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Degradation of the herbicide sulfentrazone in a Brazilian Typic Hapludox soil

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Abstract

The herbicide sulfentrazone is classified as highly mobile and persistent and this study aimed to examine degradation of this compound on a Typic Hapludox soil that is representative of regions where sulfentrazone is used in Brazil. Soil samples were supplemented with sulfentrazone (0.7 μg active ingredient (a.i.) g^{-1} soil), and maintained at 27 °C. Soil moisture was corrected to 30%, 70%, or 100% water-holding capacity (WHC) and maintained constant until the end of the experimental period. Soils without added herbicide were used as controls. Aliquots were taken after 14, 30, 60, 120, 180, and 255 days of incubation for quantitative analysis of sulfentrazone residues by gas chromatography. Another experiment was conducted in soil samples, with and without the herbicide, at different temperatures (15, 30, and 40 °C), with moisture kept constant at 70% of WHC. The sulfentrazone residues were quantified by gas chromatography after 14, 30, 60, and 120 days of incubation. Sulfentrazone degradation was not affected by soil moisture. A significant effect was observed for the temperature factor after 120 days on herbicide degradation, which was higher at 30 °C. A half-life of 146.5 days was recorded. It was observed that the herbicide stimulated growth of actinomycetes, whereas bacterial and fungal growth was not affected. The microorganisms selected as potential sulfentrazone degraders were *Rhizobium radiobacter*, *Ralstonia pickettii*, *Methylobacterium radiotolerans*, *Cladosporium* sp., *Eupenicillium* sp., and *Paecilomyces* sp.

Keywords: Herbicide; Microorganisms; Degradation; Sulfentrazone

1. Introduction

The use of herbicides for agricultural purposes represents 49% of all consumption of agrochemicals in the world. In Brazil, business involving these compounds is concentrated in crops such as soybean, sugarcane, corn, rice, and cotton (Pachione, 2004). The State of São Paulo is the second largest soybean and sugarcane producer (Governo, 2005) and the most popular herbicide used on these crops is sulfentrazone (Fairbanks, 2005).

Sulfentrazone, described chemically as a triazolinone (Leung et al., 1991; Anderson et al., 1994), allows a wide control spectrum and can be applied post-planting or at pre-emergence in relation to the weeds. Weed control occurs by inhibition of the enzyme protoporphyrinogen oxidase (Protox) (Dayan et al., 1998). It is a weak acid with a dissociation constant (pK_a) of 6.56. In agricultural soils, it can be found in neutral ($pH < 6.56$) or anionic form ($pH > 6.56$) (Grey et al., 1997). The herbicide has high persistence, is highly mobile (mean partitioning coefficient, $K_{oc} = 43$; and mean sorption coefficient, $K_d < 1$), and has both high vertical and horizontal leaching potential, being considered dangerous to the environment (Agrofit, 2004; EPA, 2004). Paraíba et al. (2003) classified the sulfentrazone leaching potential as very high in two soils of Brazil, typically Quartzipsamment and Rhodic Hapludox, and demonstrated that including soil temperature in the leaching potential index under tropical conditions is essential.

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Although most investigations have been conducted in soils under temperate climate conditions, predominantly in Europe and North America (Dores and Delamonica-Freire, 1999), the fate of pesticides and their metabolites in tropical soils and the mechanisms that govern the distribution of these products must be better studied. In tropical regions, higher temperatures are observed in the superficial layers of the soil profiles, resulting in greater organic matter decomposition and an accumulation of iron and aluminum oxides, due to high weathering rates. Predominance of high clay contents in relation to silt also occurs (Silva et al., 1998). In Brazil, there are extensive areas with oxisols which comprise approximately 50–60% of all land (Regitano et al., 2001). Laabs et al. (2002), through a pilot study of leaching and degradation of corn and soybean pesticides in an Oxisol area of the Brazilian Cerrados, have shown that the overall pesticide half-life in the studied soil conditions differs from temperate weather conditions, and leaching studies revealed low or moderate mobility. They reported a faster degradation rate of pesticides in comparison to the rate found in temperate climates. This was due to heightened microbial populations and activities under the modified environmental conditions as a result of higher temperatures in the soil.

Thus, the objective of this study was to evaluate degradation of this herbicide in Typic Hapludox soil, under different moisture and temperature conditions, and the isolation and identification of degraders microorganisms from the soil. Soil microorganisms, especially bacteria and fungi, have been reported as the most important degraders of agrochemicals, and the environmental conditions that favor microbial development in the soil are the same that favor degradation of chemical compounds, such as temperature, moisture, and aeration (Beulke et al., 2004).

2. Material and methods

The investigation consisted of two separate laboratory soil incubations. The first incubation experiment tested the moisture effect and the second tested the temperature effect, on sulfentrazone biotransformation in soil.

2.1. Soils and treatments

2.1.1. Moisture effect

The herbicide biotransformation was evaluated in a Typic Hapludox soil under different moisture levels, at the recommended concentration of herbicide for use in the field. This soil had no previous history of sulfentrazone use. The study soil presented the chemical and physical characteristics described in Table 1. Ten subsamples of this soil were taken at random and collected at 0–10 cm depth. In the laboratory, the subsamples were mixed and homogenized to form a composite sample. The samples were air-dried, sieved (2 mm-mesh) and maintained under cold storage at 4 °C until used. The soil's pH, residual moisture, and water-holding capacity (WHC) were determined according to the methodology proposed by Embrapa (1997). One hundred and fifty grams were taken from the composite sample and were incubated in Erlenmeyer flasks (500 ml). Before the herbicide addition to the experimental units, the soil remained in the flasks for 7 days, already with moisture corrected to 30, 70, and 100% WHC, and were maintained at 27 °C. At the end of this period, the sulfentrazone technical grade (91.93% purity) was then applied to the soils by misting aqueous suspension of the herbicide to obtain the soil moisture content desired. The soil was sampled periodically for moisture evaluation, and the moisture was maintained at a standard level. Soil concentration of sulfentrazone was applied at field application rates (0.7 $\mu g\,g^{-1}$ soil). Soil samples without addition of the herbicide were used as controls. Three flasks were replicated for each treatment. At 14, 30, 60, 120, 180, and 255 days after supplementation, samples were taken to evaluate the herbicide's degradability by gas chromatography.

Table 1
Chemical properties and granulometric analysis for the Typic Hapludox soil

Physicochemical properties	Measured value
V (%)[a]	46
Aluminum ($mmol\,dm^{-3}$)	1
Potential acidity ($mmol\,dm^{-3}$)	28
Cation exchange capacity ($mmol\,dm^{-3}$)	61.1
Sum of bases ($mmol\,dm^{-3}$)	23.3
Organic matter ($g\,dm^{-3}$)	16
pH in water	6.22
Clay ($g\,kg^{-1}$)	444
Silt ($g\,kg^{-1}$)	100
Total sand ($g\,kg^{-1}$)	456
Textural classification	Loamy

[a]Base saturation.

2.1.2. Temperature effect

The other experiment was conducted in soil samples, either supplemented (0.7 $\mu g\,g^{-1}$ soil) or non-supplemented with the herbicide, with moisture corrected to 70% WHC. They were maintained at different temperatures 15, 30, and 40 °C. There were three replicate flasks for each treatment. The sulfentrazone residues were quantified by gas chromatography at 14, 30, 60, and 120 days after incubation.

2.2. Extraction and analysis of residual sulfentrazone from the soils (Lee, 2003)

Soil samples (10 g) were removed from the incubated soil samples at the sampling times mentioned earlier for different treatments. Soil samples were submitted to extraction in a reflux system, using 150 ml of acetone:hydrochloric acid (3:1; v/v) solution 0.25 $mol\,l^{-1}$, for 1 h. Later, in order to separate the matrix after reaching room

temperature, the sample was filtered through a Büchner funnel, using filter paper Whatman No. 1 (pre-rinsed with 5 ml of acetone). The filtrate was quantitatively transferred to a round-bottom flask (250 ml) and then the filtrate volume was reduced to <25 ml by rotatory evaporation at 40 °C. The final volume was adjusted to 100 ml with double distilled water. The aqueous extract was purified and concentrated in two different absorbents (C_8 and silica gel), using a Manifold system.

Aliquots (25 ml) of the extract were submitted as part of a clean-up process. Initially, at the C_8 phase, pre-conditioned with 3 ml of methanol followed by 3 ml of 0.25 N HCl. The flow rate was maintained through the C_8 cartridge at 2 ml min^{-1}. The cartridge was closed and an additional 1.5 ml of 0.25 N HCL was added. The sample was then passed through the C_8 cartridge and the cartridge was blown completely dry. In this step, the analyte of interest is retained in the absorbent; the analyte was then eluted to the silica gel cartridge with a 3-ml hexane–ethyl acetate (19:1; v/v). A silica gel cartridge was conditioned with 3 ml of ethyl acetate followed by 3 ml of hexane. The flow rate was maintained through the silica gel cartridge at 2 ml min^{-1}. The cartridge was closed and 1 ml of hexane–ethyl acetate (7:3; v/v) was added. The C_8 cartridge was attached to the top of the silica gel cartridge with a reducing adapter. Then 3 ml of hexane–ethyl acetate (7:3; v/v) was added to the C_8 cartridge, and then an additional 3 ml of hexane–ethyl acetate (7:3; v/v) was added. The C_8 eluate reached the top of the silica gel cartridge packing. The C_8 cartridge was removed and discarded. The silica gel cartridge was washed with 3 ml of hexane–ethyl acetate (7:3; v/v). The analytes were eluted and collected from the silica gel cartridge with 6 ml ethyl acetate in a glass tube. The silica gel cartridge was discarded. Then the solvent was evaporated to near dryness ($\pm$0.5 ml) using an evaporation system under nitrogen flow, and the extract was resuspended to a final volume of 5 ml in acetone.

The samples were analyzed by gas chromatography with an Agilent model 6890 gas chromatograph, equipped with a splitless injection system at 250 °C and an electron capture detector, μECD, with temperature adjusted to 300 °C. The analyte was separated in a DB 608 megabore column (30 m × 0.53 mm × 0.83 μm) with a nitrogen flow of 16 ml min^{-1} and an oven temperature of 180 °C for 1 min, followed by a heating ramp at 20 °C min^{-1} until 250 °C and 5 °C min^{-1} until 260 °C. The sulfentrazone retention time was 8.8 min.

Each set of analysis contained a minimum of one control sample, two fortified control samples, and treated samples. Each treatment was replicated three times. The sulfentrazone standard curve (0.01–0.1 μg ml^{-1}) was linear with $r^2 = 0.9933$ ($n = 5$, data not shown). Chromatograms of soil extracts had no major peaks eluting in the retention window of sulfentrazone (data not shown).

The efficiency of the applied method was evaluated and validated by acceptable and reproducible recoveries of pesticide in fortified control samples. The detection limit (LOD) was fixed in 0.01 μg ml^{-1} and the limit of quantification (LOQ) was 0.05 mg kg^{-1}, the validation for soil was made at the spike level of 0.05 mg kg^{-1}.

The 3-hydroxymethylsulfentrazone (HMS) metabolite was qualified by high-performance liquid chromatography (HPLC). In this case, the organic phase (acetone) of the purified samples was evaporated with an evaporation system under nitrogen flow at 45 °C, and the samples were resuspended in acetonitrile. The chromatography conditions were as follows: Shimadzu chromatograph, C_{18} reverse-phase column, flow of 1 ml min^{-1}, room temperature, detection by a set of photodiodes (DAD), UV 254 nm, 24:75 to 60:40 gradient in 60 min, with the mobile phase consisting of 0.5% acetonitrile and acetic acid. Under these conditions, the HMS metabolite was identified as the peak that showed a mean retention time of 12.52 min.

2.3. Isolation and characterization of sulfentrazone-degraders

The enrichment soils (10 g) with and without the application of sulfentrazone, incubated for 120 days at 27 °C and 70% WHC, was aseptically suspended in 90 ml of distilled water for 2 min using a Vortex mixer. Serial dilutions of suspensions (10^{-2}, 10^{-3}, and 10^{-4}) were removed and placed in a minimal medium ($NaNO_3$ 3 g; K_2HPO_4 1 g; $MgSO_4 \cdot 7H_2O$ 0.5 g; KCl 0.5 g; $FeSO_4 \cdot 7H_2O$ 0.01 g; agar 16 g; H_2O 1000 ml) supplement with sulfentrazone (0.7 μg i.a ml^{-1} medium) as a sole carbon and energy source. Medium without herbicide was used as control. The organic compound was added at room temperature after autoclaving. After incubation for 2, 7, and 17 days at 27° C, the individual colonies of bacteria, actynomicetes and fungi, respectively, that grew were picked and transferred to new minimum liquid medium (99 ml) ($NaNO_3$ 3 g; K_2HPO_4 1 g; $MgSO_4 \cdot 7H_2O$ 0.5 g; KCl 0.5 g; $FeSO_4 \cdot 7H_2O$ 0.01 g; H_2O 1000 ml), and after serial dilution, aliquots of 1 ml of the dilution 10^{-1} were inoculated into Erlenmeyers flasks (250 ml) containing 99 ml of the same medium supplement of the herbicide in different concentrations (2.13, 4.22, and 7.0 μg i.a.ml^{-1} medium). The cultures were transferred three times over a 50-day period. The microorganisms were isolated after vortexing in Tween 80 solution 0.1% and streaking in solid medium. The purified strains were then tested in medium with sulfentrazone as the sole carbon source. The isolated bacterial and actinomycetes strains were identified by analysis of fatty acid–methyl esters (FAMEs) using the Microbial Identification System developed by Microbial ID (MIDI, 2001). Cellular fatty acids were extracted according to the method of Sasser (1990). Fatty acid methyl–esters from each strain were separated using a Hewlett-Packard gas chromatograph model fitted with a fused silica column (25 m × 0.2 mm internal diameter). FAME peaks were named by the MISsoftware, and

bacterial strains were identified using the MIS "Aerobia Library" (Version TSBA50).

The fungal stocks cultures were maintained at 4 °C on Sabourad medium (Tuite, 1969). A scanning electron microscopy was used for this, through an adaptation of Nogueira and Barroso's methodology (1998) for visualization, and the use of an identification manual (Barnett and Hunter, 1972).

2.4. Statistical analysis

The influence of moisture and incubation period on the remaining amount of sulfentrazone (RASulf) was investigated using variance analysis and Snedecor's F tests (Montgomery, 1991). The same analysis was performed to evaluate the effect of temperature on RASulf. The influence of temperature and moisture on the mean degradation rate was quantified by RASulf on the last evaluation date, since the initial amount ($0.7\,\mu g\,g^{-1}$) was constant for all treatments. To accomplish this, t-tests were performed for contrasts between the RASulf recorded means at different temperature and moisture levels.

Analysis of variance and t-tests for contrasts were performed using the MIXED Procedure of the SAS System (SAS, 2004).

Where significant differences between RASulf means arose for any of the contrasts evaluated, we selected the factor level (temperature or moisture) with the lowest RASulf (highest degradation rate) to fit degradation models and to estimate half-life values (in cases where RASulf was lower than 50% on the last evaluation date). Nonlinear, negative exponential type models were fitted (Eq (1)):

$$\mathrm{RASulf}(t) = 1 - a\exp(-bt) + e, \quad a>0, \quad b>0, \qquad (1)$$

where RASulf(t) is the fraction of sulfentrazone remaining in time t, a is the maximum degradation attained, b is the parameter related to the degradation velocity in the descending phase, and e is the random error associated with each observation. The half-life corresponds to the t value for which the predicted RASulf(t) value is equal to 0.50. The Gauss–Newton method (BARD, 1970), implemented in the SAS System's NLIN Procedure (SAS, 2004) was used to fit the models. The above negative exponential model is adequate for describing degradation process for which the velocity of degradation is not constant over time. At time zero, the remaining fraction (RASulf(t)) is equal to one; according to the proposed model, the fraction RASulf(t) decreases asymptotically to zero, which is consistent with the process being modeled.

The effect of the herbicide on the soil microbiota, measured by the number of colony-forming units (CFU), was evaluated by comparing the mean number of CFUs in soil samples with or without sulfentrazone, using Student's test with Satterthwaite's approximation (1946). The TTEST Procedure of the SAS System (SAS, 2004) was used.

3. Results

3.1. Sulfentrazone dissipation: moisture and temperature effects

There was no evidence of a moisture factor effect on sulfentrazone degradation (t-test for contrasts; $P>0.28$). Rates of degradation decreased in all treatments, under different moisture levels from 180 days of incubation, at 27 °C (Table 2). According to the observed data, the highest degradation percentage of the herbicide sulfentrazone was 69.3% (RASulf = $0.2151\,\mu g\,g^{-1}$), after 255 days of incubation, considering a moisture content of 70% WHC. An adaptation period (lag phase) of the microorganisms to the herbicide was observed, which varied with moisture content.

Sulfentrazone half-life (146.5 days) was estimated for the treatment that provided the highest degradation in the analysis in which moisture levels were compared (27 °C and 70% WHC; Fig. 1). The estimates for parameters $\acute{\alpha}$ and β of the corresponding degradation curve fitting models can be seen in Table 3.

The temperature did have an effect on sulfentrazone degradation (F-test, $P<0.013$), and herbicide degradation was higher at 30 °C. An increase in degradation velocity was observed starting at 60 days for all treatments. According to the observed data, the highest degradation rate was 52% (RASulf = $0.3149\,\mu g\,g^{-1}$), after 120 days of incubation at 30 °C (Fig. 2).

The microorganisms present in the soil were able to metabolize the herbicide. After 180 days, the presence of the HMS metabolite was observed in the soil samples incubated at 30 °C and 70% WHC.

Table 2
Mean values for the remaining amount of sulfentrazone ($\mu g\,g^{-1}$) in a Typic Hapludox soil over time, under different moisture levels

Moisture (% WHC)	Mean values for the remaining amount of sulfentrazone ($\mu g\,g^{-1}$)					
	0 days	30 days	60 days	120 days	180 days	255 days
30	0.7	0.43	0.43	0.39	0.25	0.27
70	0.7	0.57	0.57	0.39	0.23	0.22
100	0.7	0.62	0.62	0.50	0.36	0.35

3.2. Characterization of sulfentrazone degraders

It was observed that during the growth of actinomycetes stimulated by the presence of the herbicide, there was an increase of 5.5×10^5 CFUs' in relation to the control (t-test, $P = 0.007$). For fungi and bacteria there was no significative evidence of the herbicide effect in CFU's (Table 4), but there was an effect over these microorganisms biodiversity. Among the fungi, the strains isolated from the control soil were: *Aspergillus* sp., two strains of *Penicillium* sp., *Eupenicillium* sp. e *Paecilomyces*, while the strains isolated and identified from the soil with sulfentrazone ($7.0\,\mu g\,g^{-1}$) as the only source of carbon and energy were: *Cladosporium* sp., *Eupenicillium* sp. e *Paecilomyces* sp. Taking into consideration the selection made by the herbicide, these strains were identified as possible herbicide degraders. The bacterial strains *Ralstonia pickettii*, *Rhizobium radiobacter*, and *Methylobacterium-mesophilicum/radiotolerans* were selected as potential sulfentrazone degraders because they had a tolerance level of concentration up to $7.0\,\mu g\,ml^{-1}$ (Table 5).

4. Discussion

4.1. Sulfentrazone dissipation: moisture and temperature effects

The primary method of sulfentrazone soil dissipation is considered microbial degradation and the reported DT_{50} is 110–280 days depending on soil and environmental conditions (Hatzios, 1998). Sulfentrazone degradation under controlled laboratory conditions was slower in autoclaved soil than in non-autoclaved surface soil and subsurface soil (Ohmes et al., 2000). Slower sulfentrazone degradation in autoclaved soil indicated that microbial degradation was an important sulfentrazone dissipation mechanism from the soil. Although the observed degradation under sterile conditions was slower, chemical degradation also appears to be involved in sulfentrazone dissipation in soil. Detailed research results on the dissipation and degradation of sulfentrazone in surface soil are currently unavailable in referenced journals.

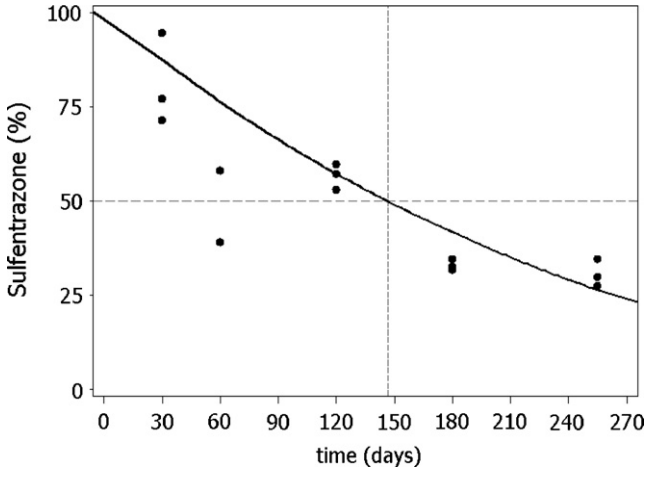

Fig. 1. Sulfentrazone degradation in a Typic Hapludox soil over time at 27 °C and 70% WHC. The dotted vertical line indicates the half-life value: 146.5 days (- - -: fitted negative exponential model and ●: observed values).

Researchers have demonstrated that the parameters such as soil moisture and temperature have impacts on the degradation rate of most herbicides due to the interference of these parameters in the soil microorganisms. Sulfentrazone biotransformation was significantly affected by temperature. The half-life value obtained in the present study was 146.5 days at 27 °C and at a moisture content of 70% WHC. Higher degradation occurred at 30 °C. Sulfentrazone dissipation under controlled laboratory conditions in non-autoclaved surface soil and subsurface soil, from Tennessee, USA, showed DT_{50} of 93 and 102 days, respectively, at 30 °C (Ohmes et al., 2000).

In this research, the results were not significant in relation to the moisture effect on herbicide degradation. With regard to moisture, degradation was higher than 50% at 30 °C and 70% WHC levels and equal to 50% at 100% WHC until 255 days. A slower degradation of the herbicide was observed for the 100% WHC, where the anaerobic microorganisms, even obligator and facultative ones, are predominant, indicating that sulfentrazona degradation occurs especially by aerobics organisms. Other studies have demonstrated a decrease in herbicide degradation rates under anaerobic conditions, as observed for the herbicide flufenacet in three types of soils submerged in water (Gupta and Gajbhiye, 2002). However, Ohmes et al. (2000) observed that sulfentrazona dissipation in the field was more rapid with a DT_{50} of 25-days when rainfall was above normal. With more rain, sulfentrazone could have moved below the sampling zone. However, application was not preceded by a heavy rain, and with a soil pH of 6.1, which is below the sulfentrazone pK_a of 6.56, there should have been minimal movement due to increased absorption at lower pH levels (Grey et al., 1997). A lower DT_{50} value

Table 3
Estimates for the $\acute{\alpha}$ and β parameters of degradation models and respective estimated herbicide half-life values in a Typic Hapludox soil under different experimental conditions

Moisture (%)	Temperature (°C)	F	P-value[a]	$\acute{\alpha}$	β	Half-life (days)
70	27	34.49	<0.001	1.2506	0.0034	146.5
70	30	147.38	<0.001	0.5000	0.0260	>120.0

[a] P-value relative to the t-test.

under these conditions is consistent with laboratory data that suggest that microorganisms degrade sulfentrazone.

Prior to degradation of many organic compounds, a period is noted in which no destruction of the chemical is evident. This time interval is designed to be an acclimation period or, sometimes, an adaptation or lag period (Alexander, 1999). Reddy and Locke (1998) demonstrated the low potential of the biotransformation of sulfentrazone in soils that were never exposed to it. The mineralization rate derived from mineralization of the phenyl ring of sulfentrazone was lower than 2.1% of that applied, from the three soils studied, over the 77-days incubation. The moisture was maintained near field capacity (30%) and the soils had no history of sulfentrazone application. The microbial population exhibits little adaptation to sulfentrazone as a substrate. Similar results were observed in this study, because the soil used had no history of sulfentrazone application and the microorganisms need an adaptation period (lag phase) with variable temperature and moisture. The microorganisms after a lag phase could degraded the herbicide under differing conditions of temperature and moisture, this is corroborated by the presence of the HMS metabolite.

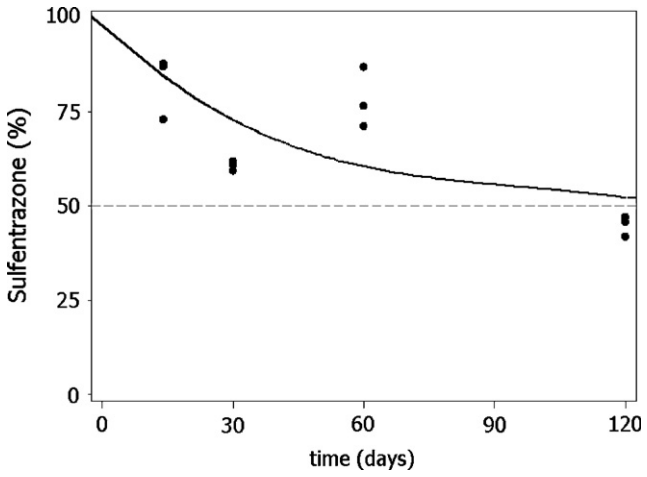

Fig. 2. Sulfentrazone degradation in a Typic Hapludox soil over time at 30 °C and 70% WHC, with a half-life higher than 120 days (---: fitted negative exponential model and ●: observed values).

The metabolism of sulfentrazone in animals and plants is similar, the major plant metabolite is HMS. Other metabolites include sulfentrazonecarboxylic acid (SCA) and 3-desmethylsulfentrazone (DMS) (EPA, 2004).

This shows that, despite of the lack of information in literature about sulfentrazone degradation route by

Table 4
Evaluation of the effect of the herbicide sulfentrazone on the mean number of microbial colony-forming units isolated in a Typic Hapludox soil

Organisms	Sulfentrazone (0.7 μg g^{-1})	Mean[a] (CFU)	Lower limit[b]	Upper limit[b]	*P*-value[c]
Actinomycete	Absent	203.33	119.923	526.59	
Actinomycete	Present	753.33	414.239	1092.43	
Actinomycete	Difference	550.00			0.00727
Bacterium	Absent	1233.33	795.721	1670.95	
Bacterium	Present	1826.67	506.953	3146.38	
Bacterium	Difference	593.33			0.18463
Fungi	Absent	7.00	−3.828	17.83	
Fungi	Present	2.00	−0.484	4.48	
Fungi	Difference	−5.00			0.18019

Application of Student's *t*-test.
[a]Difference between numbers of CFU in samples with or without sulfentrazone.
[b]Value 1000 times smaller than the observed (divided by 1000).
[c]*P*-value relative to the *t*-test.

Table 5
Identification of bacterial strains using MIS, in a Typic Hapludox soil

Soil/isolate no.	Sulfentrazone (μg ml^{-1})	Identification of single colonies	Library matches[a]
LVA 7	2.13	*Rhizobium radiobacter*	0.623
LVA 5	2.13	*Rhizobium radiobacter*	0.572
LVA 4	2.13	*Ralstonia pickettii*	0.628
LVA 2	7.0	*Rhizobium radiobacter*	0.585
LVA 10	7.0	*Rhizobium radiobacter*	0.560
LVA 1B	7.0	*Ralstonia pickettii*	0.750
LVA 12	7.0	*Methylobacterium-mesophilicum/radiotolerans*	0.732
LVA 1	7.0	*Ralstonia pickettii*	0.855

These microorganisms were isolated in a medium supplemented with sulfentrazone in different concentrations, as a sole carbon and energy source.
[a]Library matches are expressed on a scale of 0–1, with a match of 0.5 or greater considered good to the species level.

microorganisms, the disappearance of the parental compost coincident and the appearance of the metabolite at the end of the experimental period is in accordance with the metabolism of the herbicide in plants and animals. Consequently, HMS is also in the metabolic degradation route of this herbicide by microorganisms.

4.2. Characterization of sulfentrazone degraders

There have been no published studies concerning the isolation and identification of microorganisms that degrade the herbicide sulfentrazone. In this paper, degrading isolates were characterized by determining FAME profiles that present the potential for sulfentrazona degradation. The microorganisms isolated were: *R. pickettii*, *R. radiobacter*, *Eupenicillium* sp., *Cladosporium* sp., and *Paecilomyces* sp. These isolated microorganisms will undergo further testing separately in pure cultures to determine their degrading potential.

The bacteria showed excellent growth in media supplemented with high concentrations of sulfentrazone ($7.0\,\mu g\,ml^{-1}$), indicating a high degrading potential of those strains. The influence, which agrochemicals have on bacterial groups with an important function in soil nitrogen transformation, has been the focus of many studies. In general, herbicides have little influence on soil ammonification (Das and Mukherjee, 1998). According to Olson and Lindwall (1991), the herbicides glyphosate and 2,4-D, when applied at field doses or at rates 10 times higher, have not demonstrated negative effects on soil nitrification. In the present study, the bacterium *R. radiobacter* was not affected by the herbicide sulfentrazone, and grew at a dose 10 times higher than the field dose ($7.0\,\mu g\,g^{-1}$ soil).

Fungi of the genus Paecilomyces have shown the capacity to metabolize different organic compounds, such as herbicides, lignin, plastic (Oda et al., 1997), and PHAs (Estevez et al., 2005). Two soil fungi, *Paecilomyces varioti* and *Fusarium oxysporum*, grown on culture medium, degraded pendimethalin to the metabolites *N*-(1-ethylpropyl)-3,4-dimethyl-2-nitrobenzene-1,6-diamine and 3,4-dimethyl-2,6-dinitroaniline (Singh and Kulshrestha, 1991). In a study conducted by Vroumsia et al. (2005), the fungus *Cladosporium cladosporioides* degraded 49% of 2,4-dichlorophenol, a metabolite of the herbicide 2,4-D, after 5 days of incubation.

5. Conclusion

The herbicide appears to be more affected by changes in the soil temperature, while the soil water contents have no significant effect. The microorganisms showed a lag phase early in the experiments, followed by a slower degradation phase. The overall results of this paper suggest that the sulfentrazone degradation was influenced by microorganisms and the DT_{50} (146.5 days) value measured under 27 °C and 70% WHC in tropical soils may be shorter than previously reported in the temperate soil by *The Pesticide Manual* (DT_{50} 18 mo) (Tomlin, 2000). HMS was a major degradation product, albeit at significantly reduced rates. The bacteria and fungi were involved in a sulfentrazone degradation in Typic Hapludox soil.

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