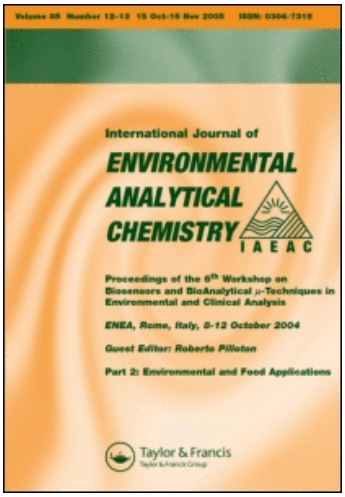

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Persistence and metabolism of Imazapyr in four typical soils of Zhejiang Province (China)

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A laboratory experiment was conducted to study the effects of two different formulations (25% Arsenal SL and 5.0% Arsenal G) and doses (equivalent to 0.5 ug A.I. g^{-1} and 1.0 ug A.I. g^{-1} soil) on persistence of imazapyr in four soils of Zhejiang province, southeastern China. Based on the first-order kinetic equation, the calculated half-lives of imazapyr were in the range 22.0–35.7 days among all the treatments. With regard to the four soils, the highest (30.9 days) and lowest (24.1 days) mean half-lives were observed in Soil C (Coastal Saline Soil, pH 8.78) and Soil B (Yellow-Red Soil, pH 5.25), respectively. The persistence of imazapyr increased in the order soil C (pH 8.78) > soil A (Silt-Loamy Paddy Soil, pH 7.86) > soil D (Fluvio-Marine Yellow Loamy Soil, pH 7.06) > soil B (pH 5.25), which demonstrated that an increase in soil pH tended to lead to higher persistence of imazapyr in soil. The difference between the mean half-lives, corresponding to 0.5 and 1.0 ug A.I. g^{-1} soil treatment for 25% Arsenal SL or for 5.0% Arsenal G, respectively, was not significant, which showed that the different initial application rates had little impact upon degradation of imazapyr. In contrast, a greater impact of the different formulation type upon persistence of imazapyr was observed. Higher persistence was observed with the granular formulation ($t_{1/2} = 28.1$ d) compared with the liquid formulation ($t_{1/2} = 26.2$ d) for the lower dose, which was statistically significant, and an identical trend also existed in the higher dose. Three major metabolites were separated by preparative TLC. On the basis of their spectral data (IR, LC-MS and ^{1}H NMR), the structure of each compound was deduced and their formation pathway was also discussed.

Keywords: Imazapyr; Soil; Formulation; Dose; Metabolism

1. Introduction

For each pesticide that comes in contact with the soil, extensive information is required about its persistence and metabolism. With herbicides, this is important for agronomic and environmental reasons. The persistence and metabolism of a herbicide in soil determines its biological efficacy and carry-over problems with rotation crops [1]. Imazapyr [2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)nicotinic acid] is a non-selective herbicide that is registered for control of most annual grasses and broadleaf

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weeds in rubber and, oil palm mutations, orchards, and on non-crop land. It kills plants by inhibiting acetohydroxyacid synthase (AHAS), the feedback enzyme in the biosynthesis of the branched-chain essential acids [2]. The herbicide was introduced into the mainland China market under the trade name Arsenal as formulations 25% SL and 5.0% G in 2002. Imazapyr is more persistent in the soil environment than are other non-selective herbicides and can control weeds for as long as five months. It is weakly adsorbed by soil and sediment and has the potential to leach to groundwater because it is very soluble (11 272 mg L^{-1} at 25° C) in water, which has raised concern about its safety to human health [3]. Several studies have confirmed that microbial transformations are the essential mechanisms responsible for imazapyrs degradation. It degrades in soil under aerobic conditions to form minor metabolites, which leads ultimately to mineralization [4]. Mallipudi *et al.* [5] reported the photolysis of imazapyr and detected four photoproducts. Metabolism of imazapyr by strepomyces (strain PS1/5) was first reported by Shelton *et al.* [6], who found imazapyr could be thus transformed but to a lesser extent than by light.

In addition, extensive research has been conducted on imazapyr with regard to its degradation, adsorption and desorption, and mobility in soil [7, 8]; availability to plants [9]; and hydrolysis and photodegradation in water [10]. However, little quantitative information is available concerning its environmental behaviour in soils under mainland Chinese conditions. The primary objective of the present work was to study the persistence and to characterize the metabolism of imazapyr applied as two formulations to four typical soils from Zhejiang province, southeastern China, under laboratory conditions.

2. Experimental

2.1 *Materials*

Imazapyr (99.6% purity) and two commercially formulated products (25% Arsenal SL and 5.0% Arsenal G) were kindly provided by Shanghai Branch, BASF (China) Co., Ltd. The purity of the chemicals were confirmed by TLC and HPLC. Solvents used in the study were HPLC grade, and all inorganic reagents were laboratory grade.

2.2 *Preparation of soil samples*

Field soils were collected from four different regions of Zhejiang province: (1) Silt-Loamy Paddy Soil from Shilifeng, Quzhou district; (2) Yellow-Red Soil from Huajiachi, Hangzhou district; (3) Coastal Saline Soil from Longyou, Jinhua district; (4) Fluvio-Marine Yellow Loamy Soil from Pinghu, Jiaxing district, and were designated as soil A, B, C and D, respectively. Soils were air-dried, ground, and passed through a 2-mm sieve. Soil pH was measured in soil + deionized water (1:2.5 by weight). The organic carbon content of soil was determined by oxidation with dichromate [11]. The cation-exchangeable capacity (CEC) of soil was determined by extracting the soil with buffered barium chloride solution at pH 8.1, adjusted with triethanolamine, following the method outlined by Dongrui *et al.* [12]. Soil texture was determined by the hydrometer method [13], and the physicochemical properties of the investigated soils are listed in table 1. The four different soils are classified as

Table 1. The basic physicochemical properties of soils used.

Soil	Type of soil	pH	Organic carbon ($g\,kg^{-1}$)	CEC ($cmol^{(+)}\,kg^{-1}$)	TN ($g\,kg^{-1}$)	WHC_{max} (%)	Texture (%)		
							Sand	Silt	Clay
A	Silt-Loamy Paddy Soil	7.86	28.7	17.50	4.2	69.41	14.4	71.2	14.4
B	Yellow-Red Soil	5.25	11.0	10.63	1.6	49.76	32.5	31.0	36.5
C	Coastal Saline Soil	8.78	5.5	9.50	1.9	43.63	23.1	72.3	4.6
D	Fluvio-Marine Yellow Loamy Soil	7.06	19.5	9.00	3.2	59.43	8.3	70.3	21.4

CEC: Cation-exchangeable capacity; TN: Total nitrogen; WHC_{max}: Maximum water-holding capacity.

Silt-Loamy Soil, Sand-Silty Soil, Silt Soil, and Sand-Loamy Soil, respectively, by international soil-classification standard [14].

2.3 *Application of imazapyr*

The pretreated soil samples (air-dried, ground, and passed through a 0.2-mm sieve) were placed into Erlenmeyer flasks. Imazapyr formulations were applied individually in two doses. The application rates of the liquid formulation (25% Arsenal SL) for treatments T_1 and T_2 corresponded to concentrations of imazapyr in the soil of 0.5 and 1.0 ug active ingredient (A.I.) g^{-1}, respectively, and the same doses of granular formulation (5.0% Arsenal G) were applied as treatments T_3 and T_4, respectively. The control soil set received only methanol. The solution was mixed well with occasional stirring and the solvent was allowed to evaporate for 2 h. The calculated amount of sterilized distilled water was then added to the soils to maintain 60% of maximum water holding capacity (WHC_{max}). The flasks were weighed and kept at 28 ($\pm$1)° C. The weight loss due to evaporation of soil moisture was maintained by periodic addition of water at intervals of 5 days during the incubation period. Each set was in four replicates and was processed for analysis of imazapyr residues at specific time intervals of 0 (2 h after application), 10, 20, 30, 45, 60, 75 and 90 days after treatment (DAT).

2.4 *Extraction and clean-up of soil samples*

Each soil sample (20 g) was mixed with 100 mL of extraction solution (methanol–water, 70:30 by volume), and the pH was adjusted to 5 by 0.1 M HCl. The solution was shaken vigorously for 2 h on a mechanical shaker and filtered through a Buchner funnel under vacuum with repeated washing using methanol. The methanol was evaporated from the filtrate using a rotary vacuum evaporator. The remaining aqueous portion was then extracted three times with dichloromethane (50 + 25 + 25 mL). The organic layer was dehydrated over anhydrous sodium sulfate and its volume reduced to about 1–2 mL with a rotary vacuum evaporator. A glass column (1.0 cm i.d.; 20 cm length), packed with Florisil (80–120 mesh)–acidic aluminium oxide (1:1 by weight), was pretreated by a rinse with methanol–ethyl acetate (2:8 by volume) and the solution was discarded. The concentrated dichloromethane extract was then transferred to the glass column and eluted with a total of 100 mL of methanol, the eluate was concentrated using rotary vacuum evaporator, and the volume was made up to the mark for estimation by HPLC.

2.5 *Recovery study*

To appraise the recovery of imazapyr residues, a recovery study was carried out by fortifying the soils at a series of concentrations (0.1, 0.5, 1.0 ug g^{-1}). The soil was extracted, cleaned up as previously described, and analyzed by HPLC as mentioned below. Results showed that the average recoveries were in the range 82.3–97.4% and that the coefficient of variance varied between 1.72 and 3.71% (data not shown). As a result, the method adopted for the analysis of imazapyr residue was satisfactory.

2.6 *Analysis of imazapyr residue by HPLC*

The residual amount of imazapyr was determined by an HP1100 Model HPLC equipped with a diode-array detector. A YWG- C_{18} reversed phase column (25 cm × 4.6 mm i.d.; 5 um particle size) was used. The mobile phase was methanol–water (55:45 by volume) at a flow rate of 1.0 mL min^{-1}. The column was thermostated at $25 \pm 1^{\circ}$ C, the detector set at 234 nm wavelength, and the injection volume was 20 uL.

2.7 *Metabolic products of imazapyr in soils*

In order to separate enough pure metabolites for analysis by ^{1}H NMR, IR and LC-MS, we increased the initial concentration of imazapyr to 50 ug g^{-1} by addition of the standard stock solution to soil. Because all of the imazapyr half-lives in the four soils were 30 days or so, we collected the samples obtained after 30 DAT as the representatives used to study its metabolic products. The samples were extracted, and cleaned up as previously described. The final concentrated samples were separated and purified by preparative TLC on precoated 0.25 mm, 20×20 cm^2 silica gel 60 F_{254} plates (Merck, Germany). The plates were developed with chloroform–methanol (65:35 by volume). The metabolites were visualized by UV light absorption. The bands on preparative TLC plates were scraped off and extracted with dichloromethane. The suspension was then filtered through a medium-porosity glass filter to yield a filtrate, which was concentrated and dried by gentle flow of nitrogen to give pure compounds. These pure metabolites were subjected to IR, MS and ^{1}H NMR.

2.8 *NMR analysis*

^{1}H NMR analysis spectra of the metabolites were recorded by using a Varian Mercury Plus 400 instrument with $CDCl_3$ as solvent. Chemical shifts are given in parts per million units relative to δ 0.00 in tetramethylsilane (TMS) as internal standard.

2.9 *IR analysis*

The absorption spectra of metabolites were measured in KBr pellets using a Nicolet AVATAR-360 model FITR spectrophotometer.

2.10 *HPLC-MS analysis*

Mass spectra analysis of imazapyr and its products was performed by HPLC-MS using an Agilent 1100 Series spectrometer (Agilent technologies, U.S.A) equipped with

a 2.1 mm (i.d.) × 250 mm column packed with 10-um YWG-C_{18} reversed-phase material and thermostated at 25 ± 1° C. A mixture of methanol and water (70:30 by volume) was used as mobile phase at a constant flow rate of 0.2 mL min^{-1}. The Agilent 1100 series mass spectrometer was also equipped with a quadrupole analyzer and atmospheric pressure chemical ionization (APCI) source operating in positive-ion mode. Parametric optimization was performed using a direct-infusion method at 5 uL min^{-1} with a solution of 50 ng mL^{-1} imazapyr concentration in methanol–water (80:20 by volume). The following optimal conditions were obtained: collision-induced dissociation (CID) 100; dry gas flow 10 L min^{-1}; nebulizer pressure 206.8 kPa; drying gas temperature 300° C; vaporizer temperature 300° C; capillary voltage 3500 V.

2.11 *Statistical analysis*

Imazapyr residue data on different days were subjected to first-order fitting to obtain the regression equation, and the residual half-lives ($t_{1/2}$) are given in table 2. The $t_{1/2}$-values were further tested using SPSS software to determine the effects of treatment (application rate and formulation type) dose (T_1–T_4) and soil type (A–D) on the half-life of imazapyr. Paired comparisons of means were also performed by the LSD test and the results are listed in table 3.

Table 2. Degradation kinetic parameters for imazapyr in soils.

Soil	Treatment[a]	Kinetic equation	R^2	$t_{1/2}$ (d)
A	T_1	$Y = -0.2657 - 0.0267x$	0.9062	26.0
	T_2	$Y = 0.4378 - 0.0290x$	0.9596	23.9
	T_3	$Y = -0.5181 - 0.0215x$	0.9905	32.3
	T_4	$Y = 0.5793 - 0.0308x$	0.9456	22.5
B	T_1	$Y = -0.0137 - 0.0304x$	0.9094	22.8
	T_2	$Y = 0.5900 - 0.0272x$	0.9231	25.5
	T_3	$Y = -0.3281 - 0.0315x$	0.9882	22.0
	T_4	$Y = 0.4801 - 0.0265x$	0.9367	26.1
C	T_1	$Y = -0.2654 - 0.0219x$	0.9397	31.6
	T_2	$Y = 0.3336 - 0.0269x$	0.9679	25.8
	T_3	$Y = -0.3908 - 0.0225x$	0.9674	30.8
	T_4	$Y = 0.2750 - 0.0194x$	0.9346	35.7
D	T_1	$Y = -0.2005 - 0.0285x$	0.9127	24.3
	T_2	$Y = 0.1359 - 0.0261x$	0.9552	26.6
	T_3	$Y = -0.4461 - 0.0256x$	0.9959	27.1
	T_4	$Y = 0.5271 - 0.0285x$	0.9436	24.3

[a]T_1 and T_2 correspond to 0.5 and 1.0 ug A.I. g^{-1} soil, respectively, for 25% Arsenal SL.
T_3 and T_4 correspond to 0.5 and 1.0 ug A.I. g^{-1} soil, respectively, for 5.0% Arsenal G.

Table 3. Analysis of variance for the half-lives of imazapyr in four different soils.

Treatment[a]	Mean half-life (days)	Statistical parameter[b] ($P < 0.05$)	Type of soil	Mean half-life (days)	Statistical parameter[b] ($P < 0.05$)
T_3	28.1	a	C	30.9	a
T_4	27.2	a	A	26.2	b
T_1	26.2	b	D	25.6	b
T_2	25.5	bc	B	24.1	c

[a]T_1 and T_2 correspond to 0.5 and 1.0 ug A.I. g^{-1} soil, respectively, for 25% Arsenal SL. T_3 and T_4 correspond to 0.5 and 1.0 ug A.I. g^{-1} soil, respectively, for 5.0% Arsenal G.
[b]Different lower-case letters (a, b, c) mark differences that 5% level of significance.

3. Results and discussion

3.1 *The persistence of imazapyr in different soils*

The measured imazapyr residues on different days after its application in soil are presented in figure 1 (T_1), figure 2 (T_2), figure 3 (T_3) and figure 4 (T_4), respectively. As can be seen from the above-mentioned figures, imazapyr residues gradually decreased with time during the study period of 90 days irrespective of the formulation and application rate used. The degradation tendencies were found to follow first-order kinetics in all the soils. As a result, the half-life ($t_{1/2}$) was calculated by the following mathematical model expressed as equation (1):

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

where C_0 is the initial concentration of imazapyr (ug g^{-1} soil), C_t is the concentration at time t (ug g^{-1} soil), t is the incubation time (days), and k is the degradation rate constant. Half-life was expressed by ln(2/k) and the degradation rate constant (k) was determined using regression of $\ln(C_t/C_0)$. The computed results indicated that R^2

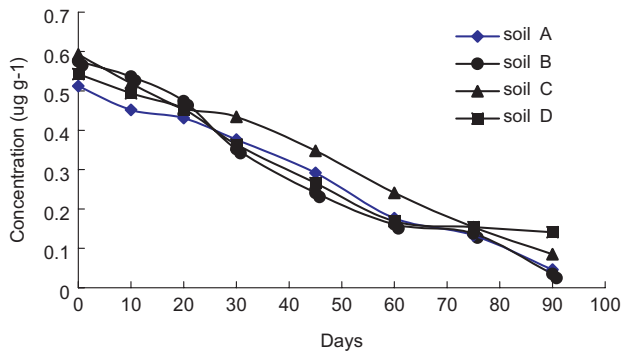

Figure 1. Degradation of imazapyr in different soils over time following the application of imazapyr (25% Arsenal SL) equivatlent to 0.5 ug A.I. g^{-1} soil (T1).

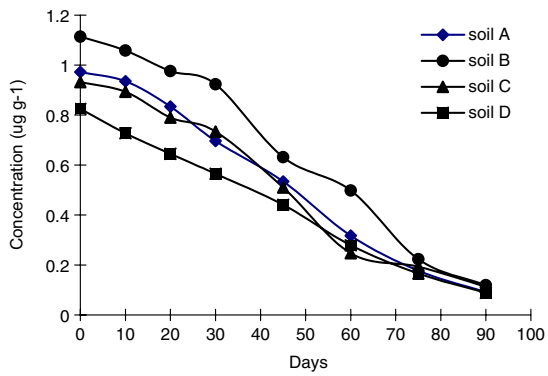

Figure 2. Degradation of imazapyr in different soils over time following the application of imazapyr (25% Arsenal SL) equivatlent to 1.0 ug A.I. g^{-1} soil (T2).

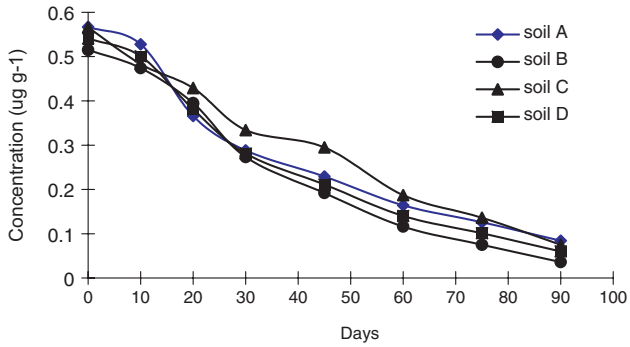

Figure 3. Degradation of imazapyr in different soils over time following the application of imazapyr (5.0% Arsenal G) corresponding to 0.5 ug A.I. g^{-1} soil (T3).

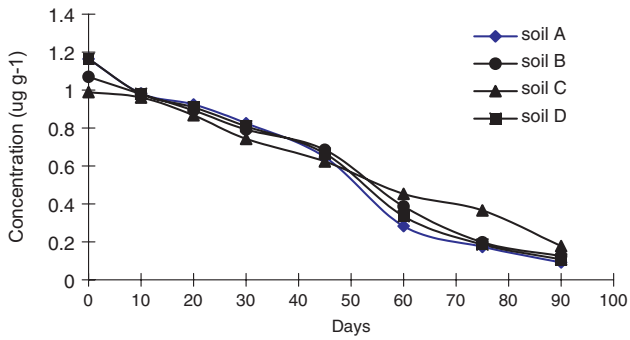

Figure 4. Degradation of imazapyr in different soils over time following the application of imazapyr (5.0% Arsenal G) corresponding to 1.0 ug A.I. g^{-1} soil (T4).

varied between 0.9062 and 0.9905, and table 2 summarizes the calculated half-lives and kinetic equations. The initial soil concentration of imazapyr residues was in the range 0.512–0.593 ug A.I. g^{-1} for T_1 (figure 1) and T_3 (figure 3) and 0.825–1.165 ug A.I. g^{-1} for T_2 (figure 2) and T_4 (figure 4) irrespective of formulation used and type of soil analyzed. However, more than 74% of the residue had dissipated after 90 days after treatment (DAT). For the initial dose, equivalent to 0.5 ug A.I. g^{-1} soil (T_1 and T_3), the half-lives were in the range 22.0–32.3 days, while value of 22.5–35.7 days were found for T_2 and T_4, equivalent to 1.0 ug A.I. g^{-1} soil, which supported the results obtained by other workers [7, 15]. Ismail and Ahmad [15] found that the residual half-lives of imazapyr under field conditions were 22 and 19 days at 25° C in the clay and clay loam soils, respectively, while Azzouzi *et al.* [7] reported that $t_{1/2}$ varied between 25 and 58 d in the red and organic soils. Overall, the preceding results were in general agreement with the data obtained in this work under laboratory conditions.

As can be seen from table 3, the mean half-lives of imazapyr in four soils were in the following order: soil C (pH 8.78) > soil A (pH 7.86) > soil D (pH 7.06) > soil B (pH 5.25). That is to say, the highest mean half-life was found in soil C (Coastal Saline Soil) possessing the highest pH value. In contrast, the lowest mean half-life was observed in soil B (Yellow-Red Soil) having the lowest pH value. As a result, it was evident from the simple order between the pH values and the mean half-lives

that an increase in soil pH appears to lead to higher persistence of imazapyr in soil. Vizantinopoulos and Lolos [1] also observed that the half-life of imazapyr tended to increase with increase in soil pH, and Sarkar [16] obtained identical results for the degradation of imidacloprid. Because Imazapyr belongs to an organic acid herbicide possessing similar properties to other organic acid and ionized pesticides, its hydrolysis and adsorption by soils were more sensitive to pH than are those of other kind of pesticide [17]. It has been reported by Qiquan [17] and by Wehtje *et al*. [18] that low pH contributes to the hydrolysis and adsorption of imazapyr by soil, thereby accelerating the degradation of imazapyr in soil. However, in different soils the degradation rate exhibits a high degree of variety as well. It is not always possible to significantly relate them to the measured soil properties. Thus this must be determined on a case-by-case basis, since different soil properties will affect imazapyr behaviour and degradation. For example, Jenkins *et al*. [8] previously determined that the most important factors in the degradation of imazapyr were temperature and moisture, not pH, in five Alabama soils. In any case, the fact that soil pH may play a significant role in the degradation of imazapyr is beyond controversy.

3.2 *Effect of the formulation type and dose on persistence of imazapyr*

For the liquid formulation (25% Arsenal SL), the different doses led to different mean half-lives, of 26.2 and 25.5 days for T_1 and T_2, days, respectively. For the granular formulation (5.0% Arsenal G), the mean half-life of T_3 and T_4 was 28.1 and 27.2 days, respectively. However, the difference between the mean half-lives of T_1 and T_2, T_3 and T_4 was not significant at the level of 5% probability which demonstrated that the different initial application rate had little impact upon degradation of imazapyr. Nevertheless, higher persistence was observed with the granular formulation (T_3, mean $t_{1/2} = 28.1$ d), compared with the liquid formulation (T_1, mean $t_{1/2} = 26.2$ d) for the lower dose, which was statistically significant, and an identical trend also existed for the higher dose of imazapyr. Significantly higher persistence was observed for the granular formulation than for liquid formulation. It was obvious from the above data that the difference in formulation type could have a greater effect on the persistence of imazapyr in soils than owing to the difference in application rate.

3.3 *Metabolites of imazapyr in four different soils*

Metabolism of imazapyr was also investigated in four different soils at the initial concentration of 50 ug g^{-1} after 30 DAT. The metabolic products were separated by preparative TLC technique according to the preceding method. The results showed that only two major metabolites (**M-1** and **M-2**) were found when imazapyr degraded in soils A and D, while another main metabolite (**M-3**) was also found along with the former two products (**M-1** and **M-2**) in soil B and C. On the basis of IR, ^{1}H NMR and HPLC-MS results, the following structures are reported for the metabolites.

3.4 (*1*) *2,3-Pyridinedicarboxamide* (*M-1*)

IR: 3372, 3189 (NH_2), 1638 (C=O); ^{1}H NMR: 7.86 (m, 3H, pyridine 4,-5,-6-H), 8.18 (br, 2H, pyridine 3-$CONH_2$), 8.42 (br, 2H, pyridine 2-$CONH_2$); MS: m/z 166 ($M + 1$), fragment ions 123 ($- CONH_2$), 80 ($- CONH_2 - CONH_2$).

3.5 (*2*) *2,3-Pyridinedicarboximide* (*M-2*)

IR: 3275 (-NH-), 1757, 1729 (C=O); ^{1}H NMR: 7.46 (dd, 1H, pyridine-5-H), 8.29 (d, 1H, pyridine 4-H), 8.49 (d, 1H, pyridine 6-H), 11.24 (s, 1H, NH); MS: m/z 149 (M + 1).

3.6 (*3*) *2-(4-Hydroxy-5-oxo-2-imdazolin-2-yl)nicotinic acid* (*M-3*)

IR: 3477 (imidazoline 4-OH), 3270 (NH), 3085, 1670 (COOH), 1637 (imidazoline 5-CO); ^{1}H NMR: 8.43 (s, 1H, NH), 5.85 (d, 1H, imidazolin-4-H), 7.52–8.75 (m, 3H, pyridine 4, 5, 6-H), 11.23 (−COOH), 9.84 (−OH); MS: m/z 222 (M + 1), fragment ions 178 (−COOH), 162 (−COOH, −OH), 101 (−nicotinic acid).

On the basis of metabolites so far isolated and characterized in thus study, a plausible metabolic pathway of imazapyr had been proposed in figure 5. It was clear that the main degradation pathways of imazapyr in soils were decarboxylation, demethylation, loss of the isopropyl group, and cleavage and rearrangement of the imidazolinone ring.

When imazapyr degraded in soils, the methyl and isopropyl group were lost, the imidazolinone ring opened, and a new dicarboxamide group was formed at the 2,3-positions of the pyridine ring, giving diamide **M-1**. In a more extended process, the imidazolinone ring detache, and the component parts rearranged to form the new dicarboximide at the pyridine 2,3-position, compound **M-2**. However, when degraded in soils B and C, another new band was found on preparative TLC besides the same

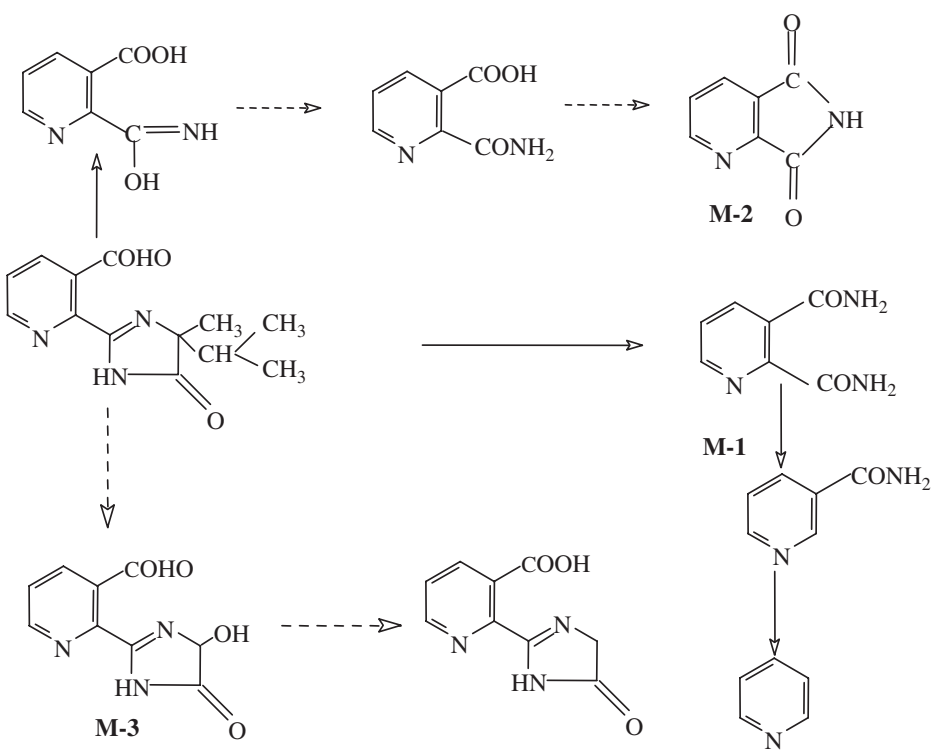

Figure 5. The proposed metabolic pathway of imazapyr in four different soils.

two bands seen in soils A and D. According to its IR, ^{1}H NMR and HPLC-MS spectral data, **M-3** at m/z 221 was formed accompanied by both the loss of the isopropyl group and the hydroxylation at the 4-position of the imidazolinone ring.

As can be seen from the preceding discussion, two main metabolites (**M-1** and **M-2**) were formed during the opening and rearrangement of the imidazolinone ring by indigenous microbial activity which was in good agreement with previous reports. Mangels *et al*. [4] reported that as imazapyr degrades in aqueous solution, the imidazolinone ring opens to form metabolites CL 252974. In addition, the hydroxylation metabolite will form as a result of conversion of the carboxylic acid group on the pyridine ring. Four photoproducts in buffer solution were detected by Mallipudi *et al*. [5], who observed that the predominant products were 7-hydroxyfuro [3,4-*b*]pyridin-5(7*H*)-one and 2,3-pyridinedicarboxylic acid, and that two cyclic compounds, 2,3-pyridinedi- carboximide and furo[3,4-*b*]pyridin-5(7*H*)-one, were also detected as minor degradation products. One of the photoproducts, 2,3-pyridinedicarboximide, was found under both photolysis in aqueous solution and degradation in soil conditions. As far as our information goes, to date, **M-3** identified in the present investigation is a new metabolite to be reported in soils.

4. Conclusions

The persistence of imazapyr decreased in the order: soil C (pH 8.78) > soil A (pH 7.86) > soil D (pH 7.06) > soil B (pH 5.25), which showed that pH plays an important role in the persistence of imazapyr in soils. The effect of the difference in application rates on the persistence of imazapyr was minute, while the greater impact of the difference in formulation type upon the persistence of imazapyr was observed. Three major metabolites in soils B and C were separated, while only two were found in soils A and D, and their respective chemical structures were also identified by LC-MS, ^{1}H NMR and IR spectral data. However, the present study can only be regarded as the first step to a better understanding of the persistence of imazapyr in soils. Indeed, laboratory experiments can't simulate many field conditions. Many case studies show that results from laboratory studies can differ greatly from results found in field studies due to the many more variables present in the latter. Therefore, more detailed information on imazapyr dissipation in soils under both laboratory and field conditions is needed.

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References

[1] S. Vizantinopoulos, P. Lolos, *Bull. Environ. Contam Toxicol*. **52**, 404 (1994).
[2] R.J. Mitchell, W. Deyou, *The Manual of the Forestry Herbicides*, J.H. Miller (Ed.) pp. 98–107, Science and Technology Press, Peking (1997).
[3] C. Cox, *J. Pestic. Reform*., **16**, 16 (1996).

[4] D.L. Shanner, S.L. Conner, *Imidazolinone Herbicides*, G. Mangels (Ed.) pp. 191–209, Academic Press, Princeton (1991).
[5] N.M. Mallipudi, S.J. Stout, A.R. Dacunha, A. Lee, *J. Agric. Food Chem.*, **39**,412 (1991).
[6] D.R. Shelton, S. Khader, J.S. Karns, *Biodegradation*, **7**, 126 (1996).
[7] M. Azzouzi, A. Dahchour, A. Bouhaouss, *Weed Res. Oxford*, **38**, 217 (1998).
[8] S.R. Jenkins, G.R. Wehtje, J.M. Morgan, A.F. Bollinger, D.G. Young, *Water, Air and Soil Poll.*, **118**, 169 (2000).
[9] C.J. Bannister, In *Aspects of Applied Biology*, R.J. Winfield (Ed.) Vol. 16, pp. 79–85, Association for Applied Biology, Warwick (1988).
[10] M. Elazzouzi, A. Bensaoud, H. Bouhaouss, *Fresenius Environ. Bull.*, **8**, 478 (1999).
[11] J. Wu, R.G. Joergensen, B. Pommerening, *Soil Biol. Biochem.*, **22**, 1167 (1990).
[12] J. Chen, *Analytical Methods of Soil*, Y. Dongrui (Ed.) pp. 37–41, China Agricultural Press, Beijing (1992).
[13] X. Yi, In *Analytical Methods of Soil Colloids*, Vol. 2, pp. 57–62, China Science Press, Beijing (1983).
[14] W. Zhangrong, Y. Zhenyu, In *The Soil Species of Zhejiang Province*, pp. 70–75, Zhejiang Science and Technology Press, Hangzhou (1994).
[15] B.S. Ismail, A.R. Ahmad, *Agric. Ecosys Environ.*, **47**, 279 (1994).
[16] M.A. Sarkar, R. Sankhajit, R.K. Kole, A. Chowdhury, *Pest Manag. Sci.*, **57**, 598 (2001).
[17] W. Qiquan, L. Weiping, *China Environ. Sci.*, **18**, 314 (1998).
[18] G. Wehtje, R. Dickens, J.W. Wilcut, B.F. Hajek, *Weed Sci.*, **35**, 858 (1987).