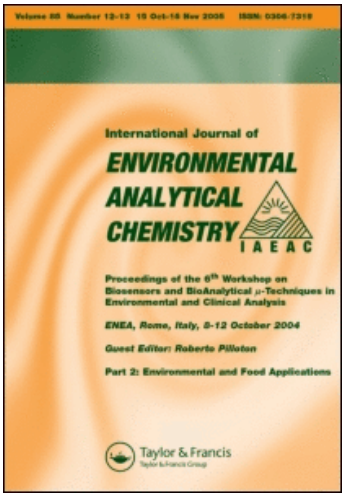


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# Degradation and metabolism of imazapyr in soils under aerobic and anaerobic conditions

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The degradation of imazapyr in four soils was investigated under laboratory aerobic and anaerobic conditions. Under aerobic conditions, imazapyr degraded faster in yellow–red soil than in other soils, and its persistence decreased depending on soil pH in the order coastal soil (pH 8.8) > silt-loamy paddy soil (pH 7.9) > fluvio-marine yellow loamy soil (pH 7.1) > Yellow–red soil (pH 5.3). However, soil pH did not affect imazapyr degradation under anaerobic conditions. The half-lives of imazapyr in soils under aerobic conditions were in the range of 26–44 days estimated by the first-order kinetics model, while 3–10 days calculated by two-stage model under anaerobic conditions. The preceding results demonstrated that anaerobic conditions contributed to imazapyr disappearance in soils. Based on the spectral data of APCI-MS, $^1$H NMR and IR, structures of the following metabolites: 2,3-pyridinedicarboxamide, 2,3-pyridinedicarboxylic anhydride and 2,3-pyridinedicarboximide for aerobic treatments; 2,3-pyridinedicarboxylic anhydride and 2-(4-hydroxy-5-oxo-2-imdazolin-2-yl) nicotinic acid for anaerobic treatments, were identified. Degradation mechanism under the different conditions was also discussed.

*Keywords:* Imazapyr; Soils; Aerobic conditions; Anaerobic conditions; Metabolites

## 1. Introduction

Organic pesticides may be used as substrates by soil microorganisms and undergo degradation resulting in the formation of new compounds. Several of these pesticides and their metabolites may have physicochemical properties that enable them to contaminate ground water. When these waters are used for drinking, the contaminants can pose a threat to human health, if present above certain levels, although the risk is difficult to assess because of a lack of toxicological data [1]. In order to obtain authorization to place new pesticides on China mainland market, an investigation

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on their environmental behaviour is mandatory on the parent compounds and their main metabolites.

Imazapyr [2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2yl) nicotinic acid] is a broad-spectrum postemergence herbicide developed by American Cyanamid Company and was registered in 2002 in China mainland. It can control annual and perennial grasses, sedges, broadleaf weeds, shrubs and deciduous trees by inhibiting acetohydroxy acid synthase, the feedback enzyme in the biosynthesis of the branched-chain essential acids [2]. Imazapyr is more persistent in the soil environment than other non-selective herbicides and can control weeds as long as 5 months. Imazapyr is weakly sorbed on soil and sediments, and has the potential to leach to groundwater because of its high solubility (11272 mg $L^{-1}$ at 25°C) in water, which raises concern about its safety to human health [2].

The previous studies have confirmed that microbial transformation is the essential mechanism responsible for the degradation of imazapyr, which accounts for 62 to 78% of imazapyr disappearance in non-sterile soils [3]. Imazapyr could form minor metabolites in soils under aerobic conditions, which was then ultimately mineralized [4]. Mallipudi *et al.* [5] reported the photolysis of imazapyr and detected four photoproducts. Metabolism of imazapyr by the isolated strain PS1/5 was first reported by Shelton *et al.* [6] who found imazapyr could be transformed by it. Additionally, Wang *et al.* [7] also reported that two isolated strains, *Pseudomonas fluorescene biotype* II and *Bacillus cereus,* from soil could rapidly degrade imzapyr in medium.

In general, previous studies mainly focused on imazapyr environmental behaviour in soils under aerobic conditions [8, 9] and in water [10]. However, little is known about its degradation and metabolism in soils under anaerobic conditions. Because this herbicide is poorly sorbed on soils, it can reach deeper horizons, where anaerobic-like conditions may occur. Therefore, the aim of this study is to compare the difference of imazapyr degradation and metabolism under laboratory aerobic and anaerobic conditions.



## 2. Experimental

### 2.1 *Materials*

Imazapyr (99.6% purity) was kindly provided by Shanghai Branch, BASF (China) Co., Ltd and its purity was 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*

Four soils were collected from the different regions of Zhejiang province, South-Eastern China, (A) silt-loamy paddy soil from Shilifeng, Quzhou district; (B) yellow–red soil from Huajiachi, Hangzhou district; (C) coastal saline soil from Longyou, Jinhua district; (D) fluvio-marine yellow loamy soil from Pinghu, Jiaxing district, and their physicochemical properties were presented in table 1. According to international soil-classification standard, the four soils were classified as silt-loamy soil, sand-silty soil, silt soil and sand-loamy soil, respectively [11]. The collected soils

Table 1. The basic physico-chemical properties of soils used.

| Soil | Type of soil | pH | Organic carbon ($g\,kg^{-1}$) | CEC ($cmol_{(+)}\,kg^{-1}$) | TN ($g\,kg^{-1}$) | WHC (%) | Texture (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Sand | Silt | Clay |
| A | Silt-loamy Paddy soil | 7.9 | 28.7 | 17.5 | 4.2 | 69.4 | 14.4 | 71.2 | 14.4 |
| B | Yellow–red soil | 5.3 | 11.0 | 10.6 | 1.6 | 49.8 | 32.5 | 31.0 | 36.5 |
| C | Coastal saline soil | 8.8 | 5.5 | 9.5 | 1.9 | 43.6 | 23.1 | 72.3 | 4.6 |
| D | Fluvio-marine yellow loamy soil | 7.1 | 19.5 | 9.0 | 3.2 | 59.4 | 8.3 | 70.3 | 21.4 |

CEC: Cation exchange capacity; TN: Total nitrogen; WHC: Water-holding capacity.

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 [12]. The cation exchange 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.* [13]. Soil texture was determined by the hydrometer method [14].



### 2.3 *Degradation of imazapyr in aerobic and anaerobic soils*

The degradation of imazapyr in soils under laboratory aerobic and anaerobic conditions was carried out in the dark and at room temperature ($20 \pm 2^{\circ}C$). Twenty-four flasks for aerobic conditions and twenty-four for anaerobic conditions, each containing 10 g of the sieved soil, were prepared and fitted with gas, air for aerobic treatments and nitrogen for anaerobic treatment, inlets and outlets according to the method reported by Morrica *et al.* [15]. Each flask was then treated with 2 mL of $50\,mg\,L^{-1}$ imazapyr solution in methanol to obtain a final concentration of $10\,mg\,kg^{-1}$. Sufficient sterilized water was added to each flask to adjust the moisture content of the soil to approximately 75% WHC (water-holding capacity) prior to incubation. These flasks were purged continuously with air or nitrogen, bubbled through distilled water to maintain the humidity at 75% WHC. For both aerobic and anaerobic treatments, the soil autoclaved for 1 h at $121^{\circ}C$ served as control. The autoclaving effectiveness was confirmed by microbial tests, which demonstrated no contamination by microorganisms at the end of each respective study. Each set was in triplicates and processed as described below for imazapyr residual analysis at intervals of 0 (2 h after spiking imazapyr solution), 1, 5, 10, 20, 30, 45 and 60 days after treatment (DAT).

### 2.4 *Extraction and cleanup of soil samples*

Each soil sample was extracted with 50 mL of methanol + water (70 : 30, v : v) solution, adjusting pH to 5 by 0.1 M HCl. The solution was shaken vigorously for 1 h on a reciprocating shaker and vacuum filtered through a Buchner funnel with repeated methanol washing. The methanol was evaporated from the filtrate using a rotary vacuum evaporator. The remaining aqueous portion was extracted 3 times with dichloromethane (50/25/25 mL). The organic layer was collected over anhydrous sodium sulphate and its volume reduced to $2 \sim 3$ mL with a rotary vacuum evaporator.

The concentrated dichloromethane extracts were transferred into a glass column (1.0 cm ID, 20 cm length) packed with Florisil (80–120 mesh) + acidic aluminum oxide (1 : 1, w : w) and rinsed with methanol + ethyl acetate (20 : 80, v : v). The eluate was evaporated to dryness on a rotary evaporator, and the residue was redissolved in methanol (5 mL) for HPLC analysis.

### 2.5 *Recovery study*

To estimate the recovery of imazapyr residue, a recovery study was carried out by spiking the soils with imazapyr stock solution (100 mg $L^{-1}$) in methanol to obtain a series of concentrations of 0.05, 0.1, 1, 10 mg $kg^{-1}$ in each soil. The average recoveries were in the range of 82.32 ~ 97.40% and the relative standard deviations (RSD) ranged from 1.66 to 3.71% (data not shown). Therefore, the method adopted for the analysis of imazapyr residue was satisfactory.

### 2.6 *Analysis of imazapyr residue*



A HP1100 model high performance liquid chromatography, equipped with diode array detector (DAD), was operated to determine the residue of imazapyr. A YWG-$C_{18}$ reversed phase column (25 cm × 4.6 mm ID) was used. The mobile phase was methanol + water (55 : 45, v : v) at a flow rate of 1.0 mL $min^{-1}$. The column was thermostated at 25 ± 1°C, the detector set at 234 nm and the injection volume was 20 μL. Under the above-mentioned conditions, the retention time of imazapyr was about 3.8 min.

### 2.7 *Metabolism of imazapyr under aerobic and anaerobic conditions*

Because the degradation half-lives of imazapyr were in the range of 26–44 and 3–10 days under aerobic and anaerobic conditions, respectively, we chose the soil samples of 40 DAT for aerobic treatment and 10 DAT for anaerobic treatment to study metabolism of imazapyr. The pretreated soil samples were spiked with imazapyr stock solution in methanol to make a final concentration of 50 mg $kg^{-1}$, incubated for the corresponding time, extracted and cleaned up as described previously in section 2.4. 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, v : v). 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 nitrogen flow to give pure compounds. Finally, these purified metabolites were subjected to IR, MS and NMR analyses.

### 2.8 *NMR analysis*

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

### 2.9 *IR analysis*

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

### 2.10 *APCI-MS analysis*

Mass spectra analysis of imazapyr and its by-products were achieved using an Agilent 1100 series mass spectrometer (Agilent technologies, USA) coupled with a quadrupole analyzer and atmospheric pressure chemical ionization (APCI) source operating in positive ion mode. The parameter optimization was performed using direct infusion method at 5 μL $min^{-1}$ with a solution of 50 μg $L^{-1}$ imazapyr concentration in methanol and water (80 : 20, v : v). The following optimized conditions were obtained: collision-induced desiccation 100; dry gas flow 10 L $min^{-1}$; nebulizer pressure 30 Psig; drying gas temperature 300°C; vaporizer temperature 300°C; capillary voltage 3500 V; corona current 25 μA.



## 3. Results and discussion

### 3.1 *Degradation of imazapyr in soils under aerobic conditions*

As can be seen from figure 1, the initial imazapyr residues were in the range of 9.28–9.78 mg $kg^{-1}$ in four soils and the concentrations of imazapyr decreased gradually with the elapse of time during the study period of 60 days. The highest (82%) and lowest (59%) degradation over 60 DAT were observed in yellow–red

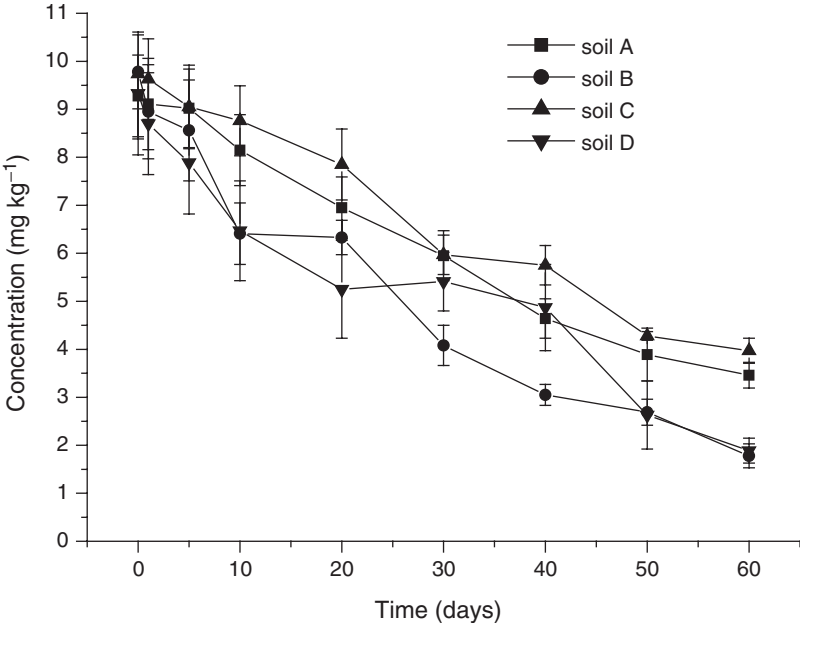


Figure 1. Degradation of imazapyr in soils under aerobic conditions.

Table 2. Degradation kinetics parameters of imazapyr in four soils under aerobic conditions.

| | Soils | | | |
|---|---|---|---|---|
| Parameters | A | B | C | D |
| $K$ | 0.0175 | 0.02676 | 0.01571 | 0.02333 |
| $T_{1/2}$ (days) | 39.6 | 25.9 | 44.1 | 29.7 |
| $R^2$ | 0.9931 | 0.9793 | 0.9776 | 0.9115 |

$K$ and $T_{1/2}$ indicate imazapyr rate constants and half-lives, respectively.

soil and coastal saline soil, respectively. The dissipation curves of imazapyr with time over 60 days were found to follow an essentially first-order kinetics in four soils and coefficients of determination ($R^2$) ranged from 0.9115 to 0.9931 (table 2). Based on the first-order kinetics, the estimated half-lives of imazapyr in different soils varied between 26 and 44 days. Ismail *et al*. [16] reported 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*. [8] reported that it varied between 25 and 58 days in the red and organic soil. All in all, the above-mentioned observations were comparable with the result in this experiment under laboratory aerobic conditions.

Based on the first-order half-lives, the persistence of imazapyr decreased in the order soil C (pH 8.8) > soil A (pH 7.9) > soil D (pH 7.1) > soil B (pH 5.3). Thus, increase in soil pH possibly led to higher persistence of imazapyr in soil. Vizantinopoulos *et al*. [17] also observed that the half-life of imazapyr tended to increase with increase in soil pH, and Sarkar *et al*. [18] got the identical conclusion on the degradation of imidacloprid. Because of belonging to an organic acid and easily ionized herbicide, imazapyr hydrolysis and sorption by soil are more sensitive to pH than other kind of pesticides [19]. It had been reported by Wehtje *et al*. [20] who found that low pH contributed to hydrolysis and sorption of imazapyr by soil, thus, accelerating its disappearance. However, Jenkins *et al*. [9] found that the most important factors for imazapyr degradation were temperature and moisture, and not pH of the soil. In different soils, the degradation rates of imazapyr exhibit a high degree of variability as well. It is not always possible to significantly relate them to the measured soil properties, but investigation of a larger variety of soils could clarify correlation between pH value and degradation rate. In spite of the preceding various debates, results obtained in this study clearly demonstrated that soil pH value was an important factor to influence imazapyr degradation.

### 3.2 *Imazapyr degradation in soils under anaerobic conditions*

As can be seen in figure 2, the residues of imazapyr also decreased with time over 60 days under anaerobic conditions, but more rapidly initially and then slowly which is suggestive of a two-stage model. On the contrary, the decline curves of imazapyr with time had a more gradual slope (figure 1) under aerobic conditions than anaerobic conditions. Based on the first-order kinetics equation, the calculated half-lives were 13.6, 31.5, 19.5 and 22.3 days, respectively in the four investigated soils. This result disagreed with the experimental data, which showed that the half-lives were clearly less than 10 days in all examined soils.

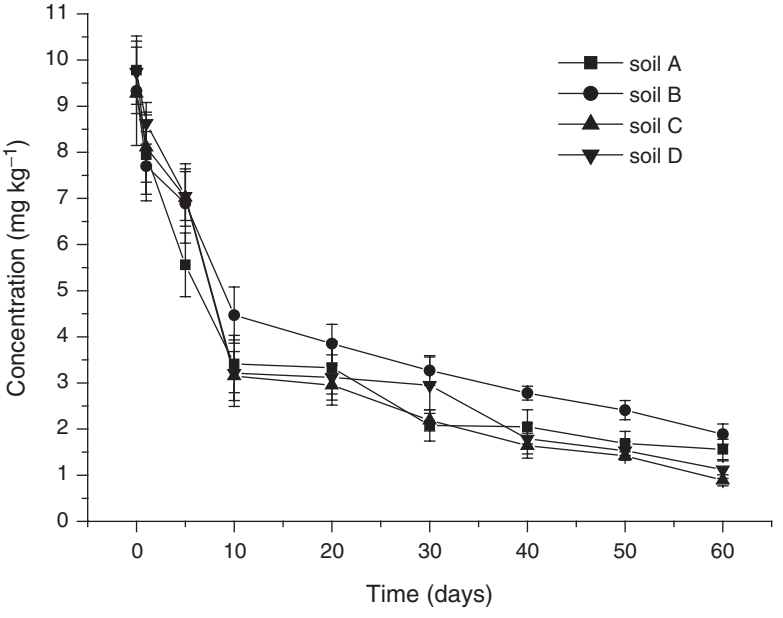


Figure 2. Degradation of imazapyr in soils under anaerobic conditions.



The experimental data were analyzed in order to establish the model between the concentration and time. In theory, the order of the reaction $n$ can be determined by assigning different values of $n$ and determining the most linear model from the experimental data [21]. Besides the first-order kinetics model, the other three kinds of model, second-order ($1/C_t = 1/C_0 + kt$), third-order ($1/C_t^2 = 1/C_0^2 + kt$) and two-stage model ($C_t/C_0 = a\mathrm{e}^{-k1t} + (1-a)\mathrm{e}^{-k2t}$), were also tested to fit the linearity of four plots in figure 2. As can be seen from table 2, the fitting results showed that the higher determination coefficient ($R^2$, $0.9267 \sim 0.9943$) and the appropriate half-lives ($3.3 \sim 9.9$ d) were achieved by two-stage model. The prominent property of two-stage model plot is that the degradation tendency is initially rapid and then slows. Heng and Webster [22] observed that the initial chlorpyrifos residues dissipated quickly with half-lives of $1.1 \sim 2.9$ days for the fast dissipation phase and the later period became much slow with the dissipation half-lives ranging from 205 to 228 days. Some authors explain this two-stage model through a decreased availability of pesticides to soil microbes as a result of shifting to a protected compartment, corresponding to the colloidal phase of soil, after sorption. In this compartment, the slower degradation is probably mainly due to chemical hydrolysis, the rate of which is generally slower than that of microbial degradation [23]. It had been reported by Wang *et al*. [3], who found that the half-lives of imazapyr in non-sterile soils were in the range of 30 to 45 days, while 81 to 133 days in sterile (by autoclaving) soils. That is to say, the rate constants of imazapyr in four soils under non-sterile conditions increased 2.3–4.4 times faster than under sterile (by autoclaving) conditions, which suggest that the indigenous microorganisms in soil play an important role in degradation of imazapyr.

According to the two-stage model, the half-life of imazapyr was calculated by the following equation: $C_t/C_0 = a\mathrm{e}^{-k1t} + (1-a)\mathrm{e}^{-k2t}$, where $C_0$ is the initial concentration

Table 3. Degradation kinetics parameters of imazapyr under anaerobic conditions.

| Kinetics model | Equation | Parameter | Type of soil | | | |
|---|---|---|---|---|---|---|
| | | | Soil A | Soil B | Soil C | Soil D |
| First-order | $\ln C_t = \ln C_0 - kt$ | $T_{1/2}$ | 13.6 | 31.5 | 19.5 | 22.3 |
| | | $R^2$ | 0.8673 | 0.9387 | 0.9348 | 0.9003 |
| | | $K$ | 0.05096 | 0.02200 | 0.03554 | 0.03108 |
| Second-order | $1/C_t = 1/C_0 + kt$ | $T_{1/2}$ | 18.1 | 20.8 | 4.2 | 8.1 |
| | | $R^2$ | 0.9570 | 0.9759 | 0.9552 | 0.9370 |
| | | $K$ | 0.00595 | 0.00492 | 0.02445 | 0.01323 |
| Two-stage model | $C_t/C_0 = ae^{-k1t} + (1-a)e^{k2t}$ | $T_{1/2}$ | 3.7 | 9.9 | 4.3 | 5.4 |
| | | $R^2$ | 0.9875 | 0.9267 | 0.9943 | 0.9761 |
| | | $K_1$ | 0.4187 | 0.1153 | 0.3639 | 0.3276 |
| | | $K_2$ | 0.0021 | 0.0017 | 0.0019 | 0.0016 |
| | | $a$ | 0.6312 | 0.7279 | 0.6284 | 0.5979 |

of imazapyr (mg kg$^{-1}$), $C_t$ is the concentration (mg kg$^{-1}$) at time $t$, $K_1$ and $K_2$ are slow and fast degradation rate constants, and $a$ is a constant. $K_1$, $K_2$, $a$, and $R^2$ were determined using an iterative non-linear regression model by STATISTICA 6.0 software. As can be seen in table 3, based on the two-stage model, the estimated half-lives of imazapyr were closer to experimental data. The half-lives in soil A, B, C and D were 3.7, 9.9, 4.3, and 5.4 days, respectively, for the faster dissipation phase, which increased on an average 6-fold over that for the aerobic treatments and proved a quick biodegradation of imazapyr by anaerobic microbes in soils under anaerobic conditions. Furthermore, a significant correlation between imazapyr dissipation and soil pH was observed for aerobic treatments. On the contrary, this was not the case in anaerobic experiment, that is to say, soil pH had a slight effect on imazapyr disappearance under anaerobic conditions and a further investigation on the mechanism for the difference was required.



### 3.3 *Metabolites of imazapyr under aerobic and anaerobic conditions*

Imazapyr, when degraded under aerobic conditions in soils, gave three main metabolites, while only two main metabolites were observed under anaerobic conditions. They were separated and purified by preparative TLC as described previously. The spectral data of IR, $^1$H NMR and APCI-MS for the four metabolites, designated as M-1, M-2, M-3 and M-4, respectively, are summarized in table 4 and the corresponding structures for them are illustrated in figure 3.

Under aerobic conditions, the methyl and isopropyl group were lost, the imidazolinone ring opened and the new carboxyl group formed, giving M-1. The imidazolinone ring became detached, and the dicarboxylic anhydride formed, yielding M-2. In a more extended process, the imidazolinone ring was detached, the component parts rearranged to form new dicarboximide in 2,3-pyridine, which resulted in M-3. 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 (7H)-one; 2,3-pyridinedicarboxylic acid and two cyclic compounds, 2,3-pyridinedicarboximide and furo[3,4-b]pyridin-5(7H)-one. One of the photoproducts, 2,3-pyridinedicarboximide, was found under both photolysis in aqueous solution and degradation in this experiment.

Table 4. The spectral data of imazapyr metabolites in soils.

| Ref. No | Nomenclature | IR | $^1$H NMR | APCI-MS |
|---|---|---|---|---|
| M-1 | 2,3-pyridine-dicarboxamide | 3372, 3189 ($NH_2$), 1638 (C=O) | 7.86 (m, 3H, pyridine-4,5,6-H), 8.18 (br, 2H, pyridine-3-$CONH_2$), 8.42 (br, 2H, pyridine-2-$CONH_2$) | $m/z$ 166(M + 1) fragment ions 123 (–CO–$NH_2$), 80 (–$CONH_2$–$CONH_2$) |
| M-2 | 2,3-pyridine-dicarboxylic anhydride | 1805, 1742 (–COOCO–) | 7.63 (dd, 1H, pyridine-5-H), 8.26 (d, 1H, pyridine-4-H), 8.89 (d, 1H, pyridine-6-H) | $m/z$ 150 (M + 1) |
| M-3 | 2,3-pyridine-dicarboximide | 3275 (–NH–), 1757, 1729 (C=O) | 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) | $m/z$ 149 (M + 1) |
| M-4 | 2-(4-hydroxy-5-oxo-2-imdazolin-2-yl) nicotinic acid | 3477 (imidazolin-4-OH), 3270 (NH), 3085, 1670 (COOH), 1637 (imidazolin-5-CO) | 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) | $m/z$ 222 (M + 1), fragment ions 178 (–COOH), 162 (–COOH, –OH), 101 (-nicoticnic acid) |



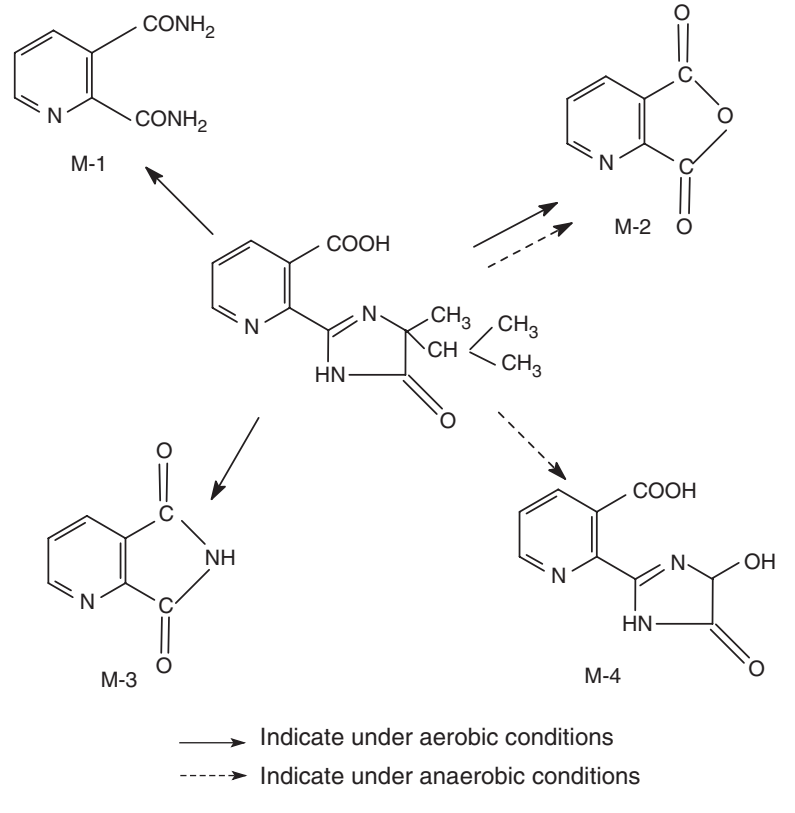


Figure 3. The main metabolites of imazapyr found in soils under different conditions.

However, under anaerobic conditions, only two main metabolites were found. The first product was found at $m/z$ 150 ($M + H^+$), which was formed through the opening of imidazolinone ring and rearrangement of the component parts. The same molecular weight and spectral data of $^1H$ NMR and IR proved the identical structure with M-2 under aerobic conditions. M-4 at $m/z$ 221 was formed accompanied by the loss of isopropyl group and hydroxylation at the 4-position of imidazolinone ring. The result showed that microorganisms having the ability to remove methyl and isopropyl groups could exist in the soil under anaerobic conditions. In addition, soil microbes under anaerobic conditions could replace methyl group by hydroxyl group at 4-position of imidazolinone ring, which was indicative of higher degrading ability than under aerobic conditions. Morrica *et al.* [24] also observed the phenomenon in the degradation of imazosulfuron.

As described above, it was clear that the main degradation pathways of imazapyr in soils under both aerobic and anaerobic conditions were demethylation, loss of the isopropyl group, cleavage and rearrangement of the imidazolinone ring.

## 4. Conclusions

The half-lives of imazapyr in four soils under aerobic conditions ranged from 26 to 44 days (by the first-order model), while varied between 3 and 10 days (by two-stage model) under anaerobic conditions, which demonstrated that a quicker biodegradation of imazapyr occurred under anaerobic than under aerobic conditions. In addition, this study also indicated that the soil pH value was one of the important factors affecting the degradation of imazapyr under aerobic conditions, while this was not the case under anaerobic conditions. On the basis of the structures of four identified metabolites, it could be concluded that they originated from loss of the methyl and isopropyl group, and cleavage and rearrangement of the imidazolinone ring. From the stand point of environmental protection, the observation in this study may be exploited further in environmental biotechnology for the quick and effective biodegradation in imazapyr-polluted areas.



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