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Atrazine degradation patterns: the role of straw cover and herbicide application history

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ABSTRACT: In Brazil, atrazine (ATZ) is widely applied to maize (*Zea mays* L.) fields for weed control. The presence of ATZ and its metabolites in soil and water matrices has become a matter of some concern for governmental authorities as well as for society at large. This study evaluated the patterns of ATZ degradation (mineralization, extractable and non-extractable ATZ residues, and metabolite formation) in a Brazilian Typic Paleudult. Soil samples from a cultivated area under a no-tillage system with a history of ATZ application were incubated with ^{14}C-ATZ in both the presence and absence of straw cover on the soil surface, and the evolved $^{14}CO_2$ was determined by liquid scintillation. Samples from an area with native vegetation, adjacent to the cultivated area, were also incubated as a control. A higher mineralization of ATZ was observed in the cultivated soil (> 85 %) in comparison with the native soil (10 %) after 85 days of incubation. In addition to the higher mineralization and hydroxyatrazine (HA) formation, a rapid decrease in the water-extractable residues was observed in the cultivated soil. When the cultivated soil was covered with straw, mineralization was reduced by up to 30 % although a small amount of remobilization to the soil occurred within the 85 days. Straw cover hindered the degradation of ATZ in cultivated soils; whereas an accelerated biodegradation was due to repeated applications of ATZ, which may have selected microbiota more skilled at biodegrading the herbicide.
Keywords: atrazine mineralization, sorption, metabolites, no-tillage

Introduction

Brazil is one of the world's largest maize (*Zea mays* L.) producers and it was estimated that production for the 2016/2017 crop season would reach 92 million tons, which is mainly cultivated under no-tillage (CONAB, 2017; Corbeels et al., 2016). The herbicide atrazine (ATZ) (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine), a broadleaf herbicide, is widely applied in maize plantations in Brazil as well as in other American countries although it has been banned in Europe since 2004 (Mudhoo and Garg, 2011; Santos et al., 2015; Sass and Colangelo, 2006). Due to its physical-chemical properties, ATZ is considered a pollutant of some environmental concern. Soil organic matter is the main soil sorbent for ATZ, followed, to a lesser extent, by the inorganic fraction, which may represent a barrier to its downward translocation of the profile (Dick et al., 2010). Concentrations of ATZ above 2 µg L^{-1}, the limit allowed by the Brazilian regulation system (Conama, 2005), and relevant concentrations of its metabolites, have been found in a number of water matrices in different regions in Brazil (Casara et al., 2012; Nogueira et al., 2012; Santos et al., 2015). Enhanced biodegradation of pesticides, due to adaptation of soil microorganisms promoted by repeated application of the chemical compound, results in a rapid use of the molecule as a carbon source of energy and/or nutrients (Arbeli and Fuentes, 2007). Agricultural soils with detected enhanced *s*-triazine degradation are to be found worldwide and the consequent short-term persistence of ATZ may mitigate its potential risk for contamination. However, it also reduces its biocide efficiency (Krutz et al., 2010).

Previous laboratory and field studies on Brazilian soils evaluated the patterns of ATZ dissipation (Aquino et al., 2013; Correia et al., 2007) while others considered the role of straw (covering or mixed into the soil (Amadori et al., 2016; Araldi et al., 2015; Silva et al., 2016) or the extent of enhanced ATZ degradation (Bonfleur et al., 2010; Martinazzo et al., 2010). Nevertheless, a simultaneous study of ATZ degradation patterns with radiolabeled herbicide, focusing on the role of the straw cover simulating a no-tillage system has not so far been reported for Brazilian agricultural soils. Thus, the role of the straw cover on the dissipation kinetics of ATZ in a Typic Paleudult with a history of ATZ application over 20 years under no-tillage was investigated. Simultaneously, ^{14}C-ATZ mineralization, extractable and non-extractable ^{14}C-ATZ residues, and metabolite formation were evaluated in both soil and straw compartments.

Materials and Methods

Field history, soil and straw sampling

The experimental field was located at Eldorado do Sul, in the state of Rio Grande do Sul, Brazil (30°50′52″ S; 51°38′08″ W) at 46 m above sea level, on a Typic Paleudult (sandy clay loam soil). The soil particle size distribution contained 496 g kg^{-1} of sand, 291 g kg^{-1} of silt, and 213 g kg^{-1} of clay, with the clay fraction composed mainly of kaolinite, hematite and goethite (Dick et al., 2010). The climate is classified as humid subtropical (Cfa) in accordance with Köppen´s climate classification (Alvares et al., 2013). The samples referred to as "cultivat-

ed soil" were collected in an area which has been under no-tillage with crop rotation (maize/oat (*Avena sativa* L.)/ soybean (*Glycine max* (L.) Merr)) since 1991 and which has been treated for the past 20 years with commercial ATZ at agronomically recommended dosages (1.0 - 1.25 kg of active ingredient ha^{-1}) to control annual weeds. The predominant weed species found in the experimental field were *Brachiaria plantaginea, Bidens* spp. and *Sida rhombifolia*. ATZ field application was carried out every other year and the last application was in Oct 2010, resulting in a total of 10 applications within 20 years. The samples referred to as "native soil" were collected in an adjacent area under field grass, approximately 50 m away from the "cultivated soil", which had no history of agricultural use of ATZ. However, the potential risk of drift caused by the ATZ spraying during its application in the cultivated soil should not be neglected. Both areas were part of a long-term experiment and approximately 2 kg of soil samples were collected from each site. Samples were taken randomly in triplicate from the upper 10 cm soil layer in Aug 2011 and were subsequently air-dried and sieved (2 mm). The main characteristics of the cultivated and native soils are given in Table 1. Samples of dry oat straw were collected in an adjacent cultivated area under no-tillage and were kept in plastic bags. Oat was chosen to simulate soil cover because it is commonly used as a winter cover crop in that region, and is usually desiccated with glyphosate (1.8 - 2.4 kg of active ingredient ha^{-1}). The oat straw contained 39 % C, 6 % H and 1 % N on air-dry matter.

ATZ mineralization

Incubation experiments were conducted for 85 days in hermetically sealed 250 mL Duran glass bottles (70 mm diameter and 143 mm height) in a dark room with a controlled temperature of 20 ± 2 °C, in Jülich, Germany. Dry soil samples were initially wetted to 20 % of the soil maximum water-holding capacity (WHCmax) and kept in the dark at 24 ± 2 °C for 24 h to reactivate soil microbiota. A spiking solution was prepared using technical-grade ATZ (99 % chemical purity) and ring-^{14}C-labeled ATZ (99 % radiochemical purity, 6.4 MBq mL^{-1}) in ethanol (Merck), and its application to the incubation vessels followed two different methods, herein referred to as "mixing" and "dropping". By the mixing application method, the spiking solution was added to 10 g of an air-dried ground aliquot of soil sample, and ethanol contained in the solution was further evaporated at an experimental temperature. At 20 °C the vapor pressure of ethanol is 5.8 kPa and that of ATZ 0.4 10^{-8} kPa (NCBI, 2017), thus the loss of ATZ during ethanol evaporation was negligible. Subsequently, these 10 g spiked aliquots were thoroughly mixed with 400 g (dry weight) of non-spiked sample (cultivated soil and native soil). Ten subsamples of 0.5 g dry weight from each soil treatment were combusted to determine total radioactivity and monitor the homogeneous distribution of ATZ in the soil. The initial herbicide concentration was 0.9 mg kg^{-1}, and initial radioactivity was 1505 kBq kg^{-1} soil. Using the dropping application method, the spiking solution was applied in small drops (0.5 µL) directly to the straw or soil surface to simulate herbicide application by spraying in the field.

Incubation experiments were performed in a complete randomized design with three replicates. To evaluate the occurrence of enhanced ATZ degradation in the cultivated soil, Microcosm 1 and Microcosm 2 were prepared with cultivated soil and native soil, respectively, and ^{14}C-ATZ solution was applied as per the mixing method. To evaluate the effect of straw cover on ATZ dissipation in the cultivated soil, Microcosm 3 and Microcosm 4 were prepared with this soil in the presence and absence of straw cover, respectively, and ^{14}C-ATZ solution was applied by dropping. An extra microcosm (Microcosm 5) with solely straw was also performed (Table 2). The final soil moisture content of each flask was adjusted to 50 % WHCmax and maintained during the 85 days of incubation by checking the weight, and adding distilled water by dropping. To monitor the evolving $^{14}CO_2$, a glass vial containing 1.5 mL of 2.0 mol L^{-1} NaOH solution was placed in a cap holder inside the incubating flasks. NaOH solution was periodically removed and replaced by fresh solution on a daily basis during the first seven days of incubation, then every two to four days during the first 30 days of incubation, and weekly thereafter. The sampling time of the trapped $^{14}CO_2$ was based on previous ^{14}C-ATZ mineralization assays (Jablonowski et al., 2010, 2013; Martinazzo et al., 2010), which showed a pattern of an increasing and rap-

Table 1 – Characteristics of the Typic Paleudult at the time of sampling.

Characteristics	Typic Paleudult	
	Cultivated	Native
Water-holding capacity (%)	53	51
Organic carbon content (%)	3.8	4.1
Soil pH (H_2O)	5.0	5.3
Clay (%)	22	14
Atrazine (ATZ)* (µg kg^{-1})	1.5	n.d.
Hydroxyatrazine (HA)* (µg kg^{-1})	1.7	0.3
Desethylatrazine (DEA)* (µg kg^{-1})	n.d.	n.d.
Deisopropylatrazine (DIA)* (µg kg^{-1})	n.d.	n.d.

*Extracted by means of accelerated solvent extraction using methanol/water solution (4:1 v/v); n.d. = below detection limit.

Table 2 – Treatments description.

Microcosm	Soil[1]	Straw cover[2]	ATZ application
1	Cultivated	Absence	Mixing with the soil
2	Native	Absence	Mixing with the soil
3	Cultivated	Presence	Dropping
4	Cultivated	Absence	Dropping
5	Absence	Presence	Dropping

[1]The soil layer (20 g dry weight) was about 3 cm high; [2]The oat straw was cut into small pieces (1 cm) and the straw layer (0.9 g dry weight) was about 2 cm high.

idly evolved $^{14}CO_2$ at the beginning of the incubation, followed by a plateau. Trapped $^{14}CO_2$ was determined by a liquid scintillation counter (LSC) using a 10 mL scintillation cocktail and 4 mL deionized water (18.0 MΩ cm) per sample.

Extractable and non-extractable ATZ residues

Extractable and non-extractable radioactivity and metabolite formation were monitored on days 0, 9, 16, 30 and 85 of incubation. Three fractions of ^{14}C activity were determined as: (1) extractable by water, (2) extractable by accelerated solvent extraction (ASE), and (3) non-extractable residues.

Water extraction

The entire soil sample contained in each flask from Microcosms 1, 2, 3 and 4 was extracted once with deionized water in a soil/solution ratio of 1:8, in accordance with Jablonowski et al. (2008). The whole straw sample from Microcosms 3 and 5 was manually collected using forceps and subsequently extracted once with 60 mL of deionized water. The soil and straw suspensions were shaken on a horizontal shaker at room temperature for 6 h at 150 rpm. The soil suspension was centrifuged at 10000 × g for 90 min. The soil and straw supernatants (~80 mL and 40 mL, respectively) were filtered through a 0.45 µm membrane and their volume measured. The filtrate was analyzed for desorbed ^{14}C activity via LSC, and for ATZ and its metabolites via liquid chromatography and mass spectrometry (LC-MS/MS). The filters used in this procedure were combusted, and the radioactivity detected was considered in the final mass balance. Water-extracted soil and straw samples were freeze-dried and ground in a mortar.

Accelerated solvent extraction

The ASE-extraction was based on the method described by Jablonowski et al. (2009). Following water extraction, 10 g subsamples of ground soil from Microcosms 1, 2, 3 and 4 were extracted four times in succession using an ASE 200 system. All the soil subsamples (10 g) were transferred to 11 mL stainless steel ASE cells, and the remaining space above the samples filled with quartz (Merck) to reduce the extract volume and avoid clogging of the ASE steel filter lid. A methanol (Merck)/water solution (4:1 v/v) was used as a solvent for extraction. Previous results have shown that this mixture yields higher residual ^{14}C-activity in the extracts than pure methanol (Gan et al., 1999; Jablonowski et al., 2009). The extraction was performed at 135 °C under 100 bar with a flush volume of 60 % of extraction cell volume. The heat-up and static times were 7 min and 15 min, respectively. Straw samples from Microcosms 3 and 5 were not ASE-extracted due to the deterioration in condition after water extraction. Water- and ASE-extracted ^{14}C activity was detected by LSC in triplicate. The water-extracted sample (5 mL) and ASE-extracted sample (1 mL plus 4 mL deionized water) were mixed with a 10 mL scintillation cocktail. An external standard was used for quenching correction using 5 mL of distilled water with 10 mL of scintillation cocktail.

Non-extractable residues

The ^{14}C activity remaining in the soil of the Microcosms 1, 2, 3 and 4 after ASE-extraction was referred to as non-extractable ^{14}C residue. The ^{14}C activity remaining in the straw of Microcosms 3 and 5 after the water extraction was referred to as non-water-extractable ^{14}C residue. The non-extractable and non-water-extractable ^{14}C residues were determined using three replicates containing 0.5 g dry weight of soil and 0.1 g dry weight of straw, respectively. Samples were weighed in porcelain vials and combusted using a biological oxidizer. The $^{14}CO_2$ produced was trapped in the scintillation cocktail and analyzed by LSC. The mass balance of ^{14}C was determined by summing the$^{14}CO_2$ evolved during mineralization, the^{14}C activity in water and ASE extracts, and the $^{14}CO_2$ recovered from the combusted samples. A mass balance between 77 % and 100 % was obtained.

LC-MS/MS analysis

ATZ and its metabolites were identified and quantified in water and ASE extracts by means of LC-MS/MS equipped with an autosampler. The standards for ATZ, and its metabolites hydroxyatrazine [2-hydroxy-4-ethylamino-6-isopropylamino-s-triazine (HA), 96 %], desethylatrazine [2-chloro-4-amino-6-isopropylamino-s-triazine (DEA), 100 %], and deisopropylatrazine [2-chloro-4-ethylamino-6-amino-s-triazine (DIA), 96 %] were purchased from Riedel-de Haën. Deuterated ATZ and its metabolites were used as internal standards in a concentration of 0.01 µg mL^{-1}. Water and ASE extracts (100 µL of each) were mixed with 40 µL of deuterated standard solution. The solid phase (2.1 mm × 125 mm × 3 µm) was used with an additional LC precolumn (2.1 mm × 10 mm × 3 µm). Solutions of 0.1 mmol L^{-1} ammonium acetate (Merck) in deionized water and acetonitrile (Biosolve) were used as LC eluents in a gradient elution mode. The flow rate was 0.15 mL min^{-1} at a column temperature of 25 °C. The injection was performed in triplicate, and the total injection volume of each sample was 10 µL. LC-MS/MS analyses were performed in a positive electrospray ionization source (ESI+), and transitions were measured in multiple reaction monitoring (MRM). The settings for the analysis and mass transfer of ATZ metabolites were based on Jablonowski et al. (2008). The analytical detection limit and retention time were, respectively, 0.03 ng mL^{-1} and 17.44 min for ATZ, 0.04 ng mL^{-1} and 13.61 min for HA, 0.7 ng mL^{-1} and 14.58 min for DIA, and 0.4 ng mL^{-1} and 13.46 min for DEA.

Data analysis

The whole ATZ mineralization data from Microcosms 1 and 4 did not fit a zero, first or second-order degradation kinetic model. However, the data, when divided into two consecutive segments, fitted a first-

order degradation kinetic model [$Y = A_0 \times (1\text{-}e^{-kt})$]. In this equation, A_0 is the maximum amount of evolved $^{14}CO_2$ (% of added radioactivity), k the mineralization rate constant (d^{-1}) and t the time (days). A mineralization rate lower than 1 % of applied ^{14}C-ATZ d^{-1} was considered as the dividing point between the two segments of the curve. The half-life ($t_{1/2}$) value for ATZ was calculated from the relationship $t_{1/2} = \ln 2/k$. Data from Microcosms 2 and 3 were fitted to a zero-order degradation kinetic model [$Y = -k \times t + C_0$], where C_0 is the initial amount of applied radioactivity, and the half-life value for ATZ was calculated from the relationship $t_{1/2} = C_0/2k$. However, due to minor ^{14}C-ATZ mineralization in these two Microcosms within the experimental time, the values for ATZ half-lives based on ^{14}C-ATZ mineralization data were not appropriate (Jablonowski et al., 2010; Martinazzo et al., 2010). Therefore, the estimated half-lives for Microcosms 2 and 3 will not be presented nor discussed.

Statistical analysis consisted of the application of an independent two-sample t test in order to compare the means as follows: cultivated soil (Microcosm 1) *versus* native soil (Microcosm 2), cultivated soil with straw cover (Microcosm 3) *versus* cultivated soil without straw cover (Microcosm 4), and straw of Microcosm 3 *versus* straw of Microcosm 5, at each sampling date. Treatment effects were considered significant at $p < 0.05$. All statistical analysis was performed using SAS (Statistical Analysis System, version 9.3).

Results and Discussion

ATZ mineralization

Figure 1A shows the cumulative $^{14}CO_2$ results. Data showed differences ($p < 0.05$) in ATZ mineralization between the cultivated soil (Microcosm 1) and the native soil (Microcosm 2) throughout the entire incubation period. By the end of the experiment, Microcosm 1 presented ATZ mineralization (86 %) higher than Microcosm 2 (10 %). By day 12 of incubation, only 25 % of the applied radioactivity remained in Microcosm 1, showing the occurrence of rapid ATZ degradation in the cultivated soil. Enhanced biodegradation of ATZ was reported in a heavy clayey subtropical Rhodic Hapludox cropped with maize under no-tillage from the south of Brazil where ATZ mineralization reached 82 % by day 85 of incubation (Martinazzo et al., 2010). In the same study, a different pattern with a clayey Xanthic Haplustox from northeastern Brazil was observed, where, in addition to a lag phase of about 7 days, approximately 74 % of the initially applied ^{14}C-ATZ was mineralized by the end of the incubation period. Results from this study, together with data from other authors indicated that, in addition to the history of herbicide application (Bonfleur et al., 2010), physical and chemical properties of the soil (Dick et al., 2010), local environmental conditions, such as rainfall (Correia et al., 2007), topography (Aquino et al., 2013) and agricultural practices, such as straw

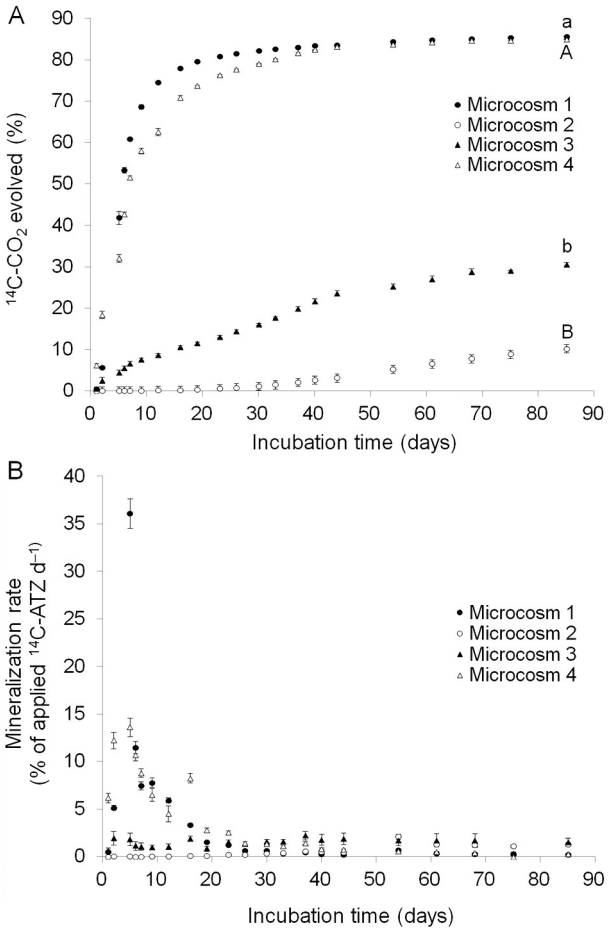

Figure 1 – Cumulative $^{14}CO_2$ (A) and rates of ^{14}C-ATZ mineralization (B) during laboratory incubations in the cultivated soil (Microcosm 1), in the native soil (Microcosm 2), in the cultivated soil covered with straw (Microcosm 3) and with no straw cover (Microcosm 4). Different upper case letters indicate significant differences between Microcosm 1 and Microcosm 2; different lower case letters indicate significant differences between Microcosm 3 and Microcosm 4, on day 85 of incubation (t test, $p < 0.05$). The error bars represent the standard deviation of the mean. n = 3.

cover (Amadori et al., 2016) and the addition of organic residues (Silva et al., 2016) may also affect the extent of ATZ dissipation.

Concerning the role of cover straw, data showed differences ($p < 0.05$) in ATZ mineralization between the cultivated soil when covered with straw (Microcosm 3) and with no straw cover (Microcosm 4) throughout the incubation period (Figure 1A). The presence of straw on the soil surface reduced ATZ mineralization from 85 % (Microcosm 4) to 31 % (Microcosm 3) by day 85 of incubation. It was concluded that the straw most likely acted as a physical barrier and prevented the herbicide from reaching the soil where it would be degraded by the adapted microorganisms. Interestingly, the cultivated soil was submitted to two methods of ATZ application,

mixing (Microcosm 1) and dropping (Microcosm 4), and in both Microcosms ATZ mineralization reached values of up to 85 %.

Figure 1B shows the mineralization rate results. By day 5 of incubation, the mineralization rate had reached its maximum, corresponding to a mineralization of 36 % and 14 % of the applied ^{14}C-ATZ d^{-1} in Microcosms 1 and 4, respectively. Thereafter, the mineralization rate decreased to 1 % d^{-1} by days 26 and 40 of incubation in Microcosms 1 and 4, respectively, and then remained fairly constant until the end of the experiment. In contrast, the mineralization rate in the native soil (Microcosm 2) and in the cultivated soil with straw cover (Microcosm 3) did not exceed values of 2 % d^{-1}. In Microcosms 1 and 4 with cultivated bare soil, the experimental data were fitted to two consecutives segments of a first-order kinetic model indicating the occurrence of two degradation kinetics within the incubation period. Regardless of the application mode, no latency time in overall mineralization was observed in the cultivated bare soil. In Microcosm 1, an initial fast degradation occurred by day 26 of incubation when 82 % of the applied ATZ had been mineralized. The estimated half-life of this phase, estimated purely from the mineralization data, was 10 days. In Microcosm 4, faster degradation kinetics occurred by 43 days of incubation when 82 % of the applied ATZ had been mineralized with an estimated half-life of 16 days. In both microcosms, the estimated half-life of the second degradation phase was 154 days. This degradation pattern may be related to the decrease in carbon sources throughout the incubation period by soil adapted microorganisms (Arbeli and Fuentes, 2007). However, a greater half-life than that calculated for the cultivated bare soil (Microcosms 1 and 4) was expected due to the lack of efficient degrading microorganisms in the native soil and also due to the physical barrier offered by the straw. The half-lives obtained in the present study for the faster degradation phase are in the same order of magnitude as the values obtained in other Brazilian Oxisols that varied between 4 and 51 days (Martinazzo et al., 2010; Silva et al., 2016).

Extractable and non-extractable ^{14}C-ATZ residues

Figures 2A and B show the water-extractable and ASE-extractable ^{14}C residues and results, respectively, obtained in Microcosms 1 and 2. Data showed differences ($p < 0.05$) in the amount of ^{14}C residues between the cultivated soil (Microcosm 1) and the native soil (Microcosm 2) throughout the incubation time. Microcosm 2 presented higher amounts of water-extractable and ASE-extractable ^{14}C residues than Microcosm 1, except on the first day of sampling. Microcosm 1 presented an amount of water-extractable ^{14}C residues of 69 % directly after ATZ application (day 0) (Figure 2A). This value decreased drastically to about 7 % on day 9 of incubation, and had reached 1 % by the end of the incubation period. The amount of ASE-extractable ^{14}C residues in Microcosm 1 varied from 16 % to 8 % during the 85 days (Figure 2B). Non-extractable ^{14}C residues were not detected in the cultivated soil. This pattern of ^{14}C residue formation was somewhat expected because of the rapid mineralization of ATZ observed in the soil with a history of ATZ application, as shown in Figure 1A. In contrast, in Microcosm 2, the amount of water-extractable ^{14}C residues decreased more slowly over time (from 62 % to 20 %) (Figure 2A), and there was a concomitant increase in the ASE-extractable ^{14}C residues from 15 % to 51 % (Figure 2B). The amount of non-extractable ^{14}C residues was about 0 % in this microcosm (data not shown). Considering the low ATZ mineralization obtained in the native soil (10 %) and the amounts of ^{14}C residues throughout the incubation

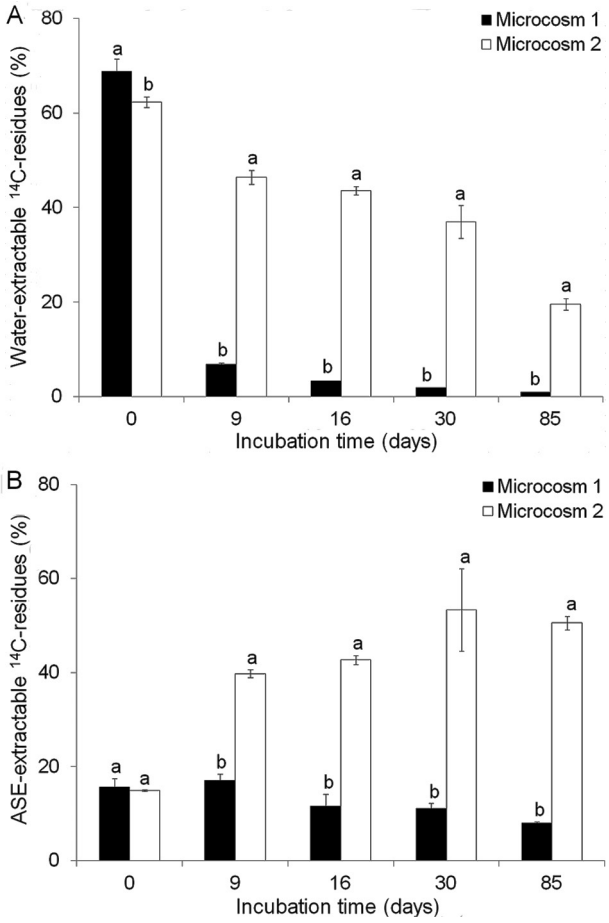

Figure 2 – Water-extractable ^{14}C residues (A) and accelerated solvent extraction (ASE)-extractable ^{14}C residues (B) in the cultivated soil (Microcosm 1) and in the native soil (Microcosm 2). Non-extractable ^{14}C residues were approximately 0 % and do not appear. Values are reported as percentages of total ^{14}C applied activity as a function of time. Different letters indicate significant differences of the water-extractable and ASE-extractable ^{14}C residues between the microcosms at a given sampling date (t test, $p < 0.05$). The error bars represent the standard deviation of the mean. n = 3.

period, it appeared that ATZ and its metabolites were mainly transferred from the more accessible soil compartment (water-extractable) to a less accessible one (ASE-extractable) as a result of its degradation. These results indicated the occurrence of an aging process of ATZ in the soil, characterized by a stronger bonding of the compound with soil components over time and a decrease in its bioavailability (Gevão et al., 2003).

Figures 3A, B and C show the water-extractable, ASE-extractable and non-extractable ^{14}C residue results, obtained in Microcosms 3 and 4, respectively. Data showed differences ($p < 0.05$) in the amount of extractable and non-extractable ^{14}C residues between the cultivated soil when covered with straw (Microcosm 3) and with no straw cover (Microcosm 4) for the entire incubation period. Microcosm 4 presented an amount of water-extractable ^{14}C residue greater than Microcosm 3 only on the first day of sampling (82 % and 23 %, respectively). In Microcosm 4 the amount of water-extractable ^{14}C residues rapidly decreased to 9 % by day 9 of incubation, reaching 10 % by day 85 of incubation (Figure 3A). Microcosm 4 also presented a higher amount of ASE-extractable ^{14}C residues than Microcosm 3 on days 0, 16 and 30 of incubation. In Microcosm 4 the amount of ASE-extractable ^{14}C residues decreased from 24 % to 9 % (Figure 3B), whilst the amount of non-extractable ^{14}C residues was approximately 5 % during the incubation period (Figure 3C). As regards Microcosm 3, the amount of water-extractable ^{14}C residues decreased from 23 % to 8 % (Figure 3A), whilst those of ASE-extractable ^{14}C residues increased from 4 % to 11 % (Figure 3B), during the incubation period. Moreover, by the end of the experiment, the amount of non-extractable ^{14}C residues (Figure 3C) was higher in Microcosm 3 (7 %) than in Microcosm 4 (4 %). On days 0 and 85 of incubation, the sum of the three ATZ compartments (water-extractable, ASE-extractable and non-extractable) in the soil of Microcosm 3 was 28 % and 26 %, respectively. These results indicated that in the soil covered with straw, ATZ was redistributed from the more accessible compartment (water-extractable) to a less accessible one (ASE-extractable and non-extractable) during the incubation period. Considering that solvent accessibility to a given ATZ soil compartment may be related to its accessibility to biodegradation or its uptake, it therefore follows that the residues in the soil tended to be less susceptible to biodegradation throughout the incubation period.

Figure 4 shows the water-extractable and non-water-extractable ^{14}C residue results obtained from Microcosms 3 and 5. The measurements of ^{14}C residues were taken only on days 0 and 85 of incubation in Microcosm 5. Therefore, there is no statistical analysis between Microcosm 3 and Microcosm 5 on days 9, 16 and 30 of incubation. Data showed differences ($p < 0.05$) in the amount of ^{14}C residue between the straw cover of cultivated soil (Microcosm 3) and solely straw cover alone (Microcosm 5) on days 0 and 85 of incubation. In the case of the straw cover alone (Microcosm 5) there

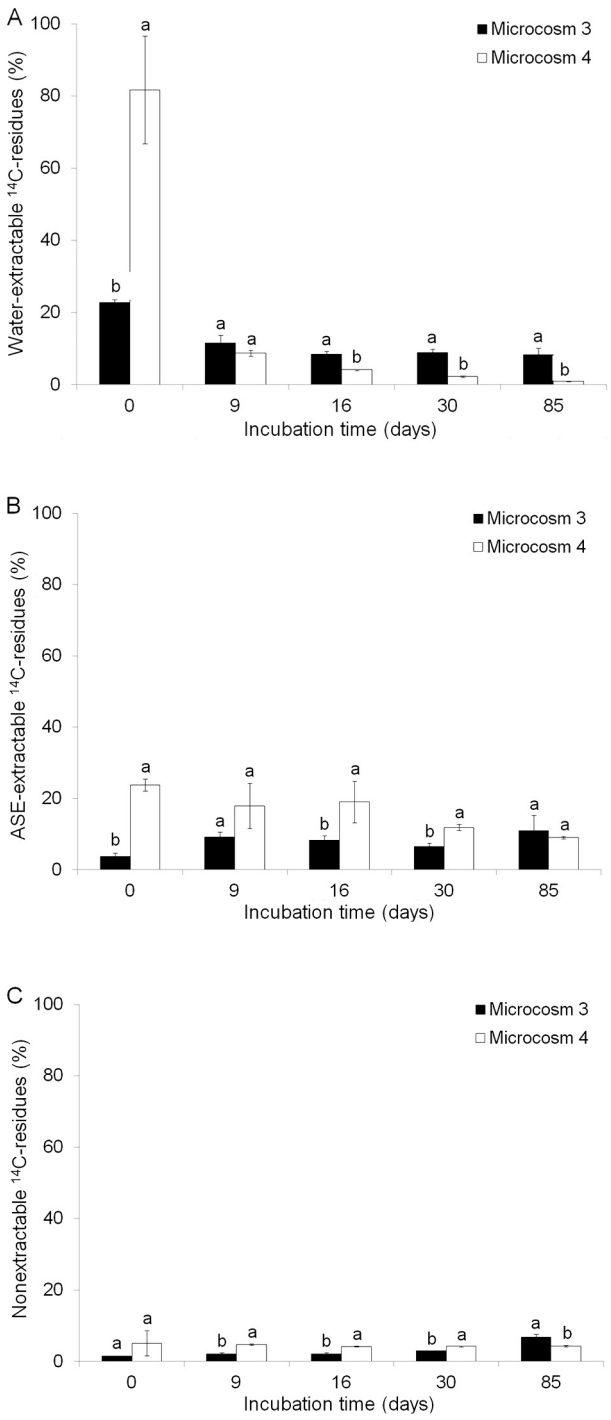

Figure 3 – Water-extractable (A), ASE-extractable (B) and non-extractable (C) ^{14}C residues in the cultivated soil covered with straw (Microcosm 3) and with no straw cover (Microcosm 4). Values are reported as percentages of total ^{14}C applied activity as a function of time. Different letters indicate significant differences in the water-extractable, ASE-extractable and non-extractable ^{14}C residues between the microcosms on a given sampling date (t test, $p < 0.05$). The error bars represent the standard deviation of the mean. n = 3.

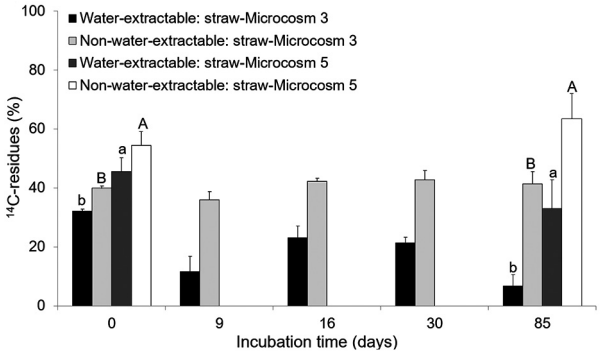

Figure 4 – Water-extractable and non-water-extractable ^{14}C residues in the straw cover of Microcosm 3 and in the straw of Microcosm 5. ^{14}C residues were extracted only on days 0 and 85 of incubation in Microcosm 5. Values are reported as percentages of total ^{14}C applied activity as a function of time. Different upper case letters indicate significant differences of the non-water-extractable ^{14}C residues between microcosms; different lower case letters denote significant differences in the water-extractable ^{14}C residues between microcosms, at a given sampling date (t test, $p < 0.05$). The error bars represent the standard deviation of the mean; n = 3.

were higher amounts of water-extractable and non-water-extractable ^{14}C residue when compared with the straw cover of the cultivated soil (Microcosm 3) on both sampling dates. In the straw cover of Microcosm 3, the amount of water-extractable ^{14}C residue ranged from 32 % on day 0 to 7 % of the applied ^{14}C ATZ on day 85 of incubation; whilst that of the non-water-extractable residue remained fairly constant oscillating around 40 %. In Microcosm 5, where straw was incubated in the absence of soil, the amount of water-extractable ^{14}C residue ranged from 46 % to 33 %; and, the amount of non-water-extractable ^{14}C residue from 54 % to 64 % during incubation. Furthermore, the sum of the amounts of the two compartments approached 100 % of the applied ATZ both on day 0 of incubation as well as on day 85. It therefore followed that no relevant ATZ mineralization (data not shown) occurred in the case of the straw cover alone (Microcosm 5). In fact, an aging process of the herbicide was observed only where it migrated to a less accessible/stronger bound form. Therefore, ATZ mineralization in Microcosm 3 may have occurred at the expense of the water-extractable ^{14}C residue in the straw cover, which was gradually transferred to the soil, where it was then degraded by the soil-adapted microorganisms (Figures 1A, 3A and 4).

Extractable ATZ and its metabolites

Figures 5A, B, C, D, E and F show the extractable ATZ and its metabolite results. The extractable ATZ and its metabolites were considered as a whole (water-extractable + ASE-extractable residues) and discussed as a proportion of the total amount extracted on each sampling date. In the water and ASE extracts, only the metabolites HA and DEA were detected. These two residues are considered the most relevant ATZ metabolites and they result from ATZ hydrolysis (HA) and N-dealkylation of the ATZ side chains (DEA) (Mudhoo and Garg, 2011).

Figure 5A, C and D show extractable ATZ and its metabolites in the microcosms with cultivated soil (Microcosms 1, 3 and 4). In general, in the cultivated soil ATZ concentration ranged from 80 % to 94 % on day 0 of incubation and dropped to values between 2 % and 19 % on day 85 of incubation. The decrease in ATZ concentration was accompanied by an increase in its extractable metabolites. As expected, a greater amount of HA was observed in comparison to DEA. At the end of the incubation period, the amount of HA varied between 78 % and 97 %; whilst that of DEA ranged from 2 % in Microcosms 1 and 4 to 10 % in Microcosm 3. This pattern indicated the occurrence of chemical hydrolysis and presence of microbial communities which degrade ATZ into hydroxylated derivatives and dealkylated metabolites, respectively (Sene et al., 2010). Similar findings of ^{14}C-ATZ degradation (mainly into HA) have been reported for other Brazilian soils (Martinazzo et al., 2010; Peixoto et al., 2000; Prata et al., 2003).

Figure 5B shows the extractable ATZ and its metabolites in the native soil (Microcosm 2). ATZ concentration decreased from 78 % to 64 %; HA concentration increased from 22 % to 32 %, and DEA concentration reached 4 % during the incubation period. Both mineralization and metabolization of ATZ (Figures 1A and 5B, respectively) were comparatively lower in the native soil. Nevertheless, the low ATZ mineralization (10 %) and this partial degradation of ATZ into HA (32 %) and DEA (4 %) in the native soil indicated the presence of soil microbiota capable of degrading ATZ. The presence of 0.3 μg kg^{-1} of HA detected in the native soil (Table 1) is most probably attributable to a previous contamination during its application in neighboring cultivated areas, whilst the agricultural use of pesticides may result in some loss of the applied compound even before it reaches the soil (Casara et al., 2012; Thurman and Cromwell, 2000). Nevertheless, the possibility of an abiotic formation of HA should not be discarded, since the studied soils presented pH levels (Table 1) that could favor the formation of hydroxylated derivatives to the detriment of DEA and DIA (Loiseau and Barriuso, 2002), which were not detected at this time.

Figures 5E and F show the extractable ATZ and metabolites in the straw of both Microcosm 3 and Microcosm 5, respectively. In the straw extracts of Microcosm 3, ATZ was the most abundant extractable compound until day 30 of incubation (> 85 %), decreasing drastically and by day 85 of incubation it represented only 12 % of the extractable residues (Figure 5E). At the end of the incubation period, the HA proportion was 77 % and that of DEA was 10 % of the extractable residues. The same pattern was observed for the straw from Microcosm 5 (Figure 5F). By day 85 of incubation, ATZ, HA and DEA concentrations had reached 24 %, 73 % and 4 %,

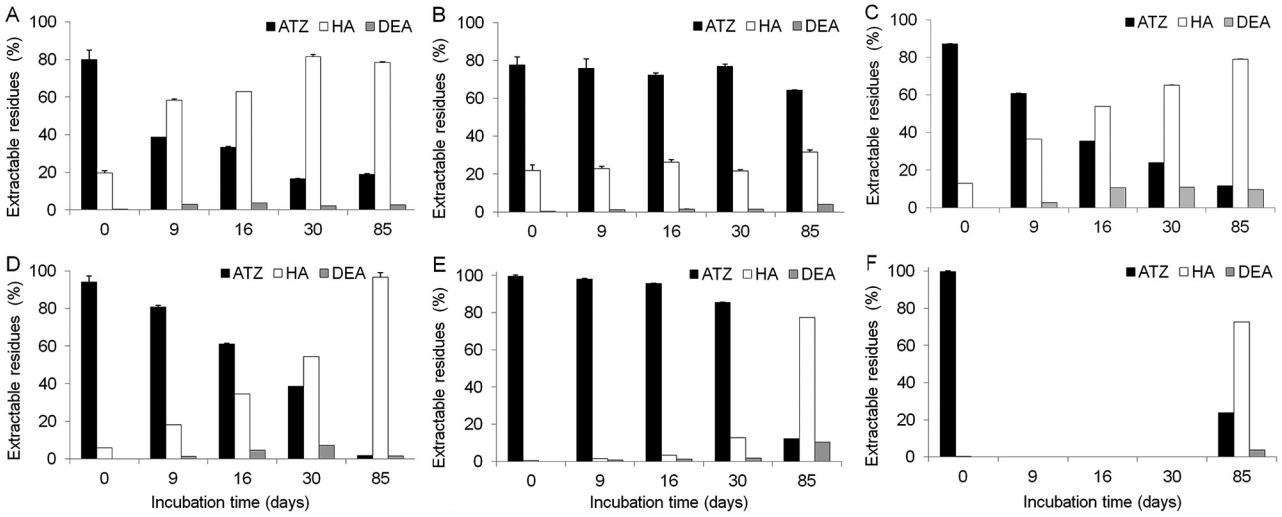

Figure 5 – Proportion of extracted atrazine (ATZ), hydroxyatrazine (HA) and desethylatrazine (DEA) in relation to the total amount extracted within 85 days of incubation in the cultivated soil (Microcosm 1) (A), in the native soil (Microcosm 2) (B), in the cultivated soil covered with straw (Microcosm 3) (C) and with no straw cover (Microcosm 4) (D), in the straw cover on the cultivated soil (Microcosm 3) (E) and in the straw (Microcosm 5) (F). The error bars represent the standard deviation of the mean; n = 3.

respectively. Regarding ATZ retention in straw and its formation of metabolites, a previous experiment performed in a maize field in Brazil (Paraná State) reported ATZ concentrations two-fold higher in comparison to DEA and DIA even after 180 days of ATZ application (Amadori et al., 2016). Degradation of ATZ into its metabolites observed in the straw from Microcosms 3 and 5 may also have been caused by fungi, since the presence of fungal colonies in both microcosms were visible to the naked eye. Sene et al. (2010) described that ATZ biodegradation is mainly due to bacteria, but it has also been observed in a number of fungi species. The authors also reported on previous incubation experiments with fungi, in which the biodegradation of ATZ resulted in the accumulation of hydroxylated and/or N-dealkylated metabolites and no mineralization of the ring-^{14}C-labeled ATZ.

Conclusion

The cultivated Typic Paleudult evaluated in this study presented greater ATZ mineralization (8.6 fold) and metabolization (2.2 fold) as compared with the native soi that was assigned to the adaptation of the soil microorganisms to the herbicide. This behavior is positive from an environmental standpoint but negative with regard to weed control. The straw cover on the soil surface decreased ATZ contact with the soil and thus its access to the ATZ-degrading microorganisms. As a result, overall ATZ mineralization in this system was also reduced. In the straw, ATZ was either transferred to a less accessible compartment or transformed, probably abiotically, into HA at the very end of the incubation period. Information about the sorption and leaching of herbicides from straw cover is needed to prevent the increase in herbicide application frequency in order to overcome the dissipation of ATZ due to these two mechanisms.

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Conceptualization: Leal, D.P.B., Dick, D.P., Burauel, P. Data acquisition: Leal, D.P.B., Stahl, A.M., Köppchen, S. Data analysis: Leal, D.P.B., Dick, D.P., Köppchen, S., Burauel, P. Design of methodology: Leal, D.P.B., Dick, D.P., Burauel, P. Writing and editing: Leal, D.P.B., Dick, D.P., Burauel, P.

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