Effect of Temperature and Moisture on Degradation of Herbicide Atrazine in Agricultural Soil

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Abstract— *Degradation rate and degraded products, deethylatrazine (DEA), deisopropylatrazine (DIA) and atrazine-2-hydroxy (HA), of herbicide atrazine in agricultural soil were determined by ultra-performance liquid chromatography–mass spectrometry. When treating soils for 60d at 25°C, the degradation rate of atrazine increased with the moisture from 5 to 20%. The degradation was fitted one-order kinetic equation and degradation rate constant (k) and Half-life ($T_{1/2}$) were obtained. $T_{1/2}$ decreased by 3–4 folds with the increasing temperature from 5 to 35°C and moisture from 5 to 20%. The species and content of the degraded products increased with the temperature and moisture. When treating soil for 60d, the sum content of three degraded products, DEA, DIA and HA is 3–6.8 times greater than atrazine residue. An updated metabolism pathway of atrazine in soil was presented.*

Keywords— Atrazine, Degradation kinetics, Degradation products, Metabolism pathways, Soil.

I. INTRODUCTION

Modern agricultural practices often require extensive use of herbicides for production of corn, soybeans, sorghum, and other row crops. Due to the continuous use of herbicides in agriculture, appreciable quantities of herbicides and their degraded products may accumulate in the ecosystem leading to serious problem to man and the environment. Herbicides, which enter the soil environment, are subject to a variety of degradation processes. The overall degradation of a pesticide from soil results from a combination of mechanisms such as microbial degradation, chemical hydrolysis, photolysis, volatility, leaching and surface runoff. The degree to which each mechanism will contribute to the overall degradation of the pesticide is in turn dependent on the physicochemical properties of the pesticide, characteristics of the soil, environmental conditions and management practices.[1] Therefore, it is essential to study the residue and degradation pattern of herbicides in soil, crops and water systematically in order to generate meaningful data from the point of view of plant protection, public health and environmental safety.

Atrazine is one of the most widely used herbicides all over the world. It is a pollutant of environmental concern due to its low biodegradability and its high potential to contaminate the surface waters and ground water. Several epidemiological cancer studies concerning atrazine and its possible association with carcinogenic effects in humans are being reviewed by the US EPA.[2] The acute toxicity of atrazine's metabolites such as deethyl- and deisopropylatrazine was found to be twice as that of atrazine.[3] Although several countries gave up the use of atrazine because of its toxicity, it is still one of the most popular herbicide in many countries.[4] Therefore, the development of ecological farming is encouraged.

The factors influencing the degradation of pesticides in soil were reviewed.[1)] An updated overview of atrazine degradation by microorganisms under different ecosystems was presented.[5] Some microbial consortia were reported for their metabolic cooperative actions by investigating the individual's contribution in atrazine degradation.[6,7] The degradation of atrazine in plant and water was reported by using biotic or abiotic method.[8-10] Biodegradation of atrazine in different soils using various bioprocessed material were investigated.[11] The best studied atrazine-degrading bacterium is Pseudomonas sp. strain ADP which was isolated from a herbicide spill site.[12] Dependence of accelerated degradation of atrazine on soil pH in French and Canadian soils was investigated, where both hydroxylated and dealkylated atrazine metabolites were detected, but no clear pattern of metabolite production could be determined.[13] Different environmental conditions obviously influence on the degradation of atrazine. The effects of temperature and moisture on the soil net nitrogen mineralization was investigated based on N content change,[14] but without degradation information before mineralization process. The degradation rates of atrazine in the control soil at three temperatures were measured by quantification with high performance liquid chromatography (HPLC).[15] This study showed that its degradation was faster in the unsterilized soil than in sterilized soil and illustrated that microbial degradation contributed to the overall degradation**,** but without any information for the degraded products.

Currently, no information is available concerning the combined effect of temperature and moisture on the degradation rate and its degraded products of atrazine in agricultural soil. The primary objective of this study was to assess simultaneously the effects of temperature and moisture upon atrazine degradation in loam soil with an ultra-performance liquid chromatography–electrospray ionization–mass spectrometry (UPLC–ESI–MS). This information will be useful in understanding the behavior of atrazine in soil in a temperature- and moisture-controlled environment, and in selecting the conditions needed for achieving optimal degradation, as well as in evaluating the potential impact of its degraded products.

II. MATERIALS AND METHODS

2.1 Chemicals and Materials

Atrazine, deethylatrazine (DEA), deisopropylatrazine (DIA), and atrazine-2-hydroxy (HA) (>98.5% for all) were purchased from Dr. Ehrenstorfer Chemical Industries (Augsburg, Germany). The methanol, acetonitrile, ammonium acetate and formic acid (liquid chromatography grade for each) were purchased from Dikma Science and Technology Co., Ltd (Beijing, China).

2.2 Instrumentation

UPLC-ESI-MS/MS analysis of soil samples were performed on a Xevo Triple Quadrupole (TQ) system (Waters, USA). This system consisted of an autosampler, a binary pump, a solvent degasser, a BEH C18 stainless steel cartridge column (100 mm ×1 mm i.d., 1.7μm; Waters) equipped with a guard column at 40°C, and a TQ mass spectrometer. A HZ-9202S water bath temperature oscillator(Equipment Factory of Science and Education, Taichang, China), a centrifuge TGL-16M (Xiangyi Centrifuge Co. Hunan, China), a RE-2000A rotary evaporator (Shanghai Yarong Biochemistry Instrument Co.), and a PHS-3C pH meter (Shanghai Precision & Scientific Instrument Co., Shanghai, China) were used in sample treatment.

2.3 Treatment of Soil Samples

The soil (sandy loam) used in this study was taken from a field in Zanhuang county, Xingtang county, and Shijiazhuang countryside, which was marked as soil 1#, soil 2#, and soil 3#. The fresh surface soil samples (0–20cm) were treated by removing gravel, grass root, beaten leaf followed by over 40 mesh sieve for use in the test. The soil had a pH of 7.5–8.1 and contained 20–24 g/kg of organic matter. After the fresh soil samples were dried at ambient temperature (20°C), a volume of 0.8 mL atrazine standard in acetonitrile (1.000 mg/mL) was added in an 80 g of the dried soil sample. The initial concentrations of atrazine for the temperature and moisture studies were 10 mg/kg. After evaporated acetonitrile in fume hood, the test soil samples contained with 10% of water were prepared with sterile deionized water. After stir to mix well, the soil samples were placed in a constant temperature incubator to investigate the degradation within 60d under treatment at 5, 25, and 35°C, respectively. The content of atrazine residue was determined in triplicate by UPLC-MS method at different treatment times.

The soil sample was brought to the desired moisture content by addition of sterile deionized water. Soil moisture content was maintained constant throughout the incubation by weighing and correcting for any weight loss by adding sterile deionized water. The soil samples were placed in a constant temperature incubator to investigate the degradation within 60d under treatment at 25°C and moisture content of 5, 10, and 20%, respectively. The content of atrazine residue was determined in triplicate by above method.

2.4 Analysis of Atrazine and Degraded Product in Soil Samples

A portion of 3 g test sample was accurately weighed and added into a 50 mL centrifuge tube. After vortex-mixing for 1 min, atrazine and three degraded products were extracted for 10minusing 12 mL of acetonitrile. The extracts were centrifuged at 9980×g for 6 min. Then, the supernatant was transferred into a heart-shaped bottle and the residue was again extracted. The extracts were concentrated to just dryness by a rotator evaporator at 35°C. The dried residue was re-dissolved into 0.5 mL acetonitrile/water (20: 80, v/v). The final solutions were filtered through a 0.22 μm filter membrane before LC–MS analysis.

Waters ACQUITY UPLCTM BEH C18 column (100 mm ×1 mm i.d., 1.7μm) was used for separation with a mobile phase consisted of aqueous solution containing 0.05% *v/v* formic acid (A) and acetonitrile (B), anda column temperature of 40°C. Sample injection volume was 5 μL. Separation was performed using gradient elution at a flow of 1 mL/min. Initial conditions were 20% acetonitrile and 80% formic acid solution (0.05%) with a linear gradient to 50% of acetonitrile within 6 min. After that, the initial conditions were reached within 2 min, and the system was equilibrated for 8 min.

The mass spectrometer used was a triple quadruple equipped with an ESI interface operating in the positive mode. Quantification was carried out by using matrix matched standards with the external calibration. The TQ parameters, retention

times, parent ions and daughter ions as well as collision energy for atrazine and its degraded products were presented in our last work.[16]

III. RESULTS AND DISCUSSION

3.1 Analytical Performance of the Method

For atrazine, DIA, DEA, and HA, positive mode was found to offer higher parent ion signal intensities and better fragmentation patterns than negative mode. The matrix effect was observed, showing enhancing effect (15%), so quantification was performed using matrix matched standards with the external calibration. Further test showed no significant differences in the mass accuracies obtained in the matrix-matched standards compared to those prepared with pure solution. The results demonstrated that the quantification using a matrix-matched standard calibration curve is the most effective method of reducing indirect matrix effects present in this method. The response was linear over two orders of magnitude with correlation coefficients (r^2) of 0.9975. The limits of quantification for atrazine and its degraded products were 0.4 µg/kg. The intra-day precision (RSD) was measured by determining a set of spiked soil samples at 0.4, 2.0, and 4 µg kg^{-1} for seven times within a day and the inter-day precision was determined by analyzing the spiked samples for a time per a day within seven days. They were 2.2–9.3%, and 5.7–17.1%, respectively. The average recovery varied from 87.1 to 99.4 % with the RSD of 1.6–11.8%. It is indicated the method has high sensitivity, repeatability, and accuracy for the quantification of atrazine and its degraded products.

3.2 Effect of Soil Temperature on Degradation Rate

In this work, three kinds of unsterilized soils (3 samples for each kind) containing 10% water were treated for 2h–60d at temperature of 5, 25, and 35°C, respectively. The content of atrazine in the control soil was determined in triplicate at different times for investigating its degradation dynamics. The degradation curves are shown in Figure 1.

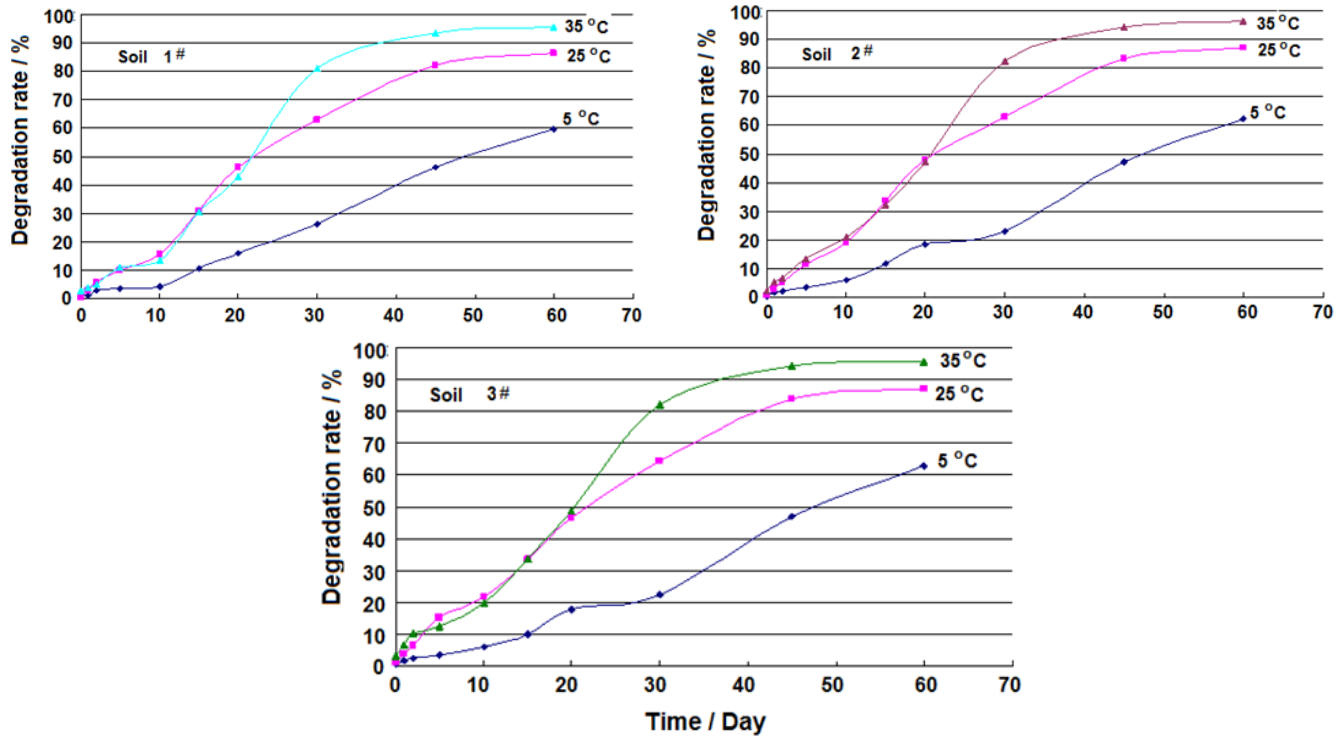

FIG. 1. DEGRADATION OF ATRAZINE IN UNSTERILIZED SOIL AT 5, 25 AND 35°C RSDS FOR EACH DEGRADATION RATE POINT LESS THAN 5%

In general, residual quantity of atrazine decreased with the increase in temperature. Degradation was faster at all three temperatures in the unsterilized soil. Among them, degradation was slowest at 5°C and fastest at 35°C with 25°C being intermediate. At treatment temperature at 5. 25, and 35 °C for 60d, the degradation rate of atrazine was 59.8, 86.4,and 95.6% for soil 1#, 62.2, 87.3, and 96.3% for soil 2# sample, and 63.1, 87.1, and 95.9% for soil 3#.The dissipation data determined for atrazine were treated by statistical analysis with SPSS software. In all cases, dissipation of atrazine was

fitted to the first-order kinetic equation: $C = C_0 e^{-kt}$, with correlation coefficient (r^2) ranged from 0.938 to 0.986, where C_0 is the concentration of atrazine in soil at time zero (mg/kg), k is the first-order rate constant (day^{-1}) and t is time (day). Half-life ($T_{1/2}$) values for atrazine in soil were calculated from the equation: $T_{1/2}$= ln2/k. Kinetic parameters are listed in Table 1.

TABLE 1
KINETIC PARAMETERS OF ATRAZINE IN SOIL AT DIFFERENT TEMPERATURES

Kinetic parameter	Soil 1#			Soil 2#			Soil 3#		
	5°C	25°C	35°C	5°C	25°C	35°C	5°C	25°C	35°C
C_0	10.54	10.64	12.06	10.57	10.56	11.88	10.63	10.40	11.614
r^2	0.961	0.984	0.956	0.950	0.986	0.962	0.938	0.983	0.959
k	0.015	0.036	0.057	0.015	0.033	0.060	0.016	0.036	0.058
$T_{1/2}$(d)	46.2	19.3	12.2	46.2	20.7	11.6	43.3	19.3	12.0

As adsorption process is exothermic and desorption process is endothermic, it is expected that adsorption reduced and herbicide solubility increased with increase in temperature. Chemical degradation of a herbicide through hydrolytic reactions is dependent on the nature of the chemical and the characteristics of the soil. The climate can directly influence the rate of hydrolysis through modulation of the temperature and moisture of the soil. Soil microorganisms play an important role in the intermediate degradation and subsequent mineralization of atrazine.[17,18]

Temperature influences obviously constant (k) of degradation rate, resulting both k and $T_{1/2}$ increased by 3–4 fold for 5 to 35°C. This is due to that the increase in temperature enhanced the solubility and hydrolysis of atrazine and stimulated microbial activity. Under 25 and 35°C, $T_{1/2}$ was less than 13d. There was no obvious difference for $T_{1/2}$ between the three kinds of soil samples due to that the soil has similar organic matter content (20–24 g/kg).Since soil microbial activities are strongly modulated by temperature, atrazine degradation would be expected to be greater in tropical soils.

3.3 Effect of Soil Temperature on Degraded Product

The catabolites of atrazine were confirmed and their contents in the soil with 10% moisture were determined in triplicate under different treatment time at 5, 25, and 35°C. In general, there was no obvious difference of degradation product content for the selected three soils under same temperature and treatment time. When treatment at 5°C, degradation product DEA was jet observed up to 15d, the content of which was a little more than its LOQ. The content of DEA increased with treatment time. DEA was firstly found in atrazine degradation products, it is due to higher deethyl rate of microorganism to atrazine than deisopropyl.[19] When up to 45d, degradation product DIA was detected, while no HA was detected up to 60d. Three degraded products, DEA, DIA, and HA, were detected when treatment at 25°C for 5d, 15d, and 20d, and when treatment at 35°C for 5d, 10d, and 15d, respectively. This indicated that the species and concentrations of the degraded products increased with the increasing temperature. Figure 2 shows chromatograms of atrazine and its degradation products in the soils with 10% moisture for different treatment times at 35°C.

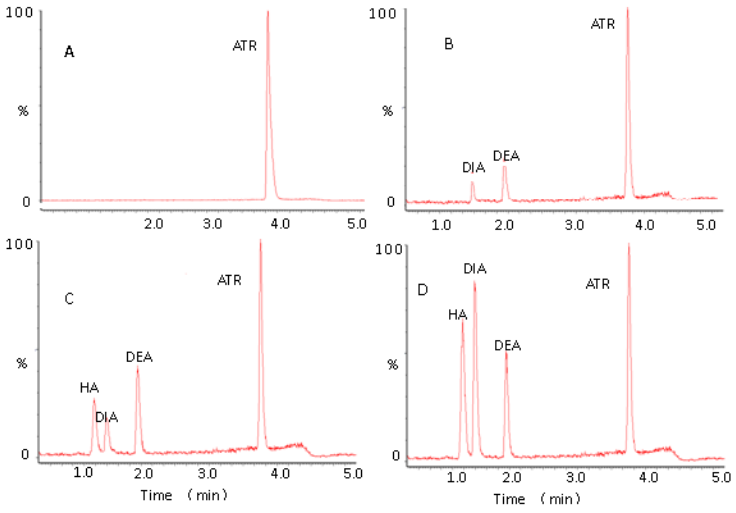

FIG. 2. CHROMATOGRAMS OF ATRAZINE AND ITS DEGRADED PRODUCTS IN SOIL 1# WITH 10% MOISTURE FOR DIFFERENT TREATMENT TIMES AT 35 °C. A—2h, B—10d, C—30d, D—60d

When treatment for 60d at 35°C, the content of atrazine, DEA, DIA, and HA was 0.44, 0.39, 0.60, and 0.34 mg/kg for soil 1#; 0.37, 0.41.1.43, and 0.53 mg/ kg for soil 2#; 0.37, 0.39, 1.42, and 0.69 mg/kg for soil 3#. In the cases for three soils, the sum content of three degraded products is 3, 6.4, and 6.8 times greater than atrazine residue, respectively. This should be caused for more concern due to that these degraded products have similar or higher toxicity than atrazine residue.

3.4 Effect of Soil Moisture on Degradation Rate

Water acts as solvent for herbicide movement and diffusion and is essential for microbial functioning. Herbicide degradation is slow in dry soils. The rate of herbicide transformation generally increases with water content. In very wet soils such as rice paddies, the rate of diffusion of atmospheric oxygen into the soil is limited and anaerobic herbicide transformation can prevail over aerobic degradation. Poor oxygen transfer at high moisture content can, however, accelerate or retard the degradation of pesticides. Atrazine disappeared more transformation rapidly under anaerobic conditions than under aerobic conditions.[1] Three kinds of soil samples containing 5%, 10%, and 20% water were placed for 2h–60d at temperature of 25°C. The content of atrazine in the control soil was determined in triplicate at different times for investigating its degradation dynamics. The degradation curves are shown in Figure 3.

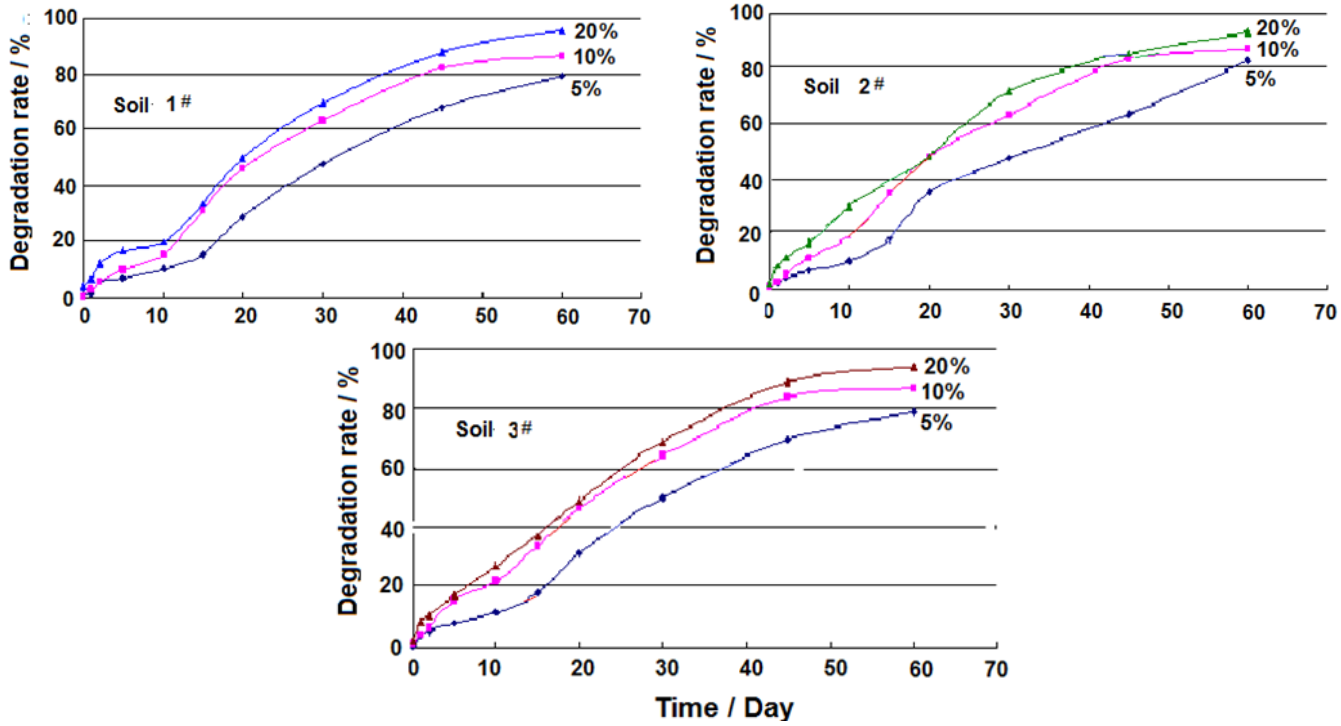

FIG. 3. DEGRADATION OF ATRAZINE IN UNSTERILIZED SOIL WITH DIFFERENT MOISTURE CONTENTS AT 35°C

Our experiments indicated that degradation rate increased with moisture content in the unsterilized soil. Among them degradation of atrazine was slowest at 5% and fastest at 20% with 10% being intermediate. For treating soil 1#, soil 2#, and soil 3# for 60 d, the degradation rate of atrazine was 79.2, 82.1, and 78.7% for 5% moisture, 86.4, 87.3, and 87.1% for 10% moisture content, and 95.7, 93.1, and 93.9% for 20% moisture content, respectively.

The dissipation data determined for atrazine were treated by statistical analysis with SPSS software. In all cases, dissipation of atrazine was fitted to the first-order kinetic equation, with correlation coefficient ranged from 0.936 to 0.980. Kinetic parameters are listed in Table 2.

TABLE 2

KINETIC PARAMETERS OF ATRAZINE IN SOIL WITH 5%, 10%, AND 20% MOISTURE CONTENT

kinetic parameter	Soil 1#			Soil 2#			Soil 3#		
	5 %	10%	20%	5 %	10%	20%	5 %	10%	20%
C_0	10.85	10.64	11.39	10.85	10.56	10.49	10.69	10.40	10.83
r^2	0.965	0.971	0.953	0.950	0.981	0.972	0.936	0.980	0.960
k	0.026	0.036	0.050	0.027	0.033	0.044	0.026	0.036	0.047
$T_{1/2}$(d)	26.7	19.3	13.7	25.7	20.7	15.8	26.7	19.3	14.8

Soil moisture content influences obviously degradation rate constant (k), resulting $T_{1/2}$ decreased by 3–4 fold when soil moisture content increased from 5% to 20%. This is due to that moisture content increased microbial mobility, atrazine diffusion, and chemical availability.[20] Since soil microbial activities are also strongly modulated by moisture content, atrazine degradation would be expected to be greater in wet soils within rainy season.

3.5 Effect of Soil Moisture on Degraded Product

The degraded products were confirmed, and their contents in soil with different moisture content were determined in triplicate under different treatment times at 25°C. In general, there was a difference of degraded product content for the three soils with different moistures and same treatment time. For the soil with the three moisture contents, when treatment for 5d, low content DEA was detected, and the content in the three soils was same (8 μg/kg). The content of DEA increased with treatment time. After treated for 15d, DIA was detected for the three soils with 20% moisture content, and for two soils with 10% moisture content; After treated for 20d, HA was detected for the three soil with 10 and 20% moisture content, while no HA was detected for the three soils with 5% moisture content after treated for up to 60d. This change is similar with that of degradation rate with moisture content. Figure 4 shows the relative contents of atrazine and its degraded species in the soils with different moistures for treatment 60d at 25°C.

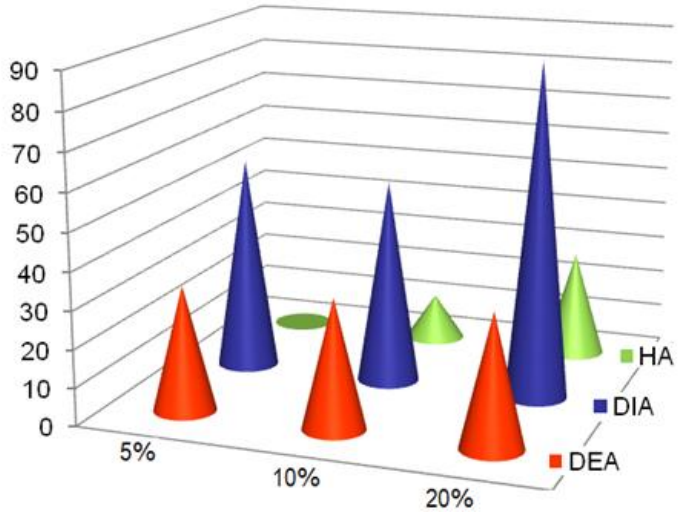

FIG. 4. THE RELATIVE CONTENT OF ATRAZINE'S DEGRADED PRODUCTS IN SOIL 1# WITH DIFFERENT MOISTURES FOR TREATMENT 60d at 25 °C.

When treatment for 60d, test results showed the content of atrazine, DEA, DIA, and HA was as follows: for the soils with 5% moisture content, 2.08, 0.3317, 0.5691 and 0 mg/kg for soil1#, 1.79, 0.3437, 0.5694, and 0 mg/kg for soil2#, and 2.13, 0.3451, 0.6013, and 0 mg/kg for soil3#; for the soils with 10% moisture content, 1.36, 0.3415, 0.5413, and 0.1218 mg/kg for soil 1#; 1.27, 0.3766, 0.7684, and 0.1731 mg/kg for soil 2#; 1.29, 0.3709, 0.6332, and 0.1122 mg/kg for soil 3#; and for the soils with 20% moisture content, 0.43, 0.3491, 0.8884, and 0.2808 mg/kg for soil 1#, 0.69, 0.3966, 1.2073, and 0.4833 mg/kg for soil 2#; and 0.61, 0.3699, 0.8421, and 0.3642 mg/kg for soil 3#. It can be seen from above data that the sum content of degraded products increased with moisture content. It is due to that moisture content increased microbial mobility, atrazine diffusion, and hydrolysis efficiency.[20,21] For the soils with 20 % moisture content, the sum content of degraded products was 3.5, 3.0, and 2.6 times greater than atrazine residue for soils 1#, 2# and 3#, respectively. This should be caused for more concern due to that these degradation products have similar or higher toxicity than atrazine residue.

3.6 Metabolism Pathways of Atrazine in Soil

Metabolism of atrazine in soil is a very complicated process, consisting four main steps: dehalogenation, *N*-dealkylation, deamination, and ring cleavage. Based on the mass spectra[16] and some relative reports,[2,6,22,23] this study summary and presents an updated metabolism pathway of atrazine in soil (Figure 5).

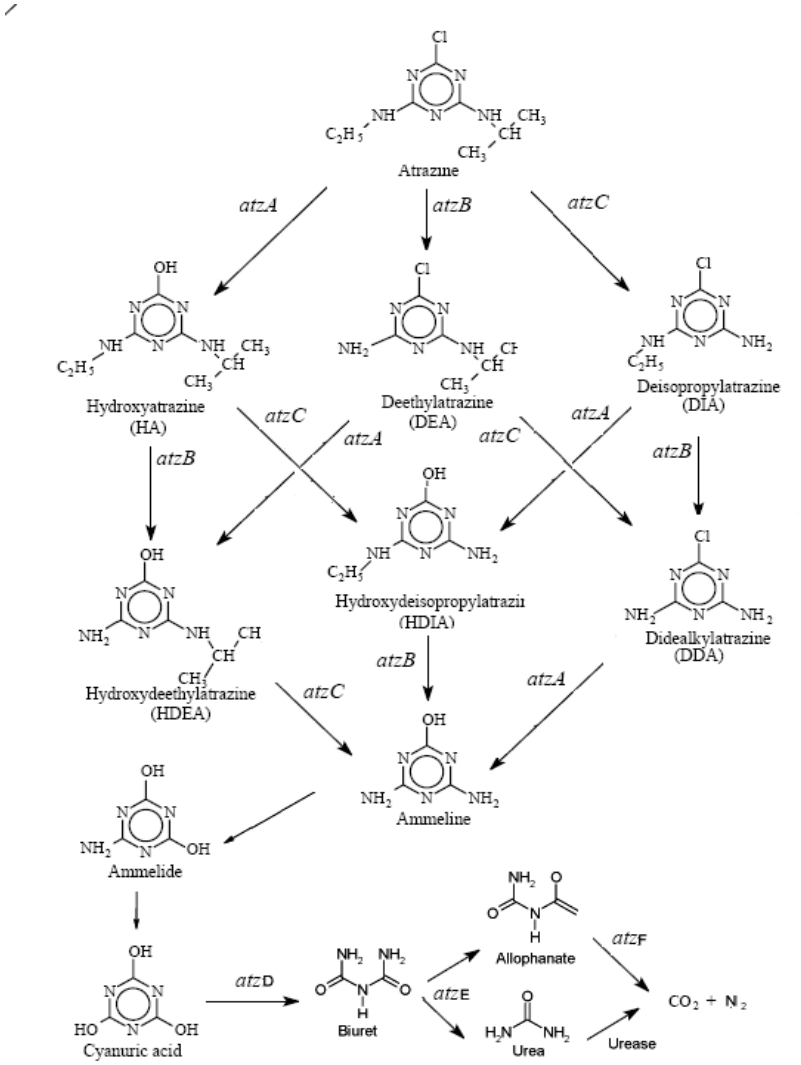

FIG. 5. METABOLISM PATHWAYS OF ATRAZINE IN SOIL

Atrazine degrades in soil through both biotic and abiotic reactions to the hydroxylated metabolite and dealkylated metabolites. The atrazine degrading bacteria generally initiate the degradation through a hydrolytic dechlorination, to hydroxyatrazine (HA) through a hydrolytic dechlorination catalysed by the enzyme atrazine chlorohydrolase (atzA). Atrazine may degrade to deethylatrazine (DEA) through a hydrolytic deethylnation catalysed by hydroxy-atrazine ethylamino-hydrolase (atzB), and to deisopropylatrazine (DIA) through N-isopropyl-ammelide isopropyl-amino-hydrolase (atzC). HA may degrade to dealkylated HDIA and HDEA.[24] DEA may further degrade to the dealkylated hydroxymetabolitesof didealkylatrazine (DDA) and hydroxydeethylatrazine (HDEA), DIA may further degrade to the hydroxymetabolites of DDA and HDIA. The three degraded products, DDA, HDIA, and HDEA, may further degrade to ammeline through hydrolytic dechlorinationc atalysed by atzA, through hydrolytic deethylnationcatalysed by at zB, and through hydrolytic deisopropylnation catalysed by atzC, respectively. Ammeline may further degrade to ammelide through hydrolyticdeamination, and ammelide further to cyanuric acid through hydrolyticdeamination. The ring of cyanuric acid was opened by cyanuric acid amidohydrolase (atzD), produced biuret, which may degrade by biuret amidohydrolase (atzE) to allophanate and urea. Allophanate may mineralize by allophanatehydrolase (atzF) to carbon dioxide and ammonia,6 and urea may mineralize to carbon dioxide and nitrogen gas.[25]

IV. CONCLUSIONS

The ultra-performance liquid chromatography–mass spectrometric method proposed can be used to investigate the degradation rate and degraded products, deethylatrazine (DEA), deisopropylatrazine (DIA) and atrazine-2-hydroxy (HA) of atrazine in soil. Atrazine degradation can be accelerated by regulating soil temperature and soil moisture, fitted one-order kinetic equation. When treating soil for 60d, the sum content of above three degraded products is 3–6.8 times greater than

atrazine residue. The information for its metabolism pathway in soil will be useful in understanding the behavior of atrazine in soil in a temperature- and moisture-controlled environment, and in selecting the conditions needed for achieving optimal degradation, as well as in evaluating the potential impact of its degraded products.

ACKNOWLEDGEMENTS

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