

Influence of Soil Factors on the Dissipation of a New Pyrimidynyloxybenzoic Herbicide ZJ0273

Wei Wang,[†] Qing-Fu Ye,^{*,†} Wei Ding,[†] Ai-Liang Han,[†] Hai-Yan Wang,[†] Long Lu,^{*,‡} and Jay Gan[§]

[†]Institute of Nuclear Agricultural Sciences, Zhejiang University, Hangzhou 310029, China, [‡]Shanghai Institute of Organic Chemistry, Chinese Academy of Science, Shanghai 200032, China, and [§]Department of Environmental Sciences, University of California, Riverside, California 92521

A novel pyrimidynyloxybenzoic herbicide (ZJ0273) uniformly labeled with ^{14}C on the benzoate ring was applied to soils under sterile and nonsterile conditions to understand the effect of soil microorganisms and selected properties on its dissipation and transformations to bound resides and $^{14}CO_2$. A significant effect of soil microorganisms was found in an acidic soil, where sterilization significantly prolonged the half-dissipation time (DT_{50}) of ZJ0273 from 15.57 to 34.31 days and decreased the total amount of mineralized $^{14}CO_2$ from 19.91 to 0.43%. However, sterilization showed limited effect on the patterns of bound residue or extractable residue levels in soils having pH \geq 6.1. In addition, a significant suppression of high pH was found on the dissipation of extractable residues and formation of bound residues. The enhancement of bound residue formation by low soil pH was attributed to increased conversion of ZJ0273 to its intermediates, which were rapidly bound to soil organic matter.

KEYWORDS: ZJ0273; herbicide; bound residue; extractable residue; mineralization

INTRODUCTION

ZJ0273, propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino)benzoate, is a novel pyrimidynyloxybenzoic herbicidal ingredient developed from the family of 2-pyrimidyloxy-*N*-arylbenzylamine derivatives (*I*). In recent years, ZJ0273 has been rapidly adopted for use in China, with the total application area reaching 533,000 ha in 2007 (*2*). Application of ZJ0273 at the rate of 45–60 g (active ingredient) per hectare showed effective preand postemergence weed control in oil rape fields (*3*). The activity of ZJ0273 involves the inhibition of acetolactate synthase (ALS) and consequently biosynthesis of three essential branch amino acids, i.e., valine, isoleucine, and leucine. Studies with ¹⁴C labeling showed negligible uptake of ZJ0273 into the rapeseed, largely due to the poor root absorption and weak translocation of this compound (*4*).

Extensive use and occurrence of negative environmental effects mandate the need to understand the fate and transport of new pesticide products (5, 6). Studies with ¹⁴C-labeled pesticides have shown that the parent compound and/or its metabolites may be converted to extractable residue (ER) or bound residue (BR) or mineralized to CO_2 after application in soil. Extractable residue is defined as the fraction that is recoverable by solvents and is considered to have bioavailability potential and susceptibility to

pubs.acs.org/JAFC

degradation (7, 8). Bound residue, defined as the fraction that is not recovered by exhaustive solvent extraction, is generally accepted as a pathway for decreased bioavailability and hazardous potential. However, studies show that a portion of BR may become bioavailable at a later time and impose effects on plants and animals. For certain herbicides, such delayed release of residues is especially problematic, as it may lead to significant injuries to sensitive plants (9–12). On the other hand, mineralization to CO₂ is an environmentally desirable pathway, as it completely removes the inherent toxicity.

In the present study, five representative soils were incubated after treatment of ZJ0273 labeled with ¹⁴C on the benzoate ring (A ring) under sterile and nonsterile conditions to understand the role of soil microbial activity in influencing the dissipation of this herbicide. The formation of BR and mineralization to ¹⁴CO₂ specific to the benzoate ring label were also studied. The role of selected soil properties in these processes was further evaluated.

MATERIALS AND METHODS

Chemicals. [A *ring*-U-¹⁴C]-ZJ0273 (**Figure 1**), propyl 4-[2-(4,6-dimethoxy-2- pyrimidinyloxy)benzyblamino]-[phenyl-U-¹⁴C] benzoate, or ¹⁴C-ZJ0273, was synthesized by the Institute of Nuclear Agricultural Sciences, Zhejiang University (Hangzhou, China), from 4-amino[phenyl-U-¹⁴C] benzonic acid through esterification, condensation, reduction, and substitution (*13*). Analyses including high performance liquid chromatography–liquid scintillation counting (HPLC-LSC) and thin layer chromatography–isotope imaging analysis (TLC-IIA) showed that both radiochemical purity and chemical purity were >98% with a specific activity of 40.29 \pm 0.41 MBq/mmol. 2,5-Diphenyloxazole (PPO, scintillation grade) and 1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP, scintillation grade)

^{*}To whom correspondence should be addressed. Qing-Fu Ye, Institute of Nuclear Agricultural Sciences, Zhejiang University, Kaixuan Road No. 268, Hangzhou 310029, Zhejiang Province, China. E-mail: qfye@zju.edu.cn. Long Lu, Shanghai Institute of Organic Chemistry, Chinese Academy of Science, No. 354, Fenglin Road, Shanghai 200032, China. E-mail: lulong@mail.sioc.ac.cn.



Figure 1. Chemical structure of [A ring-U- 14 C]-ZJ0273, with asterisks marking the position of the 14 C label.

were obtained from Acros Organics (Geel, Belgium). Other chemicals and solvents used in this study were all of analytical grade and used as received.

Soils. Five representative soils for oilseed rape growing areas were collected from the surface layer (0-15 cm) of agricultural fields and used in the present study: a red clayey soil (S_1) from Quzhou, Zhejiang; a sandy paddy soil (S_2) from Hanzhong, Shanxi; a fluvio-marine yellow loamy soil (S_3) from Hangzhou, Zhejiang; a hydromophic paddy soil (S_4) from Jingzhou, Hubei; and a coastal saline soil (S_5) from Cixi, Zhejiang. Based on the pH, we defined S_1 (pH = 4.2) as acidic soil, S_2 – S_4 (pH is from 6.12 to 6.70) as neutral soils, and S_5 (pH = 8.25) as alkaline soil. All soils were not previously exposed to ZJ0273. Soil samples were air-dried, mixed, and sieved through a 1-mm sieve before use. Selected physicochemical properties of the five soils were determined using standard methods and are given in **Table 1** (*14*, *15*).

Radioassay. Radioactivity (in dpm) was measured on a Wallac 1414 liquid scintillation counter (LSC; Wallac-1414, Wallac Co., Turku, Finland) or an ultralow liquid scintillation spectrometer (ULLSS; Wallac 1220 Quantulus, PerkinElmer, Turku, Finland) for extremely low radioactivity samples. Scintillation cocktail I was prepared by dissolving PPO (7 g) and POPOP (0.5 g) in a mixture of xylene (650 mL) and 2-methoxyethanol (350 mL). Scintillation cocktail II was prepared by dissolving PPO (7 g) and POPOP (0.5 g) in a mixture of xylene (600 mL), 2-methoxyethanol (225 mL), and ethanolamine (175 mL) and was used for trapping $^{14}CO_2$ during sample combustion. Chemoluminescence was eliminated before radioactive measurement by placing the test scintillation vials in the dark for 24 h.

Pesticide Treatment and Incubation. To determine the role of microbial activity in affecting the dissipation of ZJ0273, two sets of samples were prepared for each soil type. One set of samples was subjected to sterilization treatment to remove microbial activity while the other set of samples was preincubated at 25 ± 1 °C for 7 days to allow the microorganisms to acclimatize. Sterilization was carried out by exposing the soil samples to ⁶⁰Co γ -irradiation at an absorbed dose of 30 kGy (dose rate 1.25 kGy/h).

Subsequently, each soil (400 g, air-dry weight) was transferred into a 500-mL flask with a rubber stopper. Then 5 mL of prepared methanol solution of ¹⁴C-ZJ0273 (1.905 × 10⁴ Bq) was diluted with 15 mL of distilled water and added into the soil sample, followed by thorough mixing. The homogenized soil samples were left in a fume hood to remove methanol. The soil moisture content was then adjusted to about 60% of the water-holding capacity by adding sterilized distilled water. The treated soil samples were incubated at 25 ± 1 °C. During the incubation, the soil–water content was maintained constant by weighing the flasks and adding sterilized distilled water on a daily basis. ZJ0273 treatment and incubation for sterilized soils followed a similar procedure but were performed in a sterile environment.

Measurement of Mineralization Rate. To monitor the mineralization rate, a 20-mL glass vial filled with 10 mL of 0.5 M NaOH solution was suspended under the rubber stopper of a sample flask to trap the released ¹⁴CO₂ during incubation. The NaOH solution was changed every five days, and the NaOH solution samples were stored in refrigerator (4 °C) for further analysis. At different time intervals (5, 10, 20, 30, 45, and 60 days after ZJ0273 spiking), the NaOH solutions were combined and an aliquot of 1.0 mL was removed and mixed with 1.0 mL of distilled water and then combined with 15 mL of scintillation cocktail I. The radioactivity was measured by ULL- LSC to derive the amount of ¹⁴CO₂, from which the mineralization rate was derived.

Analysis of ¹⁴C-Extractable Residue. At different time intervals (5, 10, 20, 30, 45, and 60 days after ZJ0273 spiking), aliquots of 10 g of soil (dry weight equivalent) were removed from the sample flasks and

transferred into 100-mL polypropylene centrifuge tubes. The soils were consecutively extracted with methanol by using a previously developed method (*16*). Each soil sample was extracted by mixing with 50 mL of methanol on a horizontal shaker for 2 h, followed by centrifugation at 4000 rpm for 15 min, after which the supernatant was decanted and the remaining soil was repeatedly extracted with fresh solvents four additional times. Preliminary experiments showed that, after five consecutive extractions, no radioactivity could be detected in the extract following additional extractions. After the exhaustive solvent extraction, all supernatants were pooled and condensed to 50 mL using a vacuumed rotary evaporator (Eyela SB-1000, Eyela Co. Shanghai, China). One milliliter of the final extract was mixed with 15 mL of scintillation cocktail I to measure the associated ¹⁴C-ER radioactivity.

Analysis of ¹⁴C-Bound Residue. After the sequential extraction, the soil pellets were left in a fume hood to remove the residual methanol and were then homogenized. Aliquots of 1.0 g (triplicates) were combusted using a biological oxidizer (OX-600, R.J. Harvey Instrument, Hillsdale, NJ). The total time of oxidation was 4 min at a combustion temperature of 900 °C and a catalysis temperature of 680 °C. The evolved ¹⁴CO₂ was recovered in 15 mL of scintillation cocktail II and then measured for radioactivity by LSC as above. The recovery of this procedure was determined to above 90% by combusting ¹⁴C-ZJ0273 standards. This recovery was used to correct the above measurements to derive the fraction of BR formed in the soils.

Data Analysis. All measurements were in three replicates and the arithmetic means and standard errors of means (mean \pm SEM) were calculated from the repeated measurements. The log-transformed data for the dissipation of total ¹⁴C-ER over time was fitted to a first-order decay model to estimate the dissipation rate constant *k* (days⁻¹) and half-dissipation time (DT₅₀). Data were subjected to a linear regression analysis (In of mean % residual ZJ0273 versus time). Significance between treatments was tested using a one-way analysis of variance (ANOVA) using SigmaPlot 9.0 (Systat Software, Richmond, CA).

RESULTS AND DISCUSSION

Material Mass Balance. Material balance was calculated by adding the ¹⁴C radioactivity recovered in methanol extracts, ¹⁴CO₂ in NaOH traps, and bound residues after combustion. The material balance averaged across the different soils ranged from 98.2 ± 4.9 to $101.5 \pm 4.6\%$ for the nonsterile soils and from 97.9 ± 4.6 to $100.6 \pm 5.1\%$ for the sterilized soils. The good mass conservation suggested that the steps used for measuring the different residue forms after soil treatment of ¹⁴C-ZJ0273 were highly reproducible and quantitative.

Dissipation of Extractable Residue (ER). The dissipation of extractable residue over time in the five test soils is shown in **Figure 2.** The level of extractable radioactivity decreased significantly from 0 to 60 days after ¹⁴C-ZJ0273 application. After 5 days of incubation, depending on the soil type, the amount of ¹⁴C-ER ranged from 73.8 ± 4.6 to 92.4 ± 4.8% of the added ¹⁴C-activity in the nonsterile soils and from 80.3 ± 3.3 to 93.4 ± 2.6% in the sterilized soils. At the end of the incubation, the fractions of ER were only from 6.2 ± 0.5 to $50.0 \pm 3.2\%$ in the nonsterile soils and from 27.5 ± 1.9 to $52.1 \pm 3.9\%$ in the sterilized soils. Since the incubation was carried out using closed systems, the losses of ER over time may be attributed to the conversion of ¹⁴C residues to BR and/or mineralization to ¹⁴CO₂.

The dissipation of total ¹⁴C-ER over time was well fitted to the first-order decay model, with the coefficient r^2 ranging from 0.91 to 0.99 (**Table 2**). The estimated DT₅₀ values were 46.5, 52.3, 50.6, and 69.3 days, respectively, for the nonsterilized neutral and alkaline soils (S₂-S₄). The corresponding DT₅₀ values (51.3, 60.8, 53.7, and 73.7 days) obtained for their sterilized counterparts were no different (p > 0.24). Compared with the neutral and alkaline soils, the dissipation of ER in acidic soil (S₁) was much faster. The half-dissipation time was shortened to 15.6 days in the sterilized S₁ soil and 34.3 days in the nonsterile soil. The accelerated dissipation of ER in acidic soils was also observed in previous studies for other

Table 1.	Basic	Physical	and	Chemical	Properties	of Soil	ls Used i	n This	Study
----------	-------	----------	-----	----------	------------	---------	-----------	--------	-------

soil no.		рН (H ₂ O)	OM ^a (g kg ⁻¹)	WHC ^b (%)	CEC ^c (cmol kg ⁻¹)	particle distribution (%)		
	type					clay	silt	sand
S ₁	red clayed soil	4.20	7.8	35.2	6.62	39.0	41.1	19.9
S ₂	sandy paddy soil	6.12	26.2	52.1	12.4	44.8	47.5	7.7
S ₃	fluvio-marine yellow 10amy soil	6.50	15.2	38.4	6.40	8.0	71.2	20.8
S ₄	hydromorphic paddy soil	6.70	39.8	50.1	13.4	71.6	25.1	3.3
S ₅	coastal saline soil	8.25	24.8	39.6	16.1	24.3	71.1	4.6

^aOM = organic matter. ^bWHC = water holding capacity. ^cCEC = cation exchange capacity.



Figure 2. Dynamics of an ¹⁴C-extractable residue of $[A ring-U-{}^{14}C]$ -ZJ0273 in five soils: (**A**) nonsterile soil treatments; (**B**) sterilized soil treatments. Each point is the mean of the three replicates, and the bars represent standard errors.

pesticides. For instance, Ryan et al. observed that, at equivalent water contents, prosulfuron DT_{50} values were positively correlated with soil pH, varying from 6.5 days at pH 5.4 to 122.9 days at pH 7.9 (*17*). Ye et al. (*18*) investigated the degradation of ¹⁴C-metsulfuron-methyl and ¹⁴C-chlorsulfuron in seven soils and found that the total ER was positively related to soil pH.

Analysis of variance showed a significant effect of soil microorganisms on the dissipation rate of ER in S₁ ($p \le 0.001$). After 60 d of incubation, about 28% of the applied radioactivity was extractable in the sterilized S₁, but only 6% in the nonsterile soil. Given the good mass balance, the enhanced dissipation of ER in the nonsterile soil S₁ may be attributed to increased mineralization of ZJ0273 to CO₂, and/or microbially mediated conversion of the parent compound to intermediates that were readily bound to soil organic matter. However, the differences caused by sterilization were much smaller for the other four soils, and in fact statistical test showed no significant effect in the dissipation of ER for S₂-S₅ by sterilization (p > 0.05).

Table 2. Parameters from First-Order Regression Analysis of log-Transformed Extractable Residues of ¹⁴C-ZJ0273 in Different Soils

SOIL	<i>k</i> (day ')	DI ₅₀ " (day)	r	Р
	No	nsterilized Soil		
c	0.0424 0.0001	156 00	0.00	-0.01
S ₁	0.0434 ± 0.0001 0.0146 ± 0.0004	15.0 ± 0.0 46.5 ± 1.3	0.99	< 0.01
S ₃	0.0122 ± 0.0008	52.3 ± 3.6	0.96	< 0.01
S ₄	0.0137 ± 0.0004	50.6 ± 1.5	0.95	<0.01
S_5	0.0100 ± 0.0005	69.3 ± 3.5	0.92	<0.01
	S	Sterilized Soil		
S ₁	0.0182 ± 0.0012	34.3 ± 2.6	0.96	<0.01
S ₂	0.0121 ± 0.0013	51.3 ± 6.5	0.94	<0.01
S₃	0.0116 ± 0.0008	60.8 ± 4.0	0.95	<0.01
S_4	0.0132 ± 0.0006	53.7 ± 2.4	0.93	<0.01
S_5	0.0096 ± 0.0096	73.7 ± 2.7	0.92	<0.01

^a DT₅₀: half-dissipation time.

Formation of Bound Residue. As the fraction of ER decreased, a gradual formation of BR was observed in all soils (**Figure 3**). The fractions of BR were 5.23 ± 0.43 to $29.40 \pm 2.65\%$ of the added ¹⁴C-activity at 5 days in the nonsterile soils and 4.51 ± 0.11 to $25.00 \pm 1.35\%$ in the sterilized soils. At the end of incubation, the fraction of BR increased to 48.03 ± 1.34 to $77.57 \pm 3.15\%$ of the added ¹⁴C-activity in the nonsterile soils and 45.69 ± 0.74 to $70.04 \pm 3.15\%$ in the sterilized soils. Regardless of the sterilization treatment, the highest level of BR was always observed in the strongly acidic soil S₁, while the lowest formation was seen in the alkaline coastal saline soil S₅ (**Figure 4**). The level of BR at the various sampling times generally followed the order of acidic soil > neutral soil > alkaline soil.

In S₁, the fraction of BR was consistently smaller under sterilized conditions (p < 0.001). However, the levels of BR were of no significant difference for the sterilized and nonsterile S2-S4 soils (p > 0.05). The formation of BR for many pesticides is usually attributed to soil microbial activity and the content of soil organic matter (19). In addition, the decrease in residue extractability and the concomitant increase in BR accumulation may be caused also by physical sequestration and other abiotic binding mechanisms (20). While the parent molecules of most pesticides do not bind covalently with soil humic materials to any significant extent, covalent binding to humus is often possible after conversion of the parent compound to intermediates (21, 22). The lack of effect by sterilization in neutral and alkaline soils in the present suggested that abiotic processes were involved in the formation of BR for ZJ0273. Furthermore, it is likely that certain microbes in the acidic soil may be involved in the active transformation of ZJ0273 that led to the enhanced formation of soil BR.

The enhanced potential for BR formation in acidic soils may have several implications. Formation of BR is generally considered as a detoxification process due to the loss of biological



Figure 3. Dynamics of a ¹⁴C-bound residue of [A *ring*-U-¹⁴C]-ZJ0273 in five soils: (**A**) nonsterile soil treatments; (**B**) sterilized soil treatments. Each point is the mean of three replicates, and the bars represent standard errors.

activity (23). Therefore, the rapid formation of BR may cause a significant loss of herbicidal activity of ZJ0273, compromising its ability for weed control in acidic soils. This may lead to increased pesticide use in the form of higher application rates or application frequency. However, studies also show that microorganisms may reverse the binding of pesticides and release the parent or intermediates associated in BR. For instance, Khan and Behki (24) investigated the microbial release of bound atrazine residue and found that 30-35% of the initially bound ¹⁴Cactivity became extractable at the end of a 83 day incubation. Bartha et al. reported that humus-bound 3,4-dichloroaniline was liberated in its intact form and gave rise to crop contamination due to microbial activation (25). In our studies, significant dosedependent inhibition was observed for rice seedlings treated with 14 C-BR of ZJ0273 at a rate of 0.6, 1.2, and 1.8 nmol g⁻¹ within 14 days of treatment (26). On the other hand, if release of the biologically active parent or intermediates from BR occurs, there may be a long-lasting potential for phytotoxicity from the use of ZJ0273 in acidic soils as well as an enhanced risk for its off-site movement such as leaching or runoff.

Mineralization to ¹⁴CO₂. [A *ring*-U-¹⁴C] ZJ0273, as a tracer, indicates the cleavage of the phenyl ring of ZJ0273 and its mineralization to ¹⁴CO₂. The mineralization rate, expressed as the fraction of ¹⁴CO₂ evolved as a percentage of the initially spiked ¹⁴C activity, gradually increased with incubation time in all soils (**Figure 4**). However, in sterilized soils, the cumulative fraction of ¹⁴CO₂ after 60 days of incubation was very small, at < 1% in all soils. In contrast, in the nonsterile soils, the cumulative levels of ¹⁴CO₂ produced were significantly higher than those in the sterilized soils. The highest ¹⁴CO₂ production was found in the acidic soil S₁, with the total ¹⁴CO₂ fraction reaching nearly



Figure 4. Dynamics of the percentage of $[A ring-U-{}^{14}C]$ -ZJ0273 mineralized to CO₂ in five soils during aerobic incubation: (**A**) nonsterile soil treatments; (**B**) sterilized soil treatments. Each point is the mean of three replicates, and the bars represent standard errors.

20% of the applied radioactivity after 60 days of incubation. The production of $^{14}CO_2$ in the other four soils, however, was substantially smaller, at 3.92% for S₂, 1.92% for S₃, 0.24% for S₄, and 6.08% for S₅.

Mineralization of organic compounds in soil is known to be mediated by soil microorganisms. Many studies show that there is a good correlation between soil microbial biomass and the mineralization rate of an organic compound. For instance, Willems et al. (27) found that mineralization rates of 2,4-D and atrazine decreased with soil depth as a result of reduced microbial activity in deeper soil layers. Moreover, Winkeilmann and Klaine (28) observed 10- to 600-fold higher mineralization rates of four radiolabeled atrazine metabolites in nonsterile soil microcosms than in sterilized soil microcosms. The lack of mineralization in the sterilized soils as compared to the nonsterile soils (p <0.001) clearly suggested the involvement of soil microorganisms in the further degradation of extractable residues of ZJ0273 in soils. It may also be concluded that the microbial activity for mineralizing ZJ0273 was rather limited in the neutral and alkaline soils as compared to the acidic soil, which was consistent with the absence of a sterilization effect on ER dissipation in those soils. The enhanced mineralization rate in the acidic soil S₁ was also in agreement with the rapid dissipation of ER, suggesting that different microbial communities in this soil contributed to the enhanced transformations.

Influence of Soil Properties. Correlation analysis was conducted to characterize the effect of selected soil properties (pH, OM, CEC, and particles distribution) on the distribution of ER, BR, and fextractable and bound residues of ZJ0273, and they

Table 3. Correlation Coefficients between ¹⁴C-Extractable Residue, ¹⁴C-Bound Residue, and ¹⁴CO₂, and Soil pH^a

soil treatment	DAT ^b	pH-ER ^c	pH-BR ^d	pH-CO ₂ e
nonsterilization	5	0.88*	-0.91*	0.06**
	10	0.90*	-0.90*	0.62**
	20	0.88*	-0.93*	0.01**
	30	0.94*	-0.94*	0.83**
	45	0.94*	-0.96*	0.10**
	60	0.93*	-0.96*	0.98**
sterilizaiton	5	0.90*	-0.91*	0.97**
	10	0.90*	-0.86*	0.10**
	20	0.93*	-0.93*	0.10**
	30	0.93*	-0.93*	0.62**
	45	0.95*	-0.97*	0.00**
	60	0.97**	-0.98*	0.76**

^{*a* *} indicates significant at the 0.05 probability level; ** indicates significant at the 0.01 probability level. ^{*b*} Days after treatment. ^{*c*} Correlation coefficients between ¹⁴C-ER and soil pH. ^{*d*} Correlation coefficients between ¹⁴CO₂ and soil pH.

were found to depend closely on the soil properties. Extractable residues were positively correlated with soil pH ($0.88 \le r^2 \le 0.97$, p < 0.05) while BR showed a negative correlation with soil pH $(-0.98 \le -r^2 \le -0.86, p < 0.05)$ (Table 3). Generally, ER was characterized as the total of parent compound and extractable degradation products. In previous studies, the main metabolites of ZJ0273 were identified as 4-(2-(4,6-dimethoxypyrimidin-2yloxy)benzylamino)benzoic acid (M1), 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzamido)benzoic acid (M2), 2-(4,6-dimethoxypyrimidin-2-yloxy)benzoic acid (M3), and 4,6-dimethoxypyrimidin-2-ol (M4) (2, 29). Among the metabolites, only M1and M2 would retain the ¹⁴C-labeled phenyl group. Therefore, ¹⁴C-ER in the present study should contain mostly ZJ0273 and its benzoate ring related degradation products, M1 and M2, both of which are carboxylic acids. Acidic and anionic pesticides, such as the phenoxyacetic acids and esters, asulum and dicamba, were reported to have a tendency to interact with soil organic matter by H-bonding at pH values below their pK_a in nonionized forms through their -COOH and -COOR (30, 31). Moreover, soil humic substances contain in their structures electron-deficient moieties, such as quinones. In low pH conditions, metabolites M1 and M2 that contain electron-rich centers tend to form charge transfer complexes with the soil electron-deficient moieties via electron donor-acceptor mechanisms (9).

No apparent relationship was found between the level of extractable or bound residues and soil OM, CEC, or particle distribution (**Tables 3**). The OM, CEC, or particle distribution varied considerably among the five soils considered in the present study, but these variations apparently did not induce any detectable effect on the distribution of the different forms of ZJ0273 residues.

In summary, results from the present study showed that soil microbes influenced the formation of different forms of residues as well as the mineralization rate of ZJ0273 in an acidic soil. Sterilization of the strongly acidic soil (pH 4.2) significantly inhibited the dissipation of ER and mineralization to CO_2 derived from A ring cleavage, and formation of BR. However, a similar effect was not observed for neutral and alkaline soils. In these soils, dissipation of ER and formation of BR appeared to be mediated abiotically. It is likely that ZJ0273 was abiotically activated to its intermediates in these soils, and the formed intermediates were quickly and strongly bound to soil organic matter. The findings of this study suggested a potential for BR formation in acidic soils. In such soils, the release of ZJ0273 BR and its bioavailability to nontarget rotational crops may be a

problem meriting further investigation. Moreover, mechanisms for the formation of BR should also be explored.

ABBREVIATION USED

ER, extractable residue; BR, bound residue, HPLC, high performance liquid chromatography; LSC, liquid scintillation counting; TLC, thin layer chromatography; ULLSS, ultralow liquid scintillation spectrometer; IIA, isotope imaging analysis; PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis(5-phenyloxazol-2-yl)benzene; DT₅₀, half-dissipation time; DAT, days after treatment.

LITERATURE CITED

- Lu, L.; Chen, J.; Wu, J.; Ling, W.; Mao, L. S.; Li, M. Z. 2-Pyrimidyloxy-N-arylbenzylamine derivatives, their processes and uses [P]. U.S. Patent 6,800,590B2, 2004.
- (2) Wang, H. Y.; Ye, Q. F.; Yue, L.; Han, A. L.; Yu, Z. Y.; Wang, W.; Yang, Z. M.; Lu, L. Fate characterization of a novel herbicide ZJ0273 in aerobic soils using multi-position ¹⁴C labeling. *Sci. Total Environ.* **2009**, *407*, 4134–4139.
- (3) Wang, H. Y.; Guo, Y. L.; Lu, L. Studies of rearrangement reactions of protonated and lithium cationized 2-Pyrimidinyloxy-N-Arylbenzylamine derivatives by MALDI-FT-ICR Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 1820–1832.
- (4) Yang, Z. M.; Wang, W.; Han, A. L.; Ye, Q. F.; Lu, L. Determination of herbicide ZJ0273 residue in rapeseed by radioisotopic tracing method. *Food Chem.* 2008, *114*, 300–305.
- (5) Wolt, J. D.; Smith, J. K.; Sams, J. K. Products and kinetics of cloransulam-methyl aerobic soil metabolism. J. Agric. Food Chem. 1996, 44, 324–332.
- (6) Barriuso, E.; Houot, S.; Serra, W. C. Influence of compost addition to soil on the behaviour of herbicides. *Pestic. Sci.* 1997, 49, 65–75.
- (7) Walker, S. S.; Robinson, G. R.; Hargreaves, P. A. Weed control with atrazine and chlorsulfuron is determined by herbicide availability and persistence in soils. *Aust. J. Agric. Res.* 1997, 48, 1003– 1009.
- (8) Alexander, M. Biodegradation and Bioremediation; Academic Press: San Diego, CA, 1994.
- (9) Gevao, B.; Semple, K. T.; Jones, K. C. Bound pesticide residues in soils: a review. *Environ. Pollut.* 2000, 108, 3–14.
- (10) Hague, A.; Schuphan, I.; Ebing, W. Bioavailability of conjugated and soil-bound [¹⁴C] hydroxymonolinuron-D-glucoside residues to earthworms and ryegrass. *Pestic. Sci.* **1982**, *13*, 219–228.
- (11) Dec, J.; Haider, K.; Rangaswamy, V.; Schaffer, A.; Fernandes, E.; Bollag, J. M. Formation of soil-bound residues of cyprodinil and their plant uptake. J. Agric. Food Chem. 1997, 45, 514–520.
- (12) Enrique, B.; Pierre, B.; Igor, G. D. Formation of pesticide nonextractable (bound) residues in soil: Magnitude, controlling factors and reversibility. *Environ. Sci. Technol.* **2008**, *42*, 1845–1854.
- (13) Yang, Z. M.; Ye, Q. F.; Tang, Q. H.; Lu, L.; Chen, Z. Y. Radiolabeled synthesis and identification of a novel herbicide, propyl 4-2[2-(4,6-Dimethoxy-2-pyrimidinyloxy)benzylamino]benzoate with all six benzoic ring carbons labeled by 14C. *Acta Chim. Sin.* 2005, 63, 1999–2003.
- (14) Nelson, D. W.; Sommers, L. E. Total carbon, organic carbon, and organic matter. In *Methods of Soil Analysis, Part 3, Chemical methods*; Bartels, J. M., Ed.; American Society of Agronomy: Madison, WI, 1996; pp 539–579.
- (15) Gee, G. W.; Bauder, J. W. Particle-size analysis. In *Methods of Soil Analysis, Part 1, Physical and Mineralogical Methods*; Klute, A., Klute, A., Ed.; Soil Science Society of America: Madison, WI, 1986; pp 383–412.
- (16) Wang, H. Z.; Gan, J.; Zhang, J. B.; Xu, J. M.; Yates, S. R.; Wu, J. J.; Ye, Q. F. Kinetic distribution of ¹⁴C-metsulfuron-methyl residues in paddy soil under different moisture conditions. *J. Environ. Qual.* **2009**, *38*, 164–170.
- (17) Ryan, P. H.; Robert, J. M. H.; Gerald, K. Effects of soil pH and soil water content on prosulfuron dissipation. J. Agric. Food Chem. 2002, 50, 3236–3243.

- (18) Ye, Q. F.; Wu, J. M.; Sun, J. H. ¹⁴C-Extractable residue, ¹⁴C-bound residue and mineralization of ¹⁴C-labeled metsulfuron-methyl in soils. *Environ. Sci.* 2002, 23, 62–68.
- (19) Abdelhafid, R.; Houot, S.; Barriuso, E. Dependence of atrazine degradation on C and N availability in adapted and non-adapted soils. *Soil Biol. Biochem.* **2000**, *32*, 389–401.
- (20) Gevao, B.; Jones, K. C.; Semple, K. T. Formation and release of nonextractable ¹⁴C-Dicamba residues in soil under sterile and non-sterile regimes. *Environ. Pollut.* 2005, *133*, 17–24.
- (21) Dec, J.; Bollag, J. M. Microbial release and degradation of Catechol and chlorophenols bound to synthetic humic acids. *Soil Sci. Soc. Am. J.* **1988**, *52*, 1366–1371.
- (22) Senesi, N. Binding mechanisms of pesticides to soil humic substances. *Sci. Total Environ.* **1992**, *123*, 63–76.
- (23) Barriuso, E.; Benoit, P.; Dubus, I. Formation of pesticide nonextractable (Bound) Residues in soil: Magnitude, controlling factors and reversibility. *Environ. Sci. Technol.* 2008, 42, 1845– 1854.
- (24) Khan, S. U.; Behki, R. M. Effects of *pseudomonas* species on the release of bound C-14 residues from soil treated with [C-14]Atrazine. *J. Agric. Food Chem.* **1990**, *38*, 2090–2093.
- (25) Bartha, R.; You, I. S.; Saxena, A. In *Humus-Bound Residues of Phenylamide Herbicides: Their Nature, Persistence and Monitoring*; Bartha, R., You, I.-S., Saxena, A., Eds.; Pergamon: Oxford, U.K., 1983; Vol. 3, pp 345–350.
- (26) Han, A. L.; Yue, L.; Li, Z.; Wang, H. Y.; Wang, Y.; Ye, Q. F.; Lu, L.; Gan, J. Plant availability and phytotoxicity of soil bound residues of

herbicide ZJ0273, a novel acetolactate synthase potential inhibitor. *Chemosphere* **2009**, *77*, 955–961.

- (27) Willems, H.; Lewis, K. J.; Dyson, J. S.; Lewis, F. J. Mineralization of 2,4-D and atrazine in the unsaturated zone of a sandy loam soil. *Soil Biol. Biochem.* **1996**, *28*, 989–996.
- (28) Winkelmann, D. A.; Klaine, S. J. Degradation and bound residue formation of four atrazine metabolites, deethylatrazine, deisopropylatrazine, dealkyatrazine and hydroxyatrazine, in a western Tennessee soil. *Environ. Toxicol. Chem.* **1991**, *10*, 347–354.
- (29) Wang, H. Y.; Ye, Q. F.; Yue, L.; Yu, Z. Y.; Han, A. L.; Yang, Z. M.; Lu, L. Kinetics of extractable residue, bound residue and mineralization of a novel herbicide, ZJ0273, in aerobic soils. *Chemistry* 2009, 76, 1036–1040.
- (30) Carringer, R. D.; Weber, J. B.; Monaco, T. J. Adsorption-desorption of selected pesticides by organic matter and montmorillonite. *J. Agric. Food Chem.* **1973**, *23*, 569–572.
- (31) Katan, J.; Lichtenstein, E. P. Mechanisms of production of soil bound residues of [¹⁴C]parathion by microorganisms. J. Agric. Food Chem. 1977, 25, 1404–1408.

Received for review August 5, 2009. Revised manuscript received January 24, 2010. Accepted January 26, 2010. This research was financially funded by the Ministry of Agriculture of China National Natural Science Foundation (Projects No. 200803034, 20632070, and 10775118) and the Knowledge Innovation Program of the Chinese Academy of Sciences (KGCX3-SYW-203-3).