

# Enantioselective Degradation and Chiral Stability of Malathion in Environmental Samples

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**ABSTRACT:** The dissipation behaviors of the two enantiomers of the organophosphorus pesticide malathion (MA) in environment samples were elucidated using a normal-phase high-performance liquid chromatography with a cellulose-tris(3,5-dimethylphenylcarbamate) (CDMPC) chiral column. A validated chiral residue analysis method in soil and water was established; the average recoveries for the two enantiomers were 88–102% in soil and 81–99% in water. Racemic and enantiopure *R*-(+)- and *S*-(-)-MA were incubated in five soil and water systems. The results of the degradation of racemate in all of the environment samples showed the inactive *S*-(-)-enantiomer degraded more rapidly than the active *R*-(+)-enantiomer, resulting in a relative enrichment of the *R*-form. Moreover, when the enantiopure *S*-(-)- and *R*-(+)-MA were incubated in three well-chosen soil and water samples, respectively, inversion from one enantiomer to another was found, indicating that using the optically pure enantiomer will not help to increase the bioactivity and reduce the environmental pollution.

**KEYWORDS:** malathion enantiomers, enantioselectivity degradation, soil, water

## INTRODUCTION

The development and use of pesticides have played a significant role in the increase of agricultural productivity because of their high effectiveness and low cost.<sup>1–3</sup> Organophosphorus pesticides (OPs) are among the most important chemicals used for protection against agricultural and household pests<sup>4,5</sup> and were introduced in the 1950s for use on fruits, vegetables, and other crops.<sup>6</sup> It is estimated that OPs account for nearly 40% of the global market, and they are expected to maintain dominance for some time into the future.<sup>7</sup>

About 30% of OPs sold are chiral, and the proportion is expected to increase in conjunction with more complex structures introduced into use.<sup>8</sup> As is well-known, enantiomers from the same compound have identical physical and chemical properties but always have different biological and physiological characters in asymmetry systems.<sup>9,10</sup> Therefore, there is an increasing interest in evaluating the enantioselective behavior of chiral contaminants in the environment.

The stereogenic centers of chiral OPs are generally on three different atoms, such as a pentavalent phosphorus, a carbon, or a sulfur substituent. According to this, chiral OPs can be divided into three broad classes.<sup>8,11</sup> OP isomers have been studied in detail for their separation from racemate and the bioactivity, environmental fate. The most commonly used chromatographic separation methods are high-performance liquid chromatography (HPLC) and gas chromatography (GC). Capillary electrophoresis (CE) has also been explored and has shown a great potential for enantiomer resolution due to its high separation efficiency, versatility, and low consumption of chiral selectors. The analysis of chiral OPs by these chromatographic and electrophoretic techniques has long been known.<sup>7,8</sup>

Over the past few years, numerous studies have demonstrated that the enantiomers of chiral OPs generally possess different biological activities. As potent acetylcholinesterase (AChE) inhibitors, OPs are expected to have toxic effects on

target and nontarget species. In addition, enantioselectivity has been found in the toxicity experiments for more and more OPs both in vitro and vivo.<sup>12</sup> For instance, the acute aquatic toxicities of fonofos, profenofos, and leptophos to *Daphnia magna* were also found to be enantioselective.<sup>13</sup> Enantiomers of chiral OPs differ in in vitro inhibitory potential toward various enzymes such as acetylcholinesterase, butyrylcholinesterase, carboxyesterase, and neuropathy target esterase and in vivo acute or delayed neurotoxic effects on mammals and/or nonmammals.<sup>11</sup> Likely due to their relatively short environmental persistence, there has been little interest in the possible enantioselective transformations of chiral OPs in the environment. Enantioselectivity in degradation has been reported for several chiral OPs.<sup>14</sup> Nonetheless, the separation, analysis, and toxicity of testing of OP enantiomers remain challenging tasks.

Malathion (MA), *S*-(1,2-dicarboethoxyethyl)-*O*,*O*-dimethylthiophosphate, which is used to control Coleoptera, Diptera, Hemiptera, Hymenoptera, and Lepidoptera in a wide range of crops, including cotton, pome, soft and stone fruit, potatoes, rice, and vegetables, is one of the most commonly used chiral organophosphorus insecticides and contains an asymmetric  $\alpha$ -carbon atom on the succinyl ligand.<sup>15</sup> It is a nonsystemic insecticide and acaricide with contact, stomach, and respiratory action.

Since the report that the *R*-enantiomer of MA showed preferential bioactivity over the corresponding *S*-enantiomer, the enantioselective toxicity of MA has received continual attention. Połęcz determined the enantioselective toxicity of the synthesized MA to the rats, housefly, cockroach, granary weevil, and two-spotted spider mite using standard toxicological methods<sup>16</sup> and observed that the *R*-configuration on the chiral

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carbon atom caused the highest toxicity and the *S*-configuration the lowest toxicity. Recently, Zhang et al. investigated the stereoselective toxicity and inhibition of MA on *D. magna* and acid  $\alpha$ -naphthyl acetate esterase extracted from wheat flour and found enantiomers with *R*-configuration showed higher toxicity to *D. magna* than *S*-forms and racemic forms.<sup>17</sup>

Like most organophosphates, malathion is considered to be nonpersistent, and the degradation of rac-MA in soils,<sup>18</sup> in aquatic systems,<sup>19</sup> in terrestrial plants, and in animals has been widely reported.<sup>20–22</sup> However, information concerning its stereoselective environmental fate is limited.<sup>23</sup> To understand the difference of dissipation between the enantiomers and racemate in the actual environment, the degradation fates of *R*-MA, *S*-MA, and racemate of malathion in selected soils and natural water were investigated for this paper.

## MATERIALS AND METHODS

**Chemicals and Materials.** Rac-malathion (>95.0% purity) was provided by the China Ministry of Agriculture Institute for Control of Agrochemicals. The resolved enantiomers were individually collected at the HPLC outlet of the circular dichroism (CD) detector during the observed responses,<sup>13</sup> absolute configurations were determined according to the elution order of enantiomers on chiral column GC described in a previous study,<sup>24,25</sup> and the enantiomeric purities of the *R*-enantiomer and *S*-enantiomer were 99.0 and 97.5%. Water was purified by a Milli-Q system. The solid phase extraction (SPE) column was AccuBondSPE ODS-C18 cartridges (500 mg, 6 mL, Agilent Technologies). Ethyl ether, *n*-hexane, and 2-propanol (analytical or HPLC grade) were from Fisher Scientific (Fair Lawn, NJ), and the purity of the reagents was >99.0%. All other chemicals and solvents were of analytical grade and purchased from commercial sources.

**Soil and Water Samples.** Five soil samples representing different physicochemical properties and climatic environments were collected at 0–10 cm plow layer from geographically distinct agricultural regions of China. No MA was found at detectable levels in soils. After collection, the soil 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. More details on soil sites and specific physicochemical characteristics (particle size, texture, pH, and organic carbon) are presented in Table 1.

**Table 1. Sampling Sites and Properties of the Soils Studied**

soil site <sup>a</sup>	particle-size			soil texture	pH (water) <sup>b</sup>	C <sub>org</sub> (%)
	sand (%)	silt (%)	clay (%)			
SC Neimenggu Chifeng	74.4	21.9	3.7	sandy loam	8.1	1.9
SD Liaoning Dalian	83.2	15.0	1.7	sandy loam	7.2	1.3
SY Shandong Yanzhou	41.9	52.6	5.5	silt loam	6.9	1.1
SN Jiangxi Nanchang	30.2	28.1	41.7	clay	5.0	0.7
SW Jiangsu Wuxi	36.8	61.5	1.7	silt loam	4.8	1.7

<sup>a</sup>Sites in China. <sup>b</sup>Suspension of soil in water, 1:2.5 (w/w).

Five water samples were taken directly from different channels in Beijing from May to July in 2010, filtered by vacuum filtration to eliminate the suspended particles, then transferred to 1 L glass bottles, and stored in the dark at 4 °C until analysis within 2 weeks; the blanks showed nondetectable levels of malathion. More details on water, locations, and specific physicochemical characteristics (pH, conductivity, total bacteria count, and mineralization) are listed in Table 2.

**Incubation in Soils and Water.** Separate incubation experiments spiked with the racemic malathion were conducted in the five soils in 250 mL Erlenmeyer flasks covered with sterile cotton plugs. Before

**Table 2. Basic Data of Tested Water**

water site in Beijing	conductivity/ mS m <sup>-1</sup>	total bacteria count/cfu mL <sup>-1</sup>	mineralization/ mg L <sup>-1</sup>	pH
WJ Jingmi diversion canal	66.5	36.5	178.1	8.52
WS Shangzhuang reservoir	110.8	30283.5	330.5	8.24
WD underground water	136.2	177.0	644.6	7.80
WQ Qinhe River	100.1	46737.5	225.7	7.16
WY rain	40.3	14783.0	485.2	6.01

fortification the soils were preincubated in the dark at 30 ± 1 °C for a week to activate the microorganisms in the soils. Approximately 100 g of air-dried soil was fortified by dropwise addition of 1000 µg (1.0 mL of a 1000 µg/mL stock solution in acetone) of rac-MA (fortification level of 10 µg/g, experiments SC1, SN1, SY1, SD1, and SW1, respectively, see Table 3). The soil samples were incubated with a water content of 20–36 g per 100 g of dry soil, corresponding to about 60% of field holding capacity (w/w) at 25 °C in the dark. Occasionally, the weights were controlled, and distilled water was added to compensate for loss of water. Similar experiments were carried out with pure *R*- and *S*-MA in three selected soils (experiments SC2–SC3, SN2–SN3, and SY2–SY3, respectively, see in Table 3). Periodically, samples (10 g) were removed and placed into a 50 mL polypropylene centrifuge tube for further sample treatment.

Five water samples were spiked with rac-MA by adding the standard solution in acetone in a plastic bottle and evaporating the solvent under nitrogen (experiments WD1, WS1, WY1, WQ1, and WJ1, respectively, see Table 4). Afterward, each bottle was filled with 1 L of water samples to produce a nominal pesticide concentration of 1 mg/L. The bottle content was homogenized during 15 min by careful agitation, capped, and placed at 25 °C in the dark. Similar experiments were carried out with pure *R*- and *S*-MA in three selected water samples (experiments WD2–WD3, WS2–WS3, and WY2–WY3, respectively, see Table 4). At fixed periods, 100 mL samples were removed into beakers and extracted immediately.

All of the soil and water incubation experiments including the control treatments were carried out in triplicate.

**Extraction of Soil and Water Samples.** For soil extraction, to the polypropylene centrifuge tube was added 20 mL of ethyl acetate, and the tube was stirred for 3 min on a vortex mixer, exposed to ultrasonic vibration for 10 min, and then centrifuged at 4000 rpm for 5 min. The extraction was repeated twice with 20 mL of ethyl acetate, and the three extracts were combined and filtered through 5 g of anhydrous sodium sulfate for dehydration. The combined extracts were reduced to near dryness on a vacuum rotary evaporator at 30 °C and then reconstituted in 1 mL of isopropanol.

Malathion and its enantiomers were extracted from water using ODS-C18 SPE cartridges. The cartridges were preconditioned with 5 mL of methanol and 5 mL of Milli-Q water. The 100 mL water sample was loaded and passed through the SPE cartridges at a flow rate of about 3 mL/min. After vacuum-drying for 20 min, the SPE cartridges were eluted with 5 mL of methanol. The eluting solvent was collected and evaporated under nitrogen to dryness and then resolved to 1 mL with isopropanol.

**Enantioselective HPLC Analysis.** The HPLC system consisted of an Agilent 1200 series HPLC equipped with a G1322A degasser, a G1311A quaternary pump, a G1329A automatic liquid sampler, and a G1314B variable-wavelength UV detector; Agilent 1200 Chemstation software was used. An AT-930 heater and cooler column temperature controller (Tianjin Automatic Science Instrument Co. Ltd., China) was used to control the column temperature.

The enantiomers were baseline separated on cellulose-tris(3,5-dimethylphenyl)carbamate (CDMPC)-based chiral stationary phase in normal phase condition. The chiral column 250 × 4.6 mm (i.d.) was

**Table 3. First-Order Rate Constant ( $k$ ), Half-Life ( $t_{1/2}$ ), Correlation Coefficient ( $R^2$ ), and ES Values for the Degradation of Malathion in Soil Samples**

soil origin	experiment (incubated compound)	enantiomer	$k$ (day <sup>-1</sup> )	$t_{1/2}^a$ (days)	$R^2$	ES
Neimenggu Chifeng	SC1 (rac-MA)	R-(+)-MA	0.3659	1.89 ± 0.14	0.91	0.058
		S(-)-MA	0.4112	1.69 ± 0.11	0.91	
	SC2 (R-(+)-MA)	R-(+)-MA	0.3784	1.83 ± 0.14	0.88	
	SC3 (S(-)-MA)	S(-)-MA	0.5504	1.26 ± 0.06	0.93	
Jiangxi Nanchang	SN1 (rac-MA)	R-(+)-MA	0.1648	4.21 ± 0.08	0.93	0.018
		S(-)-MA	0.1707	4.06 ± 0.09	0.93	
	SN2 (R-(+)-MA)	R-(+)-MA	0.2859	2.42 ± 0.12	0.92	
	SN3 (S(-)-MA)	S(-)-MA	0.2361	2.94 ± 0.13	0.91	
Shandong Yanzhou	SY1 (rac-MA)	R-(+)-MA	0.3360	2.06 ± 0.01	0.93	0.020
		S(-)-MA	0.3494	1.98 ± 0.08	0.95	
	SY2 (R-(+)-MA)	R-(+)-MA	0.6305	1.10 ± 0.09	0.96	
	SY3 (S(-)-MA)	S(-)-MA	0.9074	0.76 ± 0.04	0.96	
Liaoning Dalian	SD1 (rac-MA)	R-(+)-MA	0.4930	1.40 ± 0.02	0.91	0.018
		S(-)-MA	0.5106	1.36 ± 0.03	0.94	
Jiangsu Wuxi	SW1 (rac-MA)	R-(+)-MA	0.4958	1.40 ± 0.06	0.93	0.055
		S(-)-MA	0.5537	1.26 ± 0.04	0.93	

<sup>a</sup>Values represent the mean ± SD.

**Table 4. First-Order Rate Constant ( $k$ ), Half-Life ( $t_{1/2}$ ), Correlation Coefficient ( $R^2$ ), and ES Values for the Degradation of Malathion in Water Samples**

water origin	experiments (incubated compound)	enantiomer	$k$ (day <sup>-1</sup> )	$t_{1/2}^a$ (days)	$R^2$	ES
underground water	WD1 (rac-MA)	R-(+)-MA	0.3596	1.93 ± 0.05	0.89	0.0073
		S(-)-MA	0.3649	1.90 ± 0.08	0.91	
	WD2 (R-(+)-MA)	R-(+)-MA	0.8973	0.77 ± 0.11	0.88	
	WD3 (S(-)-MA)	S(-)-MA	0.8529	0.81 ± 0.07	0.84	
Shangzhuang reservoir	WS1 (rac-MA)	R-(+)-MA	0.4142	1.67 ± 0.03	0.91	0.010
		S(-)-MA	0.4227	1.64 ± 0.04	0.88	
	WS2 (R-(+)-MA)	R-(+)-MA	0.8305	0.79 ± 0.08	0.88	
	WS3 (S(-)-MA)	S(-)-MA	0.7504	0.92 ± 0.12	0.86	
rain	WY1 (rac-MA)	R-(+)-MA	0.1757	3.94 ± 0.09	0.92	0.0028
		S(-)-MA	0.1767	3.92 ± 0.10	0.92	
	WY2 (R-(+)-MA)	R-(+)-MA	0.2114	3.28 ± 0.16	0.86	
	WY3 (S(-)-MA)	S(-)-MA	0.2192	3.16 ± 0.13	0.85	
Qinhe River	WQ1 (rac-MA)	R-(+)-MA	0.6571	1.05 ± 0.05	0.95	0.021
		S(-)-MA	0.6857	1.01 ± 0.01	0.96	
Jingmi diversion canal	WJ1 (rac-MA)	R-(+)-MA	0.4354	1.59 ± 0.07	0.93	0.013
		S(-)-MA	0.4473	1.43 ± 0.08	0.93	

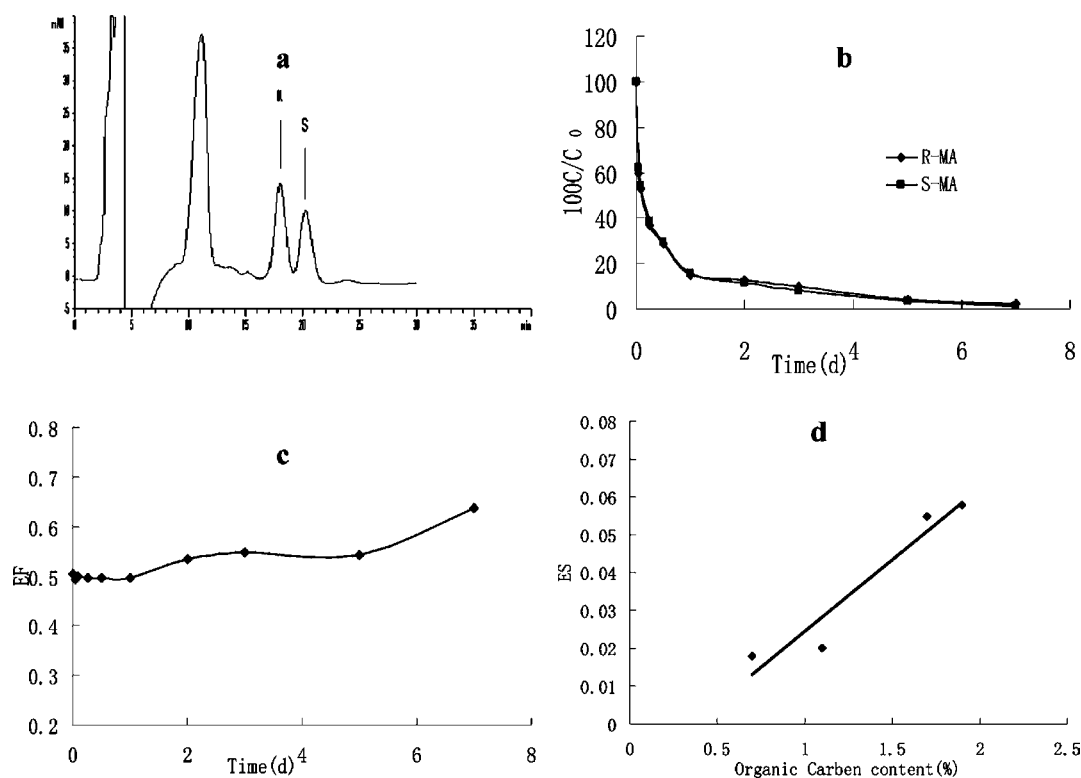
<sup>a</sup>Values represent the mean ± SD.

prepared by our group described in the previous literature.<sup>26</sup> Soil and water samples were analyzed using a mixture of *n*-hexane/2-propanol (98:2 v/v) with a flow rate of 1.0 mL/min. The wavelength for UV detection was 230 nm, and the temperature for chromatographic separation was 10 °C. The first eluted enantiomer was the *R*-(+)-form, and the second was the *S*(-)-form compared with the retention time of the enantiopure enantiomers of the malathion in the previous study.<sup>13</sup> No enantiomerization had been observed for malathion under these analytical conditions. Concentrations were determined by peak area, assuming the same response factor for enantiomers originating from the same compound.

## RESULTS AND DISCUSSION

**Assay Validation.** On the basis of the developed chiral HPLC method, enantioselective analysis methods for malathion in environmental matrices (soil and water) were developed and validated.

The series of rac-malathion standard solutions (0.5, 1, 5, 10, 50, 100, and 200 mg/L each enantiomer) for linearity of the two enantiomers were prepared in isopropanol. Good linearity was achieved within the concentration range of 0.5–200 mg/L ( $n = 5$ ) with linear equations  $y = 6.9104x - 1.6261$  ( $R^2 =$



**Figure 1.** (a) Chromatogram showing elution of malathion from the incubation of raceme in Nanchang soil after 2 days (expt SN1); (b) degradation of malathion with raceme in Wuxi soil (expt SW1); (c) enantiomeric fraction (EF) of malathion residues in Wuxi soil; (d) linear correlation between ES and organic carbon content in soils (expts SC1, SY1, SN1, SW1).

0.9997) and  $y = 6.8912x - 0.8639$  ( $R^2 = 0.9997$ ) for *R*-(+)- and *S*-(-)-MA, respectively.

Recovery estimation was carried out over three concentration levels. Blank environmental samples were amended with a given series of the standard solutions to get final concentrations equivalent to 0.1, 5, and 10  $\mu\text{g/g}$  in soil samples and 0.05, 0.5, and 1 mg/L in water samples. Recoveries of these compounds were determined immediately after fortification. In the case of soil, recoveries ranged from 88 to 99% and from 88% to 102% for *R*-(+)- and *S*-(-)-MA, respectively, with SD below 10% ( $n = 3$  for each concentration). For water, the recoveries of the two enantiomers were 81–99 and 81–98%, and the SD was not higher than 10%, too. The limit of quantification (LOQ) for these compounds was found to be 0.1  $\mu\text{g/g}$  in soil and 0.05 mg/L in water samples on the basis of an acceptable RSD of 20%.

**Analysis Parameters.** When the degradation fitted well to the first-order kinetics, the corresponding rate constants  $k$  and half-life ( $t_{1/2}$ ) of the pesticides were determined using regression plots of  $\ln(C_0/C)$  versus time ( $t$ ) with the equation

$$\ln(C/C_0) = -kt \quad (1)$$

$$t_{1/2} = \ln 2/k = 0.693/k \quad (2)$$

where  $C_0$  is the initial concentration of enantiomer ( $\mu\text{g/g}$  or mg/L),  $C$  is its concentration ( $\mu\text{g/g}$  or mg/L) at time  $t$  (days), and  $k$  is the degradation rate constant.<sup>27</sup>

Considering that the biodegradation of the chiral malathion might be enantioselective, the enantiomer fraction (EF) is used as expressed by  $\text{EF} = \text{peak areas of } R / (R + S)$  to investigate the chiral profiles. This definition is superior to other approaches because it provides a more meaningful representation in the

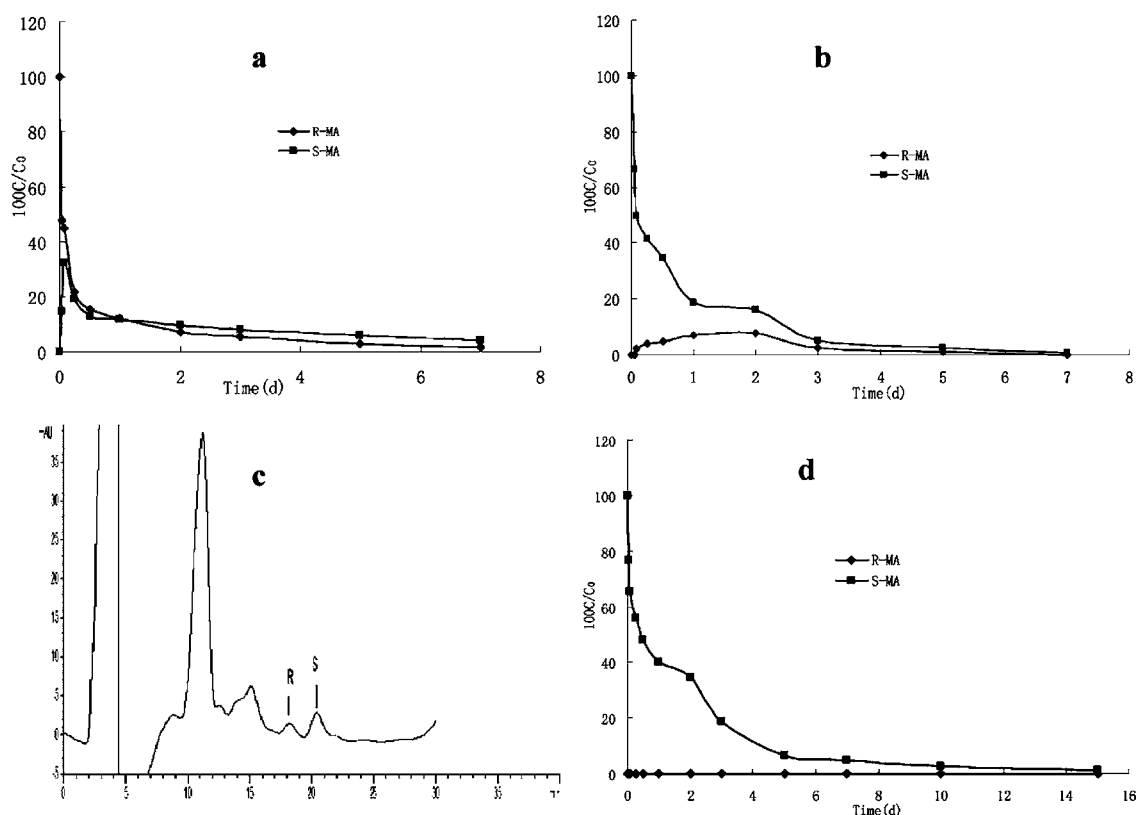
field of environmental chemistry.<sup>28</sup> The EF ranges from 0 to 1.0, and the racemate represents  $\text{EF} = 0.5$ .

ES, which reflects the overall trend in enantioselective dissipation, was defined as  $\text{ES} = (k_S - k_R) / (k_S + k_R)$  in a previous study.<sup>29</sup> Positive values ( $0 < \text{ES} \leq 1$ ) indicate a more rapid degradation of the *S*-enantiomer, whereas negative values ( $-1 < \text{ES} \leq 0$ ) indicate a more rapid dissipation of the *R*-enantiomer. At an ES value of 0, dissipation is not enantioselective, and at an ES value of 1, degradation is fully enantioselective.

**Degradation of Malathion in Soils.** Malathion transformation in the five live soils, spiked with the rac-MA, the enantiopure *R*-MA, or *S*-MA during incubation period, generally complied with first-order kinetics, with correlation coefficient,  $R^2$ , values ranging from 0.88 to 0.96 (see Table 3). The degradation rates of racemic malathion decreased in the order group SW1, SD1, SC1, SY1, and SN1, giving rise to half-lives changing from 0.76 to 4.21 days in diverse soils, which was analogous to previously reported data for other soils.<sup>23,30</sup> Distinguishingly, the persistence of malathion in Nanchang soil was 2–3 times longer than the others, and, surprisingly, we could detect a certain amount of isomers after 15 days in Nanchang soil but not in the other four soils. The observed differences in the persistence of MA in the five soils may be determined by their textural composition, which will be discussed later in this paper. The Nanchang soil was the only clay loam soil in the experiments; its porosity and ventilation must be lower than in silt and sandy loam soils. Therefore, the dissipation in the clay loam soil might be less favored than in the others.<sup>31,32</sup>

The rapid disappearance of both enantiomers of MA with time was observed in experiments, and all five samples





**Figure 2.** (a) Degradation of *R*-malathion showing concurrent formation of *S*-malathion in Chifeng soil (expt SC2); (b) degradation of *S*-malathion showing concurrent formation of *R*-malathion in Yanzhou soil (expt SY3); (c) chromatograms showing elution of malathion from the incubation of *S*-isomer in Yanzhou soil after 2 days (expt SY3); (d) degradation of *S*-malathion showing no enantiomerization in Nanchang soil (expt SN3).

degraded to levels <13% after 7 days of incubation in soil experiments. The *S*-enantiomer was degraded more quickly compared with *R*-MA in all experiments, which led to relative enrichment of the active *R*-(+)-enantiomer (Figure 1a shows the chromatogram of malathion from the incubation of raceme in Nanchang soil after 2 days). Moreover, degradation rates for the *R*- and *S*-enantiomers in the incubations with the single pure enantiomers were approximately 1.5 times higher than the ones in incubations with the raceme; this phenomenon was consistent with the results reported by Zipper.<sup>33</sup>

In Figure 1b,c, we plot the data from experiment SW1 with rac-MA, as normalized concentrations (100C/C<sub>0</sub>) and enantiomer fraction (EF) values versus time (*t*). In general, the residues of both enantiomers of malathion in Wuxi soil decreased with time elapsed. The EF values during the first day of incubations remained close to 0.5 and then increased from 0.54 to 0.64 at days 2 and 7, which indicated a faster degradation of the *S*-enantiomer in SW1. Also, analogous results were observed in the other soils, whereas the enantioselective dissipation of malathion in the five soils were different, which will be discussed in the following in depth.

**Correlation between ES and Soil Properties.** As described above, a preferential dissipation of *S*-enantiomer occurred in five soils, consequently showing positive ESs (ES from 0.018 to 0.058, Table 3) and enrichment of the *R*-malathion.

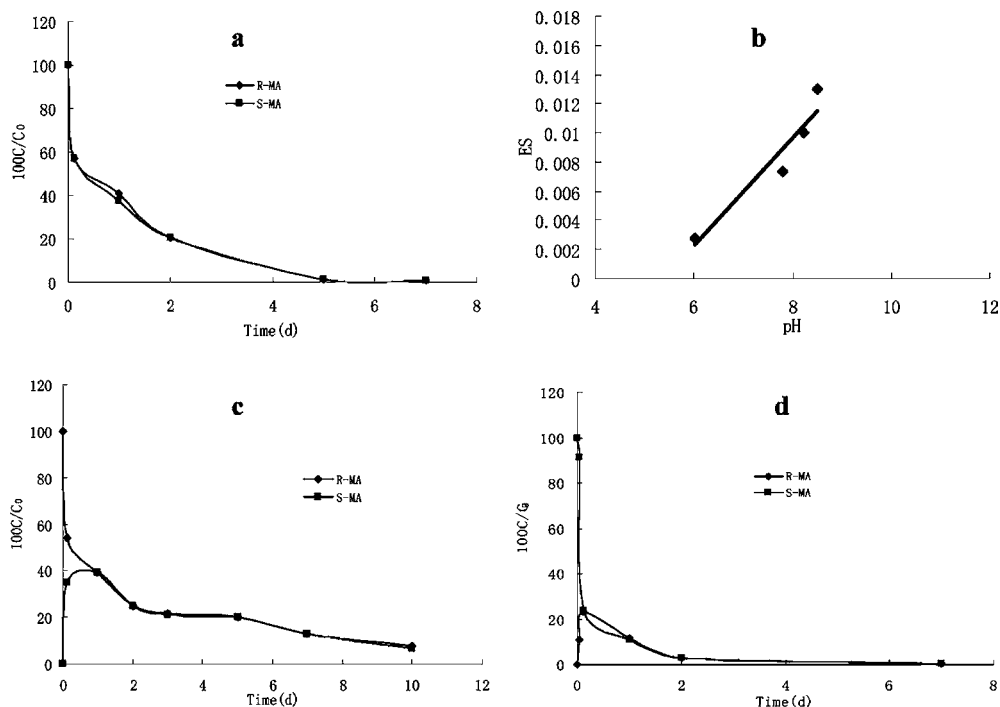
The highest ES was observed in the group of SC1, which had the highest organic carbon content. The lowest ES was found in SN1 with the lowest organic carbon content. The increase of ES values with rising organic carbon content fitted with a linear correlation ( $r^2 = 0.932$ ,  $p = 0.035$ , Figure 1d). Meanwhile, the

ES showed less positive values with lower silt and sand content. We also observed a similar linear correlation in silt and sandy loam soils ( $r^2 = 0.913$ ,  $p = 0.191$  and  $r^2 = 0.963$ ,  $p = 0.123$ ), respectively. This result was similar to a previous experiment,<sup>34</sup> showing that there was a correlation between ES value and soil content. Enantioselectivities, however, did not correlate with the pH of the soils.

From these results, it can be concluded that the rate in the dissipation of malathion was directly dependent on the soil texture and organic carbon content. This provided some support for the assertion that soil properties can influence chiral signatures by influencing the activity of the soil microbial community or the level of enzymes responsible for actual dissipation.<sup>35</sup>

**Chiral Stability of Malathion in Soils.** Enantiomerization, that is, the inversion between *S*- and *R*-malathion, was studied by separate incubations of the single pure enantiomers under native conditions.

The data for experiments SC2 and SY2 (incubation of the *R*-(+)-MA in Chifeng and Yanzhou soils, respectively) showed a continuous decrease of the concentration of *R*-(+)-MA to 3.14 and 0.78% of the initial concentrations after 7 days of incubation, respectively (see Figure 2a). At the same time, the concentrations of *S*-(-)-MA in Chifeng soil increased from initial values of 0 to 3.63 mg/kg after 3 h of incubation in experiment SC2, and then *S*-(-)-MA decreased to 0.46 mg/kg after 7 days. In detail, *R*-(+)-MA was the major enantiomer in the initial phase of the experiments of SC2 (EF = 1.0 at 0 h), and then the *S*-(-)-MA was produced by enantiomerization of *R*-(+)-MA (EF = 0.76 at 1 h). Afterward, the concentrations of the two enantiomers were equal (EF = 0.50 at 1 day); that is, a



**Figure 3.** (a) Degradation of malathion with raceme in Qinhe River (expt WQ1); (b) linear correlation between ES and pH in water samples (expts WJ1, WS1, WD1, WY1); (c) degradation of malathion in rain with R-malathion showing concurrent formation of S-malathion (expt WY2); (d) degradation of malathion in Shangzhuang reservoir with S-malathion showing concurrent formation of R-malathion (expt WS3).

50% inversion of R-(+)-MA in Chifeng was observed at 1 day; finally, R- and S-MA both decreased to a minimum concentration (EF = 0.29 at 7 days). In experiment SY2, the concentrations of S(-)-MA increased to 0.92 mg/kg after 0.5 day of incubation and then decreased. Nevertheless, the concentrations of the S-enantiomer were below those of the R-enantiomer during all of the incubation times in Yanzhou soil and EF declined from an initial value of 1.0 to the lowest value of 0.58 at 7 days.

The data for experiments SC3 and SY3 (incubation of the S(-)-MA in Chifeng and Yanzhou soils, respectively) showed a continuous decrease of the concentration of S(-)-MA to 1.63 and 0.16% of the initial concentrations after 7 days of incubation, respectively (see Figure 2b,c). Meanwhile, the concentrations of R-(+)-MA increased from initial values of 0 to maxima of 3.29 and 0.95 mg/kg after 0.25–2 days of incubation for SC3 and SY3, respectively, and then decreased again. In experiment SC3, the curves for the R- and S-enantiomers intersected after 0.25 day (EF = 0.5), indicating reversed enantiomeric compositions with higher concentrations of the R-enantiomers. However, the concentration of the R-enantiomer was below that of the S-enantiomer during all of the incubation times in experiment SY3, and the EF approached from the initial value of 0 to the highest value of 0.42 at the seventh day. The results from the above experiments illustrated translation of the R-enantiomers into the S-enantiomers for malathion in Chifeng and Yanzhou soils and vice versa.

Nonetheless, no indication for enantiomerization was found in Nanchang soil from the incubation of the pure R-(+)- and S(-)-MA (expt SN2 and SN3, respectively), revealing no conversion of R-(+)- to S(-)-MA throughout incubation (Figure 2d), and showed that the two isomers were both configurationally stable in Nanchang soil, which was not

observed in Chifeng and Yanzhou soils owing to their different type of soil as mentioned before.

From these soil experiments, we observed obvious inversion between R- and S-MA during the incubation in Chifeng and Yanzhou soils but not in Nanchang soil. Some studies suggested that soil properties such as soil pH, organic carbon, and soil texture had a major impact on the activity of the soil microbial community and then could influence chiral signatures including enantiomerization in soils.<sup>36–38</sup>

**Degradation of Malathion in Aqueous Systems.** In the five natural water samples spiked with the rac-malathion or the enantiopure R- or S-MA during the incubation period, malathion dissipation normally complied with first-order kinetics (see Table 4), with acceptable linearity (correlation coefficients,  $r^2 = 0.84–0.96$ ). Degradation of MA in laboratory water is strongly influenced by pH; that is, hydrolysis of organophosphates proceeded at higher rates under alkaline conditions.<sup>39</sup> Using the relationships described by Wolfe et al. and the pH measured, estimated degradation rates in groups WJ1, WS1, WD1, and WY1 (pH 8.52, 8.24, 7.80, and 6.01, respectively) were coincident relatively well with those observed. These consequences also supported the indication that the reaction was more effectively catalyzed by hydroxide ions than by hydronium ions or neutral water molecules.<sup>40</sup> However, if only the chemical hydrolysis were taking place, the half-life for malathion in the WQ1 experiment should be longer than that observed in the research at pH 7.16. The fact that malathion had such a short half-life at the low pH indicated that microbial action may also play a role in the degradation of malathion.<sup>41,42</sup>

Particularly, the S-enantiomer was degraded slightly faster than its antipode in all experiments and caused a bit of relative enrichment of R-malathion, which agreed with the results in the soil tests. Moreover, degradation rates for the R- and S-

enantiomers in the incubations with the single pure enantiomers were approximately 1.5–2.5 times higher than the ones in incubations with the racemic mixtures.

In Figure 3a, we plotted the data from experiments WQ1 with rac-MA, as normalized concentrations ( $100C/C_0$ ) versus time. In general, the residues of both enantiomers of malathion in Qinghe River water decreased with time elapsed. The EF values during the initial 2 days of incubations remained close to 0.5 and then increased from 0.53 to 0.56 at days 5 and 7. In the other four aqueous systems, the trends were analogous to experiment WQ1.

In these experiments, the ES values ranged from 0.0028 to 0.021. These ES values suggested that the enantioselective degradation of malathion in the five water samples was consistent with that in soils. Also, we found a linear correlation between ES values and pH ( $r^2 = 0.9645$ ,  $p = 0.488$ , Figure 3b).

**Chiral Stability of Malathion in Water.** The chiral stability of malathion in water was studied by incubating the pure R-MA or S-MA in three well-chosen water samples (WD2, WS2, WY2, WD3, WS3, and WY3, respectively).

The data for experiments WD2, WS2, and WY2 (spiked with pure R-MA) showed a continuous decrease of the concentration of R-(+)-MA (see in Figure 3c), in the meantime, the concentrations of the S-isomer increased from initial values of 0 to a maximum concentration of 0.30, 0.31, or 0.51 mg/L, respectively, and then decreased. In these experiments, the curves for the R- and S-enantiomers intersected after 3–24 h (EF = 0.5), which means that enantiomerization from R- to S-MA made the two isomers have the same concentration. In experiments WD3, WS3, and WY3 (spiked with pure S-MA), similarly, we observed the conversion of the S-(−)-MA into its antipode (see in Figure 3d).

The degradations of R-(+)- and S-(−)-isomers and racemate of malathion in selected soils and natural water samples were studied. We concluded that a preferential degradation of the S-enantiomer occurred in five soils and water samples; moreover, we found the inversion between R-(+)-MA and S-(−)-MA in two soils and three water samples. In our study, some physicochemical characteristics of soil and water samples, such as pH, organic carbon, and soil texture, had the major impact on the enantioselective behavior of malathion in the environment.

As is well-known, besides stereoselective degradations and transformations in the environment, malathion enantiomers can also differ in their toxic effects on target and nontarget species. By combining the environmental fate in our research and the acute toxicity in prevent studies,<sup>17</sup> we could have some implications for better environmental and ecological risk assessment for chiral organophosphates. In short, the study showed production of a single-isomer malathion has no effect on the reduction of environmental burden.

In the future, we will investigate the acute toxicity and dissipation behaviors of the main metabolites of malathion, which may provide some implications for better environmental and ecological risk assessment.

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