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ARTICLE

Accelerated Degradation of ^{14}C-Atrazine in Brazilian Soils from Different Regions

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The repeated use of a given pesticide may induce a selection of the soil microbial population, resulting in a rapid degradation of the respective xenobiotic. Patterns of atrazine degradation (mineralization, formation of metabolites and nonextractable residues (NER)) were evaluated in two Brazilian soils with a history of atrazine application. Results were compared with those obtained from soils that had no agricultural use or herbicide application history. ^{14}C-Atrazine mineralization in unsaturated treated soils was high. By the 85th day of incubation, 82% of the applied ^{14}C-atrazine was mineralized in the Rhodic Hapludox and 74% in the Xanthic Haplustox. Mineralization remained low in nontreated soils (≤5.1%). Incubation under slurry conditions enhanced atrazine mineralization in the treated Xantic Haplustox and surprisingly also in the nontreated Rhodic Hapludox (98 and 83% on the 85th day, respectively), whereas in the other samples the evolved $^{14}CO_2$ did not differ ($p < 0.05$) from the unsaturated conditions. The water-extractable amount of atrazine directly after ^{14}C-atrazine application was higher in both Xanthic Haplustox samples (around 80% of applied atrazine) in comparison to the Rhodic Hapludox samples (around 60%). Extractable activity and the formation of metabolites and NER varied among the studied soils according to the atrazine application history rather than the soil characteristics.

KEYWORDS: Enhanced biodegradation; metabolites; desorption; herbicide application history

INTRODUCTION

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], one of the most widely used herbicides for broadleaf control, has often been detected at high concentrations in soil, sediments, surface water, and groundwater, even in remote areas (*1*). The use of atrazine in all EU Member States was banned in September 2004, with permission to consume existing stocks until October 2005 (*2*). However, atrazine is still extensively used throughout North and South America (*3*, *4*). The widespread detection of atrazine in the environment resulted in an increasing concern regarding its impact on ecological and human health. Recent studies revealed a negative impact of atrazine on aquatic systems, amphibians, and mammals due to its endocrine-disrupting activity (*1*, *5*).

Maize (*Zea mays*), soybean (*Glycine max* L.), and sugar cane (*Saccharum officinarum* L.) represent the most important and profitable Brazilian agricultural products. In the crop season of 2009/2010, 13.0 million hectares of agricultural land was planted with maize (*6*). Considering the lowest recommended atrazine dose of 0.75 kg of active ingredient (ai) ha^{-1}, a total amount of approximately 9750 tons of this herbicide was applied in Brazil during the crop season 2009/2010.

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Following application, atrazine undergoes several dissipation processes, which are determined by the physical and chemical properties of the soil and by biotic and abiotic factors. Atrazine was demonstrated to be highly persistent in soil under field conditions (*7*). Previous studies suggested half-life values ranging from 21 to > 300 days (*8*), depending mainly on temperature, soil moisture regimen, and soil type (*9*, *10*). However, as a potentially biodegradable product, atrazine may induce selection of the soil microbial population that acquires the ability to metabolize the pesticide into a source of energy (*11*). Repeated field applications of a pesticide can improve this selection and enhance its degradation, thus drastically reducing the compound's half-life and the intended herbicidal effects (*10*, *12*). Enhanced pesticide mineralization was first described by Audus (*13*) with his pioneering research on the biodegradation of 2,4-D and has been reported for atrazine in recent years throughout the world according to Krutz's review of the literature (*14*).

Atrazine may degrade biotically and/or abiotically into CO_2 or partially into metabolites, which differ in persistence and toxicity (*15*, *16*). The most common metabolites include hydroxyatrazine, *N*-dealkyl metabolites, and hydroxy-*N*-dealkyl metabolites (*1*, *15*). Inactivation of the parent herbicide through the production of metabolites usually results in loss of phytotoxicity. However, some atrazine derivatives such as deethylatrazine and

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deisopropylatrazine are phytotoxic, with deethylatrazine being considered just as toxic as the parent compound (*17*). Therefore, the examination of these residues along with the parent compound is essential when the environmental behavior and the hazard potential of atrazine are assessed.

Considering the extensive use of atrazine in Brazilian agriculture, studies concerning the fate of this herbicide within this environment are important. Some studies were performed on Brazilian soils to evaluate atrazine dissipation in soil under unsaturated soil conditions (*9*, *16*, *18*) and wetlands (*19*). However, these experiments did not consider the history of atrazine application as a factor affecting the compound's degradation and its extractability. In the present study, the patterns of atrazine degradation (mineralization, metabolite formation, and formation of nonextractable, so-called "bound" residues) in two Brazilian soils were characterized, with the role of herbicide application history in atrazine persistence taken into consideration. The studied soils received repeated atrazine field applications over 10−20 years, and the results were compared with those obtained from soils of the same region without any history of atrazine application.

MATERIALS AND METHODS

Site History and Soil Sampling. Soils samples were collected from two Brazilian soil types.

Samples of a Rhodic Hapludox (RH-T) located in Campinas do Sul, southern Brazil (27° 43′ S, 53° 38′ W), were taken randomly in triplicate in the upper 10 cm in October 2008. The area has been under no-tillage with crop rotation (soybean/wheat/maize/oat) since 1990. It had been treated biennially for the past 20 years with commercial atrazine at agronomically recommended doses (1.0−3.25 kg ha^{-1}) to control annual weeds. The last atrazine field application before sampling was performed in October 2007. Soil samples of a Xanthic Haplustox (XH-T) from northeastern Brazil were collected in June 2008 from the upper 10 cm from a 10-year-old maize plantation under no-tillage in Correntina County (14° 05′ S, 46° 21′ W). This area has been alternately planted with maize and soybean since 2000, and it had been treated biennially for the past 10 years with atrazine at agronomically recommended doses (0.75−3.0 kg ha^{-1}). The last atrazine field application before sampling was made on October 2007. On both sites, samples from an adjacent area under native vegetation, which had neither agricultural use nor atrazine application history, were collected as control soils.

The climate at the southern Brazilian site is humid subtropical with fairly uniform precipitation over the year, whereas the northeastern region is characterized by a tropical climate with a rainy season from October to March and a dry season from April to September. Further information about soil classification, site description, main characteristics of the soils, and concentration of atrazine and its metabolites at the time of sampling is given in **Table 1**.

Atrazine Mineralization. Soil samples composed of three replicates were air-dried, sieved (2 mm), and kept at 5 ± 2 °C until further analysis. Prior to incubation, soil samples were acclimatized to the experimental temperature (20 ± 2 °C). Soil moisture content was determined separately, and distilled water was added to adjust the soil to 20% of the soil maximum water-holding capacity (WHC_{max}). After rewetting, the soil was incubated in the dark at 20 °C for 3 days, aiming to re-establish soil equilibrium and reactivate soil microflora.

A spiking solution was prepared using technical-grade atrazine (99% chemical purity, Riedel-de Haën, Seelze, Germany) and ^{14}C-atrazine (99% radiochemical purity, 6.4 MBq mL^{-1}, American Radiolabeled Chemicals Inc., St. Louis, MO) in ethanol. This solution was added to 20 g of an air-dried powdered aliquot of each soil, and ethanol was further removed by evaporation. Subsequently, these aliquots were thoroughly mixed with 400 g (dry weight equivalents) of each nonspiked soil. Ten subsamples (each 0.5 g dry weight) were combusted (Biological Oxidizer OX500, R. J. Harvey Instrument Corp.) to determine the total incorporated radioactivity and to monitor the homogeneous distribution of atrazine in the soil. The initial herbicide concentration was 3 mg kg^{-1}, and the initial radioactivity was 470 kBq kg^{-1} of soil.

Table 1. Soil Classification, Site Description, and Main Characteristics of the Rhodic Hapludox and Xanthic Haplustox Soils

	Rhodic Hapludox[a]		Xanthic Haplustox[a]	
	treated	nontreated	treated	nontreated
water-holding capacity (%)	61.5	60.1	34.4	35.3
organic carbon content (%)	3.19	3.37	1.05	0.99
nitrogen content (%)	0.28	0.29	0.07	0.05
pH (H_2O)	5.7	5.4	5.6	5.0
clay (%)	49.4	38.0	23.0	22.0
silt (%)	26.1	41.1	4.8	3.4
sand (%)	24.5	20.9	72.2	74.6
clay mineralogy[b]	Kt, Gt, Hm	Kt, Gt, Hm	Kt, Gb, Gt	Kt, Gb, Gt
MAP[c] (mm year^{-1})	1700	1700	1500	1500
MAT[d] (°C)	16	16	21	21
concentration at the time of soil sampling[e] (μg kg^{-1})				
atrazine	4.4	nd	4.3	nd
hydroxyatrazine	31.1	2.4	27.1	1.6
deethylhydroxyatrazine	62.0	nd	25.7	nd
deisopropylhydroxyatrazine	43.1	nd	nd	nd

[a] USDA-NRCS (*30*). [b] Kt, kaolinite; Hm, hematite; Gt, goethite; Gb, gibbsite. [c] MAP, mean annual precipitation. [d] MAT, mean annual temperature. [e] Extracted by means of accelerated solvent extraction using methanol/water solution (4:1 v/v); nd, below detection limit.

Mineralization of ^{14}C-atrazine was quantified during 85 days of incubation in hermetically closed 250 mL Duran glass bottles (20 g of dry soil equivalents for each soil in triplicate). The final moisture content was adjusted to 50% WHC_{max} by adding distilled water. Soil microcosms were incubated in the dark at 20 ± 2 °C. To monitor the evolving $^{14}CO_2$, a glass vial containing 1.5 mL of 2 M NaOH was placed in a cap-holder inside the flask. NaOH solution was periodically removed and replaced by fresh solution. In addition to the unsaturated microcosms, a mineralization essay employing slurry conditions was conducted to evaluate ^{14}C-atrazine mineralization within conditions of maximal availability of the herbicide to the microorganisms. Slurry conditions were achieved by using 0.01 M $CaCl_2$ solution (1:4 soil/solution ratio), following the same soil sample preparation and $^{14}CO_2$ monitoring as described above. The flasks were kept under continuous shaking on a rotary shaker at 125 rpm throughout the experimental period. The oxygen supply was ensured by a free space (around 80%) above the soil in the incubation flask and by the periodic opening of the flasks for changing NaOH solution in the trap vials. Trapped $^{14}CO_2$ was determined by a liquid scintillation counter (LSC, 2500 TR, Tri-Carb, Packard Liquid Scintillation Analyzer) using 10 mL of scintillation cocktail (Instant Scint-Gel Plus, Perkin-Elmer) and 4 mL of deionized water (18.0 MΩ/cm, Milli-Q Plus 185, Millipore, Eschborn, Germany) per sample. Sampling of soil microcosms was performed every 2−4 days in the first 30 days of incubation and, thereafter, weekly.

Extractable and Nonextractable Atrazine Residues. The monitoring of extractable and nonextractable radioactivity and of metabolite formation after 0, 8, 15, 30, and 85 days of incubation was conducted in a separate assay. For each soil, 12 flasks were filled with 20 g of atrazine-spiked soil, adjusted to 50% WHC_{max}, and handled as described above. Three fractions of ^{14}C activity with increasing binding strength to the soil matrix were determined: (1) extractable by water, (2) extractable by accelerated solvent extraction, and (3) nonextractable residues.

Water Extractions. The entire soil sample contained in each flask was extracted once with deionized Milli-Q water in a soil/solution ratio of 1:8, in accordance with Jablonowski et al. (*20*). The suspension was shaken on a horizontal shaker at room temperature for 6 h at 150 rpm and centrifuged at 10000*g* for 90 min. An aliquot (≈60 mL) of the supernatant was taken, and the volume was measured and then filtered through a 0.45 μm membrane (Sartorious Biolab). The filtrate was analyzed for desorbed ^{14}C activity via LSC and for atrazine and its metabolites via LC-MS/MS. The filters used in this procedure were combusted, and the detected radioactivity was considered in the final mass balance even though it did not exceed 0.2% of the applied activity. Water-extracted soil samples were freeze-dried (Lyovac GT2, Steris) and powdered prior to accelerated solvent extraction.

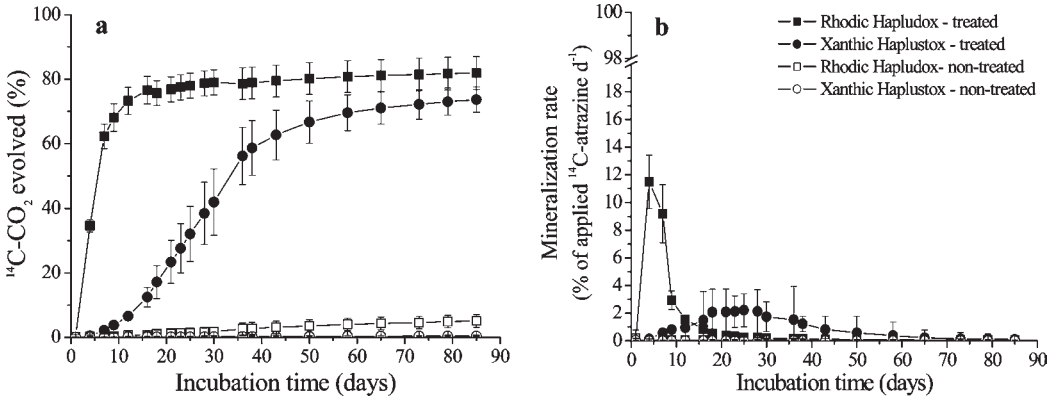

Figure 1. Cumulative $^{14}CO_2$ (**a**) and rates of ^{14}C-atrazine mineralization (**b**) during laboratory incubations at soil moisture equivalent to 50% of maximum water-holding capacity in the atrazine-treated and nontreated Rhodic Hapludox and Xantic Haplustox soils. Values are reported as percentage of total ^{14}C applied activity as a function of time. Symbols represent the mean of three replications for each of the sampling dates. Error bars indicate the SD and do not appear when smaller than the symbol for the mean.

Accelerated Solvent Extraction (*ASE*). Following water extractions, 10 g subsamples from both nontreated soils and from treated Xanthic Haplustox were extracted four times in succession using an ASE 200 system (Dionex, Dionex Co., Sunnyvale, CA) in accordance with Jablonowski et al. (*7*). Soil samples were transferred to 11 mL stainless steel ASE cells, and the remaining space above the samples was filled with quartz (Merck) to reduce the extract volume and to avoid clogging of the ASE steel filter lid. For the treated Rhodic Hapludox, 9 g of soil was mixed with 0.5 g of diatomaceous earth (Bulk Isolute Sorbent, International Sorbent Technology Ltd., Mid Glamorgan, U.K.), and the same procedure as described above was followed. This measure aimed to avoid plugging of the cell and to facilitate solvent penetration through the soil matrix due to its high clay content. A methanol/water solution (4:1 v/v) was used as a solvent, and extraction was performed at 135 °C and 100 bar with a flush volume of 60% of extraction cell volume. The heat-up and static times were 7 and 15 min, respectively.

To determine water- and ASE-extracted ^{14}C activity, a triplicate of 1 mL of each extract sample was mixed with 4 mL of scintillation cocktail, and detection of radioactivity was performed by LSC. An external standard was used for quenching correction.

Nonextractable Residues (*NER*). After water and ASE extractions, the ^{14}C activity remaining in the soil was determined using five replicates (each 1 g dry weight). Samples were weighed into porcelain vials and combusted using a Biological Oxidizer. The produced $^{14}CO_2$ was trapped in scintillation cocktail (Oxysolve C-400, Zinsser Analytic) and analyzed by LSC.

The mass balance of ^{14}C was determined by the sum of $^{14}CO_2$ evolved during mineralization, ^{14}C activity in water and ASE extracts, and $^{14}CO_2$ recovered from the combusted samples. In general, a mass balance between 91.6 and 108.2% was obtained. The RH-T soil presented a mass balance varying from 82 to 111.6%; the highest recoveries were obtained in the first 15 days of incubation and the lowest values at study termination.

LC-MS/MS Analysis. Atrazine and its metabolites were identified and quantified in water and ASE extracts by means of liquid chromatography and mass spectrometry as described previously by Jablonowski et al. (*20*) using a Thermo Electron model TSQ-Quantum 2002 equipped with a CTC-HTC-PAL sampler. The standards for atrazine, hydroxyatrazine [2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine (HA), 96%], deethylatrazine [2-chloro-4-amino-6-isopropylamino-*s*-triazine (DEA), 99.9%], deisopropylatrazine [2-chloro-4-ethylamino-6-amino-*s*-triazine (DIA), 96.1%], deethyldeisopropylatrazine [2-chloro-4,6-diamino-*s*-triazine (DEDI), 98%], deethylhydroxyatrazine [2-hydroxy-4-amino-6-isopropyl-amino-*s*-triazine (DEHA), 99%], and deisopropylhydroxyatrazine [2-hydroxy-4-ethylamino-6-amino-*s*-triazine (DIHA), 99%] were purchased from Riedel-de Haën (Seelze, Germany). Deuterated atrazine and its metabolites (Dr. Ehrenstorfer GmbH, Augsburg, Germany) were used as internal standards in a concentration of 0.01 μg mL^{-1}. One hundred microliters of each water and ASE extract was mixed with 100 μL of deuterated standard solution resulting in 0.001 μg 100 μL^{-1} of injected sample. As the solid phase, MZ Perfect Sil Target ODS-3 (2.1 mm × 125 mm × 3 μm) was used, with an additional HPLC precolumn (2.1 mm × 10 mm × 3 μm). A mixture of acetonitrile and 0.1 M ammonium acetate solution was used as the HPLC eluent. The flow rate was 0.15 mL min^{-1} at 25 °C column temperature. The injection was performed in triplicate, and the total injection volume of each sample was 5 μL. LC-MS/MS analyses were performed in a positive electrospray ionization source (ESI+), and transitions were measured in multiple reaction monitoring (MRM). All of the settings for the analysis and mass transfer of atrazine metabolites were previously described by Jablonowski et al. (*20*). The analytical detection limit was 0.03 ng mL^{-1} for atrazine, 0.04 ng mL^{-1} for HA, 0.7 ng mL^{-1} for DIA, 0.4 ng mL^{-1} for DEA, 0.6 ng mL^{-1} for DEHA, 3 ng mL^{-1} for DIHA, and 5.0 ng mL^{-1} for DEDI.

Data Treatment and Statistical Analysis. Atrazine mineralization data from the treated soils were fitted to a first-order [$Y = A_0 \times (1 - e^{-kt})$] and the data from the nontreated soil to a zero-order [$Y = -k \times t + C_0$] degradation kinetic model, where A_0 was the maximum amount of evolved $^{14}CO_2$ (% of added radioactivity), k the mineralization rate constant (day^{-1}), t the time (days), and C_0 the initial amount of applied radioactivity. The half-life ($t_{1/2}$) value for atrazine was calculated from the relationships $t_{1/2} = \ln 2/k$ and $t_{1/2} = C_0/2K$ for first- and zero-order degradation kinetics, respectively.

Mineralization data were analyzed considering a split−split plot experimental design arranged as a randomized complete block with three replications of each treatment. Soil type was the whole plot, atrazine application history the subplot, and availability of water (50% WHC and flooded) the subsubplot. Extractable and nonextractable residue formation data were similarly analyzed considering soil type as whole plot, atrazine application history as subplot, and extraction time as subsubplot. Analysis of variance and mean comparison by Tukey's test were performed using SANEST, Statistical Analysis System (*21*). All results were considered to be significantly different at $p < 0.05$.

RESULTS AND DISCUSSION

Atrazine Mineralization in Unsaturated Soils. Samples from the agriculturally used Rhodic Hapludox (RH-T) mineralized 82% of the initially applied ^{14}C-atrazine during 85 days of incubation. On day 15 after application, only 25% of the radioactivity remained in the soil microcosm (**Figure 1a**). No latency time was found in the overall mineralization, and the mineralization rate reached a maximum after 4 days of incubation, corresponding to a mineralization of 11.5% of the applied ^{14}C-atrazine day^{-1} (**Figure 1b**). Thereafter, the mineralization rate decreased to 0.84% day^{-1} on the 18th day of incubation and then remained fairly constant until the end of the experiment. The estimated half-life of 4 days was shorter than values previously reported for Brazilian soils (*9*) and is about 5−82-fold lower than those reported in Barbash's review of the literature, which ranged from 21 to 330 days under aerobic conditions (*22*). This fact demonstrates the importance of considering the specific conditions of soil and climate as well as the history of atrazine field applications for an accurate prediction of its persistence in soil.

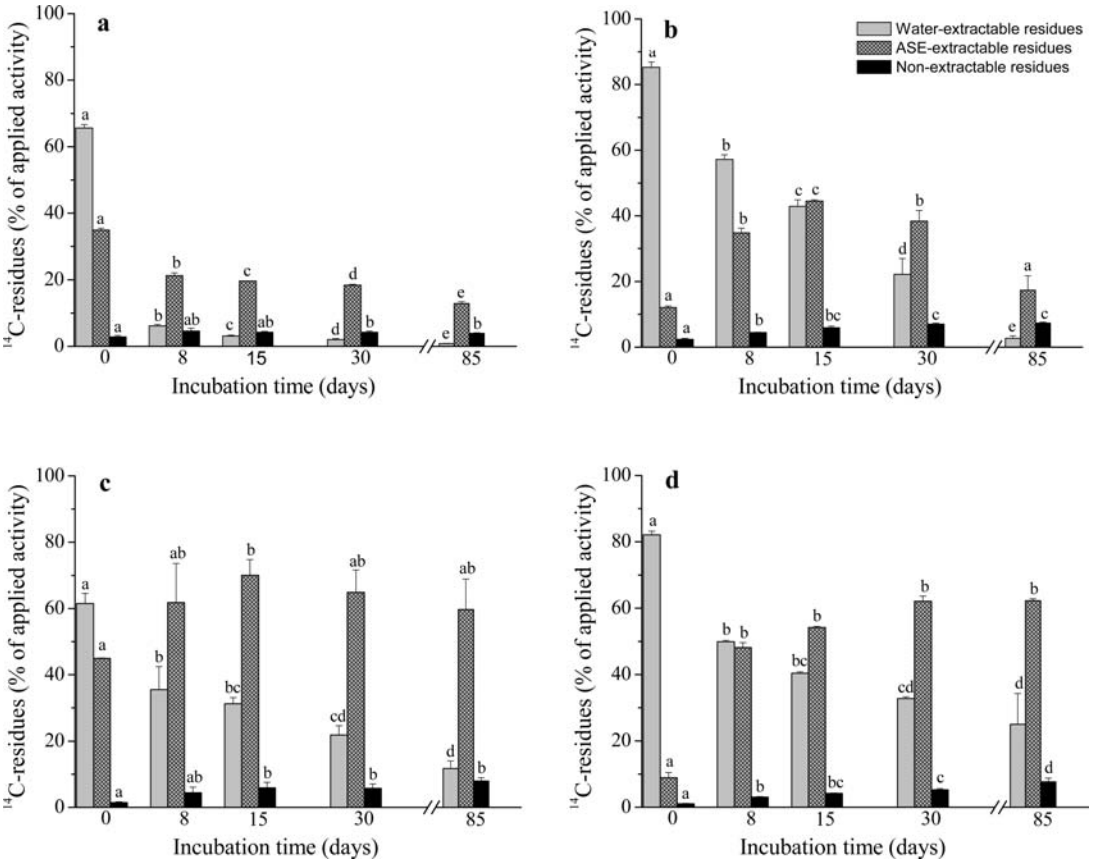

Figure 2. Extractable and nonextractable ^{14}C residues in the treated Rhodic Hapludox (**a**) and Xantic Haplustox (**b**) and in the nontreated Rhodic Hapludox (**c**) and Xantic Haplustox (**d**). Each column represents the mean of three replicates. Error bars indicate the SD and do not appear when smaller than the symbol for the mean.

In contrast, nontreated Rhodic Hapludox (RH-NT) showed very low mineralization, and during the incubation time only 5.1% of the initially applied ^{14}C-atrazine was mineralized (**Figure 1a**). The mineralization rate was fairly constant over time and ranged from 0.02 to 0.07% of applied ^{14}C-atrazine day^{-1} (**Figure 1b**). Similar behavior was reported for a Brazilian noncultivated Oxisol from São Paulo, for which atrazine mineralization did not exceed 7.3% of the applied dose after 63 days of incubation (*18*).

Similarly to the RH-T soil, treated Xanthic Haplustox (XH-T) showed high mineralization of atrazine (**Figure 1a**), reaching 73.6% of the total applied ^{14}C-labeled herbicide after 85 days of incubation. However, a lag phase of about 7 days was observed in the XH-T soil. This behavior can be attributed to both the presence of less efficient microorganisms in atrazine ring cleavage in this environment and the latent phase of the bacterial colonies (*23*, *24*). The addition of water and of a C and N source through atrazine might have restored their activity. The extent of mineralization in the XH-T soil reached a maximum between 18 and 30 days of incubation and corresponded to 1.8−2.2% of the applied ^{14}C-atrazine day^{-1} (**Figure 1b**). The calculated half-life in this soil based on the mineralization data was 51 days.

In the nontreated Xanthic Haplustox (XH-NT), mineralization did not exceed 0.5% of applied ^{14}C activity (**Figure 1a**). For the nontreated soils, the estimation of half-lives based on ^{14}C-atrazine mineralization data was not appropriate due to only minor mineralization, and thus it was not performed.

Extractable and Nonextractable ^{14}C-Atrazine Residues. The water-extractable amounts of ^{14}C activity obtained directly after ^{14}C-atrazine application (0 days of incubation) were around 62% in the RH samples and around 82% in the XH samples (**Figure 2**). Conversely, the amount of ASE-extractable activity, a chemical fraction more strongly bound to the soil matrix than the water-extractable ^{14}C activity, was higher in the RH samples (35−45%) than in the XH samples (around 10%) (**Figure 2**). This behavior can be related to the higher organic matter content of the RH samples (**Table 1**) retaining a higher amount of ^{14}C activity due to stronger sorption and/or entrapment of the chemical on organic soil constituents, in comparison to the XH samples. These results are in line with previous studies giving clear indications that organic matter in soil is a strong sorbent for atrazine and/or its metabolites (*25*).

Extractable ^{14}C activity over the incubation time in the RH-T soil varied inversely to ^{14}C-atrazine mineralization (**Figure 1a**). After 8 days of incubation, ^{14}C activity decreased from 65.6 to 6.1% and from 35 to 21.3%, respectively, in water and ASE extracts (**Figure 2a**). At study termination, around 1% of the initially applied ^{14}C activity was detected in water and 13% in ASE extracts. These results demonstrate that the adapted microorganisms were able to mineralize atrazine, although to a lesser extent, from less accessible fractions such as that determined by the ASE procedure. The nonextractable ^{14}C activity slightly increased from 2.8 to 4.6% after 8 days of incubation and tended to remain constant until study termination (**Figure 2a**).

In the XH-T soil, a gradual decrease of ^{14}C activity in water extracts over time occurred while a concomitant increase in the ASE-extractable activity was observed up to the 15th day of incubation (**Figure 2b**). Thereafter, ASE-extractable activity decreased from 38.4 to 17.3%. The nonextractable ^{14}C activity increased steadily from 2.4% at day 0 to 7.3% of applied ^{14}C activity at study termination (**Figure 2b**). These results indicate that at the beginning of the incubation, when the mineralization rate was relatively low, ^{14}C-atrazine mineralization occurred mainly at the expense of the water-soluble fraction. Simultaneously, ^{14}C activity increased in other less labile fractions, thus

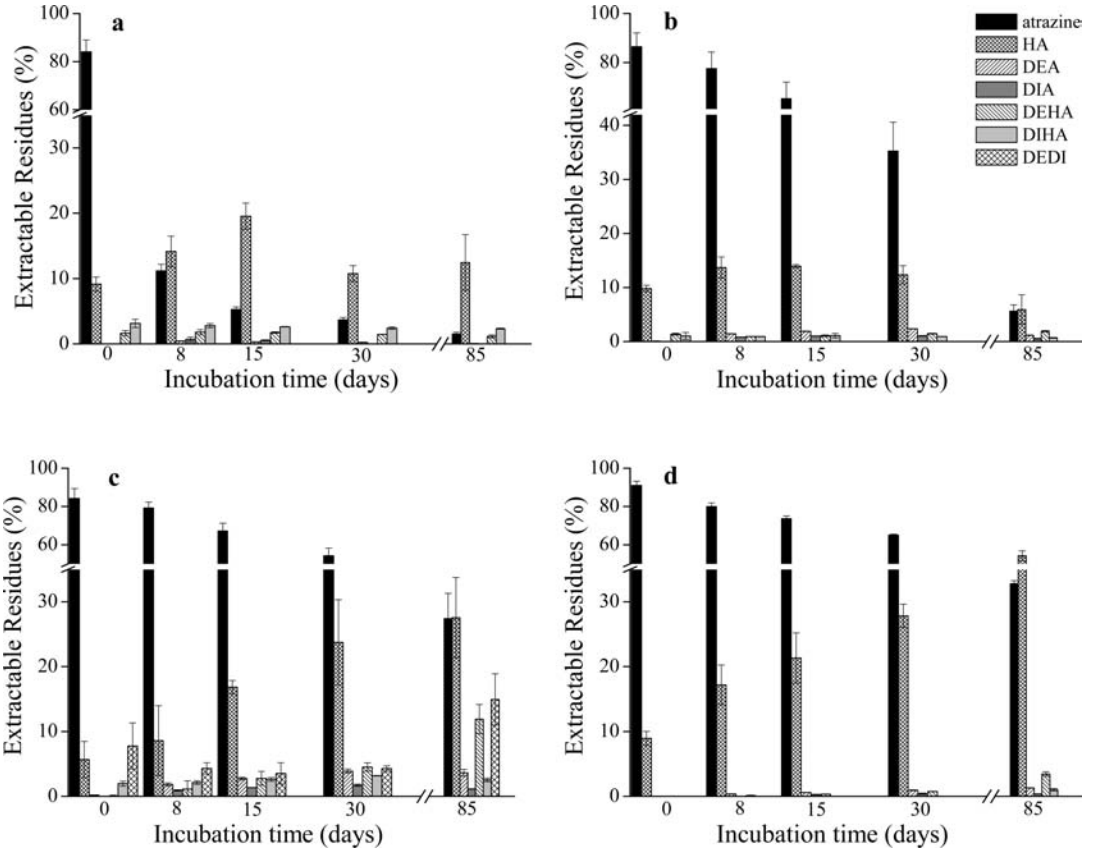

Figure 3. Contents of atrazine and its metabolites within 85 days of incubation at soil moisture equivalent to 50% of maximum water-holding capacity in the treated Rhodic Hapludox (**a**) and Xantic Haplustox (**b**) and in the nontreated Rhodic Hapludox (**c**) and Xantic Haplustox (**d**). Each column represents the mean of three replicates and indicates desorbed/extracted proportion of each compound in percentage of initially applied atrazine. Error bars indicate the SD and do not appear when smaller than the symbol for the mean.

clearly evidencing an aging process characterized by the stronger association of the compound with soil components over time (*26*). After 15 days, the mineralization rate reached a maximum (**Figure 1b**) and ^{14}C activity ASE extracts started to decrease significantly (**Figure 2b**), thus corroborating the capability of adapted microorganisms to degrade residues from less accessible fractions as observed in the RH-T soil.

In both nontreated soils, in which the mineralization rate was extremely low (**Figure 1b**), ^{14}C activity in water decreased and the ASE-extractable and nonextractable amounts increased throughout the incubation period (**Figure 2c**,**d**). This indicates a redistribution of the labeled compound from weaker to stronger adsorption sites (*26*).

Extractable Atrazine and Its Metabolites. In the present work, four atrazine derivatives (HA, DEA, DIA, and DEHA) were detected in the water extracts, whereas five metabolites (HA, DEA, DIA, DEHA, and DIHA) were observed in the ASE extracts (data not shown). DEDI was detected only in ASE extracts from RH-NT samples. Because the concentration of water-extractable metabolites did not exceed 6.3% of applied atrazine in any soil and extraction day during the incubation period, the extractable atrazine and metabolites (water-extractable + ASE-extractable residues) will be discussed as a whole.

Atrazine concentration decreased over the experimental period, and it was accompanied, in general, by an increase of its extractable metabolites (**Figure 3**). The parent compound and most of its derivatives persisted in the soils at detectable levels throughout the entire study (85 days).

In treated soils, most of the extractable metabolites were hydroxylated atrazine. The RH-T soil displayed a maximum HA concentration within 15 days of incubation, which accounted for 19.6% of the parent compound's mass (**Figure 3a**). Thereafter, the proportion of HA decreased and then remained fairly constant until the end of the assay (12.2%). In the XH-T soil, the HA concentration remained around 10−12% until the 30th day of incubation and then decreased to 5.9% at study termination (**Figure 3b**). Dealkylated atrazine derivatives were detected at low levels in both treated soils, and their sum did not exceed 5.8% of the applied atrazine for any extraction day.

Despite the low biomineralization (**Figure 1a**), nontreated soils exhibited a high metabolization of atrazine. At study termination, metabolites comprised around 60% of the initially applied atrazine (**Figure 3c**,**d**). Similar findings were reported by Nakagawa and Andrea (*16*) for a Brazilian Gley Humic soil. As shown in the case of the treated soils, HA was the main metabolite detected throughout the experimental period. These results are in agreement with those reported by Peixoto et al. (*18*) in Brazilian soils without any history of atrazine application. In both nontreated soils, HA concentration increased steadily over the incubation period accompanied by a decrease of atrazine concentration. The highest values of HA were observed after 85 days of incubation, consisting of 28% of the applied atrazine in the case of the RH-NT and 54% in the case of the XH-NT. In contrast to HA, total dealkylated atrazine derivatives were detected at low levels in the XH-NT over time, consisting of $<6.1\%$ of initial atrazine application for each extraction day (**Figure 3d**). On the other hand, RH-NT presented the highest concentration of dealkylated metabolites among the studied soils (**Figure 3c**). At the end of the study, the concentration of N-dealkylated derivatives totaled 34.1%, of which 11.9% was characterized as DEHA and 14.9% represented DEDI. This increase in

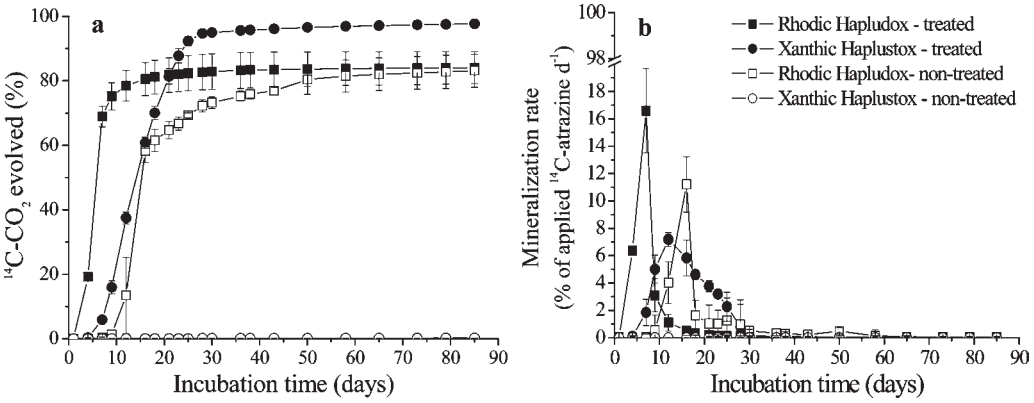

Figure 4. Cumulative $^{14}CO_2$ (**a**) and rates of ^{14}C-atrazine mineralization (**b**) during laboratory incubations under slurry conditions in the atrazine-treated and nontreated Rhodic Hapludox and Xantic Haplustox soils. Values are reported as percentage of total ^{14}C applied activity as a function of time. Symbols represent the mean of three replications for each of the sampling dates. Error bars indicate the SD and do not appear when smaller than the symbol for the mean.

dealkylated compound concentration over time suggests the growth of microbial communities possessing atrazine enzymes responsible for the conversion of atrazine and hydroxylated derivatives into dealkylated metabolites. The observed partial degradation of atrazine in both nontreated soils indicating the presence of adapted microorganisms may be explained by a previous contact of the soil microflora population with atrazine residues that probably occurred either by drifting during atrazine application or by transport of residues associated with eroded soil particles from neighboring treated areas. The occurrence of hydroxyatrazine in the nontreated soil samples prior to the incubation essay (**Table 1**) is an indication of these fields' contamination. Atrazine was already found in the air, soil, sediments and water even at great distance from urban or agricultural areas, indicating that its transport is likely to occur (*1*, *27*). Because this previous exposure to atrazine was probably sporadic and at low levels, the development of a highly adapted microbial population possessing atrazine enzymes responsible for atrazine ring cleavage might not have occurred. Another hypothesis is the possible translocation and introduction of atrazine-degrading microorganisms from the treated areas. Recent studies have proved that viable microbes can be transported by wind and in the dust among widely dispersed habitats (*28*).

Atrazine half-lives in nontreated soils, calculated from the mineralization and metabolite formation data, were 44.6 days for the RH-NT and 47.2 days for the XH-NT, whereas in the treated soils the half-lives were 2.8 and 21.2 days for the RH-T and XH-NT, respectively.

Atrazine Mineralization under Slurry Conditions. Mineralization of ^{14}C-atrazine in the RH-T soil under slurry conditions was similar to the mineralization in unsaturated soil (**Figure 4a**). At study termination, 84% of the total applied ^{14}C-atrazine was mineralized, 75% being mineralized within the first 9 days. However, the mineralization rate peak under slurry conditions was comparatively dephased, occurring on the seventh day of incubation, and corresponded to a mineralization rate of 16.6% of the applied ^{14}C-atrazine day^{-1}. The rapid atrazine mineralization observed under both moisture conditions in the RH-T soil, which contains a considerable content of soil carbon (**Table 1**), indicates that adapted microorganisms are able to compete effectively with the sorption process. This outcome contradicts the assumption reported by other authors (*16*, *29*) that a high content of soil organic matter reduces atrazine mineralization due to the decrease of its bioacessibility caused by the strong sorption of atrazine. Therefore, the use of the same sorption and persistence parameters, such as K_d coefficient and $t_{1/2}$, might be distinctive for soils with a different history of herbicide application and should therefore be considered carefully by modelers and regulatory agencies.

The mineralization kinetics in RH-NT soil under slurry conditions was significantly different from that verified in the unsaturated soil system. After a lag phase of about 10 days, the $^{14}CO_2$ evolution increased drastically, and by the 85th day of incubation, 83% of the initially applied ^{14}C-atrazine was mineralized (**Figure 4a**). The mineralization rate reached its maximum after 16 days of incubation, representing 11.2% of the applied ^{14}C-atrazine day^{-1} (**Figure 4b**). These unexpected results support the hypothesis described above concerning a previous atrazine contamination and/or introduction of atrazine degraders in the RH-NT soil. The nontreated site at the Rhodic Haplustox area was located in a lower landscape position compared to the agriculture area, increasing the possibility of contamination. The increase of atrazine accessibility ensured by the higher contact of microorganisms to the herbicide and by desorption processes under slurry conditions might have stimulated the growth of microbial communities capable of atrazine ring cleavage. According to Anderson (*23*), biodegradation can be limited by the lack of the microorganisms' ability to degrade the herbicide, by their quantity in the soil, and by the activity of enzymes responsible for the degradation of the herbicide. These variables are highly influenced by the soil and climate conditions to which the microorganisms are exposed (*24*).

In the XH-T soil, slurry conditions enhanced atrazine mineralization in comparison to incubation at 50% WHC_{max} (**Figure 4a**). The extent of mineralization was approximately 98% at study termination. The peak of the mineralization rate occurred between 9 and 25 days and consisted of 2.3−7.2% of applied ^{14}C-atrazine day^{-1} (**Figure 4b**). The higher contact between atrazine and the microorganisms promoted by the flooded condition and by the employed agitation improved the efficiency of the atrazine-mineralizing population as discussed above.

In contrast, atrazine mineralization in the XH-NT soil was not affected by the change of moisture and remained low under slurry conditions over the entire incubation time (**Figure 4a**). The absence of microorganisms able to cleave the atrazine ring in this soil is probably related to local soil conditions. The region of the Xantic Haplustox soil is relatively flat, and the nontreated area was probably less exposed to atrazine contamination in comparison to the RH-NT. Additionally, the soil pH of 5.0 (**Table 1**) might also suppress the microbial stimulation. According to Krutz et al. (*14*), pH levels lower than 5.4 can hinder microbial adaptation.

In conclusion, soils evaluated in these studies presented different behaviors with respect to atrazine metabolism, which has implications for its fate. Treated soils showed rapid mineralization of atrazine and sharp variations in the concentrations of extractable residues; on the contrary, nontreated soils presented very low mineralization under unsaturated conditions and more gradual variation in the extractable compound concentrations. After 30 days of incubation, which is a period when weed control relies on herbicide residual power, only 3.7 and 35.2% of added atrazine was detected as parent compound in the RH-T and XH-T, respectively, whereas 54.3 and 65% remained extractable in the RH-NT and XH-NT, respectively. From this total amount of extractable atrazine, only 0.4 and 18% of the added atrazine remained extractable by water in the RH-T and XH-T, respectively (data not shown). Two opposite effects may result from this behavior: whereas the short-term persistence of atrazine mitigates its potential risk for soil and water contamination, this may also reduce the efficiency of its weed control properties, resulting in both economic losses to farmers and a possible increase in the frequency of herbicide applications.

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