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Laboratory Degradation Studies of ^{14}C-Atrazine and -Isoproturon in Soil from Sugarcane Cultivated Fields Under Kenyan Tropical Conditions

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Abstract A study to compare the degradation rates of atrazine (6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diammine) and isoproturon [3-(4-isopropylphenyl)-1,1-dimethylurea] in soils from sugarcane fields with different practices of herbicides application was carried out. ^{14}C-atrazine was poorly mineralized to $^{14}CO_2$ (1.10% ± 0.22%) after 139 days of incubation in soil without previous exposure to atrazine. In the same soil also with no previous isoproturon exposure isoproturon was mineralized to $^{14}CO_2$ by 7.70% ± 0.94%. Atrazine mineralization after 98 days was 13.4% ± 0.30% in soil which discontinued the use of atrazine in 1997 while it was 89.9% ± 1.23% in soil in which atrazine is currently being used. The isoproturon mineralization values were 7.24% ± 0.85% and 22.97% ± 0.96% in soil which discontinued atrazine and soil currently using atrazine, respectively.

Keywords Soil · Mineralization · Isoproturon · Atrazine

Both phenylurea and s-triazine herbicides are used worldwide for pre- and post-emergence weed control in corn, sorghum, sugarcane and other crops (Sørensen et al. 2003; Khadrani et al. 1999; Shaner et al. 2007; Wackett et al. 2002). Degradation of chemicals can involve biotic and abiotic processes, where microbially facilitated biodegradation is especially interesting, as it is a major process in the complete mineralization of aromatic compounds to harmless inorganic products (Alexander 1981). The halogen, methylthioether, and *N*-alkyl substituents on the s-triazine ring of the herbicides impede facile microbial metabolism (Wackett et al. 2002). This has also been observed in some of the halogenated phenylura herbicides such as methabenzthiazuron, diuron, metobromuron and monuron (Berger 1999). As result some of the compounds most frequently used such as atrazine and isoproturon are frequently detected in surface and ground waters (Yassir et al. 1999; Mahia and Diaz-Ravina 2007; Sørensen et al. 2003).

However, enhanced degradation has been observed for atrazine and isoproturon in soils where they have been applied repeatedly and used for a long time with subsequent isolation of the bacterial strains which metabolized the pesticides to get C, N and energy for growth (Barriuso and Houot 1996; Sørensen et al. 2001). Enhanced degradation is a phenomenon whereby, a soil-applied pesticide is rapidly degraded by a population of microorganisms that has developed the ability to use the compound as a C, energy and or nutrient source because of previous exposure to it or its analogue (Krutz et al. 2008).

In Kenya both s-triazine and phenylurea herbicides have been used in various sugarcane fields to control weeds for more than 20 years. One of the s-triazine herbicides that is frequently used and has been used for a long time is atrazine. Isoproturon is not among the phenylurea herbicides used in the sugarcane fields in Kenya. In a previous laboratory study of atrazine degradation in soil from one of the sugarcane fields atrazine mineralization was enhanced by adding organic amendments (Getenga 2003). There has

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been no study before to ascertain if the soils in the Kenyan sugarcane fields have developed enhanced degradation of the soil–applied herbicides. The objective of our study was to find out if both atrazine and isoproturon could be degraded rapidly in the soils from the sugarcane fields.

Materials and Methods

Soils were collected in December 2006 from three sugarcane fields with different practices in herbicides application. In field (N) both atrazine and isoproturon had not been used. In field (D), both s-triazine and phenylurea herbicides including atrazine but not IPU had been used for the past 20 years until 1997 when atrazine was discontinued. In field (U), both phenylurea and s-triazine herbicides which include atrazine but not isoproturon are currently being used. The pH, total C and N for the soils were determined (Table 1).

Uniformly ^{14}C-ring labeled atrazine (specific activity 1.628 MBq/mg; radio-purity of 98%) was purchased from Sigma–Aldrich (St. Louis Missouri, USA). The labeled atrazine was mixed with unlabeled one to give new specific radio-activity of 7 Bq/μg. ^{14}C-ring-labeled isoproturon with specific radioactivity of 3.96 MBq/mg and radio-purity of 98% was mixed with commercial available product arelon to a final concentration of 500 mg/mL according to guidelines provided by the pesticide producer Agrevo (Frankfurt, Germany), and resulting in final specific radioactivity of 686 Bq/μg. Analytical standards and their metabolites were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Scintillation cocktails were obtained from Packard (Dreieich, Germany). All other chemicals and solvents were of analytical grade and were purchased from Merck (Darmstadt, Germany).

Aliquots of 50 g-dry soil samples spiked with uniformly ^{14}C-ring labeled and non-labeled pesticides (atrazine and isoproturon) were placed in double walled 100 mL round flasks and incubated at 20 ± 1°C in darkness. Before laboratory incubation, the soils at the soil density of 1.3 g/cm^3 were moistened to optimum soil water contents of 24.6%, 24.9% and 21.4% for the soils N, D, and U, respectively. The atrazine-amended soil had an initial pesticide concentration of 24.6 μg/g and radioactivity of 2,097.2 Bq/g dry soil. The isoproturon-amended soil had initial isoproturon concentration of 24.6 μg and radioactivity of 2,815.5 Bq/g dry soil. Soils sterilized with 1,000 μg $HgCl_2$/g dry soil were used as controls. The flasks with pesticide-spiked soils were connected to a closed laboratory trapping system and aerated thrice per week for 1 h at an air exchange rate of 1 L/h to trap $^{14}CO_2$. The trapping system and sampling of the trapping solutions have previously been described (Schroll et al. 2004). The incubation for each soil was conducted in quadruplicates.

Table 1 Measured values of pH, total N and C for the soils

Source of soil	pH	Total N (%)	Total C (%)
Soil from field N	6.5	0.08	4.2
Soil from field D	4.8	0.13	1.2
Soil from field U	5.6	0.10	1.0

Isoproturon and its metabolites in the methanol extracts from soil after incubation in the laboratory were extracted, cleaned and analyzed by HPLC (Berthold, Wildbad, Germany) as previously described (Getenga et al. 2004). Atrazine and its metabolites in methanol extracts were obtained from soil as previously described (Gan et al. 1999) in an accelerated solvent extractor, ASE 200 (Dionex, Germany). The methanol extracts were cleaned and concentrated through triazine SPE columns before analysis by HPLC (Berthold, Wildbad, Germany). The analysis was performed by HPLC equipped with both Hitachi UV detector L-7400 and Berthold radioflow detector LB 506Cl for radioactivity measurement. The measurements were conducted at the following conditions. Mobile phase composition; A = acetonitrile (HPLC grade, Riedel-de Haen, Seelze, B = buffer (0.003 M KH_2PO_4 at a pH of 3). At time (min) = 0 (T0), A = 20%, B = 80%, T2: A = 38%, B = 62%, T16: A = 75%, B = 25%, T25: A = 20%, B = 80%. The UV detector was set at 220 nm and the flow rate of the mobile phase was 1.0 mL/min.

Results and Discussion

Mineralization of ^{14}C-atrazine to $^{14}CO_2$ after 139 days of soil incubation was very low (1.10% ± 0.22%) in soil from the field (N) where the two herbicides had not been used. However, ^{14}C-isoproturon mineralization in the same soil was higher (7.70% ± 0.94%) for the same period of soil incubation. There was enhanced atrazine mineralization (89.9% ± 1.23%) in soil from the field (U) where atrazine is currently being used but isoproturon has not been used before. In the same soil ^{14}C-isoproturon mineralization to $^{14}CO_2$ was 22.97% ± 0.97% after 98 days of soil incubation in the laboratory. In soil from the field (D) where atrazine was discontinued in 1997, ^{14}C-atrazine mineralization was 13.4% ± 3.0% while ^{14}C-isoproturon mineralization was (7.24% ± 0.85%) after 98 days (Fig. 1). The extractable and non-extractable residues of the herbicides remaining in the soils after the experiments are shown (Table 2).

Many studies on atrazine metabolism in soil using ^{14}C-ring labeled atrazine have shown that $^{14}CO_2$ is produced. However, the amount of $^{14}CO_2$ produced from some soils could not be attributed to the applied radio-labeled molecule

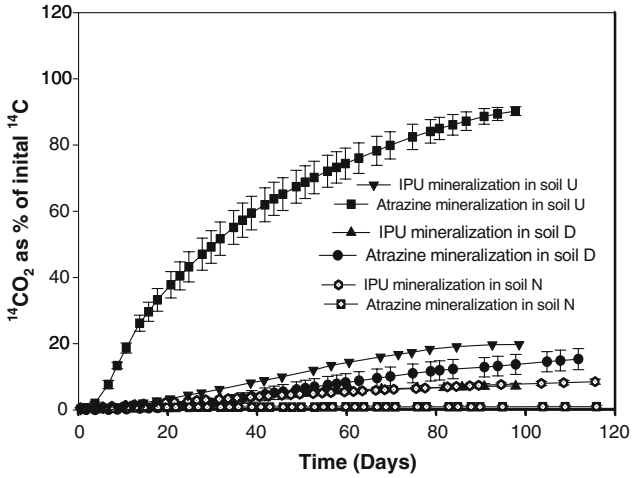

Fig. 1 Mineralization of ^{14}C-atrazine and ^{14}C-isoproturon in the soils

(atrazine), because the percentage of ^{14}C evolved as $^{14}CO_2$ was equal or less than the concentration of ^{14}C impurities in the radio-labeled atrazine (Winkelmann and Klaine 1991). Langenbach et al. (2000) observed after 95 days of soil incubation 0.2% mineralization of atrazine to $^{14}CO_2$ from ^{14}C-labeled atrazine with a radio-purity of 99%. Shapir and Mandelbaum (1997) found that ^{14}C-labeled atrazine with radio-purity of 98.6% could only be mineralized to $^{14}CO_2$ by about 1% in upper soil layer after 30 days. Dousset et al. (1997) observed that the quantity of $^{14}CO_2$ (0.8%) evolved from the soils with ^{14}C-labeled atrazine of radio-purity of 98.6% could not be attributed to atrazine mineralization but to impurities. In our study, the ^{14}C-labeled atrazine had a radio-purity of 98%, with 2% being impurities. The 1.10% ± 0.22% of $^{14}CO_2$ produced during atrazine mineralization in soil with no history of previous atrazine use could be due to the impurities in radio-labeled atrazine. The slow mineralization of atrazine by native soil microbes in most soils has been attributed to the halogen on the atrazine ring which impedes facile microbial metabolism (Wackett et al. 2002). However, isoproturon mineralization was higher with an average value of 7.47% ± 0.9% for the two soils where isoproturon has not been used before. The absence of a halogen substituent on the phenyl-urea ring of isoproturon may explain the higher mineralization by native soil microbes as observed by Sørensen et al. (2003). However, halogenated phenylurea herbicides such as diuron, methabenzthiazuron, metobromuron, monuron, linuron and fluometuron have been found to be slowly mineralized in agricultural soils (Berger 1999).

In the soil from the field (D) where the use of atrazine had been discontinued, the recorded total atrazine mineralization of 13.40% ± 3.0% with a lag phase of 10 days could be attributed to the presence of adapted atrazine degraders developed when the soil was exposed to the herbicide. Due to the exposure of the soil to atrazine again in the laboratory, the adapted atrazine degraders were reactivated and therefore atrazine mineralization was higher than in the soil where atrazine had not been used. The mineralization rate increased to a maximum value of 0.92% $^{14}CO_2$/day after 17.7 days (Fig. 2). However, atrazine mineralization in most soils has been reported to be poor where adapted atrazine degraders have not developed (Winkelmann and Klaine 1991; Langenbach et al. 2000; Shapir and Mandelbaum 1997; Dousset et al. 1997). The isoproturon mineralization in all the soils with no exposure to isoproturon can only be attributed to the presence of

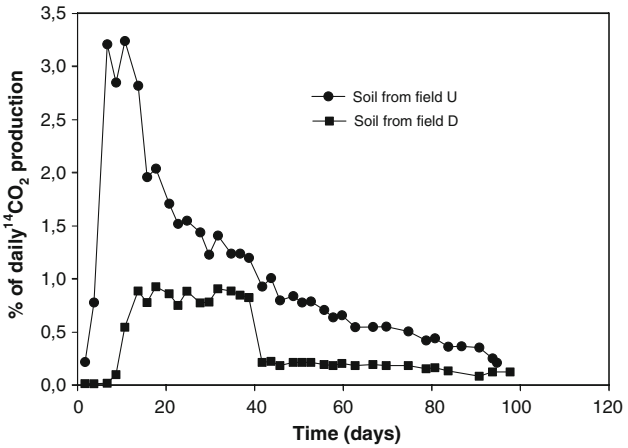

Fig. 2 Rate of $^{14}CO_2$ production from ^{14}C-atrazine with time in soils from fields D and U

Table 2 Mass balance for ^{14}C-atrazine and ^{14}C-isoproturon in the soils

Soil source	Herbicide	Accumulated $^{14}CO_2$	Extractable residue (%)	Non-extractable residue (%)
Field N	Atrazine	1.10 ± 0.22	65.8 ± 2.5	33.2 ± 2.6
	Isoproturon	7.70 ± 0.94	28.0 ± 4.31	66.3 ± 2.23
Field D	Atrazine	13.40 ± 3.0	45.3 ± 12.9	41.3 ± 12.3
	Isoproturon	7.24 ± 0.85	57.83 ± 1.66	34.88 ± 1.27
Field U	Atrazine	89.9 ± 1.23	2.65 ± 1.17	7.4 ± 0.30
	Isoproturon	22.97 ± 0.96	27.36 ± 2.45	49.76 ± 1.59

non-specific native soil microbes which appeared to co-metabolize isoproturon readily.

Enhanced atrazine mineralization (89.9% ± 1.23%) occurred in the soil from the field (U) where atrazine is being used with other herbicides. Atrazine mineralization started immediately after incubation of the soil. Maximum mineralization rate of 3.24% $^{14}CO_2$/day for atrazine was attained after 6.7 days (Fig. 2). The high atrazine mineralization in the soil can only be attributed to the presence of highly adapted atrazine degraders in the soil developed by long exposure of the microbes to atrazine. The mineralization curve for the soil did not show a lag phase because the adapted atrazine degraders were already used to atrazine as a substrate. The isolation and characterization of the adapted atrazine degraders from the soil through liquid culture enrichment techniques is still going on in our study. Many previous studies have reported enhanced atrazine mineralization in soil with subsequent isolation and characterization of numerous atrazine-degrading strains belonging to diverse bacteria genera (Marion et al. 2005).

The five metabolites from atrazine in the soil (Table 3) are due to both chemical and microbial activities. In soils which do not have adapted atrazine degraders, atrazine is normally hydrolyzed chemically to hydroxyatrazine (HA), which itself can be acted upon by nonspecific monooxygenases on the ethyl side chain to form desethylhydroxyatrazine (DEHA; Wackett et al. 2002). However, DEHA was not detected in this study. The monooxygenases from native soil microbes can also act directly on atrazine converting it into desethylatrazine and desisopropylatrazine (Marion et al. 2005), hence the observed metabolites. The other metabolite desisopropylhydroyatrazine (DIHA), which was also detected in this study, resulted from the initial chemical hydrolysis of atrazine into HA which was then acted upon by the monooxygenases through the isopropyl side chain to form DIHA. We could not detect any metabolite of atrazine in the soil from field U where atrazine was metabolized rapidly. The extractable residue in the soil at the end of the experiment was only 2.65% ± 1.17% of the initially applied atrazine and therefore, the metabolites could not be detected.

Table 3 Metabolites of atrazine and isoproturon in the soil field N

Metabolite	Unidentified	Atrazine	HA	DIA	DEA	DIHA
Metabolites from atrazine						
R_t (min)	13.80	11.70	10.13	7.32	6.30	3.10
Metabolite	IPU	MDIPU	DDIPU			
Metabolites from isoproturon						
R_t (min)	35.87	34.37	32.90			

HA hydroxyatrazine, *DEA* desethylatrazine, *DIA* Desisopropylatrazine, *DIHA* desisopropyl hydroxyatrazine, *MDIPU* Monodesmethyl isoproturon, *DDIPU* didesethylisoproturon

The metabolites from isoproturon were monodesmethyl isoproturon and didesethyl isoproturon only. The degradation pathway of isoproturon has been found to proceed via initial cometabolic steps followed by metabolic processes. The initial attack is demethylation resulting in the removal of one methyl group followed by removal of another methyl group from N of the urea side chain (Lehr et al. 1996). Isoproturon like any other phenylurea has been shown to be stable to chemical degradation within the pH range of 4–10. Consequently, chemical degradation of isoproturon in soils is of minor importance (Sørensen et al. 2003). The two metabolites observed in this study therefore resulted from microbial attack of isoproturon.

Results from the study showed that atrazine could not be mineralized in soil where atrazine had not been used before. The moderate atrazine mineralization which was observed in soil which discontinued the use of atrazine in 1997 showed that adapted atrazine degraders could lose their ability to degrade atrazine and reactivated on exposure to the herbicide. The high mineralization in soil with adapted atrazine degraders substantially reduced atrazine residues in the soil thus minimizing the threat of contamination of surface and ground water by the herbicide. Isoproturon appeared to be easily mineralized in all soils which had not been exposed to the herbicide. The absence of a halogen on the phenyl ring of isoproturon may explain why the native soil microbes could easily mineralize isoproturon.

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