

# Enantioselective Bioaccumulation and Degradation of Sediment-Associated Metalaxyl Enantiomers in *Tubifex tubifex*

Shanshan Di, Tiantian Liu, Jinling Diao, and Zhiqiang Zhou\*

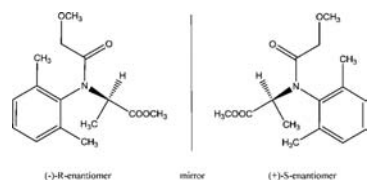
Department of Applied Chemistry, China Agricultural University, Beijing, People's Republic of China

**ABSTRACT:** Knowledge about the enantioselective bioavailability of chiral pesticides in aquatic organisms facilitates more accurate interpretation of their environmental behaviors. In this study, the enantioselective bioaccumulation of metalaxyl enantiomers in *Tubifex tubifex* was detected in two uptake pathways. For the spike water treatment, a 16 day exposure experiment was employed and the enantiomer fractions (EFs) in tubifex tissue were maintained approximately at 0.47 during the experiment. For the spike sediment treatment, a 14 day bioaccumulation period indicated the concentrations of (–)-(R)-metalaxyl were higher than those of (+)-(S)-metalaxyl. Therefore, the bioaccumulation of metalaxyl in worms was enantioselective for these treatments. With the presence of tubifex, higher concentrations of metalaxyl in overlying water and lower concentrations in sediment were detected than in worm-free treatments. This means that tubifex has positive functions in metalaxyl's diffusion from the sediment to overlying water and in the degradation of the sediment-associated metalaxyl.

**KEYWORDS:** enantioselectivity, bioaccumulation, metalaxyl, *Tubifex tubifex*

## INTRODUCTION

Metalaxyl (*R,S*)-methyl-*N*-(2'-methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate is an important acetanilide fungicide with systemic residual action and moderate toxicity. It is widely used to fight and prevent fungal diseases in a variety of crops worldwide.<sup>1</sup> Metalaxyl is C-chiral due to the presence of the stereogenic center in the alkyl moiety (Figure 1) and consists of a pair of



**Figure 1.** Structures (absolute configurations) of (R)- and (S)-metalaxyl.

enantiomers; (–)-(R)-metalaxyl is about 3–10 times more fungicidally active than (+)-(S)-metalaxyl.<sup>2</sup> In some countries, racemic metalaxyl has been replaced by metalaxyl-M, consisting of >97% of (–)-(R)-metalaxyl, thus allowing reduction of application rates and potential environmental damage.<sup>3</sup>

Many pesticides are chiral compounds and consist of two or more enantiomers. It has been shown that the enantiomers of chiral pesticides differ in biological activity, toxicity, effects on beneficial and nontarget organisms, and environmental fate. Thus, pesticide stereoselectivity or degradation studies make important contributions in improving pesticide safety to humans and animals and in minimizing contamination of the environment.<sup>3</sup> This has led to a recent surge of research interest in enantioselective behavior of chiral compounds, including metalaxyl. Several studies have shown that enantiomers of metalaxyl behave significantly differently during biodegradation and bioaccumulation in the environment. For instance, (+)-(S)-metalaxyl showed a faster degradation in plants, whereas (–)-(R)-metalaxyl showed a faster degradation in soil.<sup>4</sup> The

stereoselective degradation of metalaxyl and its enantiomers in rat and rabbit hepatic microsomes *in vitro* has been reported.<sup>5</sup> Xu et al. reported the bioaccumulation of metalaxyl in earthworm was enantioselective with preferential accumulation of (+)-(S)-metalaxyl, and the acute toxicity of metalaxyl to earthworm was different, with *rac*-metalaxyl exhibiting >2 times the toxicity of (–)-(R)-metalaxyl.<sup>2</sup> However, other aspects such as enantioselective bioaccumulation and degradation of metalaxyl in tubifex were not investigated.

*Tubifex tubifex* worms and, more generally, oligochaetes Tubificidae, are some of the most abundant and ubiquitous groups in freshwater ecosystems.<sup>6,7</sup> These oligochaete worms play an important role in many aquatic systems because they are very widely distributed and frequently dominant in freshwater benthic communities;<sup>8</sup> they are closely associated with superficial sediments, their anterior part burrowing into the substrate and the posterior part undulating in the overlying water. Therefore, tubifex worms are particularly exposed to environmental pollutants, via sediment, pore water, and water column, through ingestion and epidermal contact,<sup>9</sup> and they can cause an intensive exchange of material at the sediment–water interface and play a major role in the decomposition of organic matter.<sup>10</sup> Meanwhile, these organisms may accumulate these sediment-associated chemicals and then pose a risk to higher trophic level organisms via the food chain and help these compounds input into the environment again through bioturbation. The bioturbation of worms generally favors the exchange of matter between the water column and the sediment.<sup>11</sup> In conclusion, tubifex and several other oligochaete species have been proven suitable for the evaluation of acute and chronic toxicity and bioaccumulation, as well as benthic in

**Received:** March 8, 2013

**Revised:** April 30, 2013

**Accepted:** May 1, 2013

**Published:** May 1, 2013

situ indicators.<sup>10,12–17</sup> Although tubifex has been recommended for ecotoxicological studies, few data concerning the bioaccumulation of pollutants on this worm were available.<sup>8,14,18,19</sup> To evaluate the environmental risk of metalaxyl comprehensively, it is necessary to consider the effects on benthic organisms.<sup>19</sup>

The main objective of our study was to fill the knowledge gap regarding the differences in bioaccumulation behavior of individual enantiomers of metalaxyl in tubifex tissue. In this study, suitable methods for extraction and detection of metalaxyl enantiomers in tubifex tissue, water, and sediment samples were developed. We compared the influence of two different contamination sources, including spike water and spike sediment, on the bioaccumulation of metalaxyl, and the results showed that bioaccumulations of metalaxyl were enantioselective in these contamination treatments. In addition, we evaluated the effects of the tubifex on diffusion and degradation of metalaxyl in sediment.

## MATERIALS AND METHODS

**Chemicals and Reagents.** The fungicide *rac*-metalaxyl (>99.0% purity) was provided by the Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA). 2-Propanol (HPLC grade) and *n*-hexane (HPLC grade) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals and solvents were of analytical grade and purchased from commercial sources.

**Worms.** *T. tubifex* was obtained from Beijing Da Senlin Flower Market (Beijing, China). Worms were maintained in 2 L plastic tanks containing uncontaminated soil and deionized water at  $20 \pm 1$  °C with 12 h of light/12 h of darkness. The water was continuously aerated and 75% replaced weekly. The worms were fed TetraMin Flakes (Tetra Werke, Melle, Germany) weekly. For the experiments, adult *T. tubifex* (aged 5–7 weeks) was used. Before the worms were introduced to the treatments, they were allowed to live in this kind of uncontaminated environment for 1 week to acclimate.

**Sediment Collection, Handling, Spiking.** The sediment was collected from a location along Ulla Gail Lake and collected from the 0–10 cm surface layer. No detectable metalaxyl was found at detectable levels in the sediment. After collection, the sediment samples were air-dried at room temperature, homogenized, passed through a 2 mm sieve, and kept in the dark until use within a few days. The physicochemical properties of the sediment are listed in Table 1.

**Table 1. Properties of Sediment Used in Bioaccumulation Study**

parameter	sediment
soil type	silty loam
organic matter, g/100 g dry wt	$2.89 \pm 0.18$
clay (<2 $\mu\text{m}$ ), %	$6.0 \pm 0.02$
silt (<20–2 $\mu\text{m}$ ), %	$88.4 \pm 0.6$
sand (2000–2 $\mu\text{m}$ ), %	$5.6 \pm 1.5$
pH	$9.9 \pm 0.02$

To disperse the test substance metalaxyl homogeneously within the 100 g dry wt sediment, we did the dilution spike procedure in steps.<sup>19</sup> First, a stock standard solution containing a 1000 mg/L concentration of metalaxyl was used to make working-strength solutions by appropriate dilution as required. Then 5 mL of acetone solution was added dropwise into the dry soil (100 g). Meanwhile, the manual mixing continued for about 5 min with a stainless steel lab spoon. The final soil concentration of metalaxyl was 20 mg/kg dry wt. The spiked sediment was left in a fume cupboard overnight. After complete evaporation of the solvent, the contaminated sediment (100 g dry wt) was transferred to a 500 mL beaker and rehydrated with 100 g of deionized water, the height of the bottom substrate was 2–3 cm, and

the overlying water was 2–3 cm. Prior to adding tubifex (10 g), four 50 mL samples of wet soil were weighed, dried at room temperature until the weight did not change any longer, and then reweighed to estimate the moisture content.

**Bioaccumulation Experiment.** To assess the influence of different uptake pathways of metalaxyl on the total bioaccumulation in tubifex, two types of uptake kinetics were examined resulting from spike water and spike sediment treatments. The first scenario was called +TWA, in which metalaxyl was accumulated from spiked water. For each individual experiment, acclimated tubifex worms (10 g) were placed into beakers (21 beakers, 7 sampling points, triplicates for one sampling point), and the spiking solutions were made by adding metalaxyl dissolved in acetone to deionized water so that the final concentration was 5 mg/L. After an exposure period (1, 3, 5, 7, 9, 12, and 16 days), worms (5 g) were removed from the beaker, gently passed through a 500  $\mu\text{m}$  sieve to a clean pan, and then washed with deionized water three times. Finally, the peripheral water of the worm samples was dried using absorbent paper, and samples were weighed before being stored at  $-20$  °C. In this study, uptake from the aqueous phase was studied for 16 day exposure, and this treatment was carried out in semistatic conditions with the metalaxyl solution renewed every day.

The second scenario including tubifex, water, and spiked sediment was called +TSE, in which metalaxyl may be accumulated from overlying water, pore water, and ingestion of sediment particles. Acclimated tubifex worms (10 g) were added to the test beaker containing unspiked water and spiked sediment (24 beakers, 8 sampling points, triplicates for one sampling point). For this treatment, test organisms, overlying water, and sediment were also sampled after an exposure period (1, 2, 3, 5, 7, 9, 11, and 14 days). At each sample point, overlying water was gently poured off and sampled first. Then the beakers were placed on ice for 2 h; in this period tubifex worms climbed to the sediment surface and intertwined together slowly; at this moment worms' aggregation was sampled with forceps and rinsed in deionized water. Water on the surface of the worms was dried by absorbent paper cautiously. Finally, aliquots of 10 g of sediment (based on dry weight) were removed from each treatment and transferred into 50 mL plastic centrifuge tubes for extraction and analysis; all of the samples were frozen at  $-20$  °C.

To compare with the second scenario, a separate experiment (negative control) including only water and spiked sediment was carried out (24 beakers, 8 sampling points, triplicates for one sampling point), which was called –TSE. The sampling times were also 1, 2, 3, 5, 7, 9, 11, and 14 days, respectively. At these sampling points, overlying water and aliquots of 10 g of soil (based on dry weight) were removed from each beaker and transferred into 50 mL plastic centrifuge tubes for extraction and analysis.

For these +TSE and –TSE treatments, the test beakers were weighed daily, and the loss of water resulting from evaporation was compensated by addition of deionized water. All of the beakers were cultured in the environmental chamber with 12 h of light and 12 h of darkness; temperature was controllable to  $20 \pm 2$  °C. Each treatment beaker was arranged in a randomized block design.

**Chemical Analysis.** All of the samples were thawed for about 15 min at room temperature. For the sediment, overlying water samples (after centrifuging at 3500 rpm for 3 min) were extracted by 25 mL of ethyl acetate in a 50 mL polypropylene centrifuge tube. The tube was stirred for 3 min on a vortex mixer. This extraction was repeated twice using fresh solvent. The combined solvent phase was filtered through 5 g of anhydrous sodium sulfate for dehydration, transferred to a pear-shaped flask, and then evaporated to dryness at 35 °C by vacuum rotary evaporator. The dry extract was redissolved to 1.0 mL with 2-propanol for analysis on liquid chromatography.

Sediment samples were mixed with 5 g of anhydrous sodium sulfate and 25 mL of ethyl acetate in a 50 mL polypropylene centrifuge tube. The tube was vortexed vigorously during 3 min, extracted in an ultrasonicator for 10 min, and then centrifuged at 3500 rpm for 3 min. The extraction was repeated again following the same step. The combined extracts were filtered through 5 g of anhydrous sodium sulfate for dehydration and evaporated to dryness on a vacuum rotary

at 35 °C. The residue was reconstituted in 1.0 mL of 2-propanol and filtered through a 0.45  $\mu\text{m}$  filter prior to HPLC analysis.

For analysis of the tubifex worms, the samples (4 g) were blended with 25 mL of ethyl acetate and homogenized with an Ultra-Turrax T18 homogenizer for 30 s. The mixture was vortex-mixed for 3 min, exposed to ultrasonic vibration for 10 min, and then centrifuged at 3500 rpm for 3 min. The upper organic phase was passed through a funnel with about 5 g of anhydrous sodium sulfate to a pear-shaped flask. The organic phase was then evaporated to dryness at 35 °C. Next, the residue was reconstituted in 5 mL of acetonitrile, and then 2  $\times$  5 mL of *n*-hexane was added for liquid–liquid partition to extract most of the lipid. The upper layer of *n*-hexane was discarded, and the layer of acetonitrile was evaporated to dryness by vacuum rotary evaporation. Thereafter, the residue was purified by silica-SPE column (500 mg, 3 mL, Agilent SampliQ Products). The column was preconditioned by rinsing with 5 mL of ethyl acetate followed by 5 mL of *n*-hexane and equilibrated with 5 mL of 40% ethyl acetate *n*-hexane. The sample of dry extract was dissolved in 2 mL of 40% ethyl acetate in *n*-hexane, and then the solution was passed through the column. The SPE column was eluted with 14 mL of 40% ethyl acetate in *n*-hexane, and the following 8 mL of eluates was collected in a glass tube, evaporated to dryness under a stream of nitrogen, and diluted to 1.0 mL with 2-propanol.

For HPLC analysis with UV detection, the work was performed using an Agilent 1200 series HPLC (Agilent Technology) equipped with a G1322A degasser, a G1311A pump, a G1314B VWD, and a G1329AALS. An AT-930 heater and cooler column attenuator (Tianjin Automatic Science Instrument Co. Ltd., China) was used to control column temperature. The signal was received and processed by Agilent chemstation software. A cellulose-tri(3,5-dimethylphenylcarbamate)-based chiral stationary phase (CDMPC-CSP, provided by the Department of Applied Chemistry, CAU, Beijing) was used to separate metalaxyl in this study. The CSP and column were prepared according to the procedure described in the literature.<sup>19</sup> A mixture of *n*-hexane and 2-propanol (65:35, v/v) was used as mobile phase at a rate of 0.5 mL  $\text{min}^{-1}$ . The injection volume was 20  $\mu\text{L}$ , and the UV detection wavelength was 210 nm. The chromatographic separation was conducted at 20 °C. The elution order was established by comparison with the retention time of the (–)-(R)-metalaxyl; (+)-(S)-metalaxyl was the first eluted enantiomer and (–)-(R)-metalaxyl the second. No enantiomerization was observed for metalaxyl under this analytical condition. The average recoveries for both enantiomers at levels between 0.5 and 25 mg/kg ranged from 74 to 105% in overlying water, between 70 and 73% in tubifex tissue, and between 86 and 95% in sediment with SD below 10% ( $n = 3$  for each sample type). The limits of detection (LODs) for both enantiomers, defined as the concentration that produced a signal-to-noise ratio of 3, were 0.1 mg/kg in overlying water and sediment and 0.5 mg/kg in tubifex tissue.

**Data Analysis.** The data of the residual concentrations of the two enantiomers were used for estimating the enantiomer fraction (EF) values during these experiments. EF was used to measure the enantioselectivity during the experiment. The EF values defined ranged from 0 to 1, with EF = 0.5 representing the racemic mixture. EF was expressed as follows:  $\text{EF} = \text{peak area of (+)} / [(-) + (+)]$ , where (+) is the first eluted chromatograph peak for (+)-(S)-metalaxyl and (–) is the second eluted peak for (–)-(R)-metalaxyl. The data presented correspond to means  $\pm$  standard deviations of three independent experiments ( $N = 3$ ). Statistical analysis for the enantioselectivity of metalaxyl enantiomers was performed using SPSS 16.0 and Origin8.5. A one-sample *t* test was used to compare the means of the EF values in tubifex and sediment samples with EF = 0.501 (the actual EF of the racemic metalaxyl).

The resulting ratio, termed the accumulation factor (AF) at each specific sampling date, was plotted against time. The term accumulation factor or AF is used for any time point of uptake when the steady state has not been reached, whereas the bioaccumulation factor (BAF) is defined for steady state conditions only.<sup>20</sup> As the steady state was not reached, we chose AF to express the

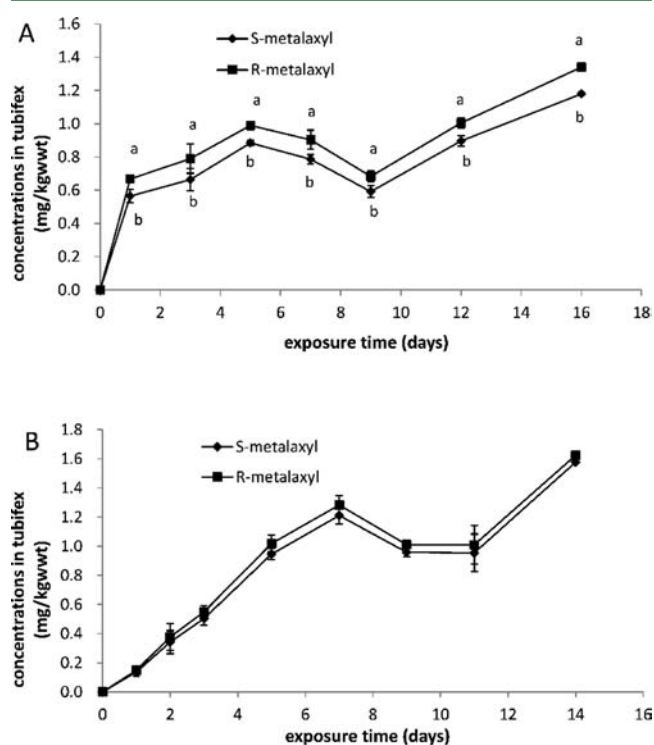
relative sorptive capacities of the organism versus the surrounding environmental. AF was defined as

$$\text{AF} = C_{\text{worm}} / C_{(\text{water/sediment})}$$

where  $C_{\text{worm}}$ ,  $C_{\text{water}}$ , and  $C_{\text{sediment}}$  are concentrations of metalaxyl enantiomers in tubifex, water, and sediment, respectively. The concentrations and AFs of the two enantiomers of metalaxyl were analyzed using one-way analysis of variance (one-way ANOVA), and a pairwise multiple-comparison procedure (S–N–K test) was used to compare results at  $p < 0.05$ .

## RESULTS AND DISCUSSION

**Bioaccumulation of Metalaxyl in Spike Water Treatment.** For the treatment +TWA, in which water was the sole source of contamination and living environment, concentrations of metalaxyl enantiomers in tubifex tissue were detected. The accumulation curves are shown in Figure 2A;



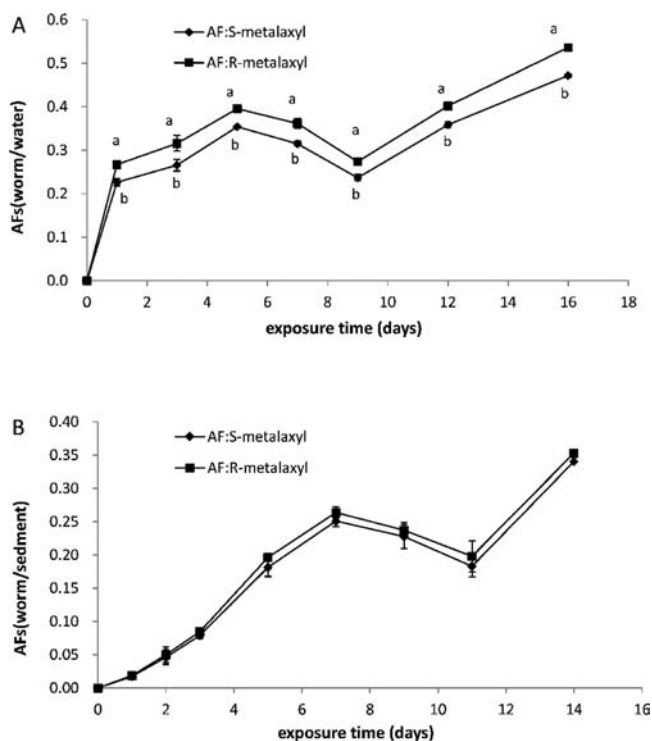
**Figure 2.** Accumulation curves for metalaxyl enantiomers in tubifex tissue in spike water treatment (A) and spike sediment treatment (B). Bars are standard error. Different letters indicate significant difference between the two enantiomers at the same time point ( $p < 0.05$ , S–N–K test).

concentrations of the two enantiomers both reached a higher level on the fifth day, and in the following days, they showed the same tendency, which could be described as a “decrease–increase” process. The decrease of concentration between days 5 and 9 of exposure may be concerned with a development of detoxification in tubifex tissue to metabolize and excrete metalaxyl. In addition, a significant difference was observed between the concentrations of two enantiomers in tubifex tissue at the same sample point, with concentrations of (–)-(R)-metalaxyl higher than those of (+)-(S)-metalaxyl. Meanwhile, the EF values were calculated, and the data are shown in Table 2. The EFs in tubifex tissue were deflected from 0.501 in the bioaccumulation experiment. A one-sample *t* test was carried out to compare the means of the EF values in tubifex with EF =

**Table 2.** EF ( $EF = S/(R + S)$ , Mean  $\pm$  SD) of Metalaxyl Accumulated in Tubifex Tissue in Spike Water Treatment and Spike Sediment

	exposure time						
	1 day	3 days	5 days	7 days	9 days	12/11 days	16/14 days
spike water EF	0.458 $\pm$ 0.007	0.456 $\pm$ 0.001	0.472 $\pm$ 0.001	0.465 $\pm$ 0.004	0.463 $\pm$ 0.002	0.471 $\pm$ 0.001	0.465 $\pm$ 0.005
spike sediment EF	0.479 $\pm$ 0.008	0.478 $\pm$ 0.003	0.481 $\pm$ 0.005	0.485 $\pm$ 0.003	0.486 $\pm$ 0.001	0.486 $\pm$ 0.001	0.492 $\pm$ 0.001

0.501, and a significant difference ( $p < 0.001$ ) between EFs and 0.501 was detected. At the same time, the AF value of (–)-(R)-metalaxyl was larger than that of (+)-(S)-metalaxyl (Figure 3A),



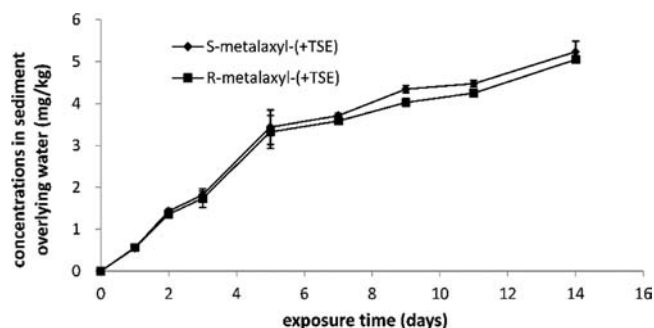
**Figure 3.** Calculated accumulation factors (AFs) for the two enantiomers of metalaxyl: (A) +TWA; (B) +TSE. Bars are standard error. Different letters indicate significant difference between the two enantiomers at the same time point ( $p < 0.05$ , S–N–K test).

indicating that (–)-(R)-metalaxyl was preferentially accumulated over (+)-(S)-metalaxyl in tubifex tissue, and a significant difference was observed between the two enantiomers. Therefore, enantioselectivity occurred when the two enantiomers of metalaxyl were accumulated by tubifex.

**Enantioselective Bioaccumulation Detection in Spike Sediment Treatment.** To simulate the living environment of tubifex preferably, the spike sediment treatment was carried out. Figure 2B shows the time course of uptake in tubifex under sediment exposure, and a difference was observed between the two enantiomers of metalaxyl. The concentrations of (–)-(R)-metalaxyl were slightly higher than those of (+)-(S)-metalaxyl, resulting in bioaccumulation enriched with (–)-(R)-metalaxyl. A one-sample  $t$  test was carried out to compare the means of EF values in tubifex with  $EF = 0.501$ . As a result, the EF values deviated from 0.501 (Table 2), and the deviation was significant ( $p < 0.001$ ). The deviation of EFs from 0.501 in tubifex tissue showed that the bioaccumulation of metalaxyl to tubifex was enantioselective. The accumulation model was analogous to that in the spike water treatment, and the tendency of

bioaccumulation was approximate to N-type. Concentrations in tubifex tissue reached the highest level on the seventh day. After a short-time decrease, the concentrations grew continuously. As shown in Figure 3B, the AF values of (–)-(R)-metalaxyl were slightly larger than those of (+)-(S)-metalaxyl, indicating that the enantioselective bioaccumulation of metalaxyl in the +TSE treatment was similar to that in the +TWA treatment, whereas the AFs of the two groups were different. It was concluded that bioaccumulation of metalaxyl may be mainly through overlying water, compared to ingestion of sediment particles. Thus, the AFs in the +TWA treatment were higher than that in this treatment during the whole exposure period, because in the +TSE group only the posterior part of tubifex undulates in the overlying water. AFs increased as the concentrations of metalaxyl in overlying water increased. That is, the deviation level of EF, equilibrium time, and AFs were distinct in these two groups because of the difference in exposure route.

**Influence of Bioturbation.** The concentrations of metalaxyl enantiomers in overlying water samples for the +TSE treatment are shown in Figure 4. As the exposure time

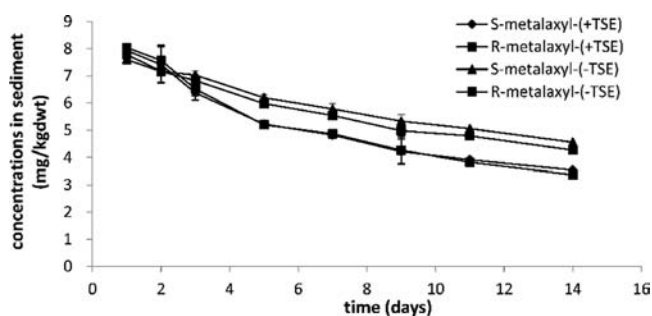


**Figure 4.** Concentrations of metalaxyl enantiomers in the overlying water for the +TSE treatments. Bars are standard error.

increased, the concentration of metalaxyl increased gradually, and no significantly enantioselective phenomenon of metalaxyl enantiomers appeared in these treatments. After the worms had been added to the system, the equilibrium of metalaxyl between sediment, pore water, and overlying water may take a long time;<sup>20</sup> thus, the concentrations of metalaxyl in the overlying water were the conjunction of diffusion and bioturbation processes for the +TSE treatment. It could be concluded that bioturbation has a power-assisted effect when metalaxyl diffused from spiked sediment into overlying water and altered the partitioning of metalaxyl, resulting in the increase of bioavailability to other aquatic organisms. The existence of worms could change the distribution of metalaxyl in the sediment environment.

**Dissipation of Metalaxyl in Sediment.** Because the  $K_{oc}$  of metalaxyl is in a realistic range of 30–300 mL/g, the partition equilibrium of metalaxyl between sediment and water was slow and metalaxyl would be moderately bound to sediment. The decline of metalaxyl concentration in sediment

over time is plotted in Figure 5 for worm-free and worm-present treatments. No significantly enantioselective behavior



**Figure 5.** Degradation curves of metalaxyl enantiomers in +TSE and -TSE. Bars are standard error.

was detected in the dissipation of the metalaxyl in the sediment for these treatments. These results indicate that the enantioselective bioaccumulation of metalaxyl in tubifex tissue was decided by the enantioselective absorption or degradation, rather than the selective dissipation of metalaxyl in sediment. Under the -TSE experimental conditions, metalaxyl displayed high persistence in sediment; 57.34% of (-)-(R)-metalaxyl and 54.45% of (+)-(S)-metalaxyl were dissipated after incubation for 14 days. For the +TSE treatment, (-)-(R)-metalaxyl and (+)-(S)-metalaxyl in sediment dissipated 66.31 and 64.62%, respectively. The dissipation kinetics of metalaxyl enantiomers in sediment followed the first-order kinetic equation  $C_{(t)} = C_{t=0} \times e^{-kt}$ , where  $C_{(t)}$  is the concentration at time  $t$  (days). The corresponding half-life was calculated as  $t_{1/2} = \ln 2/k = 0.693/k$ , where  $k$  is the dissipation rate constant, and a significant difference was observed between the half-life of *rac*-metalaxyl in +TSE (9.57 days) and that in -TSE (15.71 days) treatments (Table 3). Therefore, the dissipation of metalaxyl under worm-

**Table 3.** Kinetic Parameters of Metalaxyl Enantiomer Dissipation Processes in Sediment Resulting from Data Fitting with First-Order Kinetics Equation

	$k$ (day <sup>-1</sup> )	SE <sup>a</sup>	$C_{t=0}$	SE	$t_{1/2}$ (days)	R <sup>2</sup>
S-metalaxyl (+TSE)	0.070	0.007	8.19	0.30	9.85	0.95
R-metalaxyl (+TSE)	0.075	0.006	8.14	0.29	9.30	0.96
S-metalaxyl (-TSE)	0.041	0.002	7.80	0.09	17.09	0.99
R-metalaxyl (-TSE)	0.048	0.003	7.91	0.15	14.49	0.98
<i>rac</i> -metalaxyl (+TSE)	0.072	0.007	16.61	0.59	9.57	0.95
<i>rac</i> -metalaxyl (-TSE)	0.044	0.002	15.71	0.23	15.71	0.98

<sup>a</sup>Standard error.

present condition was faster than that under worm-free condition. This suggested that the existence of tubifex was an important factor influencing metalaxyl's dissipation in sediment, and tubifex played an important role in refining the contaminated sediment.

In this study, we found that enantioselectivity occurred in +TWA and +TSE treatments when the chiral compound metalaxyl was accumulated in tubifex tissue. The results showed that (-)-(R)-metalaxyl was preferentially accumulated over

(+)-(S)-metalaxyl in tubifex tissue, and significant differences were observed between the two enantiomers in +TWA treatment. The bioturbation of tubifex could facilitate metalaxyl diffusing from spiked sediment into overlying water and altered the partitioning of metalaxyl. Moreover, the dissipation rate of metalaxyl in +TSE treatment was higher than that in -TSE treatment. The dissipation processes followed first-order kinetics, and the enantiomers showed different dissipation rate constants. Therefore, data-obtained uptake routes may be interpreted as conservative with respect to an environmental risk assessment.

## AUTHOR INFORMATION

### Corresponding Author

\*Mail: Department of Applied Chemistry, China Agricultural University, Yuanmingyuan west road 2, Beijing 100193, China. Phone: +8610-62733547. Fax: +8610-62733547. E-mail: zqzhou@cau.edu.cn.

### Funding

This work was supported by a fund from the National Natural Science Foundation of China (Contract Grants 21177154 and 41201499) Program for Changjiang Scholars and Innovative Research Team in University.

### Notes

The authors declare no competing financial interest.

## REFERENCES

- Bermúdez-Couso, A.; Fernández-Calviño, D.; Pateiro-Moure, M.; Garrido-Rodríguez, B.; Nóvoa-Muñoz, J. C.; Estévez, M. A. Adsorption and desorption behavior of metalaxyl in intensively cultivated acid soils. *J. Agric. Food Chem.* **2011**, *59*, 7286–7293.
- Xu, P.; Diao, J.; Liu, D.; Zhou, Z. Enantioselective bioaccumulation and toxic effects of metalaxyl in earthworm *Eisenia foetida*. *Chemosphere* **2011**, *83*, 1074–1079.
- Qiu, J.; Wang, Q.; Wang, P.; Jia, G.; Li, J.; Zhou, Z. Enantioselective degradation kinetics of metalaxyl in rabbits. *Pestic. Biochem. Physiol.* **2005**, *83*, 1–8.
- Marucchini, C.; Zadra, C. Stereoselective degradation of metalaxyl and metalaxyl-M in soil and sunflower plants. *Chirality* **2002**, *14*, 32–38.
- Zhang, P.; Shen, Z.; Xu, X.; Zhu, W.; Dang, Z.; Wang, X.; Liu, D.; Zhou, Z. Stereoselective degradation of metalaxyl and its enantiomers in rat and rabbit hepatic microsomes in vitro. *Xenobiotica* **2012**, *42*, 580–586.
- Mosleh, Y. Y.; Paris-Palacios, S.; Biagianti-Risbourg, S. Metallothioneins induction and antioxidative response in aquatic worms *Tubifex tubifex* (Oligochaeta, Tubificidae) exposed to copper. *Chemosphere* **2006**, *64*, 121–128.
- Wiederholm, T.; Wiederholm, A.; Milbrink, G. Bulk sediment bioassays with five species of fresh-water oligochaetes. *Water, Air Soil Pollut.* **1987**, *36*, 131–154.
- Ciutat, A.; Gerino, M.; Mesmer-Dudons, N.; Anschutz, P.; Boudou, A. Cadmium bioaccumulation in Tubificidae from the overlying water source and effects on bioturbation. *Ecotoxicol. Environ. Saf.* **2005**, *60*, 237–246.
- Nie, X.-B.; Huang, T.-L.; Zhang, J.-S.; Li, W.; Li, X.; Zhang, S.; Wang, B. Study on inactivation effect and toxic mechanism of chlorine for the aquatic worm *Tubifex tubifex* (Oligochaeta, tubificidae). *Sustain. Environ. Res.* **2010**, *20*, 314–346.
- Smutná, M.; Hilscherová, K.; Pašková, V.; Maršálek, B. Biochemical parameters in *Tubifex tubifex* as an integral part of complex sediment toxicity assessment. *J. Soils Sediments* **2008**, *8*, 154–164.
- Matisoff, G. Effects of bioturbation on solute and particle transport in sediments. *Metal Contaminated Aquatic Sediments*; CRC Press: Boca Raton, FL, 1995; pp 201–272.

(12) Pasteris, A.; Vecchi, M.; Reynoldson, T. B.; Bonomi, G. Toxicity of copper-spiked sediments to *Tubifex tubifex* (Oligochaeta, Tubificidae): a comparison of the 28-day reproductive bioassay with a 6-month cohort experiment. *Aquat. Toxicol.* **2003**, *65*, 253–265.

(13) Vidal, D. E.; Horne, A. J. Inheritance of mercury tolerance in the aquatic oligochaete *Tubifex tubifex*. *Environ. Toxicol. Chem.* **2003**, *22*, 2130–2135.

(14) Paris-Palacios, S.; Mosleh, Y. Y.; Almohamad, M.; Delahaut, L.; Conrad, A.; Arnoult, F.; Biagianti-Risbourg, S. Toxic effects and bioaccumulation of the herbicide isoproturon in *Tubifex tubifex* (Oligochaeta, Tubificidae): a study of significance of autotomy and its utility as a biomarker. *Aquat. Toxicol.* **2010**, *98*, 8–14.

(15) P. Rodríguez, P. F. M. V. Aquatic oligochaete biology VIII. In *Development in Hydrobiology*; Pilar, R., Piet, F. M. V., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2000; pp 18–22.

(16) Lagauzere, S.; Terrail, R.; Bonzom, J. M. Ecotoxicity of uranium to *Tubifex tubifex* worms (Annelida, Clitellata, Tubificidae) exposed to contaminated sediment. *Ecotoxicol. Environ. Saf.* **2009**, *72*, 527–537.

(17) Chapman, P. M. Utility and relevance of aquatic oligochaetes in ecological risk assessment. *Hydrobiologia* **2001**, *463*, 149–169.

(18) Redeker, E. S.; Blust, R. Accumulation and toxicity of cadmium in the aquatic oligochaete *Tubifex tubifex*: a kinetic modeling approach. *Environ. Sci. Technol.* **2004**, *38*, 537–543.

(19) Rinderhagen, M.; Butte, W. Kinetics of accumulation and elimination of isomeric hexachlorocyclohexanes by tubificids. *SAR QSAR Environ. Res.* **1995**, *4*, 131–138.

(20) Egeler, P.; Meller, M.; Roembke, J.; Spoerlein, P.; Streit, B.; Nagel, R. *Tubifex tubifex* as a link in food chain transfer of hexachlorobenzene from contaminated sediment to fish. *Dev. Hydrobiol.* **2001**, *158*, 171–184.