# Enantioselective sorption of the chiral fungicide metalaxyl on soil from non-racemic aqueous solutions: Environmental implications

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## HIGHLIGHTS

- Sorption of metalaxyl enantiomers on soil from non-racemic solutions was determined.
- Total $(R+S)$-metalaxyl sorption increased for solutions enriched with $R$-enantiomer.
- The more abundant $R$-metalaxyl enantiomer was preferentially sorbed by the soil.
- Sorption enantioselectivity was proposed to be caused by sorbate–sorbate interactions.
- Important environmental implications are discussed.

## GRAPHICAL ABSTRACT
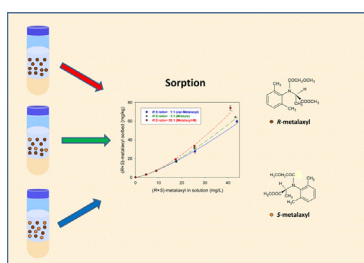


## ARTICLE INFO

## 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.

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## 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

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a list of 1693 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.

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 $20 \pm 2$ °C for 24 h. After equilibration, the suspensions were centrifuged at $5000 \times g$ for 15 min, and 4 mL of the supernatant solution (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