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Leaching of sulfentrazone in Brazilian "Cerrado" soils by chromatographic and biological methods

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ABSTRACT

Sulfentrazone is a mobile herbicide in the soil that can reach groundwater. The objective of this study was to verify the leaching of sulfentrazone in samples from three Brazilian "Cerrado" soils, and compare the biological with the chromatographic method to determine the leaching. The soils were PVC columns (10 cm in diameter x 50 cm in length), 1.5 kg ha^{-1} of sulfentrazone was applied to the top of the columns. Twelve hours after the application of the herbicide, they were submitted to simulated rainfall (60 mm). To the herbicide leaching, in each column, soil samples were collected every 5 cm, being the experiment mounted in subdivided plots (plots: columns, subplots: depths). From these, a fraction was sent to the laboratory for analysis by high performance liquid chromatography (HPLC). Another fraction was placed in pots, performing bioassay with *Sorghum bicolor*. Sulfentrazone leached more in treatments Tillage System - Sandy Soil - High acidity and Tillage System - Red Latosol - Low acidity, being quantified by HPLC at depths of 5, 10 and 15 cm and detected in the same depths by bioassay. In treatment, Native Forest - Red Latosol - High acidity the herbicide leached up to 10 cm, also being detected by HPLC and bioassay. The biological method, when compared to the chromatographic, presents good sensitivity to sulfentrazone, being able to be used for leaching study of this herbicide.

Key words: bioassay; environmental contamination; herbicide

Lixiviação do sulfentrazone em solos característicos do Cerrado brasileiro pelos métodos cromatográfico e biológico

RESUMO

Sulfentrazone é um herbicida móvel no solo podendo atingir águas subterrâneas. Objetivou-se com este trabalho estudar a lixiviação do sulfentrazone em amostras de três solos do Cerrado brasileiro e comparar o método biológico com o cromatográfico para determinação da lixiviação. Os solos foram acondicionados em colunas de PVC (10 cm de diâmetro x 50 cm de comprimento), foi aplicado 1,5 kg ha^{-1} de sulfentrazone no topo das colunas. Doze horas após a aplicação do herbicida, essas foram submetidas à chuva simulada (60 mm). Para a confirmação da lixiviação do herbicida em cada coluna, foram coletadas amostras de solo a cada 5 cm, sendo o experimento montado em parcelas subdivididas (parcelas: colunas; subparcelas: profundidades). Dessas, uma fração foi enviada ao laboratório para analise por cromatografia líquida de alta eficiência (CLAE). Outra fração foi colocada em vasos, realizando bioensáio com a espécie *Sorghum bicolor*. O sulfentrazone lixiviou mais nos tratamentos Plantio direto – Neossolo Quartzarênico – acidez alta e Plantio direto – Latossolo Vermelho – acidez baixa, sendo quantificado por CLAE nas profundidades de 5, 10 e 15 cm e detectado nas mesmas profundidades por bioensaio. No tratamento Mata nativa – Latossolo vermelho – acidez alta o herbicida lixiviou até 10 cm, também sendo detectado por CLAE e pelo método biológico. O método biológico apresenta boa sensibilidade à presença do sulfentrazone, podendo ser utilizado para estudo de lixiviação desse herbicida.

Palavras-chave: bioensaio; contaminação ambiental; herbicida

Introduction

The presence of weeds in crops is one of the main problems faced by farmers. To avoid this problem in the various stages of the production process, weed management is carried out using mechanical, cultural, physical and chemical methods, either isolated or combined (Inderjit, 2004). In chemical control, herbicides applied without knowledge of soil characteristics and climatic conditions can result in surface and groundwater contamination (Andrade & Stigter, 2009; Andrade et al., 2010), and compromise the agronomic efficiency of these products (Passos et al., 2015, Silva et al., 2016).

Most soils in the Cerrado region are Latosols and Neosols, covering approximately 46% and 15% of the area, respectively (Adámoli et al., 1986). Latosols are deep, well–drained and acid soils with aluminum toxicity and low base saturation. There are also soils with mean and even high base saturation. Neosols, in turn, comprise soils composed of mineral material or thin organic matter, with high or low base saturation, eventually acid and with high aluminum and sodium contents, according to the Brazilian Soil Classification System - SiBCS (Embrapa, 2006). Florido et al. (2015) evaluated the mobility of imazaquin in different soils and noticed that this herbicide moves more easily in the Quartzarenic Neosol and less easily in the Red Latosol. Similarly, Inoue et al. (2014) concluded that ametryne leached more in the Quartzarenic Neosol and less in Red Latosol.

In the cerrado, crops such as soybean and sugar cane widely use sulfentrazone, N-[2,4-dicloro-5-[4-(difluorometil)-4,5-dihidro-3-metil-5-oxo-1H-1,2,4-triazol-1-il]fenil] metanosulonamida, which belongs to the chemical group of aryl triazolinones, as herbicide. Its action can be via xylem or by contact; it can be absorbed by the roots and leaves, with primary apoplastic translocation and limited movement in the phloem. Sulfentrazone acts on the inhibition of PROTOX, an enzyme involved in chlorophyll biosynthesis, causing the accumulation of protoporphine IX and consequent oxygen peroxidation and destruction of cell membranes (Reddy & Locke, 1998).

Sulfentrazone is classified as little volatile (vapor pressure 1 x 10^{-9} mmHg at 25°C). Microbial degradation is considered the main form of dissipation. Solubility in water changes according to pH, being 110, 780, and 1600 mg L^{-1} at pH 6.0; 7.0 and 7.5, respectively. Sulfentrazone is a weak acid with dissociation constant (pKa) of 6.56 and partition coefficient (Kow_{pH7}) of 9.79 (Tomlin, 2015). High solubility combined with low Kow causes most of the herbicide to remain in the soil solution, being more subject to leaching. Thus, this herbicide may present high persistence and mobility in soil and aquatic systems (EPA, 2015), and may cause serious environmental problems.

Analytical methods such as high performance liquid chromatography (HPLC), which allows the quantification of herbicides, are employed in studies aiming to quantify the leaching of these substances in the environment. However, the quantification of herbicides in the soil requires the determination of quantification and detection thresholds for these may vary between different types of soils, depending on their characteristics.

Methods based on biological assays may also be employed in herbicide leaching studies. These have the advantage of being simpler and cheaper, but do not allow the quantification of herbicides with the same precision as that of the chromatographic method. This technique is based on the use of plants that are sensitive to the tested herbicides, so that the presence of residues in the soil is evidenced by changes in physiological and morphological characteristics of the key-plant species. In the literature, several studies involving bioassays for determination of leaching of herbicides such as amethrin (Silva et al., 2012), sulfentrazone (Silva Junior et al., 2016), imazethapyr, imazapyque and imazapyr (Bundt et al. 2014) have been reported. On the other hand, to test the efficiency of this method, it would be interesting to compare it with already consolidated methods such as HPLC.

In view of the above, the objective of the present work was to study the leaching of sulfentrazone in Brazilian Cerrado soils and to compare the results of chromatographic and biological methods of leaching determination.

Materials and Methods

Samples of three soils collected at depths of 0 to 20 cm, which were chemically and physically characterized, were used to perform the experiment (Table 1). Twenty samples were collected from each soil, and then mixed, air dried, sieved in 4-mm mesh sieves, and homogenized to obtain composite samples (undeformed). The soils are from different regions of the southwest of the state of Goiás. The soil used in the treatment "Direct planting - Quartzarenic Neosol - high acidity" was collected in the municipality of Jataí; the soil for the treatment "Direct planting - Red Latosol - low acidity" was collected in Quirinópolis; and the soil for the treatment "Native

Table 1. Chemical and physical attributes of the studied soils.

Soil	pH	P	K	Ca	Mg	Al	H+Al	SB	CTC	V	m	OM
	$CaCl_2$	mg dm^{-3}				$cmol_c$ dm^{-3}				%		g kg^{-1}
A	5.0	37.3	27	2.4	0.5	0.1	3.2	3.0	6.16	48	3.2	28.0
B	5.9	28.1	340	5.5	0.2	0.0	2.0	8.6	10.40	81	0.0	32.2
C	4.4	0.74	91	1.3	1.0	0.6	6.3	2.5	8.81	29	19.4	26.1

Soil	Sand	Silte	Clay	Textural class
	g kg^{-1}			
A	880	40	80	Sandy
B	380	100	520	Argillose
C	550	80	370	Argillose

Soil A: Direct planting - Quartzarenic Neosol - high acidity; soil B: Direct planting - Red Latosol - low acidity; soil C: native forest - Red Latosol - high acidity.
Analyses carried out at the Soil Analysis Laboratory of Viçosa LTDA. SB: sum of bases, CEC: cation exchange capacity, V: saturation by bases, m: saturation by aluminum, OM: organic matter.

forest - Red Latosol - high acidity" was collected in Rio Verde. Soils were classified according to the SiBCS (Embrapa, 2006) and the classification of acidity pH $CaCl_2$ was made according to Raij et al. (1997). The soils "Direct planting - Quartzarenic Neosol - high acidity" and "Direct Planting - Red Latosol - low acidity" were collected in areas with no history of sulfentrazone use, and the soils "Native forest - Red Latosol - high acidity" came from native forest without a history of sulfentrazone application.

Each treatment was conditioned in 4 PVC columns (replicates) of 10 cm diameter by 50 cm length, previously prepared and waxed in the inside to reduce percolation of the water by the walls. All columns were marked and sectioned every 5 cm and had a removable side cover.

After filling the columns with soil samples, they were saturated in water. At this stage, they were placed in a container with water up to 80% of the column height for a period of 48 hours, promoting the upward moistening and avoiding formation of air bubbles trapped in the pores. Subsequently, they were left standing for 72 hours to drain the excess water until reaching the field capacity (100%).

Subsequently, a dose of 1500 g ha^{-1} of the active ingredient sulfentrazone (500 g L^{-1} a.i.) was applied to the top of the columns. In this step, a CO_2 pressurized sprayer, equipped with XR 110.02 tips, was set to apply 150 L ha^{-1} of the herbicide mixture. Twelve hours after the herbicide application, with the columns still upright, a precipitation of 60 mm was simulated for a period of 3 hours using a rain simulator equipped with TT 110.03 tips. Pluviometers were coupled to the side walls of the columns to measure the precipitation applied.

After this step, the columns remained standing for a further 72 hours in an upright position and then placed in horizontal position. In this occasion, the columns' lateral was opened and the soil was sectioned every 5 cm.

To confirm the leaching of the sulfentrazone in each treatment, soil samples were collected at the depths of 0-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35- 40, 40-45, 45-50 cm. In each sample, a fraction (50g) was collected, air dried, sieved in a 2-mm mesh sieve and sent to the laboratory for analysis by high performance liquid chromatography (HPLC) to quantify the herbicide in the soil. Another fraction (250g) of each treatment was placed in pots of 300 cm^3 capacity, where the bioindicator *Sorghum bicolor* was planted. *S. bicolor* was selected as an indicator of sulfentrazone residues in soil based on several studies (Faustino et al., 2015, Silva Junior et al., 2016, Madalão et al., 2017) that confirmed the high sensitivity of this species to sulfentrazone.

Bioassay

We transferred 250 g of soil from the samples of each layer to plastic pots of 300 cm^3capacity, after which ten seeds of the bioindicator *S. bicolor* were seeded, leaving six seedlings per pot after thinning. The experiment had a completely randomized block design with four replications.

At the 21st day after emergence (DAE), the shoot dry matter (SDM) of the key-plant was quantified after all the plants were cut close to the soil surface and dried in a forced circulation air oven (70 ± 2°C) until reaching constant weight.

Quantification of sulfentrazone residues in soil by high performance liquid chromatography (HPLC)

Herbicide quantification in soil samples collected in each layer was carried out using solid-liquid extraction with low-temperature partitioning (SLE/LTP), according to methodology proposed by Vieira et al. (2007) and Goulart et al. (2008) and adapted by Paula (2007) for determination of herbicides in soil.

Sulfentrazone determination was performed using a high performance liquid chromatography system, model Shimadzu LC 20AT, photodiode arrangement detector (Shimadzu SPD-M20A) and C_{18} stainless steel column (Shimadzu VP-ODS Shim-pack 250 mm x 4.6 mm d.i., 5 µm particle size). Stock solution of the herbicide was prepared from the standard 92.01% purity available from FMC Corporation at the concentration of 1,000 µg mL^{-1} in acetonitrile and work solutions were prepared therefrom.

Chromatographic conditions for analysis were: mobile phase composed of water (acidified with 0.01% phosphoric acid) and acetonitrile in the proportion of 45:55 (v/v) for the treatments "Direct planting - Quartzarenic Neosol - high acidity" and "Native forest - Red Latosol - high acidity"; and 50:50 for the treatment "Direct planting - Red Latosol - low acidity"; 1.0 mL min^{-1} flow rate; injection volume of 20 µL; column temperature of 30°C and wavelength of 207 nm. Identification of the sulfentrazone signal was made by comparing retention time (Figures 1, 2 and 3) and quantification performed by the external calibration method (Table 2).

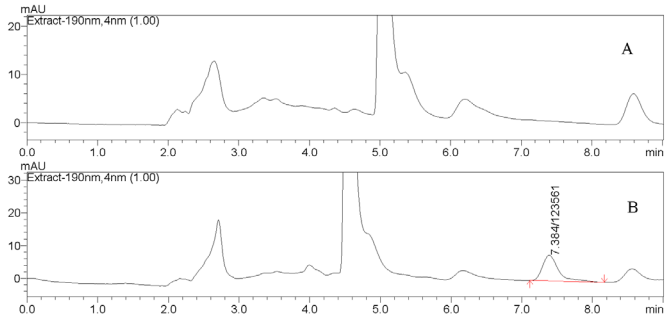

Figure 1. (A) Chromatogram of the extract obtained from the treatment "Direct planting - Quartzarenic Neosol - high acidity" free of herbicide and (B) chromatogram of the extract of the same treatment plus 5.0 mg kg^{-1} of sulfentrazone.

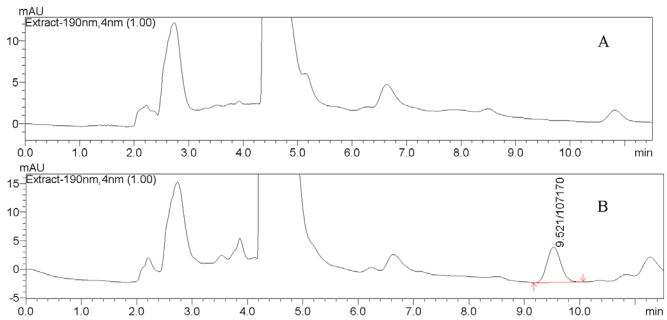

Figure 2. (A) Chromatogram of the extract obtained from treatment "Direct planting - Red Latosol - low acidity" free of herbicide and (B) chromatogram of the extract of the same treatment plus 5.0 mg kg^{-1} of sulfentrazone.

Statistical analysis of data

The experiment was subdivided in plots where each column corresponded to a plot and each depth to a subplot.

Table 2. Linearity curves of the proposed method for the treatments "Direct planting - Quartzarenic Neosol - high acidity", "Direct planting - Red Latosol - low acidity" and "Native forest - Red Latosol - high acidity".

Analytic curves of the chromatographic method		
	Linearity curve	**Correlation coefficient**
Direct planting - Quartzarenic Neosol - high acidity	$\hat{Y}$ = 1437.35 + 121.19x	0.9999
Direct planting - Red Latosol - low acidity	$\hat{Y}$ = 5941.19 + 113.46x	0.9999
Native forest - Red Latosol - high acidity	$\hat{Y}$ = 852.90 + 142.81x	0.9999

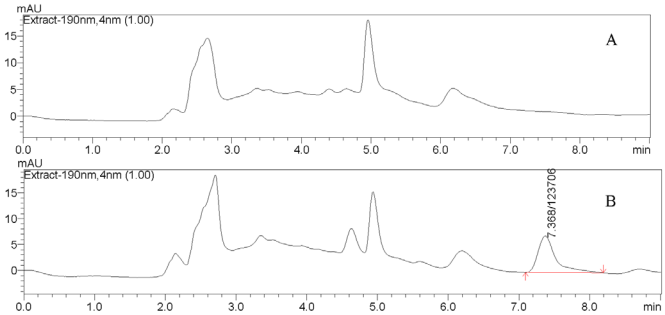

Figure 3. (A) Chromatogram of the extract obtained from treatment "Native forest - Red Latosol – high acidity" free of herbicide and (B) chromatogram of the extract of the same treatment plus 5.0 mg kg^{-1} of sulfentrazone.

At the end of the tests, data were submitted to analysis of variance, and when the effects were significant, a regression analysis was performed. The choice of the model was based on the significance of regression coefficients and on the correlation coefficient at 5% probability.

Results and Discussion

Sulfentrazone leached little in all soils, being detected only up to 15 cm deep (Figure 4). Low leaching of sulfentrazone in the studied soils is due to organic matter content, good cation exchange capacity (CEC) and/or high clay content (Alvarez et al., 1999). Thus, although the herbicide presents high solubility, the soil characteristics made sulfentrazone to remain retained in the initial layers, reducing its potential for environmental contamination in these soils.

Sulfentrazone is a weak acid herbicide with dissociation constant (pK_a) of 6.56. The lower pH of the soil in relation to the pK_a of the herbicide, the greater is the tendency of the herbicide to keep in the molecular form and, possibly, the lower is its ability to be adsorbed in the colloidal particles of the soil. When the pH of the medium is higher than the pK_a of the herbicide, most of its molecules will be in the dissociated form and its retention capacity in the soil will be lower (Inderjit, 2004).

Sulfentrazone leached more in soils of the treatments Direct planting - Quartzarenic Neosol - high acidity and Direct planting - Red Latosol - low acidity, being detected in the depths of 0-5, 5-10 and 10-15 cm (Figure 4) and with lower Sorghum SDM (Figure 5). The highest leaching of sulfentrazone in the soil corresponding to the treatment Direct planting - Quartzarenic Neosol - high acidity is due to the lower clay content, and in the soil of the treatment Direct planting - Red Latosol - low acidity, was due to higher pH. This observed pH value (5.9) is close to the pK_a value of the herbicide, so that a greater part of sulfentrazone, when compared to other soils, prevails in dissociated form. As a consequence, most of the molecules of the herbicide prevails with negative charges, and as negative charges normally also prevail in the soil, repulsion occurs between molecules, favoring the leaching. Low clay content also contributes to lower sorption and consequently higher herbicide leaching in the soil (Firmino et al., 2008).

Sulfentrazone leached less in the soil of the Native forest - Red Latosol - high acidity treatment, being detected in the depths of 0-5 and 5-10 cm (Figure 4) with smaller reduction of SDM of the sorghum in the other depths (Figure 5). Lower sulfentrazone leaching in this treatment is due to low pH of the soil; in this condition, the herbicide tended to remain in the molecular form, increasing its capacity to be adsorbed in colloidal particles, being retained in the first layers. In general, the herbicide binds to hydroxyl and carboxylic groups (Liao et al., 2014) interacting with soil colloids by hydrogen bonds and Van der Waals interactions (Clausen et al., 2001; Kovaios et al., 2006; Rohit & Kailasa, 2017).

OM is one of the main components that influence the activity of herbicides registered in tropical soils, interfering with all sorting processes. In the case of Brazilian soils, the properties that most correlate with sorption of herbicides are CEC and organic carbon content. Since most of CEC is related to organic matter, this characteristic can be considered the most important for herbicides (Silva et al., 2007).

In the treatments Direct planting - Quartzarenic Neosol - high acidity and Direct planting - Red Latosol - low acidity,

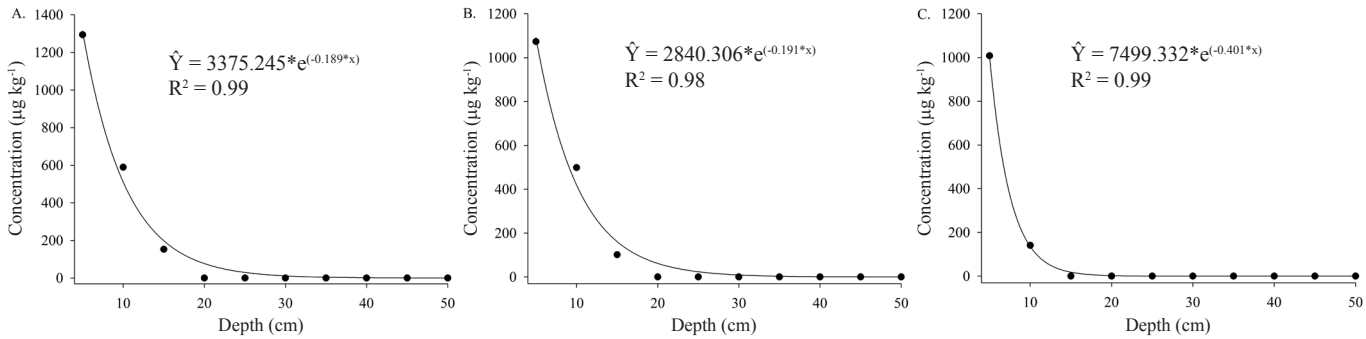

Figure 4. Concentration of sulfentrazone determined by HPLC in (A) Direct planting - Quartzarenic Neosol - high acidity, (B) Direct planting - Red Latosol - low acidity and (C) Native forest- Red Latosol - high acidity at different depths.

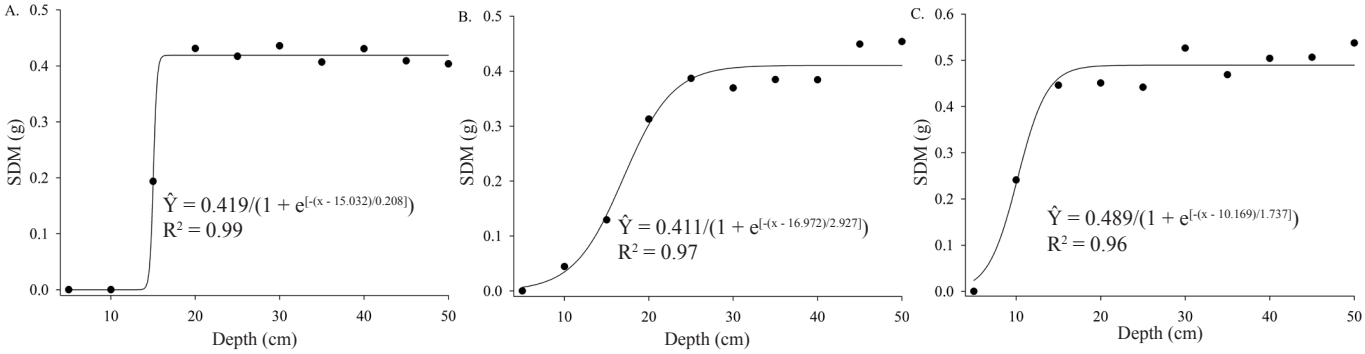

Figure 5. Shoot dry matter (SDM) of *Sorghum bicolor* cultivated in soil samples of (A) Direct planting - Quartzarenic Neosol - high acidity, (B) Direct planting - Red Latosol - low acidity and (C) Native forest - Red Latosol - high acidity at different depths.

despite presenting good OM contents, leaching was more intense than in Native - Red Latosol - high acidity. This is due to the higher pH values of these soils. However, leaching in these soils did not exceed 15 cm, which can be attributed to the clay (Direct Planting - Red Latosol - low acidity) and OM contents and CEC. Thus, in soils with similar characteristics to the studied here, there is a lower risk of contamination of the water table. Passos et al. (2015) evaluating the leaching of sulfentrazone in different Brazilian soils detected the presence of the herbicide until the last section tested (30 cm).

The chromatographic and biological methods were complementary to the study of sulfentrazone leaching (Figures 4 and 5). Comparing figures, it is observed that in the Figure 4A, the herbicide is detected at depths of 5 cm, 10 cm and 15 cm, with the highest amount detected at 5 cm, followed by the depth of 10 cm, and the lowest quantification occurred in the depth of 15 cm. The herbicide was not detected in the other depths. In relation to the Figure 5A, corresponding to the Figure 4A, it can be observed that sorghum presented dry matter reduction up to 15 cm. A similar behavior was observed in the data obtained by HPLC, with highest reduction in dry matter at the depth of 5 cm, followed by the depths of 10 cm and 15 cm; no dry matter loss of sorghum was seen in the other depths. When comparing the other figures (Figures 4B and 5B, Figures 4C and 5C), the same behavior is observed.

The sensitivity of the biological method to detect the presence of sulfentrazone in the soil makes it a possible option to use in leaching tests of this product. The association between instrumental and biological methods has the advantage of reducing the cost of labor, as well as the number of chemical analyses (Silva et al., 2012).

Chromatographic analysis require sophisticated laboratories and highly skilled personnel, a large amount of reagents and other chemical compounds, which besides being expensive has a great potential of waste production and environmental contamination. However, this technique allows quantifying the herbicide in the soil whereas the biological assay does not. The later, though, is a much simpler and cheaper process and does not demand sophisticated infrastructure.

Conclusions

Sulfentrazone leached more in the soils corresponding to the treatments Direct planting - Quartzarenic Neosol - high acidity and Direct planting - Red Latosol - low acidity, reaching up to 15 cm depth; in the soil corresponding to the treatment Native forest - Red Latosol - high acidity, the herbicide leached up to 10 cm.

Soils with higher pH proved to be more amenable to leaching.

The biological method is efficient to detect the presence of the herbicide in the soil.

Acknowledgements

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