being degraded more rapidly than *S*-metalaxyl in aerobic soils with $\text{pH} > 5$ (Buerge et al., 2003; Buser et al., 2002; Chen and Liu, 2009). However, the factors influencing the enantioselectivity of metalaxyl degradation in soils with similar pH values remain less understood. Furthermore, there is no published data on the enantioselectivity of sorption and leaching processes affecting metalaxyl in soil or on the linkages between these processes, although this information is essential to understand the overall enantioselective behavior of chiral pesticides in the environment. The objectives of this work were: i) to characterize the enantioselectivity of metalaxyl sorption by three slightly alkaline, agricultural soils from Southern Spain with different textures, their clay fractions, and a series of model particles simulating naturally-occurring soil colloids, ii) to determine the enantioselectivity of metalaxyl dissipation in the soils and relate it to the soil characteristics and sorptive properties, and iii) to characterize the enantioselectivity of metalaxyl leaching through soil columns and relate it to metalaxyl sorption and degradation data.

2. Materials and methods

2.1. Fungicide

High-purity, analytical standard grade, racemic-metalaxyl (chemical purity $> 99.5\%$) purchased from Sigma-Aldrich (Spain) was used in the experiments. Metalaxyl is an acylanilide fungicide with molecular mass 279.3 g/mol, water solubility 8.4 g/L (22 °C), and vapor pressure 0.75 mPa (25 °C) (Tomlin, 2006).

2.2. Soils, clay fractions, and model sorbents

Three slightly alkaline, Mediterranean agricultural soils from Seville (SW Spain) were used in this study. The soils were selected for having similar pH and organic carbon contents, but different textures and clay mineralogies. The soils were sampled (0-20 cm), air-dried, and sieved to pass a 2 mm mesh before use. The main physicochemical properties of the soils are summarized in Table 1 and are discussed in detail in Gámiz et al. (2012).

The clay fraction ($< 2 \mu\text{m}$ particle-size fraction) of the soils was separated by sedimentation after carbonate elimination (Jackson, 1975). Elemental analysis of the soil clay fractions was carried out in a Perkin Elmer, model 1106, elemental analyzer (Perkin-Elmer Corp., Norwalk, CT), whereas their phyllosilicate mineralogy was determined by X-ray diffraction analysis on oriented specimens of Mg^{2+} - and K^{+} -saturated clay samples, solvated with liquid ethylene glycol and calcined at 550°C , respectively (Brown, 1961). X-ray diffractograms were obtained in a D-5000 diffractometer (Siemens, Stuttgart) using $\text{CuK}\alpha$ radiation. A detailed discussion of the characteristics of the clay fractions of the soils, which are summarized in Table S1 of the Supplementary material, can also be found in Gámiz et al. (2012).

The model sorbents used in metalaxyl sorption experiments were those used in earlier studies by Cruz-Guzmán et al. (2003) and Celis et al. (2005, 2006), and contained montmorillonite (SW), poorly crystallized ferrihydrite (Ferrih), and soil-extracted humic acid (HA). The most relevant characteristics of the model particles are given in the Supplementary material (Table S2). The montmorillonite (SW) was the $< 2 \mu\text{m}$ fraction of SWy-2 Wyoming montmorillonite, supplied by the Clay Minerals Repository of the Clay Minerals Society (Purdue University, IN). A SW-Ferrih binary association was prepared by neutralizing with 0.1 M NaOH (final pH = 7.5) a suspension containing 500 mL of 30 mM $\text{Fe}(\text{NO}_3)_3$ and 10 g of SW. The resulting SW-Ferrih complex was shaken for 16 h, washed with deionized water, and finally freeze-dried. A SW-Ferrih-HA ternary model sorbent was obtained by shaking 10 g of the freshly precipitated SW-Ferrih binary association in 400 mL of a dissolved HA solution (1

g/L, pH 6.5), then dialyzing against distilled water and freeze-drying (Celis et al., 2005; Cruz-Guzmán et al., 2003).

2.3. Sorption experiments

Sorption experiments were conducted by adding 8 mL of an aqueous solution of *rac*-metalaxyl (1 mg/L) to aliquots of 4 g of soil, 1 g of clay fraction or 0.05 g of model sorbent in glass centrifuge tubes lined with screw caps. The suspensions were equilibrated by shaking in an end-over-end shaker (30 r.p.m.) at 20 ± 2 °C for 24 h. After equilibration, the suspensions were centrifuged at $5000 \times g$ for 15 min, and 4 mL of the supernatant solution was removed, filtered using glass fiber filters (pore diameter= 0.45 μ m), and analyzed by chiral high-performance liquid chromatography (HPLC) to determine the solution concentration of each metalaxyl 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 (C_{ini} = 0.5 mg/L) and equilibrium (C_e) solution concentration of each enantiomer. Tubes with the initial *rac*-metalaxyl solution without sorbent were also shaken for 24 h and revealed no loss of the fungicide due to processes different from sorption to the solids during the sorption experiment. All sorption measurements were conducted in triplicate. Sorption distribution coefficients, K_d (L/kg), were calculated by dividing the amount of each enantiomer sorbed (C_s) by its equilibrium solution concentration (C_e). The enantiomer fraction (EF) in the equilibrated solutions during the sorption experiments was calculated according to Harner et al. (2000) as:

$$EF = C_{e-S(+)} / (C_{e-S(+)} + C_{e-R(-)}) \quad (1)$$

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

2.4. Dissipation experiment

The enantioselective dissipation of metalaxyl in the soils was studied under aerobic conditions by means of an incubation experiment in which samples of 200 g of soil were spiked with *rac*-metalaxyl at a rate of 2 mg/kg soil, and then incubated in glass jars at 20 ± 2 °C for 42 days. The soil moisture content was maintained at a constant level close to the water content held by the soil columns used in the leaching experiments after they were saturated with water and allowed to drain for 24 h (see below), i.e., 40% for soil 1, 30% for soil 2, and 25% for soil 3 on a weight basis. Periodically, triplicate 3 g soil aliquots were sampled using a sterilized spatula and immediately frozen until analyzed. The amounts of *S*- and *R*-metalaxyl in the 3 g soil aliquots at each sampling time were determined by extraction with 8 mL of methanol (24 h shaking), followed by centrifugation, and enantioselective analysis of the supernatant by chiral HPLC. Preliminary experiments had shown that this extraction procedure recovered > 90% of the *S*- and *R*-metalaxyl freshly applied to the soils.

At each sampling time, the enantiomer fraction (EF) was calculated by dividing the soil concentration of *S*(+)-metalaxyl by the sum of the soil concentrations of the *S*(+)- and *R*(-)-metalaxyl enantiomers (Harner et al., 2000). In addition, single first-order (eq. 2) and double first-order in parallel (eq. 3) equations were used to describe *S*- and *R*-metalaxyl dissipation data (FOCUS, 2006):

$$C = C_0 e^{-k \cdot t} \quad (2)$$

$$C = C_{0,1} e^{-k_1 \cdot t} + C_{0,2} e^{-k_2 \cdot t} \quad (3)$$

where C (mg/kg) is the enantiomer concentration in soil at time t (days). In eq. 2, C_0 (mg/kg) is the enantiomer concentration in soil at time zero and k (days⁻¹) is the single first-order dissipation rate. In eq. 3, $C_{0,1}$ and $C_{0,2}$ are the initial enantiomer concentrations in compartments 1 and 2, and k_1 and k_2 their respective dissipation constants. Model fitting was achieved by using nonlinear regression software available with SigmaPlot Version 8.0. Other kinetic equations such as the empirical square root first-order model, which has previously been used to describe metalaxyl dissipation data in the field (Monkiedje et al., 2007), were also tested, but resulted in poor fitting of the experimental data to the model.

2.5. Column leaching experiment

Leaching experiments were conducted in 30 cm length $\times$ 3.1 cm internal diameter (i.d.) glass columns filled with air-dried soil. Glass wool plus 10 g of sea sand was placed on the bottom of the columns (5 cm) to avoid losses of soil and contamination of leachates with soil particles. Next, the columns were hand-packed with the soil (20 cm), and 10 g of sea sand was placed on the soil surface. The columns were saturated with water, allowed to drain for 24 h, and then 0.15 mg of *rac*-metalaxyl dissolved in 1 mL of water was applied to the top of the soil columns, to give an application rate of 2 kg/ha. The amount of water held by the columns after saturation and 24 h-drainage (column pore volume, V_p) was 64 ± 1 mL for soil 1, 55 ± 2 mL for soil 2, and 57 ± 2 mL for soil 3. Daily, 15 mL of distilled water was applied to the soil columns and the leachates were collected, filtered, and analyzed by HPLC to determine the *S*- and *R*-metalaxyl enantiomer concentrations.

The position of the maximum concentration peaks of the *S*- and *R*-metalaxyl breakthrough curves (BTCs) was compared with the predicted retardation factor (RF), which was calculated for each soil assuming linear, instantaneous, and reversible sorption conditions using the following equation (Rao et al., 1985):

$$RF = 1 + (\rho/\theta) K_d \quad (4)$$

In Eq. 4, ρ is the bulk density of the soil column (g/cm^3); θ is the soil column volumetric water content (cm^3/cm^3) and K_d (L/kg) is the sorption distribution coefficient for *S*- and *R*-metalaxyl in the soil column. The K_d values measured in the batch sorption experiments were assumed for the calculation of the RF values using Eq. 4. The RF value represents the number of water pore volumes (V_p) at which the maximum concentration of chemical is expected to appear in leachates (Flores-Céspedes et al., 2002).

2.6. Enantioselective analysis of metalaxyl

Metalaxyl enantiomers were determined by chiral HPLC using a Waters 600E chromatograph coupled to a Water 996 diode-array detector. The conditions used were: Chiralpak IB column (150 mm length $\times$ 4.6 mm i.d., 5 μm particle size), 60:40 water:acetonitrile eluent mixture at a flow rate of 1 mL/min, 50 μL 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. Under the analytical conditions used, the retention times for the *S*(+)- and *R*(-)-enantiomers of metalaxyl were 4.9 and 6.1 min, respectively, and *rac*-metalaxyl yielded an exact peak area ratio of 1:1 ($EF = 0.500 \pm 0.003$) in both standard solutions and freshly-spiked aqueous and methanolic soil extracts (see Fig. S1 of the Supplementary material). 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.

3. Results and discussion

3.1. Sorption experiments

Table 2 shows the distribution coefficients (K_d) measured for the *S*- and *R*-enantiomers of metalaxyl after a 24 h-equilibration of *rac*-metalaxyl with the soils, clay fractions, and model sorbents. The enantiomer fractions (EFs) in solution after the 24 h-equilibration are also included in Table 2. There was no statistical difference in the K_d values measured for the *S*- and *R*-enantiomers and the EF values remained 0.50 ± 0.01 after the 24 h-equilibration (Table 2), indicating that the sorption of metalaxyl by the soils, clay fractions, and model sorbents was non-enantioselective. Considering the variability associated with the sorption experiments conducted in this work, our data indicated that, if it occurred, enantioselectivity accounted for less than 5% of the total metalaxyl sorption by the soils, clay fractions, and model sorbents, which made it non-detectable during the sorption experiments. Consequently, in contrast to the strong enantioselectivity reported for the sorption of other chiral compounds on natural materials (Liu and Lee, 2007; Oravec et al., 2010; Wedyan and Preston, 2005), it appeared that the wide variety of soil materials used in this work were either mostly non-enantioselective in their interaction with metalaxyl or at least net "isomer-blind" at the macro-scale with regard to metalaxyl sorption.

The sorption coefficients obtained for *S*- and *R*-metalaxyl on the three Mediterranean soils selected for this study (Table 2) are similar to those obtained in previous non-enantioselective sorption studies of *rac*-metalaxyl on low organic carbon content soils, where the fungicide was analyzed as if it was an achiral single compound (Bermúdez-Couso et al., 2011; Fernandes et al., 2003; Marín-Benito et al., 2009). In such previous studies, organic matter was identified as the most important single soil component affecting the sorption of metalaxyl, but the role of mineral soil constituents was also pointed out and related to the polar character of this fungicide (Fernandes et al., 2003; Marín-Benito et al., 2009). For example, very high sorption of metalaxyl on a clay soil from Southern Spain was attributed to its content in highly sorptive, altered illite (Fernandes et al., 2003). For the three soils used in the present study, which had

similar and low organic carbon contents (Table 1), the distribution coefficients measured for *R*- and *S*-metalaxyl at an initial *rac*-metalaxyl concentration of 1 mg/L (Table 2) increased with the clay content of the soils: soil 3 < soil 2 < soil 1 (Table 1) and with the smectite + illite content of the soil clay fractions: soil 2 < soil 3 < soil 1 (Table S1), supporting the importance of clay mineral constituents on the sorption of metalaxyl enantiomers in low organic carbon content soils. In addition, sorption of metalaxyl enantiomers on model sorbents (Table 2) revealed greater sorption on montmorillonite (SW) and montmorillonite-ferrihydrite (SW-Ferrih) mineral sorbents than on the ternary sorbent containing humic acid (SW-Ferrih-HA). This behavior, which has also been observed for other very polar pesticides and attributed to blockage of the mineral surfaces by the organic material (Celis et al., 1997), further illustrated the importance of mineral soil constituents in the sorption of metalaxyl enantiomers.

3.2. Dissipation experiment

The dissipation of *rac*-metalaxyl in the three soils selected for this study was enantioselective, with the *R*-enantiomer being degraded faster than the *S*-enantiomer in the three soils, particularly in soils 2 and 3 (Fig. 1). Accordingly, EF values, $EF = [S]/([S] + [R])$, rapidly became > 0.5 during the incubation experiment, reaching a value of 0.80 at the end of the experimental period for soils 2 and 3 (Fig. 1). A value of EF = 0.80 represents that as much as 80% of the metalaxyl residues were in the form of *S*-metalaxyl, and only 20% were in the form of *R*-metalaxyl. These data are consistent with those obtained by Buerge et al. (2003) indicating that in aerobic soils with pH > 5, the fungicidally active *R*-enantiomer of metalaxyl degraded faster than the *S*-enantiomer, leading to residues with a composition $[S] > [R]$. Buerge et al. (2003) indicated that, although the underlying processes are unknown, this result may be rationalized on the predominance of microbial populations or consortia preferentially degrading the *R*-enantiomer at certain pH levels or on the activation/inhibition of enantiomer-specific

enzymes depending on pH. Since racemization may have a great influence on enantioselectivity, it is also important to note that previous studies have proven that metalaxyl was configurationally stable in soil, showing no interconversion of *R*- to *S*-enantiomers, and vice-versa (Buerge et al., 2003; Buser et al., 2002).

In general, the dissipation of *S*- and *R*-metalaxyl in the coarse-textured soils (soils 2 and 3) was very well described by single first-order kinetics (eq. 2), with coefficients of determination $R^2 = 0.98$ -1.00 (Table 3, Fig. 1). The first-order half-lives ($t_{1/2} = 0.693/k$) obtained for *S*- and *R*-metalaxyl in soils 2 and 3 (Table 3) were in the range of those reported by Buerge et al. (2003) for aerobic surface soils with pH values of 6.4-7.1, that is, close to the pH values of the soils used in the present study (Table 1). However, the clay soil used in this work (soil 1) had a markedly different behavior, yielding considerably greater half-lives, less enantioselectivity, and a poorer fitting of the experimental dissipation data to single first-order kinetics ($R^2 = 0.92$ -0.96) compared to soils 2 and 3 (Fig. 1, Table 3).

Fitting of the dissipation data to a two-compartment, bi-exponential model (eq. 3) was also tested. A visual assessment of the goodness of the fits indicated that while the two-compartment model was not strongly superior to the single first-order equation in describing the dissipation of metalaxyl enantiomers in soils 2 and 3, it provided a better fit than did the simple first-order equation for soil 1, particularly at shorter incubation times (Fig. 1). Since the determination coefficient R^2 has a limited value when comparing the fits of two models with different numbers of parameters, the chi-square (χ^2) error value test recommended by the FOCUS degradation kinetics guidance was used for model comparison (FOCUS, 2006). Only for soil 1, the two-compartment model resulted in smaller χ^2 minimum error value (at which the test is passed at the 5% significance level) than the single first-order model for both enantiomers (Table 3). The two bi-exponential equations describing the dissipation of *S*- and *R*-metalaxyl in soil 1 were:

$$S\text{-metalaxyl:} \quad C = 0.07 \cdot e^{-0.165t} + 0.79 \cdot e^{-0.005t} \quad (\text{eq. 5})$$

$$R\text{-metalaxyl:} \quad C = 0.07 \cdot e^{-0.666t} + 0.77 \cdot e^{-0.009t} \quad (\text{eq. 6})$$

The superior fitting of metalaxyl dissipation data to the two-compartment model for the clay soil (soil 1) suggests that a fraction of the added pesticide in this soil could have displayed reduced bioavailability, thus dissipating at a relatively slower degradation rate, than the more available fraction typically present in the accessible soil solution phase (Beulke and Brown, 2001). This behavior would also explain the reduced enantioselectivity of metalaxyl degradation in soil 1, because the protected, less bioavailable fraction is expected to be more racemic than the fraction readily available to enantioselective biodegradation, since it is supposed to have undergone less microbial attack (Ulrich et al., 2009; Wong et al., 2012).

A possible explanation for the soil-dependent dissipation pattern of metalaxyl enantiomers in the three soils selected for this study could be the variability in the amount and activities of specific microbial species taking part in the degradation of the pesticide molecule (Banerjee et al., 2006). Degradation is slower in the clay soil than in the other soils, so that the difference between the two enantiomers is expected to be less pronounced. However, the performance of the two-compartment model to describe the dissipation of metalaxyl enantiomers in the clay soil strongly suggests that differences in the physicochemical properties of the soils could have also played an important role. In particular, the differences in pore-size distribution between the clay soil (soil 1) and the coarse-textured soils (soils 2 and 3) should be noted (Fig. 2), since it is well known that sorption and entrapment in small-size pores is a major mechanism making organic chemicals unavailable to soil microorganisms and protecting them from biodegradation (Barriuso et al., 1997; Pignatello and Xing, 1996). For example, as the diameters of the smallest bacteria in soils, sediments, and rocks are considered to be larger than 200 nm, they

cannot directly access the organic contaminants entrapped or sorbed in these tiny pores (Cheng et al., 2012). In this regard, mercury intrusion porosimetry measurements showed that while only 9-15% of the total porosity of soils 2 and 3 corresponded to pores with a pore radius $r_p < 100$ nm, 50% of the total porosity of soil 1 corresponded to such small pores (Table 1). Pores with r_p around 10 nm were particularly abundant in soil 1 (Fig. 2), and the high smectite+illite content of this soil (Table S1) should have provided the soil with additional clay interlayer space upon swelling of the clay minerals in contact with water (Nagpal et al., 1972). Therefore, sorption and entrapment in the abundant small-size pores of soil 1 are likely to have reduced the availability of metalaxyl enantiomers for microbial degradation, and also to have reduced the enantioselectivity of the metalaxyl degradation process, the latter because the less bioavailable pesticide fraction is supposed to remain racemic longer than the readily biodegradable fraction (Ulrich et al., 2009; Wong et al., 2012).

It is also interesting to note that, using the batch sorption distribution coefficients measured for *S*- and *R*-metalaxyl on the three soils (Table 2), it can be calculated that under the experimental conditions of the incubation experiment, the total amount of metalaxyl sorbed, i.e. associated with the soil's solid phase, would represent about 80% of the added fungicide in soil 1, 65% in soil 2, and 55% in soil 3. The fact that the enantioselectivity of degradation was smaller in the soil with the largest K_d value suggests that the strength of sorption may have influenced the racemic nature. Furthermore, most of the extensive sorption of metalaxyl in soil 1 should have occurred in the abundant small-size pores present in this soil, resulting in a large portion of low-availability pesticide fraction. This large amount of less available fraction is also predicted by the large $C_{0,2}$ coefficients obtained for the two bi-exponential equations describing the dissipation of *S*- and *R*-metalaxyl enantiomers in soil 1 (eq. 5 and 6).

The main assumptions of the bi-exponential model used to fit metalaxyl dissipation data in soil 1 are that a fraction of the added pesticide is placed immediately into a compartment with

reduced bioavailability, when it comes into contact with the soil, and that in this compartment degradation occurs slowly. Thus, the bi-exponential model uses an empirical simplified concept to match a bi-phasic dissipation behavior which is not likely to occur in reality, because sorption and entrapment are time-dependent processes which occur slowly. Sorption gradually increases over time as the pesticide moves from the available to the less available state via diffusion (Pignatello and Xing, 1996). Nevertheless, although the fit to the two-compartment model cannot be used as a direct evidence for entrapment, it suggests the presence of an important pesticide fraction with reduced bioavailability in the clay soil. It has recently been observed that chiral organochlorine pesticides in sequestered and bioavailable soil compartments, which were differentiated by means of chemical extraction methods, were subjected to different degrees of enantioselective degradation (Wong et al., 2012).

3.3. Leaching experiment

Relative and cumulative breakthrough curves (BTCs) for *S*- and *R*-metalaxyl applied to soil columns as a racemic mixture are shown in Fig. 3. The enantiomer fractions (EFs) in the leachates collected during the column experiment are also included in the relative BTC graphs. For all three soils, relative BTCs show that the *S*- and *R*-enantiomers of metalaxyl were similarly retarded in the soil columns, that is, the maximum concentration of both enantiomers appeared in leachates after applying a similar amount of water of 240 mL for soil 1, 120 mL for soil 2, and 98 mL for soil 3, which corresponded to 3.7, 2.2, and 1.7 times the pore volume (V_p) of the soil columns, respectively (Fig. 3 and Table 4). This similar retardation of both enantiomers observed in the column leaching experiment with the three soils strongly supported the batch sorption data indicating insignificant enantioselectivity of the metalaxyl sorption process in soil (Table 2), because significant enantioselective sorption would have resulted in one enantiomer being leached earlier than the other, as schematically depicted in

Fig. 4b and 4c. The leaching behavior for metalaxyl in our soils corresponded to that shown in Fig. 4a, where both enantiomers are equally sorbed but one enantiomer is degraded to a greater extent than the other. Nevertheless, both enantiomers leached from the soil columns slightly earlier than predicted by the retardation factors (RF) theoretically calculated from the batch sorption distribution coefficients, assuming linear, instantaneous, and reversible sorption conditions (Eq. 4), which were RF= 4.8 (soil 1), 2.7 (soil 2), and 2.0 (soil 3) (Table 4). Non-attainment of sorption equilibrium during the column leaching experiment as well as non-linear sorption could have made the movement of metalaxyl enantiomers faster than predicted from batch sorption K_d values (Delphin and Chapot, 2006).

Despite both enantiomers being similarly retarded in the soil columns, in all leachates collected the concentration of *S*-metalaxyl was greater than that of *R*-metalaxyl (Fig. 3). This resulted in leachates with EF values > 0.5 and, as a consequence, the total cumulative amounts of *S*-enantiomer leached were greater than those of *R*-enantiomer (Fig. 3, Table 4). Given that enantioselective retention of metalaxyl in the soil column can be discarded as a cause for its enantioselective leaching behavior, the latter can be attributed mainly to the preferential degradation of the *R*-enantiomer inside the soil column (Fig. 4a), as supported by the results of the incubation experiment (Fig. 1). It is interesting to note that the enantioselectivity of metalaxyl leaching was particularly noticeable in soil 2, in which metalaxyl enantiomers displayed moderate sorption (Table 2) and high enantioselective degradation (Fig. 1). The low retention capacity and coarse texture of soil 3 resulted in the shortest residence time of metalaxyl in the soil column (Fig. 3, Table 4), which appeared to reduce the extent of degradation and the enantioselective leaching of the fungicide during the column experiment. The low enantioselectivity of metalaxyl degradation in soil 1 (Fig. 1) also resulted in little leaching enantioselectivity (Fig. 3), even though the high sorption capacity of this soil increased the residence time of the fungicide inside the soil column compared to soils 2 and 3.

These effects were also reflected by the leachate EF values, which were particularly high for soil 2 (0.55-0.70). In this soil, the latter leachates collected contained 70% of *S*-metalaxyl and only 30% of the *R*-metalaxyl enantiomer.

4. Conclusions

The results presented in this work strongly indicate that sorption of metalaxyl in soil is non-enantioselective, although the question remains as to whether metalaxyl sorption is a “true” non-enantioselective process at the molecular scale or whether different enantioselectivities of soil components could have compensated to each other to result in the lack of enantioselectivity we measured at the macroscopic scale. Incubation experiments confirmed previous work indicating that, under aerobic conditions and slightly alkaline pH values, the dissipation of metalaxyl in soil is expected to be an enantioselective process with the *R*-enantiomer being degraded faster than the *S*-enantiomer, and go further indicating that the extent of such enantioselectivity is soil-dependent even for soils with similar pH. It is proposed that sorption and entrapment in small-size pores could protect chiral pesticide enantiomers from biodegradation in soil and prolong their presence in racemic form. Our data also indicate that, once applied to the aerobic topsoil layer, enantioselective leaching of metalaxyl is likely to occur and to be influenced by the residence time of the fungicide in the topsoil layer, where degradation is usually faster than in the subsoil (Reichenberger et al., 2007), as well as by the effects exerted by other processes, such as sorption and nanopore entrapment. The fact that degradation of the biologically-active *R*-metalaxyl enantiomer is faster than that of the non-active *S*-enantiomer indicates the potential to greatly reduce the environmental impact of metalaxyl in the soils studied by reducing the fraction of *S*-metalaxyl added to the soils.

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FIGURE CAPTIONS

Fig. 1. *S*- and *R*-metalaxyl dissipation curves and change in the enantiomer fraction (EF) with time during the incubation experiment of *rac*-metalaxyl in soils. In the dissipation curves, symbols represent experimental data, whereas lines correspond to the fittings to single (solid) and bi-exponential (dashed) first-order dissipation kinetics. Error bars correspond to standard errors of triplicate measurements. The insert in the graph for soil 1 shows the same data at a different scale.

Fig. 2. Relative pore-size distributions of the soils determined by mercury intrusion porosimetry. Error bars correspond to the standard error of triplicate measurements. The vertical dotted line indicates the size limit accessible to the smallest microorganisms according to Cheng et al. (2012).

Fig. 3. Relative and cumulative breakthrough curves (BTCs) for *S*- and *R*-metalaxyl enantiomers in hand-packed soil columns. Enantiomer fractions (EF) in the leachates are also indicated in the relative BTCs. Error bars correspond to standard errors of triplicate measurements.

Fig. 4. Hypothetical breakthrough curves (BTCs) for a racemic mixture of two enantiomers in soil columns where: (a) the two enantiomers are equally sorbed but enantiomer 2 is degraded to a greater extent than enantiomer 1 during leaching, (b) the two enantiomers are equally degraded but enantiomer 2 is sorbed to a greater extent than enantiomer 1, and (c) enantiomer 2 is sorbed and degraded to a greater extent than enantiomer 1. Sorption was assumed to be reversible in all cases.

Table 1

Physicochemical properties of the soils used.

soil	texture	sand ^a	silt ^a	clay ^a	OC ^b	pH ^c	Hg pore volume ^d	
							$r_p > 100 \text{ nm}$	$r_p < 100 \text{ nm}$
		(%)	(%)	(%)	(%)		(mm ³ /g)	(mm ³ /g)
Soil 1	clay	7	34	59	0.73	7.7	55	55
Soil 2	sandy loam	73	8	19	0.52	7.7	120	22
Soil 3	loamy sand	83	7	10	0.54	7.5	159	16

^a Sand: > 0.05 mm; silt: 0.05-0.002 mm; clay: < 0.002 mm.^b OC: organic carbon content measured by dichromate oxidation.^c Measured in 4 g soil : 8 mL water suspension.^d Volume of pores with radius (r_p) > 100 nm and < 100 nm measured by mercury intrusion porosimetry (r_p measurement range: 4 – 40 000 nm).

Table 2

Distribution coefficients (K_d) for *S*- and *R*-metalaxyl sorption on soils, clay fractions, and model sorbents and enantiomer fractions (EFs) in the equilibrated solutions.

Soil		K_d (L/kg)		EF
		<i>S</i> -metalaxyl	<i>R</i> -metalaxyl	
Soils	Soil 1	1.54 ± 0.01^a	1.51 ± 0.04	0.498 ± 0.003
	Soil 2	0.58 ± 0.02	0.59 ± 0.04	0.499 ± 0.004
	Soil 3	0.31 ± 0.02	0.31 ± 0.02	0.501 ± 0.004
Clay	Soil 1	2.48 ± 0.20	2.53 ± 0.07	0.499 ± 0.002
fractions	Soil 2	1.43 ± 0.06	1.54 ± 0.05	0.501 ± 0.001
	Soil 3	1.72 ± 0.06	1.84 ± 0.02	0.501 ± 0.001
Model	SW	81.0 ± 0.5	79.9 ± 1.3	0.500 ± 0.001
sorbents	SW-Ferrih	94.1 ± 0.5	94.4 ± 4.1	0.502 ± 0.004
	SW-Ferrih-HA	67.2 ± 4.8	66.6 ± 3.3	0.501 ± 0.009

^a Mean $\pm$ standard error.

Table 3

Single first-order dissipation constants for *S*- and *R*-metalaxyl in soils.

		k (d ⁻¹)	$t_{1/2}$ (d)	R^2	χ^2 error (%)
Soil 1	<i>S</i> -metalaxyl	0.007 ± 0.001 ^a	99 ± 13	0.92 (0.96) ^b	2.3 (2.1) ^b
	<i>R</i> -metalaxyl	0.010 ± 0.001	68 ± 7	0.96 (0.99)	2.4 (1.5)
Soil 2	<i>S</i> -metalaxyl	0.018 ± 0.001	39 ± 2	0.98 (0.99)	2.6 (2.6)
	<i>R</i> -metalaxyl	0.059 ± 0.001	12 ± 1	1.00 (1.00)	2.8 (2.0)
Soil 3	<i>S</i> -metalaxyl	0.020 ± 0.001	35 ± 2	0.99 (0.99)	2.8 (3.0)
	<i>R</i> -metalaxyl	0.048 ± 0.002	14 ± 1	1.00 (1.00)	1.7 (2.0)

^a Value ± standard error.

^b For comparison purposes, the coefficients of determination (R^2) and χ^2 minimum error values at which the χ^2 test is passed at the 5% significance level (χ^2 error) for the fits to a two compartment, bi-exponential model (eq. 3) are given in parentheses.

Table 4

Summary of *S*- and *R*-metalaxyl column leaching data extracted from the relative and cumulative breakthrough curves (BTCs).^a

soil		C _{max}	position of C _{max}		RF	total leached
		(mg/L)	(mL)	(×V _p)		(%)
Soil 1	<i>S</i> -metalaxyl	0.53	240	3.7	4.8	79
	<i>R</i> -metalaxyl	0.46	240	3.7	4.8	68
Soil 2	<i>S</i> -metalaxyl	0.96	120	2.2	2.7	90
	<i>R</i> -metalaxyl	0.72	120	2.2	2.7	62
Soil 3	<i>S</i> -metalaxyl	1.27	98	1.7	2.0	92
	<i>R</i> -metalaxyl	1.17	98	1.7	2.0	82

^a $C_{\max}$: maximum concentration of *S*- and *R*-metalaxyl in leachates; position of $C_{\max}$: mL or number or pore volumes (V_p) of water added at which $C_{\max}$ appeared in leachates; RF: expected retardation factor for *S*- and *R*-metalaxyl calculated using Eq. 4; total leached: total amount of *S*- and *R*-metalaxyl recovered in leachates.

Fig. 1. S- and R-metalaxyl dissipation curves and change in the enantiomer fraction (EF) with time during the incubation experiment of *rac*-metalaxyl in soils. In the dissipation curves, symbols represent experimental data, whereas lines correspond to the fittings to single (solid) and bi-exponential (dashed) first-order dissipation kinetics. Error bars correspond to standard errors of triplicate measurements. The insert in the graph for soil 1 shows the same data at a different scale.

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