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Biodegradation of imazapyr in typical soils in Zhejiang Province, China

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Abstract: The degradation of imazapyr in non-sterile and sterile soils from four sampling sites in Zhejiang, China was studied. The results showed that the half-lives of imazapyr in non-sterile soils were in the range of 30 to 45 d, while 81 to 133 d in sterile(by autoclaving) soils. It means the rate constants of imazapyr under non-sterile conditions were 2.3—4.4 times faster than that under sterile(by autoclaving) conditions, evidently indicating that the indigenous microorganisms in soil play an important role in the degradation of imazapyr. The different sterilization methods could result in different degradation rates of imazapyr. The heat of sterilization of soil largely decreased the degradation. However, the sterile treatment of soil by sodium azide had a different effect from that by autoclaving. Further more, the mechanism was also discussed. Biodegradation in four non-sterile soils accounted for 62% to 78% of imazapyr degradation. In contrast, less than 39% of imazapyr degradation was associated with chemical mechanisms. Therefore, the degradation mechanism was predominantly involved in biology including organisms and microorganisms in soil. Two imazapyr-degrading bacterial strains were isolated in enrichment culture technique and they were identified as *Pseudomonas fluorescenes* biotype II (ZJX-5) and *Bacillus cereus* (ZJX-9), respectively. When added at a concentration of 50 μg/g in mineral salts medium(MSM), ZJX-5 and ZJX-9 could degrade 81% and 87% imazapyr after 48 h of incubation. For the treatment of incorporation of ZJX-5 or ZJX-9 into soil, the degradation rate enhanced 3—4 fold faster than that for control samples, which showed an important value in quick decontamination of imazapyr in soil.

Keywords: biodegradation; imazapyr; sterile soil; non-sterile soil; imazapyr-degrading bacteria

Introduction

Organic pesticides applied to soil may be used as substrates by organisms and undergo degradation resulting in the formation of new compounds(Screenivasulu, 2001). It is generally accepted that this is due to proliferation of soil organisms that use pesticides as C or N sources, or both, for growth, which forms the foundation of bioremediation technique (Racke, 1990). Currently, the biological decontamination of pesticide wastes or spills has become an increasing important area of research, and it is preferable if a microbial or biological method of degradation is available for the various pesticidal compounds.

Imazapyr [2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid] is a non-selective herbicide, which was registered for control of most annual grasses and broadleaf weeds in rubber, oil palm, orchards, and on non-crop land (Miller, 1997). It kills plants by inhibiting acetohydroxyacid synthase(AHAS), the feedback enzyme in the biosynthesis of the branched-chain essential acids. The herbicide was introduced into China mainland market under the trade name Arsenal as 25% SL and 5.0% G in 2002. Imazapyr is more persistent in the soil environment than other non-selective herbicides and can control weeds as long as five months. Additionally, it is weakly adsorbed to soil and sediment and has the potential to leach to groundwater because it is very soluble in water (11272 mg/L at 25℃) which has raised concern about its safety to human health (Cox, 1996). Several studies have confirmed that microbial transformations are the essential mechanisms responsible for the degradation of imazapyr. It is degraded in soil under aerobic conditions to form minor metabolites, which leads ultimately to mineralization (Mangles, 1991). Mallipudi *et al.* (Mallipudi, 1991) reported the photolysis of imazapyr and detected four photoproducts. Metabolism of imazapyr by streptomyces(strain PS1/5) was first reported by Shelton *et al.* (Shelton, 1996), who found imazapyr could be transformed by it but to a lesser extent.

In addition, extensive research has been conducted on imazapyr with regard to its degradation, adsorption and desorption, mobility in soil (Azzouzi, 1998; Jenkins, 1998); availability to plants (Winfield, 1988); hydrolysis and photodegradation in water (Bouhaouss, 1998; Mansour, 1999; Elazzouzi, 1999). However, little quantitative information is available concerning its environmental behavior in soils under China mainland conditions. The primary objectives of the work were to study the contribution of biodegradation to imazapyr dissipation in four typical soils from Zhejiang Province, southeastern China and to elucidate the predominant kind of imazapyr-degrading bacterial strains in soil.

1 Materials and methods

1.1 Chemicals

Imazapyr (99.6%) 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.

1.2 Preparation of soil samples

Four different soils were collected from the different regions of Zhejiang Province, southeastern China: (1) Silt-loamy paddy soil from Shilifeng, Quzhou district; (2) Yellow-red soil from Huajiachi, Hangzhou district; (3) Coastal saline soil from Chixi, Jinhua district and (4) Blue clayed paddy soil from Jiashan, Jiaxing district, and they were numbered in this paper as soil A, B, C and D, respectively. Following the standard methodology of soil sampling, soils were air-dried, ground and passed through a 2-mm sieve. Selected physico-chemical properties of the soils were listed in Table 1. Soil pH was measured in slurry of soil and deionized water(1:2.5 by weight). The organic carbon content of soil was determined by oxidation with dichromate (Wu, 1990). The cation exchangeable capacity (CEC) of

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soil was determined by extracting the soil with buffered barium chloride solution at pH 8.1, adjusted with triethanolamine, following the method outlined by Yao *et al*. (Yao, 1992). The four different soils were classified as Silt-loamy soil, Sand-silty soil, Silty soil and Clay-loamy soil, respectively, by international soil-classification standard(Yu, 1994).

Table 1 The basic physico-chemical properties of soils used

Location	Soil No.	Type of soil	pH	Organic carbon, g/kg	CEC, cmol/kg	TN, g/kg	WHC, %
Shilifeng	A	Silt-loamy paddy soil	7.86	28.7	17.50	4.2	69.41
Huajiachi	B	Yellow-red soil	5.25	11.0	10.63	1.6	49.76
Chixi	C	Coastal saline soil	7.12	12.8	16.32	2.9	54.55
Jiashan	D	Blue clayed paddy soil	9.04	8.6	7.11	2.3	43.57

Notes: CEC—cation exchangeable capacity; TN—total nitrogen; WHC—water-holding capacity

1.3 Degradation of imazapyr in sterile (by autoclaving) and non-sterile soils

The degradation of imazapyr in sterile and non-sterile soils was conducted at an incubation temperature of 28 ± 1℃ in the dark under static conditions. Thirty-six flasks for sterile soil treatment and another thirty-six flasks for non-sterile soil treatment were prepared. For the sterilized soil treatment, each flask containing 10 g of the pretreated soil was sterilized by autoclaving for 1 h at 121℃. The flasks were then spiked with an imazapyr standard solution in methanol(50 μg/ml) to obtain a final concentration of 10 μg/g soil. The solution was mixed well with occasional stirring and the solvent allowed to evaporate for 2 h. The calculated amount of sterilized distilled water was then added aseptically to the soils to maintain 60% of water holding capacity(WHC). The flasks were weighed and kept at 28 ± 1℃. The weight loss due to evaporation of soil moisture was maintained by periodical addition of water at intervals of 5 d during the incubation period. The above-mentioned operations were carried out by aseptic techniques to maintain sterility. The effectiveness of the aseptic procedures performed was confirmed by microbial tests, which demonstrated no contamination by microorganisms at the end of each respective study. Four flasks were removed for each set and processed for analysis of imazapyr residues at specific time intervals of 0(2 h after spiking), 1, 5, 10, 20, 30, 40, 50 and 60 d after treatment(DAT). If the samples were not analyzed immediately, they were stored at −20℃ until analyzed.

1.4 Different sterile treatment

In the first case soil samples were autoclaved for 1 h at 121℃. In the second case the inhibitor(sodium azide) was mixed into non-sterile soil at 1 g/kg soil, the moisture of soil was adjusted to 60% of WHC and the subsequent operation was the same as the method described above.

1.5 Extraction and clean-up of soil samples

Each soil sample was spiked with 50 ml extraction solution(methanol: water, 70 : 30 by volume), and the pH was adjusted to 5 by 0.1 mol/L HCl. The solution was shaken vigorously for 1 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 1—2 ml with a rotary vacuum evaporator. The concentrated dichloromethane extracts were transferred to a glass column (1.0 cm ID, 20 cm length) packed with a mixture of Florisil (80—120 mesh) and acidic aluminum oxide(1 : 1 by weight), and followed by a rinse with a mixing solvent of methanol and ethyl acetate(20 : 80 by volume). The eluate was evaporated to dryness on a rotary evaporator, and the residue was redissolved in methanol (5 ml) for estimation by HPLC.

1.6 Recovery study

To estimate the recovery of imazapyr residue, a recovery study was carried out by spiking the soils with imazapyr standard solution (50 μg/ml) in methanol to obtain a final concentrations of 0.05, 0.1, 1.0, 10 μg/g in each kind of soil. The results showed 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%. Therefore, the method adopted for the analysis of imazapyr residue was satisfactory(data not shown).

1.7 Analysis of imazapyr residue

A HP1100 model high performance liquid chromatography(HPLC), equipped with diodearray detector (DAD), was performed 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 by volume) at a flow rate of 1.0 ml/min. The column was thermostated at 25 ± 1℃, the detector set at 234 nm wavelength and the injection volume was 20 μl. Under the above-mentioned conditions, the retention time of imazapyr was about 3.8 min.

1.8 Enrichment, isolation and identification of imazapyr-degrading bacteria

The imazapyr-degrading bacterial strains were isolated by enrichment culture technique, from Yellow-red soil receiving imazapyr application repeatedly to control railway weeds in 2002. Soil enrichments were developed by eight subsequent additions of 50 μg/ml of imazapyr at the day 7 intervals(Wang, 2003). Soil suspensions were prepared with the preceding enriched soil sample. Dilutions of soil suspension were prepared up to 10^{-7} dilution. The suspensions of 10^{-7} dilution were transferred to the sterilized nutrient agar medium (NAM), containing 3.0 g of beef extract, 5.0 g of peptone, 5.0 g sodium chloride and 15.0 g of agar dissolved in 1 L of distilled water(pH, 7.0—7.2) and incubated at 28℃ for 24 h. After 24 h of incubation, single colony was selected according to the different morphological and cultural characteristics, then purified by spread-plate technique and tested for their ability to use imazapyr as the sole source of carbon in mineral salts medium (MSM) as described below. MSM consisted of 1.0 g of sodium nitrate, 1.0 g of dipotassium hydrogen phosphate, 3.0 g of potassium dihydrogen phosphate and 0.5 g of magnesium sulfate in 1 L of distilled water(pH, 7.0—7.2). The isolated strains which exhibited a greater potential in degrading imazapyr than other isolates were isolated and identified by VITEK-AMS(Automatic Bacterial Identification System) as previously reported by Wang *et al*. (Wang, 2003).

1.9 Imazapyr degradation in soil by ZJX-5 and ZJX-9

A rate kinetics study was carried out using the imazapyr-degrading pure isolates, ZJX-5 and ZJX-9, at 20 μg/g imazapyr concentration. The suspensions of two isolated strains were prepared by growing the culture to high cellular densities at 28℃. The cells were harvested by centrifugation at 10000 g for 10 min, the supernatant was discarded and the

bacterial cells were washed three times with 0.1 mol/L phosphate buffer(pH 7.2) to make cells free from residual carbon sources. The phosphate buffer(pH 7.2) was used as control to measure bacterial cell densities at 486 nm(OD_{486}). The cell density was diluted to OD_{486} = 0.2 corresponding to 1.84×10^6 colony-forming units per ml for ZJX-5 and 1.23×10^6 CFU for ZJX-9, respectively, as the inoculation cell suspension for degradation studies.

Ten gram of the pretreated soil was transferred to conical flask and inoculated with 1 ml of the preceding cell suspension including by ZJX-5 or ZJX-9 for each treatment. The control soil set was maintained without bacterial inoculation. The soil was mixed well with occasional stirring for even distribution of the cells, allowed to air-dry for 2 h and then amended with imazapyr(20 μg/g dry soil weight). The calculated amount of sterilized distilled water was then added to the soil to maintain 60% of WHC. The flasks were weighed and incubated at 28(±1)℃ for 60 d in the dark as mentioned above. Each set was in four replicates and processed for analysis of imazapyr residues at specific time intervals of 0(2 h after application), 10, 20, 30, 45 and 60 d after treatment(DAT).

2 Results and discussion

2.1 Degradation of imazapyr in non-sterile soils

The disappearance of imazapyr with time-course over 60 d in non-sterile soils was shown in Fig. 1. The data at different intervals of time were modeled using first-order kinetics and the r values(−0.8946—−0.9900) for the data fit indicating that degradation of imazapyr was well described by first-order kinetics. Based on the first-order degradation, the estimated rate constants (k), half-lives ($T_{1/2}$) and coefficient of correlation(r) were given in Table 2. The half-lives of imazapyr in four non-sterile soils ranged from 30 to 45 d, which supported the results obtained by other workers. Ismail and his co-workers (Ismail, 1994) found that the residual half-lives of imazapyr under field conditions were 22 and 19 d at 25℃ in the clay and clay loam soils, respectively. Additionally, Azzouzi *et al.* (Azzouzi, 1998) 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.

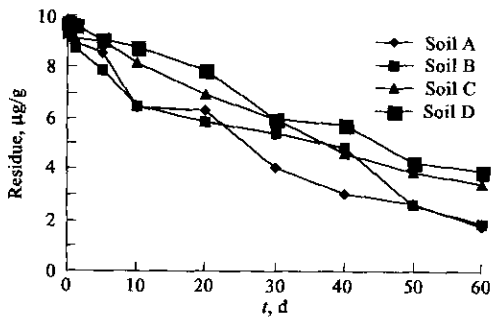

Fig. 1 Degradation curve of imazapyr in four non-sterile soils

As shown in Table 2, the highest half-life(44.1 d) in non-sterile treatment was found in soil D possessing the highest pH value(9.04), followed by soil B(39.6 d), with the lowest pH values (5.25) in four soils. In contrast, the lowest half-life(29.7 d) was observed in soil C, whose pH was close to neutral conditions(7.12). The preceding results revealed that the higher and lower soil pH values appeared to lead to higher persistence of imazapyr, while the neutral soils might be contributable the degradation of imazapyr. In neutral soils, microorganisms capable of degrading or contributing to degrading imazapyr are presumably in an optimal growth conditions, accelerating their propagation speed and thus enhance the degradation of imazapyr. Vizantinopoulos *et al.* (Vizantinopoulos, 1994) also observed that the half-life of imazapyr tended to increase with increase in soil pH. Sarkar (Sarkar, 2002) obtained the identical conclusion on the degradation of imidacloprid.

Table 2 Degradation kinetic data of imazapyr in four soils

Type of soil	Treatment	$T_{1/2}$, d	k	r
Soil A	Sterile(autoclaving)	133.3	0.0052	−0.9900
	Non-sterile	30.5	0.0227	−0.9341
	Sodium azide-treated	58.7	0.0118	−0.9812
Soil B	Sterile(autoclaving)	103.6	0.006689	−0.9554
	Non-sterile	39.6	0.0175	−0.9450
	Sodium azide-treated	55.1	0.0126	−0.9582
Soil C	Sterile(autoclaving)	81.5	0.008503	−0.9658
	Non-sterile	29.7	0.0233	−0.9547
	Sodium azide-treated	64.2	0.0108	−0.9911
Soil D	Sterile(autoclaving)	128.3	0.005401	−0.8946
	Non-sterile	44.1	0.0157	−0.9887
	Sodium azide-treated	74.5	0.0093	−0.9931

Notes: $T_{1/2}$, k, and r represent half-life, rate constant and correlation coefficient, respectively

2.2 Sterilization (by autoclaving) effect on imazapyr degradation

The degradation half-lives of imazapyr varied between 81 and 134 d and the corresponding rate constants ranged from 0.0052 to 0.0085 in four sterile(by autoclaving) soils(Table 2). Similarly, the trend of imzapyr degradation was also observed to be dependent on their corresponding pH values as described for the non-sterile treatment, i.e. the neutral soil C possessed the highest rate constants(0.008503) and the lowest half-life(81.5 d). Additionally, for each kind of soil, imazapyr degradation was faster in non-sterile soils than in sterile soils and the rate constants of imazapyr in soil A, soil B, soil C and soil D under non-sterile conditions increased 4.37, 2.62, 2.74 and 2.91 fold, respectively, faster than that under sterile conditions. Namely, the highest increasing rate was found in Silt-loamy paddy soil, followed by Blue clayed paddy soil, while the lowest was found in Yellow-red soil. Degradation in non-sterilized soil is a combination of biological and chemical reactions. However in sterilized soils by autoclaving, the main degradation process was attributable to chemical reactions. Sterilization by autoclaving killed microorganisms and altered the number of active enzymes capable of degrading or contributing to the degradation of imazapyr and the amount of some useful nutrient ingredients in soil. As a result, the degradation of imazapyr becomes very slow. The preceding analyses further confirmed that the indigenous microorganisms played an important role in degradation of imazapyr. and also supported the previous reports by Ismail *et al.* (Ismail, 1994). Thirty years ago, Beestman and Deming (Beestman, 1974) had found the mechanism that microbial decomposition was the major avenue for dissipation of most herbicides from soils, with chemical degradation accounting for less than 2% of the observed field losses under field conditions.

2.3 Influence of sterile method

The degradation rate of imazapyr was largely decreased by the heat of sterilization of soils. However, the treatment of soils by sodium azide had a different effect from that by autoclaving. As shown in Fig. 3, a greater slope for the

degradation curve of sodium azide-treatment was observed than that by autoclaving (Fig.2), which indicated that the degradation for sodium azide-treated soils was quicker than in the heated sterilized soils. The half-lives of imazapyr in four soils for sodium azide-treatment were 58.7, 55.1, 64.2 and 74.5 days, respectively, and the corresponding rate constants were 0.0118, 0.0126, 0.0108 and 0.0093, respectively, which increased 1.4—2.2 times over that for the autoclaving treatment. The corresponding highest and lowest increase of the degradation between autoclaving and sodium azide treatment were observed in soil A (2.27 fold) and soil C (1.27 fold), respectively. This suggested that the different sterile methods could lead to different degradation rates. Sodium-azide effectively suppressed microbial respiration and activity of the relevant enzymes, but did not bring about their death. However, sterilization by autoclaving killed microorganisms and altered the number of present, which ultimately resulted in the slower imazapyr degradation. Cambon *et al*. (Cambon, 1998) and Nicholls *et al*. (Nicholls, 2000) obtained the identical conclusions in the investigation of degradation of thifensulfuron-methyl and arylamide, respectively, in soils. In addition, another sterilization method using ethylene oxide was studied by Brown *et al*. (Brown, 1997) who reached an analogous conclusion with that of sodium azide.

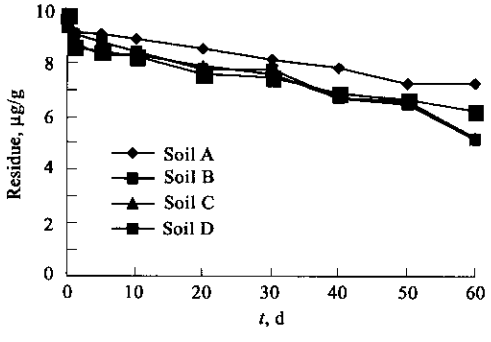

Fig.2 Degradation curve of imazapyr in four sterile(autoclaving) soils

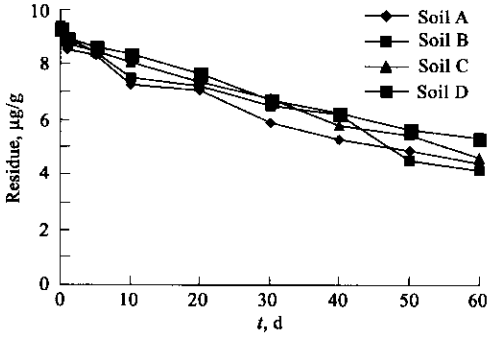

Fig.3 Degradation curve of imazapyr in four sterile(azide-treated) soils

2.4 Contribution of biodegradation to imazapyr transformation

As mentioned previously, the degradation of imazapyr in non-sterile soil involved biological and chemical transformation, while only chemical process occurred in sterile (by autoclaving) soil. It is generally accepted that chemical degradation is mainly involved in hydrolytic and photolytic transformation. The photolysis in this experiment could be negligible because the study was performed in the dark. As a result, the main chemical transformation was hydrolysis in this experiment and differences between rate constants of the sterile (by autoclaving) and non-sterile soil were assumed to be attributable to biological degradation.

As can be seen from Fig.4, biodegradation mechanisms in four non-sterile soils accounted for 57.7% to 77.1% of imazapyr degradation. The greatest percentage of biological degradation occurred in soil A, followed in soil D, while the lowest was observed in soil B. It is an interesting finding that the contributions from biodegradation in four soils generally agree with the order of the content of organic carbon except soil B, i.e. the higher organic carbon content generally lead to the greater contributions from biological transformation. It was obvious that high organic carbon content contained a great quantity of microorganisms capable of preferentially degrading imazapyr. In contrast, only 22.9% to 42.3% of the degradation was chemically associated and the percentage of chemical degradation mechanisms, amounting to imazapyr dissipation in four soils, was in the following order: soil B (42.3%) > soil C(36.5%) > soil D(34.4%) > soil A (22.9%). Overall, chemical mechanisms were the minor degradation process because less than 42.3% of imazapyr degradation was associated in each kind of soil. Consequently, the predominant degradation was involved in the biological mechanism, which further supported the results obtained by Dungan *et al*. (Dungan, 2001) who found that more than 60% of the 1,3-dichloropropene degradation was biologically associated and the degradation was also enhanced by organic amendment.

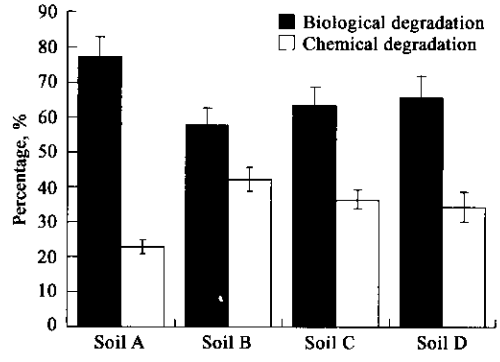

Fig. 4 Contribution of biological and chemical degradation to imazapyr dissipation

2.5 Isolation and identification of the imazapyr-degrading bacterial strains

Based on the different morphological and cultural characteristics, 14 bacterial strains numbered as ZJX-1, ZJX-2…ZJX-14, respectively, were isolated. Their degrading abilities to use imazapyr as the sole source of carbon were tested by its degradation rate after 48 h of incubation. The results in Table 3 showed that among 14 isolated strains only ZJX-5 and ZJX-9 were capable of degrading imazapyr quickly. When added at a concentration level of 50 μg/g in MSM, ZJX-5 and ZJX-9 could degrade 81% and 87% imazapyr, respectively, after 48 h of incubation which were significantly higher than that of other isolated strains and control. The strains were identified as *Pseudomonas fluorescenes* biotype Ⅱ and *Bacillus cereus* (Wang, 2003) by means of VITEK-AMS (automatic bacterial identification system). The identified confidence probabilities were 90% and 79%, respectively.

2.6 Effect of the addition of ZJX-5 or ZJX-9 to soil A on imazapyr degradation

The experiment was carried out to investigate the rate kinetics by incorporation of each imazapyr-degrading isolates into soil A. The amount of imazapyr recovered from soil with the herbicide (20 μg/g) at different time intervals was presented in Fig. 5, For the treatment of incorporation of

ZJX-5 into soil, the initial residue of 17.53 μg/g dissipated to 10.22, 5.96, 2.03, 0.69, 0.23 μg/g in 5, 10, 20, 30, and 40 d, respectively. In the first 5 d, as much as 41.70% of the added imazapyr was metabolized. This was followed by 24.3% in the next 5 d. At the end of the experimental period of 60 d, the residue was below detectable level. Overall, the degradation was very rapid in the first 10 d, then gradually declined with the passage of time. The rate of degradation was found to follow first-order kinetics with a half-life of 6.47 d.

Table 3 Degradation of imazapyr by the isolated strains

No.	Degradation rate in 48 h, % ± *SD*	No.	Degradation rate in 48 h, % ± *SD*	No.	Degradation rate in 48 h, % ± *SD*
CK	4.9 ± 0.1	ZJX-5	81.4 ± 5.9**	ZJX-10	10.5 ± 0.7
ZJX-1	4.3 ± 0.3	ZJX-6	2.2 ± 0.1	ZJX-11	9.9 ± 0.6
ZJX-2	5.8 ± 0.4	ZJX-7	6.6 ± 0.4	ZJX-12	6.9 ± 0.5
ZJX-3	10.4 ± 0.8	ZJX-8	7.4 ± 0.5	ZJX-13	7.1 ± 0.6
ZJX-4	7.8 ± 0.8	ZJX-9	86.6 ± 6.4**	ZJX-14	12.7 ± 1.0

Notes: *SD* represents standard deviation; ** marked significant difference at $P < 0.01$

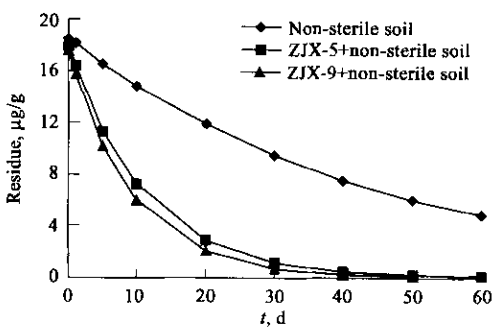

Fig. 5 Degradation curve of imazapyr by the incorporation of ZJX-9 into soil A

Similarly, when ZJX-9 was added to soil A, the initial residue of 17.91 μg/g dissipated to 11.38, 7.24, 2.93, 1.18, 0.48 μg/g in 5, 10, 20, 30 and 40 d, respectively. In the first day, only 8.65% of the added imazapyr was metabolized, which was followed by 36.46% and 59.58% in the 5 and 10 days. After 60 d of incubation, only 0.08 μg/g of the applied imazapyr was recovered from soil, that is to say, more than 99% degradation of imazapyr occurred. The rate of degradation was also found to follow first-order kinetics ($r = -0.9245$) with a half-life of 7.65 d, which was slightly slower than that by ZJX-5 treatment. But the half-life of control set was 30.9 d(Table 4), namely, the degradation rate for the addition treatment(ZJX-5 or ZJX-9) enhanced 3—4 fold faster than that for control. Although the intrinsic microorganisms in soil could degrade imazapyr, the degradation rate was rather slow, whereas the addition of the effectively imazapyr-degrading bacteria could lead to its quick dissipation.

Table 4 Kinetic parameters of imazapyr degradation by ZJX-5 or ZJX-9 in soil

Treatment	$T_{1/2}$, d	k	r	C_0
ZJX-5 + non-sterile soil	6.47	0.10788	-0.9149	17.53
ZJX-9 + non-sterile soil	7.65	0.090588	-0.9245	17.91
Non-sterile soil	30.9	0.02242	-0.9359	18.55

Note: C_0 represents initially recovered concentration

3 Conclusions

The results indicated the degradation of imazapyr increased 2.6—4.4 times faster in non-sterile soils than in sterile(by autoclaving) soils. The intrinsic microbes in soil played an important role in degradation of imazapyr, which represented 57.7% to 77.1% of degradation in four non-sterile soils. Two isolated bacterial strains, by enrichment culture technique, showed high degrading capacity for imazapyr. When ZJX-5 or ZJX-9 was added to soil, the degradation accelerated 3—4 times faster than that for control set. Consequently, although the intrinsic microorganisms in soil could degrade imazapyr, the degradation was rather slow, whereas the addition of the effectively imazapyr-degrading bacteria could lead to its quick dissipation. Thus, the results obtained in this experiment showed an important application value of bioremediation of imazapyr in soil.

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