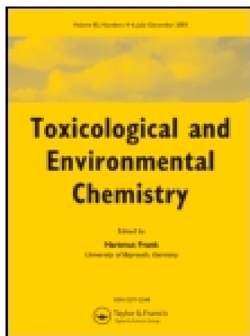

Toxicological & Environ Chemistry

ISSN: 0277-2248 (Print) 1029-0486 (Online) Journal homepage: https://www.tandfonline.com/loi/gtec20

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To cite this article: Z.M. Getenga , U. Doerfler & R. Schroll (2009) Study of atrazine degradation in soil from Kenyan sugarcane-cultivated fields in controlled laboratory conditions, Toxicological & Environ Chemistry, 91:2, 195-207, DOI: 10.1080/02772240802144497

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Toxicological & Environmental Chemistry
Vol. 91, No. 2, March 2009, 195–207

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Study of atrazine degradation in soil from Kenyan sugarcane-cultivated fields in controlled laboratory conditions

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(*Received 26 September 2007; final version received 19 April 2008*)

A study to compare the extent of atrazine mineralization in soils from Kenyan sugarcane-cultivated fields with and without history of atrazine use was carried out in the laboratory under controlled conditions. The study was testing the hypothesis that repeated atrazine application to soil will not result in enhanced atrazine mineralization. The study was carried out with ^{14}C-uniformly ring-labeled atrazine in a laboratory under controlled conditions. Atrazine mineralization to $^{14}CO_2$ in soil with no history of atrazine use was negligible (0.16%) after 163 days of soil incubation. The three metabolites hydroxyatrazine, desisopropylatrazine, and desethylatrazine in the proportion of 17.7%, 1.3%, and 2.6%, respectively, were in the soil after 75 days. In the soil from the sugarcane-cultivated field with history of atrazine use, atrazine mineralization was 89.9% after 98 days. The same soil, amended with mature compost, showed a lag phase of eight days before rapid atrazine mineralization was observed.

Keywords: atrazine; metabolites; mineralization; lag phase; compost

Introduction

Atrazine [6-chloro-N^2-ethyl N^4-isopropyl-1,3,5-triazine-2,4-diamine] is the most widely used worldwide among the *s*-triazine compounds as a herbicide to control numerous broad-leaved weeds in crops such as corn and sorghum, among other crops (Abdelhafid, Houot, and Barriuso 2000). It is a major herbicide used extensively in sugarcane production (Selim and Zhu 2005). Due to its widespread and frequent application, persistence in soil and dispersion to surface and ground waters, atrazine is frequently detected in water at concentrations above the permissible levels. For instance, in 1987 a study of Iowa public water systems by Struthers, Jayachandran, and Moorman (1998) revealed the presence of atrazine and other pesticides at concentrations of $10\,\mu g\,L^{-1}$ in surface water and $1500\,\mu g\,L^{-1}$ in ground water.

Atrazine can be degraded by both chemical and biological processes in soil environments, resulting in the formation of metabolites hydroxyatrazine (HA), desethylatrazine (DEA), and desisopropylatrazine (DIA) (Shin and Cheney 2005). DIA and DEA are more toxic than atrazine, while HA is less mobile than atrazine (Munier-Lamy, Feuvier, and Chone 2002). The degradation of atrazine to HA proceeds

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ISSN 0277–2248 print/ISSN 1029–0486 online
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DOI: 10.1080/02772240802144497
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chemical transformations at low pH in the presence of humic acids. Microbial *N*-dealkylation of the ethyl and isopropyl side chains, which is accomplished by nonspecific monooxygenases from indigenous soil microbes, produces DEA and DIA, respectively (Smalling and Aelion 2004). Atrazine was considered to be poorly biodegradable during the 1960s to 1980s as soil indigenous microbes could not utilize atrazine as a source of C or N and energy source (Wackett et al. 2002). However, in subsequent years, acclimation of the soil microflora to atrazine mineralization after repeated applications of this herbicide under field conditions was observed (Abdelhafid, Houot, and Barriuso 2000). Complete biodegradation of atrazine to ammonia and CO_2 has been obtained. The studies showed a wide variation in the kinetics and extent of atrazine degradation and the ability of the bacteria to grow on atrazine. In addition, the stimulatory or inhibitory effects of additional carbon or nitrogen sources on atrazine degradation vary among the bacteria described in the literature (Struthers, Jayachandran, and Moorman 1998). In order to minimize dispersion of atrazine outside the agricultural environments, several attempts have been undertaken in laboratory studies in the past to increase the degradation of atrazine in soils (Wackett et al. 2002). Metabolization of atrazine by indigenous soil bacteria (bio-stimulation) has been enhanced while applying appropriate and formerly limiting nutrient amendments to soils without adapted microflora in laboratory studies (Alvey and Crowley 1995; Gan et al. 1996; Getenga 2003; Houot, Barriuso, and Berghead 1998). Furthermore, soils without adapted microflora to enhanced atrazine mineralization have been stimulated by bioaugmentation whereby nonindigenous microorganisms that are capable of catabolizing atrazine are added to soils to enhance the degradation of the chemical (Aislable et al. 2005; Rousseaux et al. 2003). The results showed that atrazine-degrading bacteria applied as single strains or as a consortium could increase degradation of atrazine in soil, but the treatments varied in their effectiveness (Krutz et al. 2007).

In the present study, we investigated the kinetics of atrazine degradation by soils from two sugarcane-cultivated fields in Kenya, one without history of atrazine use and the other field with history of atrazine use. Atrazine had been applied to soil six months prior to soil samples collection from the other field. The purpose of the study was to compare the atrazine mineralization in soils from the two sugarcane-cultivated fields and to study the effect of mature compost on the soil which exhibited enhanced atrazine mineralization. The hypothesis being tested is "repeatedly used atrazine in a field does not result in adapted atrazine degraders which enhance atrazine mineralization in soil. Soil amended with mature compost will not enhance atrazine mineralization in the soil."

Materials and methods

Soils and experimental plots

Soil samples were collected from two different sugarcane-cultivated fields, one with no history of atrazine use (WNH) and the other with history of atrazine use (WH). The sugarcane cultivated field (WNH)) is located within 34°50′49″E to 35°35′41″E longitude and 0°4′55″N to 0°20′11″S latitude. The other field (WH), which is located at latitude 0°41′S and longitude 34°48′E and is 100 km away from the other field, was treated with atrazine six months prior to sampling. The soils were partially characterized and their characteristics are presented in Table 1.

Soil–water retention curves were determined generally according to Hartge and Horn (1989) with the following modification: air-dried and sieved soil samples ($\leq$2 mm) were

Table 1. Characteristics of the soils spiked with ^{14}C-ring labeled atrazine and IPU for mineralization studies.

Source of soil	pH	Total N (%)	Total C (%)
Soil with no prior exposure to atrazine	6.5	0.08	4.2
Soil with current atrazine use	5.6	0.10	1.0

pressed into small metal rings ($10\,cm^3$) to achieve a soil density of $1.3\,g\,cm^{-3}$ and then rewetted to yield water holding capacity (WHC). Using a sand/kaolinbox (08.02 Eijkelkamp, NL) the lowest limit of water tension measured was −0.05 MPa. Further measurements (≤ -1.5 MPa) were obtained using a pressure extractor (1500 F1, Eijkelkamp, NL) with a ceramic plate. Samples were allowed to equilibrate under pressure, after which the gravimetric water content was determined by weighing. Measurements were collected from five independent experiments and the resultant water-retention curves per soil were pooled to a mean curve that was used for comparing water contents to water potentials.

Chemicals

Uniformly ^{14}C-ring labeled atrazine (specific activity 9.5 mCi $(mmol)^{-1}$; radio-purity of 98%) was purchased from Sigma Aldrich (St. Louis Missouri, USA). The labeled compound was mixed with the nonlabeled one to give new specific radioactivity of 0.416 mCi $(mmol)^{-1}$ atrazine. Analytical standards of atrazine and of its 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).

Incubation experiments

We studied the mineralization of ^{14}C-labeled atrazine under laboratory conditions, as previously described by Schroll et al. (2006) Soils (50 g, dry weight equivalent, each) were incubated in 100 mL double-wall flasks in the dark at $20°C \pm 1°C$. Humidified air ($1.0\,L\,h^{-1}$) was drawn via a pump through the system at three intervals per week. After passing through the flasks, the air was trapped in four subsequent absorption tubes, the first two of which were filled with ethyleneglycolmonomethylether to fix volatile ^{14}C-substances, and the following two were filled with 0.1 M NaOH solution to fix $^{14}CO_2$ from mineralization processes separately.

Prior to experiments, aliquots of 46.5 g soil (dry weight equivalent) were equilibrated at soil water content equivalent to soil water tension of −35 KPa for two weeks at $20°C \pm 1°C$. ^{14}C-atrazine was dissolved in acetone prior to application. The pesticide (1.23 mg; 1.05×10^5 Bq) was applied drop-wise onto 3.5 g of previously dried (105°C, 24 h) grounded soil with a Hamilton syringe and then mixed intensively. The soil aliquots were transferred to equilibrated soils, mixed well with a spatula and filled into incubation flasks. Subsequently, soils were compacted with a spatula to a volume equivalent to a soil density of $1.0\,g\,cm^{-3}$. According to soil water tension curves, drops of water were applied to soil to achieve a final soil water tension of −15 KPa. Schroll et al. (2006) showed that the

Table 2. Characteristics of the compost used as an exogenous organic amendment.

Parameter	C	N	P	Cu	Mn	Fe	Zn
Value	34.9%	1.14%	0.72	140	2217	1272	755

Note: Concentrations of the elements P, Cu, Mn, Fe, and Zn were expressed in $\mu g\,g^{-1}$.

microbial processes in soil were optimized at the water tension of −15 KPa. All our degradation experiments in different soils were conducted at a soil–water tension of −15 KPa. Soil moisture was controlled at the beginning and end of experiments, gravimetrically (balance-Kern, 572, Germany, precision of measurement ±10 mg), weighing the double walled flasks.

Experiments were set up to kinetically follow the biodegradation of atrazine in soil by quantifying the extractable, bound residues, $^{14}CO_2$ evolved and metabolite formation from atrazine in soil where atrazine mineralization was negligible at varying reaction periods in 250 mL amber biometer flasks. Each biometer flask was filled with soil previously moistened to optimum water content as explained above. A 15 mL plastic vial containing 10 mL of 0.1N NaOH solution was suspended inside the biometer to trap the $^{14}CO_2$ evolved. A solution of NaOH was replaced with a fresh one whenever sampling was done. The evolved $^{14}CO_2$ was trapped by the 10 mL of 0.1N NaOH, which was sampled periodically. An aliquot of 2 mL of the 0.1N NaOH was taken and mixed with 3 mL of Ultima Flo scintillation cocktail before being radio-assayed by a liquid scintillation counter (Tricarb 1900TR, Packard, Germany). The experiments were conducted in quadruplicates.

In another set of experiments with soil in which enhanced atrazine mineralization was observed, the effect of exogenous organic amendments on atrazine mineralization was investigated. Mature compost made from municipal organic waste was added to soil at different concentrations ranging from 1000 μg to 10,000 $\mu g(g)^{-1}$ dry soil. The data on the quality of the compost used in the study is shown in Table 2. Mineralization was monitored by trapping$^{14}CO_2$ in 0.1N NaOH solution as described above. At the end of experiments, extractable and nonextractable ^{14}C-pesticide residues were quantified. Aliquots of soil material were extracted by accelerated solvent extraction (ASE 200, Dionex, Germany) as previously described by Gan et al. (1999). All degradation experiments were run in quadruplicates. An aliquot of 1 mL of the methanol extract was mixed with 4ml of Ultima Gold cocktail and radio-assayed to quantify extractable ^{14}C residue of atrazine. To determine nonextractable (bound) residue, an aliquot of 250 mg of the air-dried extracted soil was placed in paper cones mixed with sucrose solution before combustion in the materials oxidizer (Oxidzer 306, Packard, Germany).The $^{14}CO_2$ released during combustion was trapped in 8 mL of Carbsorb E (Packard, Germany), which was mixed with 12 mL of permafluor E (Packard, Germany) prior to scintillation counting. A ^{14}C-mass balance at each reaction period was computed.

Metabolites isolation and identification in soil

Atrazine and its metabolites in methanol extracts were obtained from soil as previously described by 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 a Hitachi UV detector L-7400 and a Berthold radioflow detector LB 506Cl for radioactivity measurement. The measurements were conducted at the following conditions. Mobile phase, A = acetonitrile (HPLC grade, Riedel-de Haen, Seelze, Germany), B = buffer (0.003M 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$(min)^{-1}$.

Basal respiration rate determination at different soil water tensions

We carried out an experiment to find out the effect of varying soil water tensions on basal respiration rate in soil. The soil moisture at different soil water tensions was measured as already described above. Aliquots of 100 g of dry soil was moistened to achieve the desired soil moisture content at a given soil water tension, as measured by both the sand/kaolin box and pressure palate extractor. The 100 g of soil aliquots were compacted in 250 mL polyethylene containers to give a soil density of 1.3 g cm^{-3}. The compacted soils in the containers were placed in a respirometer (Respicond IV, Nordgren, Innovations, Sweden). The evolved CO_2 from respiration was trapped by 0.1N KOH solution. The reaction between KOH solution and the evolved CO_2 as a result of the basal respiration of the indigenous soil microbes resulted in changes in conductivity, which was measured and monitored continuously. The measurements were conducted in a water bath maintained at 20°C. The rate of CO_2 formation (CO_2 μg h^{-1} g^{-1} dry soil was computed from the amount of CO_2 collected for 10 days. At the end of the experiment, microbial biomass in the soil at different moisture contents was determined by vacuum chloroform fumigation extraction method.

Microbial biomass determination in soil at different soil water tensions

Soil microbial carbon was measured by a slight modification of the original method proposed by Jenkinson and Powlon (1976). Aliquots of 5 g equivalent of dry soil were taken in glass vials for chloroform fumigation. Two sets of soil samples were prepared, one set for fumigation and the other set as a control. The nonfumigated (control) set of soil samples was immediately extracted with 0.5 M K_2SO_4 solution by shaking for 45 min on an oscillating shaker. After shaking, the mixture was filtered using Whatman No. 42. The filtrate was frozen until the total organic carbon (TOC) was determined. The other set of soil samples was fumigated as follows. The soil samples in duplicates were placed in a vacuum desiccator lined with moist paper towels at the bottom. Twenty-five milliliter of methanol-free chloroform was placed in flask with boiling chips. Another flask with soda lime pellets was placed on the moist paper towel. The soil samples were placed in the vacuum desiccator, which was sealed in a laboratory hood and evacuated with a vacuum pump, allowing the chloroform to boil for 2 min. The desiccator was then saturated with chloroform vapor and left with the soil samples for 24 h. At the end of the incubation period the desiccator was air-flushed eight times in order to remove the chloroform. The glass vials with the fumigated samples were removed from the vacuum desiccator. An aliquot of 20 mL of 0.5 M K_2SO_4 solution was added to each fumigated soil sample in the glass vial and shaken for 45 min. The mixture was filtered and frozen until TOC was determined.

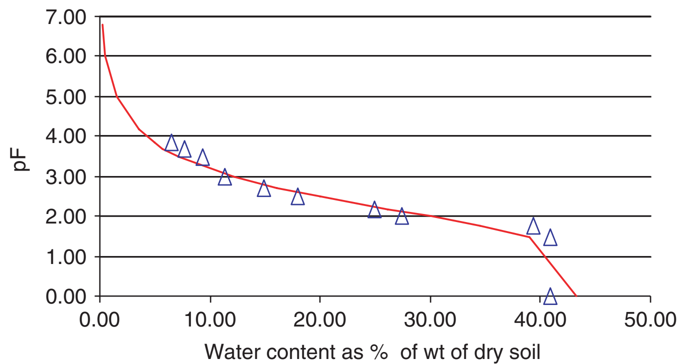

Figure 1. Water retention curve for soil without atrazine use (WNH).

TOC was analyzed by the TOC Analyzer (Dimatec 100 Analysentechnik, Germany). Calibration curve for total carbon was prepared using potassium hydrogen phthalate. To check on the reliability of the data generated, the total inorganic carbon (TIC) and total carbon (TC), a mixture of sodium carbonate and sodium hydrogen carbonate at concentrations of 15 and 5 $mg\,L^{-1}$, respectively, were included in the sample measurements. An aliquot of 1 mL of the K_2SO_4 solution extracts of the fumigated and nonfumigated soils was diluted four and two times, respectively, in plastic vials. The samples were measured together with TIC and TC standards. From the measurements, the carbon released by microbes during chloroform fumigation was computed from which microbial biomass was determined from the following equation C (biomass) = 2.64*E_C, where C is the carbon associated with the biomass cells, 2.64 is K_C factor commonly used for aerobic soils as was described by Valence, Brookes, and Jenkinson (1987). E_C is the difference between C extracted from the fumigated and nonfumigated treatments.

Results and discussion

Optimum water contents for the soils from water retention curves

Figure 1 shows the water retention curve for soil (WNH). The gravimetric water content (Wg) retained by soil, expressed as percentage of the dry soil at the soil water tension of −15 KPa, was computed from the curve and was found to be 24.6%. The water holding capacity (WHC) for the soil was 40.9%. Wg expressed as the percentage of WHC was 60%. The corresponding values of Wg, WHC, and Wg expressed as the percentage of WHC for the soil (WH) were 21.5%, 55.8% and 38.5%, respectively. It is widely recognized that any study on microbial activity needs to consider the water relation of the soil with microorganisms. However, there has been no agreement on methods for water measurement, and consequently, even though quite a number of reports from soils around the world have been published, little consensus has followed (Ilstedt, Nordgren, and Malmer 2000). Ilstedt, Nordgren, and Malmer (2000) showed that basal respiration and substrate induced respiration occurred at different soil water contents. Schroll et al. (2006) investigated the effect of soil moisture on aerobic microbial mineralization of selected pesticides in different soils. Their results demonstrated a conclusive correlation between pesticide mineralization and soil water content. Furthermore, at the soil water tension of −15 KPa, different soils retained different water contents and it was at this soil

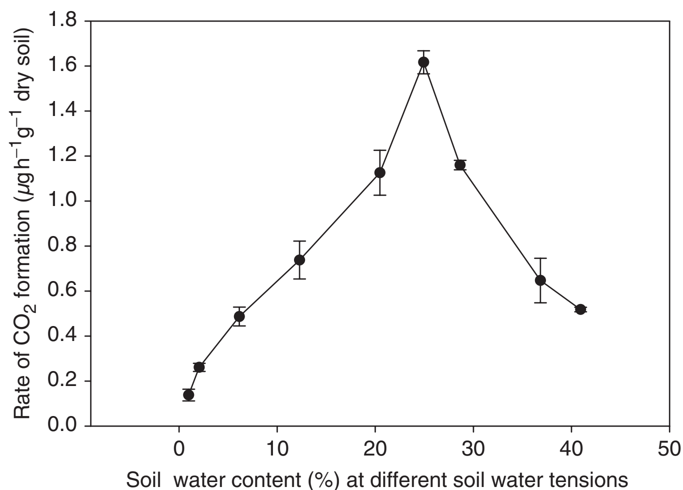

Figure 2. Basal respirations in soil (WNH).

water tension that there was maximum pesticide mineralization for the selected pesticides by various soils.

In the present study, we found that the basal respiration in the soil (WNH) correlated with the soil water retained at different soil water tensions (Figure 2). The basal respiration increased as the water retained by soil increased with increase in soil water tension. A maximum value of 1.617 CO_2 μg h^{-1} g^{-1} dry soils for basal respiration was attained at the soil water tension of −15 KPa, which is the value found by Schroll et al. (2006) as the soil water tension for maximum pesticide mineralization. At different soil water tensions, the amount of biomass C determined by CFE was the same with an average value of 633 ± 51 μg C (100 g)$^{-1}$ dry soil ($n = 7$). However, at soil water tensions of −1.9 and −4.4 KPa, biomass C was found to be abnormally high 1305 and 1430 μg C (100 g)$^{-1}$ of dry soil, respectively. This was attributed to the fungal growth under water logged conditions. The variation in basal respiration in soil with water contents at different soil water tensions has been linked to the forces tending to retain water in soil, which the microbes must overcome and is energy-linked. The other factor is the availability of the water in which solutes are dissolved and have to move to the microbes through diffusion (Ilstedt, Nordgren, and Malmer 2000). In a water logged soil condition, aerobic processes will be reduced due to limited amount of soil air.

Kinetics study of atrazine degradation in the laboratory

Atrazine mineralization in the soil (WNH) was negligible with only 0.16% being mineralized to $^{14}CO_2$ after 163 days of incubation under controlled conditions (Figure 3). There was 68.9% of atrazine that could be extracted from soil with methanol solvent. This constitutes the form of atrazine, which is labile and can potentially cause pollution to ground and surface waters. Only 31% of atrazine had become bound to soil and could not be extracted. The low atrazine mineralization in the soil was due to lack of specific atrazine degraders, which have been shown in many studies to utilize atrazine as C and energy sources for growth in soil. Low atrazine mineralization has been observed previously in soil without adapted atrazine degraders. Langenbach, Schroll, and Paim (2000) found that

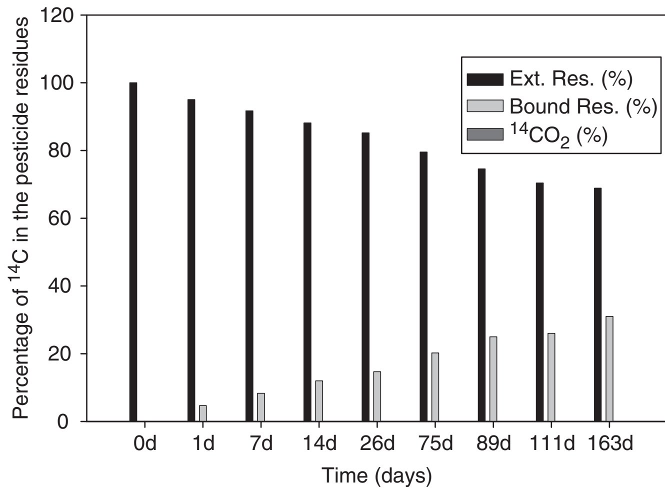

Figure 3. Kinetic study of atrazine degradation in soil (WNH) at different reaction periods.

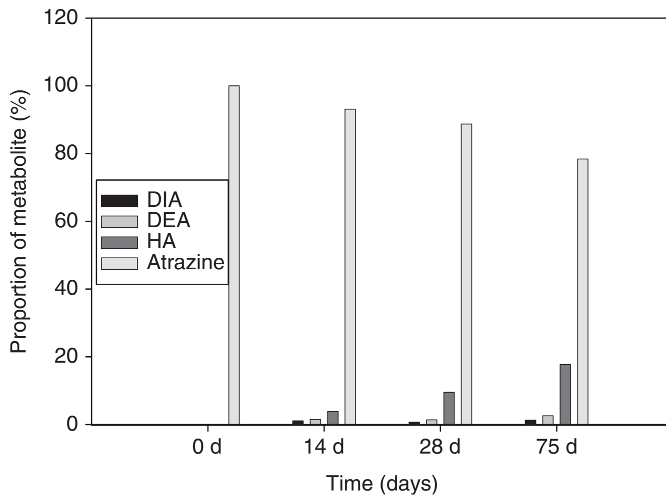

Figure 4. Metabolite formations from atrazine in soil (WNH) during degradation.

atrazine could only be mineralized to $^{14}CO_2$ up to 0.2% in a Brazilian tropical oxisol soil. Abdelhafid, Houot, and Barriuso (2000) also found that atrazine degradation in adapted soil was enhanced, while in nonadapted soil it was degraded slowly. Several studies have shown enhanced atrazine mineralization with subsequent isolation of specific atrazine degraders during liquid culture enrichments with soils previously impacted by atrazine use (Barriuso and Houot 1996; De Souza et al. 1998; Garcia-Gonzalez et al. 2003; Pussemier et al. 1997).

The degradation of atrazine in soil led to the formation of the metabolites HA, DEA and DIA (Figure 4). After 14 days of soil incubation, the metabolites composition was HA (3.9%), DIA (1.1%) and DEA (1.5%). The rest was the parent compound atrazine, which made up 93.5%. After 75 days of soil incubation, the metabolites proportion rose to 1.3%, 2.6%, and 17.7% for DIA, DEA, and HA, respectively.

The atrazine proportion reduced to 78.4%. That HA formed the highest proportion of the metabolites is consistent with previous studies which showed that HA is entirely formed by chemical degradation (Smalling and Aelion 2004). The low atrazine mineralization to $^{14}CO_2$ by only 0.16% clearly showed that the indigenous soil microbes could not utilize atrazine as C and energy source, but degraded the atrazine ring by *N*-dealkylation through the side chains ethyl and isopropyl groups to form metabolites. It appeared that the *N*-dealkylation of the ethyl group was faster kinetically than *N*-dealkylation of the isopropyl group. This is deduced from the higher proportion of DEA than DIA as metabolites resulting from *N*-dealkylation of the atrazine *s*-triazine ring. This could be attributed to steric hindrance, which is higher in the isopropyl group because it is more bulky than the ethyl group.

Effect of mature compost on the atrazine mineralization in soil (WH)

Atrazine which was ^{14}C-uniformly ring labeled was rapidly mineralized in the soil where atrazine is currently being used, and had been applied to the soil six months before soil samples were collected for the present study. Atrazine was mineralized to $^{14}CO_2$ up to $89.9 \pm 1.23\%$ with only $2.65 \pm 1.17\%$, and $7.4 \pm 0.34\%$ remaining in soil as extractable and bound residues, respectively. The soil, which was sterilized with solid $HgCl_2$ at a concentration of $1000\,\mu g\,(g)^{-1}$ dry soil, did not mineralize atrazine to $^{14}CO_2$. The extractable and bound residues in the sterilized soil were 54.3% and 45.7%, respectively. The amount of bound residue of atrazine in the sterilized soil was high because there was no other process by which the atrazine could dissipate or degrade. The bound residue has been elucidated by spectroscopic methods in a number of studies. The mechanisms by which the bound residue is formed in soil have been postulated to be by physical entrapment and covalent bonds formation (Barraclough, Kearney, and Croxford 2005). Although no attempt was made to elucidate the mechanism behind the bound residue formation in our present study, we attribute the bound residue formation entirely to the chemical process, because the microbes had been eliminated in the sterilized soil. In our present study, we showed that atrazine degraders did not incorporate any ^{14}C in their biomass cells in liquid culture media (data not shown).

The rapid atrazine mineralization observed in nonsterilized soil was due to the presence of adapted atrazine degraders, which were later isolated and identified in liquid culture enrichment experiments (data not shown). However, addition of compost that had been characterized (Table 2) did not enhance atrazine mineralization in the soil at all the concentration ranges of between 1000 and $10{,}000\,\mu g\,(g)^{-1}$ dry soil. The addition of compost at all the concentrations to the soil caused a lag phase of eight days in the mineralization of atrazine, whereas the same soil without compost did not show any lag phase during atrazine mineralization (Figure 5). The mineralization curves for soil spiked with compost at different concentrations showed sigmoid curves with three distinct phases: lag phase, rapid phase and a plateau. However, the same soil without compost exhibited atrazine mineralization curve without the distinct three phases. Mass balances for ^{14}C-ring labeled atrazine in the soils amended with compost at different concentrations showed that they had almost the same amounts of the extractable residues in soil ranging from $3.9 \pm 0.9\%$ to $4.9 \pm 0.42\%$, while the bound residues ranged from $10.7 \pm 2.93\%$ to 14.9% after 82 days of incubation under controlled conditions in the laboratory (Figure 6). Previous studies showed that application of raw or composted amendments to soils generally increased organic carbon (OC) content and increased the retention

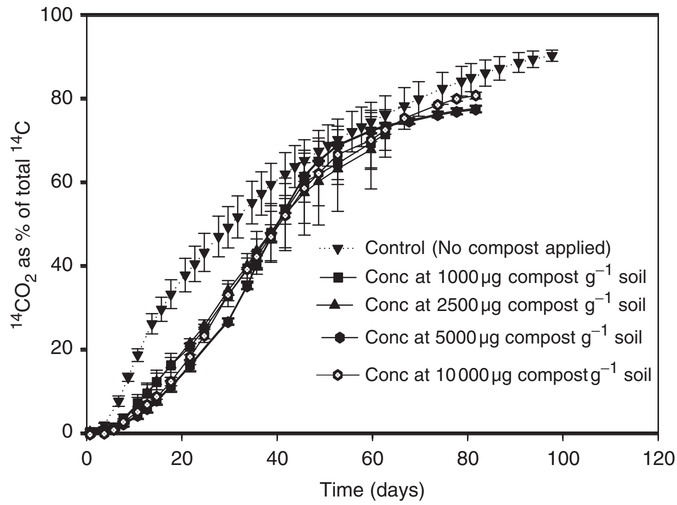

Figure 5. Effect of compost on atrazine mineralization in soil (WH).

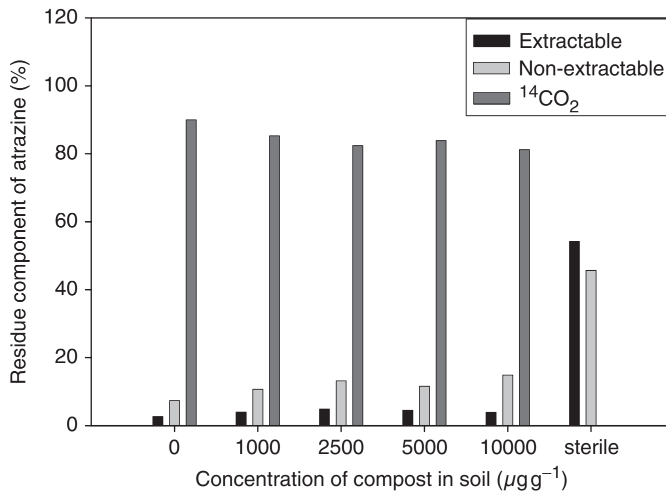

Figure 6. Mass masses for ^{14}C-ring labeled atrazine in soil (WH) amended with compost.

of pesticides (Celis, Barriuso, and Houot 1998). Ling, Xu, and Gao (2006) found that dissolved organic matter (DOM) at relatively lower concentrations significantly enhanced the sorption of atrazine by soil, while it inhibited the atrazine sorption at higher concentrations. Furthermore, a critical DOM concentration, below which DOM enhanced atrazine sorption, was established (Ling, Xu, and Gao 2006). The lag phase of eight days associated with soil amended with compost at various concentrations could be attributed to the adsorption of the atrazine by the compost, thus reducing its availability to the microbes. As time progressed, the atrazine became bio-available and was readily accessed by the microbes. In an earlier study with soil from another field with a history of atrazine use, addition of the same compost resulted in enhanced atrazine mineralization. Atrazine mineralization in the soil increased as the compost concentration in the

soil increased. The soil without compost mineralized atrazine to $^{14}CO_2$ up to 30.7% after 112 days. However, the addition of compost at concentrations ranging from 1000 to 5000 $\mu g\,g^{-1}$ resulted in increased atrazine mineralization ranging from 41.8% to 55.1% (Getenga 2003). The increased atrazine mineralization was attributed to the additional C from the compost which provided the optimum C ratio necessary for the microbial growth, as C on the atrazine ring could not be utilized as shown in previous studies (Radosevich et al. 1995; Rhine, Fuhrmann, and Radosevich 2003). Additional C in liquid culture media inoculated with the soil enhanced atrazine mineralization. However, additional N in the same liquid culture media, with atrazine as a substrate, inhibited atrazine mineralization (data not shown). The mass balances for ^{14}C-ring labeled atrazine in the soil, amended with compost at different concentrations showed that the amount of extractable and bound residues remaining in the soil after 82 days did not show much difference (Figure 6). The cause of the lag phase in the soil amended with compost was attributed to the adsorption of the atrazine to the OC in the compost. However, after some time the adsorbed atrazine became bio-available to microbes, which started utilizing atrazine as a source of both C and energy.

Conclusions

Atrazine could not be mineralized in soil from the sugarcane-cultivated field without history of atrazine use. The major degradation process in the soil was by chemical, which resulted in the formation of HA as a major metabolite. HA is known to be strongly adsorbed in soil by the humic acids. This could lead to atrazine persisting in the soil for a long time. The *N*-dealkylation of the atrazine ring resulting in the metabolites DEA and DIA could only be attributed to biotic processes as previously reported in many studies. However, rapid atrazine mineralization was observed in the soil from the sugarcane-cultivated fields with history of atrazine use. This was direct evidence of the soil having developed adapted atrazine degraders due to repeated applications of atrazine to the field. Atrazine is not expected to persist in soils of this kind. It is even possible for the atrazine to be mineralized completely before it can exert its herbicidal activities in soils where adapted atrazine degraders have developed. Application of mature compost that had enhanced atrazine mineralization in a previous study, reduced the rate of atrazine mineralization when it was added to the soil. The compost apparently adsorbed the atrazine, initially retarding its movement to the sites of microbial activities in the soil. The adsorption appeared to have been reversible, thus explaining the rapid atrazine mineralization which was observed later. It is possible that other sugarcane-cultivated fields have developed specific atrazine degraders and is the subject of our on-going research work. Furthermore, the sugarcane-cultivated field where we observed the enhanced atrazine mineralization may have developed other specific pesticide degraders for the other compounds being used in the field.

Acknowledgements

We thank the Alexander Von Humboldt foundation for the purchase of uniformly ^{14}C-ring labeled atrazine and the fellowship granted to Z.M. Getenga to be able to reside at GSF to be part of the team in the present study. We also thank the GSF for providing the facilities and consumables for the study.

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