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Enantioselective Degradation of Metalaxyl in Anaerobic Activated Sewage Sludge

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Abstract Enantioselective degradation occurs frequently for chiral pesticides in field matrices and under laboratory conditions. Metalaxyl is a chiral compound and important fungicide. In this study, the degradation of *Rac*- and *R*-metalaxyl under anaerobic conditions was studied. The degradation processes were found to follow first-order kinetics. The degradation of the *S*-enantiomer ($T_{1/2} = 2.10$ days) was much faster than the *R*-enantiomer ($T_{1/2} = 8.44$ days). The half-life of the *Rac*-metalaxyl mixture was 4.05 days, which was higher than the *S*-enantiomer, but lower than the *R*-enantiomer. These results confirm the need for emphasizing the importance of examining the fate of the stereoisomers in an environmental system.

Keywords Metalaxyl · Chiral pesticides · Enantioselective separation · Biodegradation

Many pesticides are chiral compounds and consist of two or more enantiomers/stereoisomers. It has been shown that the enantiomers/stereoisomers of chiral pesticides differ in biological activity, toxicity, effects on beneficial and non-target organisms, and environmental fate (Williams 1996; Lewis et al. 1999; Liu et al. 2004). Although pesticides are often designed to be applied to and affect specific types of plants, they are often widely dispersed throughout the ecosystem and consequently pesticides and their degradation products can be found in various portions of the food chain. In order to better understand the distribution, effect, and fate of chiral agrochemicals, it is useful to perform enantioselective biodegradation studies (Liu and Gan 2004; Liu et al. 2005a; Müller and Kholer 2004).

Metalaxyl is an important acetanilide fungicide widely used in the control of plant diseases caused by pathogens of the *Oomycota* division. Metalaxyl contains an asymmetrically substituted C atom ("C"-chirality) and has two enantiomers. The anti-fungicidal activity mostly originates from the *R*-enantiomer (Fig. 1) (Buerge et al. 2003; Kurihara and Miyamoto 1997).

The degradation of metalaxyl in the soil is a microbiogically-mediated process (Buerge et al. 2003; Buser et al. 2002; Liu et al. 2005b). As well metalaxyl shows different enantioselectivity with the *S*-enantiomer being degraded faster in sewage sludge while the *R*-enantiomer is degraded faster in soil (Müller and Buser 1995; Buser et al. 2002). In soil, the chiral degradation could be correlated with pH. In aerobic soils, the *R*-enantiomer was degraded faster when the $\mathrm{pH} > 5$; while under conditions when the $\mathrm{pH} < 4$, the *S*-enantiomer was degraded faster (Buerge et al. 2003). In addition, the *S*-enantiomer has shown a fast degradation rate in sunflower (Marucchini and Zadra 2002; Zardra et al. 2002). Little work has been done on the degradation of the *R*- and *S*-enantiomer of metalaxyl in sewage sludge under anaerobic conditions. To better understand of the fate of metalaxyl in the environment, it is necessary to examine the fate of both enantiomers of metalaxyl. The purpose of this study is to investigate the degradation of *Rac*-Metalaxyl and the *R*-enantiomer in anaerobic sewage sludge and the enantioselective differences in the degradation pattern.

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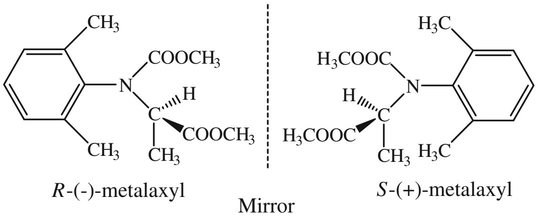

Fig. 1 Chemical structure of *R*-metalaxyl and *S*-metalaxyl

Materials and Methods

Rac-metalaxyl and *R*-metalaxyl (purity ≥98.0%) were purchased from Zhejiang Heben Pesticide & Chemicals Co., Ltd. Other solvents and chemicals used in this study were of HPLC reagent grade.

The concentration of the *Rac*-metalaxyl and *R*-metalaxyl was determined with a reversed phase HPLC system. The reversed phase HPLC system consisted of a SPD-M10A photodiode array detector set to 220 nm, a SIL-10AD automatic sampler, two LC-10AD pumps, a CTO-10A oven, a SCL-10A system control box, and a Class-VP data collection system. The column was Kromstar (C18, 250 mm × 4.6 mm i.d, 5 μm). The mobile phase was a degassed solution of 80/20 methanol/water (80/20 v/v) with flow rate of 0.80 mL/min.

The enantiomers of metalaxyl were resolved on a Chiralpak AS-H column (5 μm, 25 cm × 4.6 mm i.d.; Daicel Chemical Industries, Tokyo, Japan). The HPLC system consisted of a PU-2089 quaternary gradient pump, an AS-2055 autosampler, a CD-2095 circular dichroism detector set to 220 nm, and a column oven CO-2065 set to 30°C. The injection volumes were 20 μL. The mobile phase consisted of a solution of n-hexane/2-propanol (85/15, v/v) with a flow rate of 0.9 mL/min.

The sewage sludge, which was obtained from the wastewater treatment plant of the Hangzhou Pesticides Factory, was put in transparent containers covered with aluminum foil and a lid. A total of 200 g portions of the sewage sludge were placed in each container and then a portion was added to the container with *Rac*-metalaxyl or *R*-metalaxyl to get a concentration of 100 and 65 mg/L, respectively. The containers were incubated at 40°C. Containers with the same amount of *Rac*- or *R*-metalaxyl without sewage sludge were treated as references. Samples were collected from each container at the same intervals and were analyzed by reverse phase HPLC to determine the concentration of the metalaxyl. The samples were then extracted to determine the enantiomeric excess (ee, ee = $(C_R - C_S)/(C_R + C_S)$, where $C_R = C_S$, ee = 0; where $C_S = 0$, ee = 1).

Two gram sample were placed in an Erlenmeyer flask. Twenty milliliter of water was added and the mixture was sonicated for 1 h. After sonication, the samples were centrifuged at 10,000 rpm for 15 min. Aliquots of 2 mL were taken from the upper part of the centrifuged solution and added into 2 mL n-hexane. The mixture was sonicated and then left to for the sediments to settle. A sample of N-hexane was taken from the mixture and then was filtered with a 0.45 μm unit and transferred to 1.5 mL autosampler vials for normal HPLC analysis.

Results and Discussion

The enantiomers of metalaxyl were investigated by enantioselective analysis using chiral HPLC. To determine the elution order of each enantiomer, the racemic samples were added together with standards of *R*-(−) enantiomer. As a secondary means of confirming the elution order, a chiral detector (circular dichroism, CD) was placed in series with the UV detector. A CD detector operates on the differential absorbance between right circular polarized light and left circular light and it interacts only with optically active samples. The elution order of each enantiomer was determined by the CD spectra of the enantiomers and by comparison to the standard material of the (−)-(*R*)-enantiomer. The result (Fig. 2) shows that the retention time for the (+)-(*S*)-enantiomer was 9.2 min, while it was 12.0 min for the (−)-(*R*)-enantiomer. From Fig. 2, it can be shown that the (*S*)-enantiomer has a negative cotton effect and the (*R*)-enantiomer has a positive cotton effect at 220 nm. The retention time of metalaxyl analyzed by reverse phase HPLC was 5.0 min. The linearity of the calibration curve was 4–120 mg/L. The detection limits was 0.5 mg/L.

In order to compare the degradation of enantiomers, the degradation rate constants were calculated by assuming first-order kinetics. In sewer sludge, before degradation, *Rac*-metalaxyl has a lag phase of about 3 days after which time rapid decomposition occurs. Because of the initial lag phase, the analysis period was confined to the third day which was divided into two parts. The residual concentration will change with time *t* according to the following relationship:

$$C = C_0 e^{-kt} \tag{1}$$

where C_0 is the initial concentration and k is the degradation rate constant. From Eq. 1, the half-live time τ can be estimated from k using:

$$\tau = 0.6931/k \tag{2}$$

The half-life time of the *Rac*-metalaxyl was shown in Table 1, which is 4.05 days. The results from Fig. 3 show that *Rac*-metalaxyl was degraded, but it cannot be determined from this information whether there was enantioselective degradation of the enantiomers of *Rac*-metalaxyl or not.

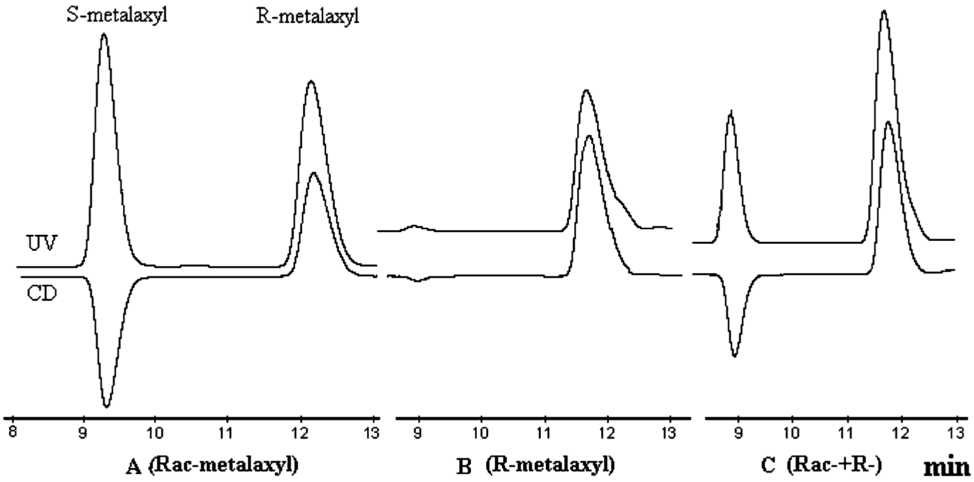

Fig. 2 Chiral HPLC chromatograms of *Rac*-metalaxyl (**a**), *R*-metalaxyl (**b**) and *R*-+*Rac* metalaxyl (**c**)

Table 1 Degradation rate constants *k* and half-life of different fungicides

Fungicides	*k* (day^{-1})	$T_{1/2}$ (day)	$T_{1/2}$ + lag phase (day)	*r*
Rac-metalaxyl	0.1711	4.05	7.11	0.9806
R-Metalaxyl	0.0795	8.71	11.71	0.9989
R-metalaxyl in *Ra*c-metalaxyl	0.0821	8.44	11.44	0.9860
S-metalaxyl in *Rac*-metalaxyl	0.3301	2.10	5.75	0.9506

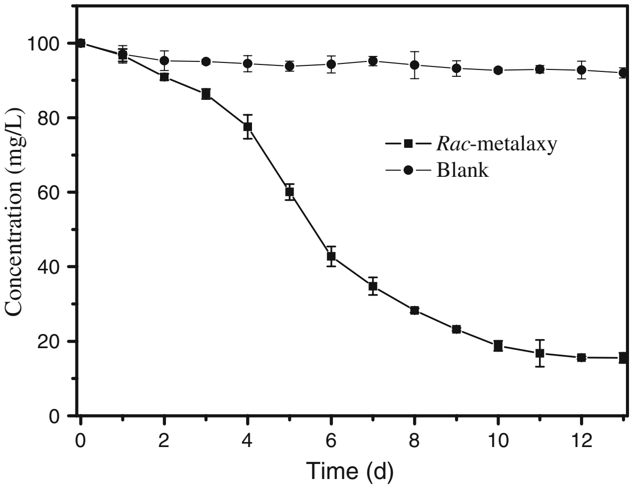

Fig. 3 Degradation of *Rac*-metalaxyl in the presence of sewage sludge

From the results shown in Fig. 4, it was determined that *R*-metalaxyl was also degraded by the sewage sludge. From the calculation, the half-life of *R*-metalaxyl is 8.71 days and rate constant is 0.0795. Compared with *Rac*-metalaxyl, *R*-metalaxyl was degraded more slowly. Samples initially containing only *R*-Metalaxyl were analyzed by chiral HPLC and no inversion of the configuration of the *R*-enantiomer was observed, indicating that metalaxyl was configurationally stable, with no interconversion of the *R*- to the *S*-enantiomer and vice versa.

As shown in Fig. 5, it appears that *Rac*-metalaxyl was racemic on the first day, and then both enantiomers were degraded. It is can be seen that the *R*-metalaxyl contained in the racemic metalaxyl was degraded more slowly than *S*-metalaxyl in the racemic mixture. This result also indicated that the degradation process was enantioselective and is likely the result of stereospecific metabolism and enzymatic transport processes. In order to compare the fate of the two enantiomers, their degradation rate constants and half-lives were calculated by assuming first-order kinetics. The results showed that the (+)-(*S*)-enantiomer ($T_{1/2}$ = 2.10 days) was degraded faster than the (−)-(*R*)-enantiomer ($T_{1/2}$ = 8.44 days) (Table 1). The half-life time of *Rac*-metalaxyl ($T_{1/2}$ = 3.11 days) was between that of the (+)-(*S*)-enantiomer and the (−)-(*R*)-enantiomer.

The enantioselective degradation also can be elucidated from the change in ee from the *Rac*-metalaxyl mixture. From the results shown in Figs. 6 and 7, it was evident that the ee changed from 0% to 85%. This indicates that the concentration of the (+)-(*S*)-enantiomer decreases faster than the (−)-(*R*)-enantiomer resulting ultimately in a higher concentration of the *R*-enantiomer than the *S*-enantiomer. This result agreed with those of Müller who found that water from a drainage canal in an agricultural area of Switzerland and ground water from Portugal contained residues of metalaxyl with a composition [*R*] > [*S*] from the application of *Rac*-metalaxyl (Müller and Buser 1995).

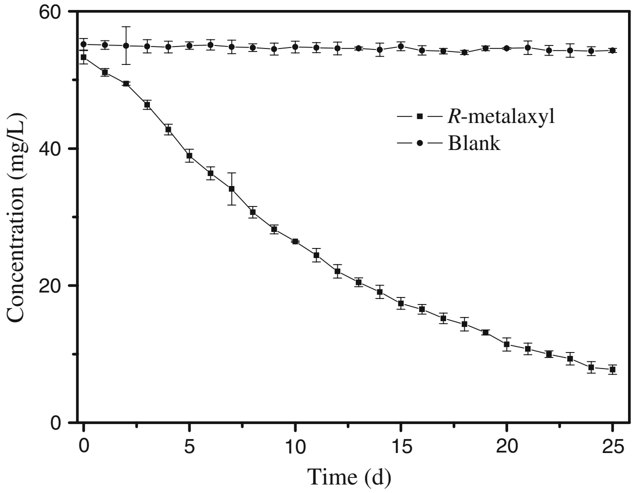

Fig. 4 Degradation of *R*-metalaxyl in the presence of sewage sludge

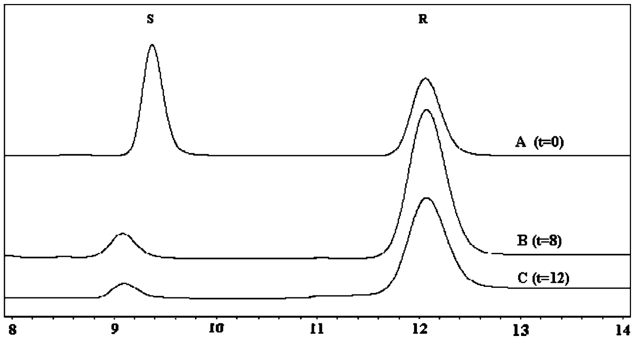

Fig. 5 Enantiomeric separations of *Rac*-metalaxyl at different incubated time (a, $t = 0$ days; b, $t = 8$ days; c, $t = 12$ days)

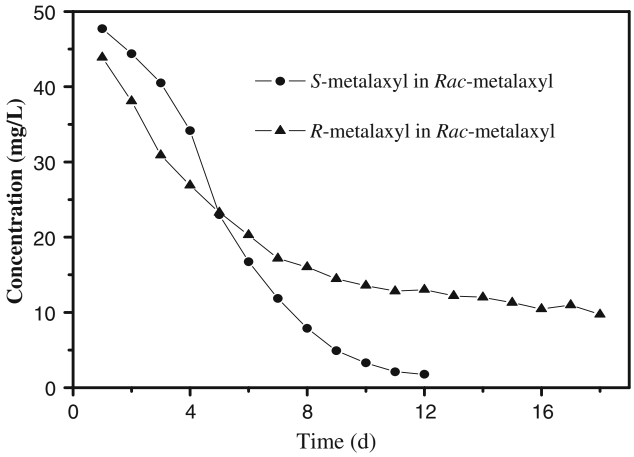

Fig. 6 Degradation of *R*-, *S*-enantiomers of *Rac*-metalaxyl in the presence of sewage sludge

The chiral degradation of metalaxyl was obviously different in soils versus sewage sludge. In this study, the sewage was sealed and thus was under anaerobic conditions, with the result that the *S*-enantiomer was preferentially degraded faster than the *R*-enantiomer.

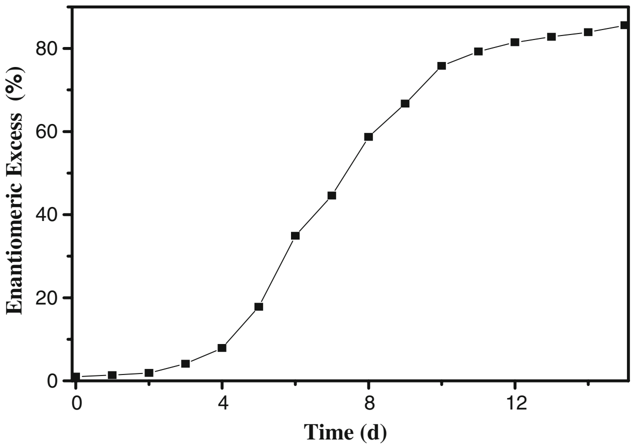

Fig. 7 Changes in enantiomeric excess of *Rac*-metalaxyl

The present study on *Rac*-metalaxyl and *R*-metalaxyl showed evidence of a stereo and enantioselective degradation of metalaxyl. Their degradation by sewage sludge showed that metalaxyl behaved differently with *Rac*-metalaxyl degraded faster than *R*-metalaxyl. Indeed, the (−)-(*R*)-enantiomer showed a longer half-life than the (+)-(*S*)-enantiomer. The degradation processes of metalaxyl in sewage sludge were followed using first-order kinetics. The degradation of the *S*-enantiomer ($T_{1/2} = 2.10$ days) was much faster than the *R*-enantiomer ($T_{1/2} = 8.44$ days). The half life of the *Rac*-metalaxyl is 4.05 days, which is higher than the *S*-enantiomer alone but is lower than the *R*-enantiomer. These results confirm the need for emphasizing the importance of examining the fate of stereoisomers of chiral agrochemicals in an environmental system.

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