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Degradation of Flumorph in Soils, Aqueous Buffer Solutions, and Natural Waters

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Flumorph is an oomycete fungicide that is now used extensively in China. A residue analysis method for the determination of flumorph in environmental samples was developed with solid-phase extraction (SPE) for sample preparation and high-performance liquid chromatographic (HPLC) for separation. An environmental fate study was performed concerning the degradation of flumorph in soils, aqueous buffer solutions, and natural waters under laboratory-controlled conditions. The degradation of flumorph in three Chinese soil samples followed a first-order kinetics, with half-lives all longer than 100 days. No degradation of flumorph occurred in aqueous buffer solutions having different pH values or in natural waters with different physical and chemical properties. The data generated from this study could be helpful for risk assessment studies of the pesticide in the environment.

KEYWORDS: Flumorph; soil; aquatic; natural waters; degradation

INTRODUCTION

The use of pesticides in agriculture has increased substantially during the past few decades, contributing to a general crop yield increase. However, in recent years, pesticide use has become a controversial issue because of its potential for posing major environmental contamination problems, causing unwanted biotoxicity and contributing to human health hazards (*1*). Such concerns have led to increased interest in the dissipation pathways of pesticides and their fate in soil and water environments. The oomycete fungicide flumorph is a recently introduced fungicide. It was developed by Shenyang Research Institute of Chemical Industry China and has been granted patents in China (ZL.96115551.5), the United States (US6020332), and Europe (0 860 438B1). Its commercial production started in 1999 (*2*).

Flumorph [CAS Registry No. 211867-47-9, (*E*,*Z*)-3-(3,4-dimethoxyphenyl)-3-(4-fluorophenyl)-1-morpholinopropenone] has two isomers [(50% (*E*)-isomer, 50% (*Z*)-isomer)], which both have good fungicide activities against *Peronospora* and *Phytophthora* at a dose of 100−200 g of ai ha^{-1} (*3*). **Figure 1** illustrates the chemical structure of flumorph.

Although flumorph is extensively used in China, studies on the environmental behavior of the pesticide are scarce. Up to now only Luo et al. (*4*) reported a degradation study of flumorph in soils, but no data were available about sandy brown soil (collected from Beijing) and aqueous solution. In this project, residue analysis was carried out by solid-phase extraction (SPE) using high-performance liquid chromatography with UV detection system. Using this method, the half-lives of flumorph in three agricultural soils and different kinds of water were determined. The influence of the types of soil, soil microbe, water pH, and water temperature on the degradation of flumorph under controlled conditions was also investigated. Degradation studies in soil and water are essential for the evaluation of the persistence of pesticide. Data on the rate of degradation are extremely important as they permit prediction of the levels likely to remain in soil and water and allow assessment of the potential risk associated with exposure. The results will provide comprehensive information on their environmental fate in soil and water.

MATERIALS AND METHODS

Chemicals. Flumorph standard (purity = 99.5%; composition of the mixture about *Z*/*E* = 50:50) was obtained from Shenyang Research Institute of Chemical Industry China. HPLC grade methanol was procured from Dikma Limited (China). Other solvents and chemicals used were of analytical grade from Dikma Limited (China). SPE columns were Dikma Limited Sample Preparation Products (C-18, 500 mg, 3 mL).

Soils. The three types of soil with different physicochemical characteristics (**Table 1**) from fields under plant cultivation in the provinces of Heilongjiang (sandy black soil), Jiangxi (silty red soil),

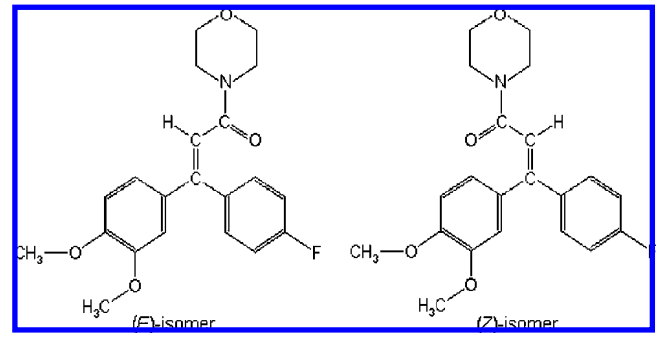

Figure 1. Chemical structures of flumorph isomers.

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Table 1. Textural and Chemical Properties of Soils Used in the Study

soil parameter	soil type: Beijing (sandy brown soil)	Heilongjiang (sandy black soil)	Jiangxi (silty red soil)
clay, <0.002 mm (%)	2.29	3.34	22.4
silt, 0.002−0.02 mm (%)	33.6	39.7	48.5
sand, 0.02−2.0 mm (%)	64.1	57.0	29.1
CEC[a] [*c* mol(+)/kg]	29.7	29.9	66.5
pH H_2O	6.73	8.72	5.06
OM[b] (%)	2.70	10.4	1.80

[a] Cation exchange capacity. [b] Organic matter content (%).

Table 2. Physical and Chemical Properties of the Natural Water Samples

sampling site	TOC[a] (mg L^{-1})	TSS[b] (mg L^{-1})	pH	EC[c] (mS cm^{-1})
Jingmi River	14.5	20.3	7.10	1.34
Guishui Reservoir	20.1	19.4	8.00	2.12
Ming Tombs Reservoir	10.0	11.0	6.90	2.01

[a] Total organic carbon. [b] Total suspended solids. [c] Electrical conductivity.

Table 3. Buffer Solutions Prepared for the Aqueous Hydrolysis of Flumorph

buffer solution	pH	preparation for 100 mL of buffer solution
A	2.0 ± 0.1	25 mL of 0.2 M KCl + 6.5 mL of 0.2 M HCl
B	4.5 ± 0.1	100 mL of 0.07 M KH_2PO_4
C	7.4 ± 0.1	2.0 mL of 0.5 M KH_2PO_4 + 6.0 mL of 0.5 M Na_2HPO_4
D	9.2 ± 0.1	5.0 mL of 1 M HCl + 5.5 mL of 2 M NH_3
E	12.3 ± 0.1	2.5 mL of 0.2 M KCl + 6.5 mL of 0.2 M NaOH

Table 4. Recoveries of Flumorph Residues in Soils, Buffer Solutions, and Nature Waters

sample	fortifn level (mg kg^{-1})	av recovery (%)	CV (%)
soils	0.02	99.9	6.3
	0.1	99.2	6.0
	0.5	99.5	1.8
buffer solutions	0.02	100.4	5.2
	0.1	98.9	2.9
	0.5	99.5	2.7
natural water	0.02	99.9	2.2
	0.1	99.9	2.0
	0.5	98.8	3.0

and Beijing (sandy brown soil), respectively, were collected from the surface (0−15 cm) horizons. The soil samples were collected from field sites that had no history of flumorph application. These collected samples were mixed, air-dried, and sieved through a 2 mm mesh. The prepared soil samples were then incubated for 14 days prior to the addition of flumorph. Water was added to the soil samples until approximately 60% of the field capacity was achieved.

Natural Waters. Water samples were collected from the suburbs of Beijing, China: Jingmi River, Guishui Reservoir, and Ming Tombs Reservoir. Natural waters were sampled at each site by dipping a clean stainless steel can into the top 1 m of water until the can was full. After the water had been transported back to the laboratory, all water samples were stored for 1 week at 4 °C before the experiment was begun. Samples were subsampled prior to the beginning of the experiment for measurements of dissolved total organic carbon (TOC), total suspended solids (TSS), pH, and electrical conductivity (EC). Data are given in **Table 2**.

Buffer Solutions. Five buffer solutions were used to study the aqueous hydrolysis of flumorph. **Table 3** shows the procedures followed for their preparation according to ref *4*. To avoid microbial degradation, buffer solutions were sterilized by filtration and all glass apparatuses by autoclaving for 20 min at 121 °C. Aseptic techniques were adopted throughout the study to maintain sterility.

Table 5. Recoveries of Flumorph Residues in Aged Soil

time elapsed after application (days)	av recovery[a] (%): fortifn 1[b]	CV (%)	fortifn 2[c]	CV (%)	fortifn 3[d]	CV (%)
0.5	100.2	2.32	96.3	5.80	99.8	2.33
1	99.8	4.67	99.5	4.13	98.9	1.90
3	100.1	5.02	98.6	3.09	97.9	1.89
5	99.4	3.73	99.0	3.06	100.2	2.40
7	97.6	4.50	97.3	2.98	99.3	1.99

[a] $n = 3$. [b] Fortification level at 0.02 mg kg^{-1}. [c] Fortification level at 0.1 mg kg^{-1}. [d] Fortification level at 0.5 mg kg^{-1}.

Instrumentation and Experimental Conditions. An HPLC (Agilent 1100) equipped with an analytical column (250 mm × 4.6 mm i.d., 5 μm ODS) was attached to a UV detector. The chromatographic conditions used for the analysis of flumorph residues were as follows: the mobile phase was methanol/water (70:30 v/v) with a total flow of 0.8 mL min^{-1}. The injection volume was 20 μL; detection was performed at 242 nm (*5*). Under these conditions, the retention time of flumorph was about 6.7 min for the *E*-isomer and 7.5 min for the *Z*-isomer. All measurements were carried out at room temperature. For HPLC-MS analysis, an Agilent 6130 single-quad MSD system was employed. Acquisition parameters were as follows: column, Agilent Zorbax SB-Aq, 2.1 mm × 100 mm, 1.8 μm; flow rate, 0.2 mL min^{-1}; temperature, 25 °C; mobile phase (A) 0.1% formic acid, (B) methanol, A/B = 50:50; gas flow, 8 mL min^{-1}; gas temperature, 350 °C; capillary voltage, 3 kV; fragmentor, 70.

Preparation of the Pesticide Standard Solutions. Standard solutions (1000 mg L^{-1}) of flumorph were prepared in a mixture of methanol and water (7:3 v/v).The solutions required for preparing a standard curve (0.25, 0.5, 1.0, 2.5, 5, 15, and 20 μg mL^{-1}) were prepared from the stock solution by serial dilutions. All solutions were protected against light with aluminum foil and were stored in a refrigerator at 4 °C.

Sample Preparation. Soil samples (20 g, passed through a 2 mm sieve) were extracted twice (2 × 50 mL) by ultrasonic extraction for 30 min with a mixture of methanol/water (50:50, v/v). The combined extracts were transferred to a separatory funnel (500 mL) with 50 mL of 10% NaCl aqueous solutions and then 60 mL of dichloromethane as the rinse. After the mixture had been shaken for 2 min, the dichloromethane layer was collected, and the aqueous layer was extracted two more times, using 20 mL of dichloromethane each time. The combined dichloromethane extract was dried over anhydrous sodium sulfate (2 cm bed), followed by the removal of the solvent in a vacuum rotary evaporator at 40 °C. The residue of the extracts was redissolved with 3 mL of methanol/water (50:50, v/v) and prepared for C-18 cartridges. The C-18 cartridges were conditioned with methanol (5 mL), followed by distilled water (5 mL). The methanol/water extract above (2 mL) was loaded onto the cartridge and then washed with methanol/water (2 mL, 40:60, v/v). Analytes were eluted with methanol (2 mL), and the eluate was dried under a gentle stream of nitrogen. The residue was reconstituted in the mobile phase for HPLC analysis. For water sample analysis, C-18 cartridges were conditioned with methanol (5 mL), followed by distilled water (5 mL). Immediately after, a 10 mL water sample was passed through the cartridge at a flow rate of approximately 1 mL min^{-1}. After loading, the analytes were eluted with methanol (2 mL). The eluate was dried under a gentle stream of nitrogen. The residue was reconstituted in the mobile phase for HPLC analysis.

Recovery Studies. Validation of the method was performed in terms of fortification and recovery studies. Recovery experiments were carried out, in five replicates, at three fortification levels (0.02, 0.1, and 0.5 mg kg^{-1}) by adding known volumes of the pesticide standards in methanol to three kinds of soil samples, buffer solutions, and natural water samples. Blank analyses were performed to check interference from the matrices.

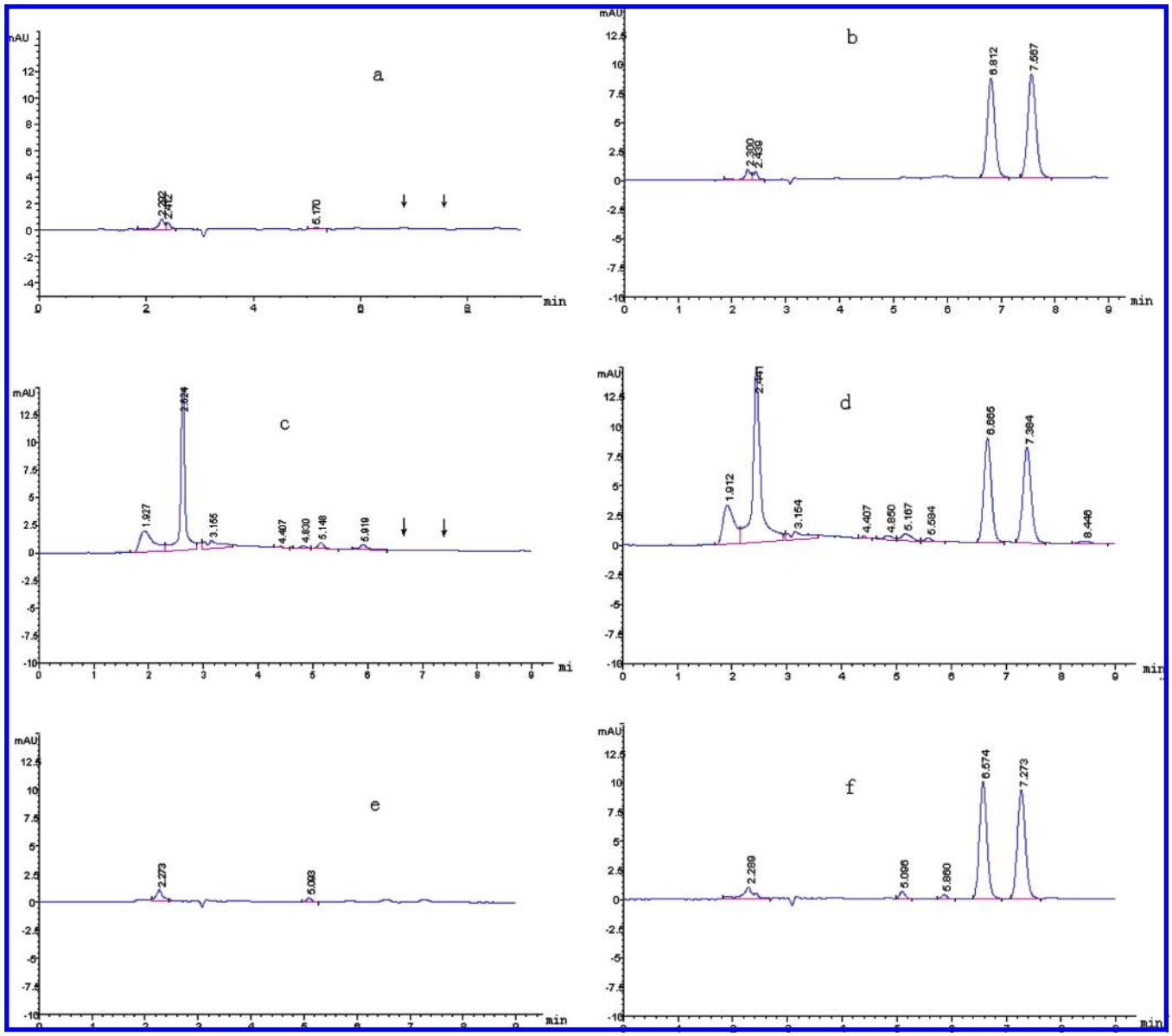

Figure 2. HPLC chromatograms of flumorph: **a**, soil blank; **b**, soil spiked at 0.5 mg kg^{-1}; **c**, buffer solution blank; **d**, buffer solution spiked at 0.5 mg kg^{-1}; **e**, natural water blank; **f**, natural water spiked at 0.5 mg kg^{-1}.

Another experiment was conducted to determine the potential of the extraction scheme for recovery of flumorph residues from aged soil. Homogenized soil samples (20 g) moistened to 60% water-holding capacity were sterilized by autoclaving at 121 °C for 1 h for 2 consecutive days. The appropriate standard solution was aseptically added to the sterilized soils to obtain concentrations of 0.02, 0.1, and 0.5 mg kg^{-1}, and the prepared samples were incubated at room temperature (22 ± 3 °C) under abiotic conditions. The residues of flumorph in soil samples were extracted and analyzed 0.5, 1, 3, 5, and 7 days after application.

Degradation Experiment of Flumorph in Soil. Nineteen grams of soil (dry weight equivalent) was placed in 250 mL flasks, and the soil−water content was adjusted to about 60% of field-holding capacity of each soil (w/w) by drying or adding deionized water. Two sets of 42 soil flasks were prepared for each soil type. One set of soil samples was autoclaved twice at 121 °C for 60 min, with a 24 h interval between the first and second autoclaving, to remove microbial activity. The second set was not autoclaved. For treatment, 50 g of oven-dried soil was treated with 10 mL of acetone solution containing flumorph at 500 μg mL^{-1} in a small beaker. The soil samples were placed in the fume hood to allow evaporation of the acetone. After the soil had been thoroughly mixed using a glass rod, a 1.0 g aliquot was removed from the beaker and mixed into the previously prepared soil samples. The soil flasks were thoroughly mixed by rotating and shaking. The initial flumorph concentration in the soil was 5 μg g^{-1}. All flasks were covered with aluminum foil and placed in the incubator at 25 ± 2 °C. Water was aseptically added to soils to maintain the required 60% of field-holding capacity. On alternate days during the incubation, three replicate flasks were removed from each treatment on days 0, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 80, 100, and 150 after the treatment to measure the flumorph residues.

Incubation of Flumorph in Aqueous Buffers. Triplicate 500.0 mL samples, each containing 1.0 mg L^{-1} of flumorph, were obtained by adding the appropriate volume of the stock solution into the buffer solution. The treated buffer solutions were stored in the dark at ambient temperature (25 ± 2 °C) in Erlenmeyer flasks. Another set of triplicate 500.0 mL distilled water samples at pH 2.0−12.3, containing 1.0 mg L^{-1} of the pesticide, was stored in the dark at 50 ± 2 °C to test the effects of temperature on hydrolysis. In all trials, the pH of each sample was periodically measured and did not vary by >0.1 unit. Initially, one 10 mL sample was taken from each bottle to determine the starting concentration. Samples (10 mL) were collected from each bottle on days 5, 10, 20, 30, 40, 60, and 90. Experimental bottles were left static in the laboratory and shaken every 8−12 h. With each set of samples collected a 10 mL deionized water blank was also extracted.

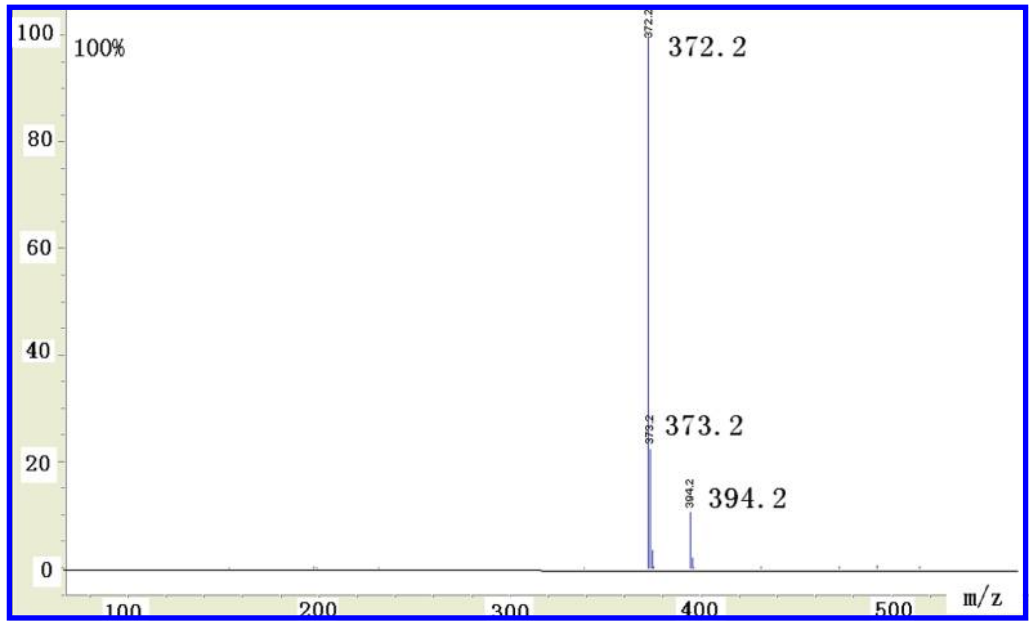

Figure 3. LC-MS spectrum of (*E* or *Z*)-flumorph.

Persistence Studies of Flumorph in Natural Water. Triplicate 500.0 mL samples from each location along with three control samples containing organic-carbon-free deionized water were kept in clean amber glass bottles. After each bottle was filled, the water was quickly spiked with flumorph standard solution. The starting concentration was approximately 1.0 mg L^{-1}. Samples (10 mL) were collected from each bottle on days 5, 10, 20, 30, 40, 60, and 90. Experimental bottles were left static in the laboratory and shaken every 8−12 h. With each set of samples collected a 10 mL deionized water blank was also extracted.

RESULTS AND DISCUSSION

Analytical Method. A standard calibration curve of each isomer of flumorph was constructed by plotting analyte concentration against peak area, separately. At 242 nm, for each isomer, the calibration range was linear from 0.25 to 20 μg mL^{-1}. The standard curve equation was $y = 35.92x + 0.3626$ ($R^2 = 0.9998$) for the (*E*)-isomer and $y = 37.679x + 1.8595$ ($R^2 = 0.9999$) for the (*Z*)-isomer.

The mean recoveries of the pesticide ($n = 5$) at spiking levels (0.02, 0.1, and 0.5 mg kg^{-1}) in soil, buffer solution, and natural water samples are given in **Table 4**. **Table 5** also indicates that the sample prepared according to our procedure could recover flumorph residues from aged soils.

Satisfactory results were found in the three instances, with recoveries between 98.8 and 100.4%. Confirmation tests by HPLC-MS were used to determine whether peaks detected at the retention times of the analyte were in fact flumorph. Each isomer of flumorph was identified by its retention time and the specific molecule ion peak $[M + H]^+$ at *m*/*z* 372.2 in HPLC-MS according to the proposed conditions. **Figures 2** and **3** illustrate the LC and LC-MS chromatograms of flumorph.

The coefficient of variation of the methods (CV %) for repeatability ranged from 1.8 to 6.3%. The limit of quantification (LOQ) for this method was defined as the lowest concentration of compounds in a sample that could be quantitatively determined with suitable precision and accuracy. The LOQ was determined as the sample concentration of the pesticide at peak heights of 10 times the baseline noise. The LOQ of flumorph was found to be 0.02 mg kg^{-1}, and that for each isomer was 0.01 mg kg^{-1}.

Degradation of Flumorph in Soils. The arable soils used in this study covered a wide range of common texture and carbon content (**Table 1**). The degradation of flumorph in the three soil samples followed a first-order kinetics according to linear regression analysis. $C_t = C_0\ e^{-kt}$, where C_t is the concentration of pesticide at time t, C_0 represents the initial concentration, and k is the rate constant $dt = -k\ dc/c$. **Figure 4** illustrates the plot of ln concentration versus time $\ln C_t = -kt + \ln C_0$, and the fit of data to the first-order decay model was good for all treatments, with R^2 ranging from 0.84834 to 0.9315. In nonsterile soils, the disappearance of flumorph was generally slow, especially in sandy brown soil, and the scope k for the degradation of flumorph in sandy black soil, silty red soil, and sandy brown soil was 0.0065, 0.0048, and 0.0045 day^{-1}; the half-lives ($t_{1/2} = \ln 2/k$) calculated for the degradation of flumorph were 106.7, 144.4, and 154.0 days, respectively. The fate of pesticides in soils is influenced significantly by the texture of the soil and also by the presence of organic matter (*7*). Accordingly, the biodegradation of flumorph was studied in three different types of soil that have contrasting properties in terms of their texture, pH, organic content, etc., when inoculated. The degradation of flumorph was faster in sandy black soil > silty red soil > sandy brown soil (**Figure 4**). It degraded most quickly in the sandy black soil in which microbes were more active, because sandy black soil has much more organic matter and is alkaline. The results obtained corresponded with many studies that demonstrated a positive influence of pH on total microbial biomass and activity, and consequently degradation of many neutral compounds has been shown to be faster at high pH (*8*). The half-lives observed for flumorph degradation in three types of soil were all longer than 100 days, which indicated longer persistent nature compared with some commonly used pesticides under aerobic conditions (*9*). Sterilization generally resulted in a decrease in degradation rate, or an increase in persistence, and flumorph in the sterile selected soils exhibited half-lives all longer than 350 days (**Figure 4**). Comparison of degradation of flumorph at a fortification of 5 μg g^{-1} under aerobic conditions in sterile and nonsterile soils revealed that flumorph persisted longer in sterile soil than in nonsterile soil. About 15.6−18.4% disappearance of flumorph from sterile soil occurred as against over 40.2% from nonsterile

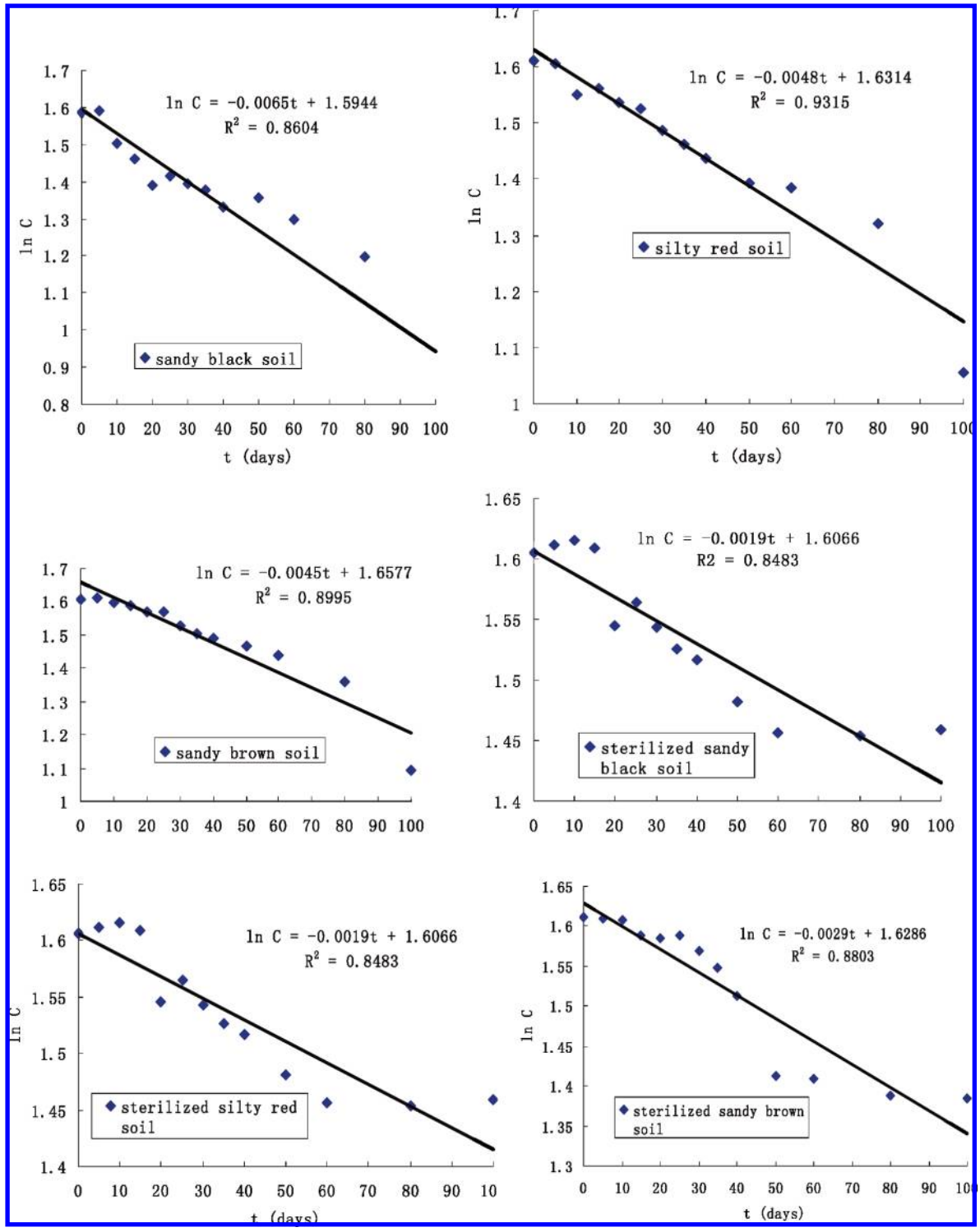

Figure 4. Degradation of flumorph in the three soils: nonsterilized soils and sterilized soils.

soil within 150 days after its application. The inhibition by sterilization suggests that microbial transformations partly contributed to the overall degradation of flumorph in the soils.

Degradation of Flumorph in Buffer Solutions. The degradation of flumorph in aqueous solution in the absence of light at ambient temperatures (25 ± 2 or 50 ± 2 °C) was monitored at different pH values. Data observed at different pH and temperature gradients are presented in **Table 6**. No degradation occurred with respect to pH and temperature. The results indicate that flumorph has substantial chemical stability in the buffer solutions.

Persistence of Flumorph in Natural Water. Flumorph concentrations measured after different time intervals of incubation in different water samples are shown in **Table 7**. The flumorph showed no detectable decrease in concentration in any of the waters over the 90 day duration of the experiment. It is noteworthy that the presence of dissolved organic carbon and suspended solids (**Table 2**) had no catalytic effect on the transformation of the flumorph. These data suggest that abiotic and biotic hydrolyses are both insignificant for flumorph at the pH and dissolved organic matter concentrations typical of most natural waters.

In conclusion, a rapid and simple HPLC method was developed and validated for the simultaneous determination of

Table 6. Dissipation of Flumorph at 25 ± 2 and 50 ± 2 °C in Aqueous Buffer Solutions[a]

	residue (μg mL^{-1})									
	25 ± 2 °C					50 ± 2 °C				
days	pH 2.0	pH 4.5	pH 7.4	pH 9.2	pH 12.3	pH 2.0	pH 4.5	pH 7.4	pH 9.2	pH 12.3
0	0.989 ± 0.021	1.012 ± 0.018	0.989 ± 0.013	0.978 ± 0.011	1.001 ± 0.031	0.976 ± 0.022	1.002 ± 0.019	0.991 ± 0.020	1.001 ± 0.011	0.999 ± 0.0008
5	1.010 ± 0.023	1.001 ± 0.022	0.987 ± 0.025	0.988 ± 0.009	1.000 ± 0.019	0.987 ± 0.014	0.995 ± 0.023	0.992 ± 0.021	1.000 ± 0.012	0.998 ± 0.0012
10	0.991 ± 0.032	0.977 ± 0.017	0.989 ± 0.030	0.999 ± 0015	1.005 ± 0.0008	0.988 ± 0.024	0.987 ± 0.015	0.998 ± 0.027	1.005 ± 0.0009	0.996 ± 0.0029
20	0.975 ± 0.030	1.006 ± 0.045	0.990 ± 0.022	0.999 ± 0.024	0.992 ± 0.030	0.998 ± 0.041	0.987 ± 0.020	0.998 ± 0.019	0.998 ± 0.0008	0.995 ± 0.0027
30	0.989 ± 0.021	0.979 ± 0.018	1.010 ± 0.043	0.995 ± 0.025	1.004 ± 0.022	0.986 ± 0.011	0.988 ± 0.018	0.995 ± 0.012	0.993 ± 0.0011	1.001 ± 0.0006
40	1.034 ± 0.045	0.973 ± 0.035	0.985 ± 0.017	0.993 ± 0.017	1.003 ± 0.034	0.965 ± 0.030	0.987 ± 0.030	0.994 ± 0.034	1.004 ± 0.0013	0.997 ± 0.0016
60	0.991 ± 0.034	1.012 ± 0.033	0.989 ± 0.022	1.009 ± 0.032	1.000 ± 0.025	0.967 ± 0.023	1.000 ± 0.023	0.993 ± 0.023	0.979 ± 0.0014	0.996 ± 0.0011
90	0.969 ± 0.034	1.003 ± 0.023	0.989 ± 0.030	0.993 ± 0.014	1.004 ± 0.034	0.999 ± 0.021	0.990 ± 0.021	0.996 ± 0.021	1.000 ± 0.0021	1.006 ± 0.0015

[a] Flumorph concentrations are denoted as the means ± standard deviation (for $n = 3$).

Table 7. Persistence of Flumorph in Natural Water at Room Temperature[a]

	residue (μg mL^{-1})		
days	Jingmi River	Guishui Reservoir	Ming Tombs Reservoir
0	0.998 ± 0.023	1.002 ± 0.039	1.000 ± 0.054
5	0.997± 0.047	1.003 ± 0.019	0.997 ± 0.034
10	0.996 ± 0.018	1.005 ± 0.020	0.996 ± 0.036
20	1.011 ± 0.012	0.998 ± 0.020	0.998 ± 0.021
30	0.995 ± 0.021	0.998 ± 0.045	1.001 ± 0.022
40	1.005 ± 0.039	0.997 ± 0.043	1.004 ± 0.011
60	0.990± 0.023	1.003 ± 0.051	0.997 ± 0.044
90	0.987 ± 0.024	0.999 ± 0.021	1.002 ± 0.056

[a] Flumorph concentrations are denoted as the means ± standard deviation (for $n = 3$).

two isomers of flumorph residues in soils, buffer solutions, and natural waters. The method developed shows satisfactory validation parameters in terms of linearity, lower limits, accuracy, and precision. The average recoveries in the studied samples for the pesticide ranged between 98.8 and 100.4%. The uncertainty associated with the analytical method, expressed as CV, was lower than 6.3% for the pesticide tested in all matrices. The LOQ of flumorph was found to be 0.02 mg kg^{-1}, and for each isomer it was 0.01 mg kg^{-1}. The degradation of flumorph in three Chinese soil samples followed a first-order kinetics, with half-lives all longer than 100 days. Sterilization treatment of flumorph in the selected soils resulted in half-lives of all longer than 350 days. The inhibition by sterilization suggests that microbial transformations partly contributed to the overall degradation of flumorph in the soils. Flumorph is quite stable in aqueous buffer solutions and natural waters, with no degradation occurring under various conditions. The results indicate that the environmental stability and persistence of flumorph deserve further scrutiny.

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