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Leaching potential of S-metolachlor in a medium-textured Oxisol soil with bioindicator plants[1]

Potencial de lixiviação do S-metolachlor em um Latossolo de textura média com plantas bioindicadoras

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HIGHLIGHTS:

The leaching of the herbicide S-metolachlor in medium-textured Oxisol depends on rainfall.
High natural rainfall can leach S-metolachlor down to 0.12-0.15 m depth in a medium-textured Oxisol.
Cucumber was the most sensitive species to the presence of S-metolachlor in the soil profile.

ABSTRACT: Information about the impact of herbicides in the soil based on the growth of bioindicator species is extremely useful in developing crop management strategies. Therefore, this study aims to evaluate the leaching potential of the herbicide S-metolachlor under different natural precipitations in medium-textured Oxisol using bioindicator plants. A completely randomized experimental design was adopted, with four replicates and treatments arranged in a 3 × 8 factorial scheme [three indexes of precipitation occurred in the environment before the collection of the samples (50, 91, and 131 mm) and eight depths in the soil profile (0-0.03; 0.03-0.06; 0.06-0.09; 0.09-0.12; 0.12-0.15; 0.15-0.20; 0.20-0.25; 0.25-0.30 m)]. PVC columns were used, maintaining the original soil integrity during sampling after accumulating the stipulated natural precipitation. Longitudinal sections separated the columns to sow the bioindicator species (cucumber, lettuce, Alexander grass, and sorghum). The phytotoxicity symptoms of bioindicator plants were evaluated, adopting a phytotoxicity visual scale between 0 and 100%, at 5, 7, 9, and 11 days after seeding. The responses of the bioindicator species to the residual effect of the herbicide S-metolachlor were variable and depended on the rainfall level. Generally, in a medium-textured Oxisol, the higher values of concentration of S-metolachlor occurs in depths ranging between 0 and 0.06 m. The maximum leaching depth detected was 0.12-0.15 m with 131 mm of precipitation. Cucumber was the most sensitive species to the presence of S-metolachlor in an Oxisol of medium-texture since it presents symptoms of phytotoxicity at higher depths.

Key words: herbicide, mobility, phytotoxicity, textured soil

RESUMO: Informações sobre o impacto dos herbicidas no solo baseado no crescimento de espécies bioindicadoras são extremamente úteis no desenvolvimento de estratégias de gerenciamento de culturas. O objetivo deste estudo foi avaliar o potencial de lixiviação do S-metolachlor em precipitações pluviométricas naturais em um Latossolo (Oxisol) de textura média, através do uso de plantas bioindicadoras. Adotou-se o delineamento experimental inteiramente casualizado, com quatro repetições e os tratamentos dispostos em esquema fatorial 3 × 8 [três índices de precipitação ocorrida no ambiente antes da coleta das amostras (50, 91 e 131 mm) e oito profundidades no perfil do solo (0-0,03; 0,03-0,06; 0,06-0,09; 0,09-0,12; 0,12-0,15; 0,15-0,20; 0,20-0,25; 0,25-0,30 m)]. Colunas de PVC foram introduzidas mantendo a integridade original do solo durante a amostragem, após o acúmulo de precipitação natural estipulado. As colunas foram separadas por seção longitudinal para a semeadura das espécies bioindicadoras (pepino, alface, capim-marmelada e sorgo). Os sintomas de fitotoxicidade nas plantas bioindicadoras foram avaliados adotando-se a escala visual de fitotoxicidade entre 0 e 100%, aos 5, 7, 9 e 11 dias após a semeadura. As respostas das espécies bioindicadoras ao efeito residual do S-metolachlor foram variáveis e dependeram da precipitação. Em um Latossolo de textura média, as maiores concentrações de S-metolachlor ocorrem em profundidades que variam entre 0 e 0,06 m. A profundidade máxima de lixiviação detectada foi de 0,12-0,15 m com 131 mm de precipitação. Por apresentar sintomas de fitotoxicidade em maiores profundidades, o pepino foi a espécie mais sensível à presença de S-metolachlor em Latossolo de textura média.

Palavras-chave: fitotoxicidade, herbicida, mobilidade, textura do solo

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Introduction

Weeds are one of the main biotic factors present in the sugarcane production system, interfering with the development and yield of crops (Paula et al., 2018). The primary method of controlling these species in sugarcane is chemical because the herbicides used have a high control efficiency (Concenço et al., 2016). However, the main concern regarding the adoption of this method is related to the behavior of herbicides in the environment (Silva Junior et al., 2015a; 2018).

The herbicide dynamics and residual effect on crops are determined by the physicochemical properties of each active ingredient (Amim et al., 2014). These properties, when interacting with climate (precipitation, temperature, and luminosity) and edaphic (soil water availability, granulometry, and organic matter content) conditions will determine the availability of the herbicide in the soil solution (Rocha et al., 2013).

The downward movement of herbicides in the soil or along the soil water is called leaching or percolation (Mancuso et al., 2011; Franceschi et al., 2019; Mendes et al., 2019). It is known, for instance, that the leaching potential of herbicides such as the S-metolachlor can be influenced by natural precipitation and/or artificial irrigation or by soils that have large pores or are poor in organic matter. In addition, natural precipitation and/or artificial irrigation can influence the leaching rate of S-metolachlor in groundwater, as it is relatively soluble in water and moderately absorbed by soil particles (Dollinger et al., 2019; Peña et al., 2019; Sigmund et al., 2019).

Thus, information about the dynamics of herbicides in different types of soils based on growth analyses of bioindicator species is beneficial for developing weed management strategies that reduce risks of environmental contamination (Borowik et al., 2017). Therefore, this study aims to evaluate the leaching potential of the herbicide S-metolachlor under different natural precipitations in medium-textured Oxisol using bioindicator plants.

Material and Methods

This research was installed under field conditions, in a medium-textured soil classified as Oxisol, in an area situated in Guatapará-SP (21°29'53" S, 48°2'1" W., with an altitude of 516 m). The soil samples of the area were collected in the layers of 0-0.10, 0.10-0.20, and 0.20-0.30 m of depth and were analyzed in the laboratory to determine chemical and granulometric properties (Silva Júnior et al., 2015a) (Table 1).

A completely randomized experimental design was adopted, with four replications and treatments arranged in a 3 × 8 factorial scheme. Three precipitation indexes occurred in the environment before the samples were collected (50, 91, and 131 mm), and eight soil depths were evaluated (0-0.03; 0.03-0.06; 0.06-0.09; 0.09-0.12; 0.12-0.15; 0.15-0.20; 0.20-0.25; 0.25-0.30 m).

It should be noted that the area was fallow for an approximate period of three years and that the only activity carried out, six months before the installation of the experiment was the pulverization of the herbicide glyphosate (3.5 L ha^{-1}) to eliminate the weeds present at the site, and the plant debris present in the soil were removed at the time of installation of the study.

PVC tubes 0.3 m long and 0.15 m in diameter were introduced up to the surface level of soil profiles for sampling, using hammers and wooden structures to assist the penetration of the columns into the ground. The dose of the herbicide S-metolachlor was according to the recommendation of the commercial product Dual Gold® (c.p.) (960 g L^{-1} EC, Syngenta). For medium-textured soils, the pulverization of 1.5 L ha^{-1} is recommended, equivalent to a dose of 1440 g a.i. The herbicide pulverization was made at the top of the columns (on the soil surface), maintaining a 0.50 m space between the top edge of the column and the bar, moving at a speed of 1 m s^{-1}. A CO_2 pressurized sprayer was used, equipped with flat jet nozzles Teejet® XR 11002VS, at a working pressure of 2 kgf cm^{-2}, which provided a spray volume of 200 L ha^{-1}. The environmental conditions at the spraying moment were 75% of relative humidity, the temperature of 23.5 °C, and the wind speed of 3 km h^{-1}. There was no precipitation before the pulverization of the herbicide within three days.

After the pulverization of the herbicide, monitoring of daily rainfall was conducted in the areas, with pre-established breaks between 40-60, 80-100, 120-140 mm of rain, regardless of the days after the pulverization (DAP) of S-metolachlor. In this way, the accumulated rainfall of 50, 91, and 131 mm of rain was obtained at 6, 11, and 14 DAP, respectively.

The sample collection was performed, digging carefully around the PVC tube columns to maintain the original soil integrity. The bottom edge of the columns was protected with a fine mesh to retain the soil after the collection. The PVC tubes columns were taken to a greenhouse located in the Department of Plant Production of the Faculty of Agricultural and Veterinary Sciences – FCAV, São Paulo State University

Table 1. Chemical and particle-size analysis of collected samples in different soil depths

Depth (m)	pH $CaCl_2$ (0.01 mol L^{-1})	OM[1] (g dm^{-3})	P_{resine} (mg dm^{-3})	K	Ca	Mg	H + Al	SB[2]	CEC[3]	BS[4] (%)
				(mmol$_c$ dm^{-3})						
0-0.10	5.0	22	18	1.2	21	8	25	30.2	55.2	54.7
0.10-0.20	5.1	16	23	0.6	18	7	21	25.6	46.6	54.9
0.20-0.30	5.1	13	17	0.4	13	6	18	19.4	37.4	51.8

Particle size (g kg^{-1})	Depth (m)		
	0-0.10	0.10-0.20	0.20-0.30
Clay	286	297	316
Silt	85	65	81
Coarse Sand	204	207	180
Fine Sand	425	431	423
Total Sand	629	638	603
Soil Texture	Medium	Medium	Medium

[1] Organic Matter; [2] Sum of Bases; [3] Cation Exchange Capacity; [4] Base Saturation

in Jaboticabal-SP (21°14'43.42" S and 48°17'32.80" W, with an altitude of 583 m). These columns were divided into two equal parts, making a longitudinal cut with a metal wire. A galvanized sheet was put between the cut at the moment of division to maintain the soil intact in each half obtained.

The sowing of bioindicator species was made in each part of the columns so that an equal number of seedlings could emerge along the 0.30 m of the soil profile at each 0.03 m interval. The germination percentage of each species was used according to the descriptive instructions provided by the seed companies to choose the sowing rate. The bioindicator species evaluated in this experiment were: cucumber (*Cucumis sativus* L.), lettuce (*Lactuca sativa* L.), Alexander grass (*Urochloa plantaginea* (Link) Hitchc.), and sorghum (*Sorghum bicolor* L. Moench.). The soil in the columns was kept moist during the experimental period using automated sprays set to dispense water in an amount similar to the soil field capacity.

The effect of the treatments was evaluated by observing the visual symptoms of phytotoxicity at 5, 7, 9, and 11 days after sowing (DAS) the bioindicator plants, conferring as evaluation criterion values between 0 (no damage observed) and 100% (death plant) (Marques et al., 2021). The observed values were submitted to the normality test by the Shapiro-Wilk at 5% significance and to the analysis of variance by F-Test. The effects of treatments were compared by the Tukey test at 0.05 probability level using the statistical software SISVAR 5.3 (Ferreira, 2019).

Results and Discussion

It is observed that, at the 5 DAS, the spraying of the herbicide S-metolachlor in pre-emergence in a medium-textured soil (on average approximately 299 g kg^{-1} of clay in the 0 - 0.30 m soil layer (Table 1)) promoted visual symptoms of phytotoxicity in cucumber plants in all rainfall levels studied (Table 2). When the accumulated rainfall values of 50 and 91 mm occurred, the visual symptoms were observed in plants grown in the 0.03-0.06 m soil layer. In the accumulated rainfall of 131 mm, the herbicide leaching occurred down to 0.12-0.15 m soil layer (Table 2), demonstrating that S-metolachlor leaching increases with high rainfall. The attributed notes of plant injury were lower than those registered in the 50 and 91 mm, probably by carrying the product to deeper layers, diluting it.

At 7 DAS, the occurrence of visual damage was registered in cucumber plants in the same depths in which they were initially found (5 DAS) but with higher values of plant injury, which demonstrates an increase in the herbicidal effect on this bioindicator plant over time (Table 2) When cucumber plants were evaluated at the 9 and 11 DAS, the S-metolachlor herbicide was found in the same depths of previous evaluations (5 and 7 DAS) for three studied rainfalls (50, 91, and 131 mm), however, with more intense visual symptoms of phytotoxicity (Table 2).

Similar results were found by Inoue et al. (2010) when studying the leaching of S-metolachlor (Dual Gold®) herbicide in sandy soil (60 g kg^{-1} of clay). They verified that

Table 2. Visual symptoms of phytotoxicity in cucumber plants (*Cucumis sativus* L.) after spraying the herbicide S-metolachlor in medium-textured soil with different accumulated rainfall levels at 5, 7, 9, and 11 days after sowing (DAS)

Depth (m)	Accumulated precipitation - mm					
	50	91	131	50	91	131
		5 DAS			7 DAS	
0-0.03	30.0 aB	56.3 aA	10.0 abC	50.0 aB	60.0 aA	15.0 bC
0.03-0.06	28.8 aB	38.8 bA	13.0 aC	51.25 aA	41.2 bB	31.2 aC
0.06-0.09	0.0 bB	0.0 cB	5.0 bcA	0.0 bB	0.0 cB	20.0 bA
0.09-0.12	0.0 bB	0.0 cB	5.0 bcA	0.0 bB	0.0 cB	20.0 bA
0.12-0.15	0.0 bB	0.0 cB	5.0 bcA	0.0 bB	0.0 cB	20.0 bA
0.15-0.20	0.0 bA	0.0 cA	0.0 cA	0.0 bA	0.0 cA	0.0 cA
0.20-0.25	0.0 bA	0.0 cA	0.0 cA	0.0 bA	0.0 cA	0.0 cA
0.25-0.30	0.0 bA	0.0 cA	0.0 cA	0.0 bA	0.0 cA	0.0 cA
F Precipitation (P)		56.29**			0.74^{ns}	
F Depth (D)		289.19**			682.7**	
F (P x D)		48.51**			107.08**	
LSD (P)		4.6			4.01	
LSD (D)		6.0			5.24	
CV (%)		34.0			18.5	
		9 DAS			11 DAS	
0-0.03	68.7 aA	60.0 aA	20.0 bC	72.5 aA	54.5 aB	56.2 aB
0.03-0.06	64.2 aA	42.7 bB	37.5 aB	71.7 aA	40.5 bC	60.0 aB
0.06-0.09	0.0 bB	0.0 bB	40.0 aA	0.0 aB	0.0 cB	60.0 aA
0.09-0.12	0.0 bB	0.0 bB	40.0 aA	0.0 aB	0.0 cB	60.0 aA
0.12-0.15	0.0 bB	0.0 bB	40.0 aA	0.0 aB	0.0 cB	60.0 aA
0.15-0.20	0.0 bB	0.0 bB	0.0 cA	0.0 aA	0.0 cA	0.0 bA
0.20-0.25	0.0 bB	0.0 bB	0.0 cA	0.0 aA	0.0 cA	0.0 bA
0.25-0.30	0.0 bB	0.0 bB	0.0 cA	0.0 aA	0.0 cA	0.0 bA
F Precipitation (P)		58.96**			463.65**	
F Depth (D)		420.36**			613.18**	
F (P x D)		109.6**			123.84**	
LSD (P)		5.9			5.8	
LSD (D)		7.6			7.6	
CV (%)		20.1			15.4	

Means of visual symptoms of phytotoxicity followed by the same lowercase letter in the column and uppercase letter in the row do not differ statistically from each other according to the Tukey test ($p > 0.05$). ** - Significant at 0.01 level of probability; ns - Not significant herbicide molecules were taken to depths greater than 0.05 m independently of the precipitation. When the precipitation was equal or greater than 80 mm, a more significant movement of the herbicide was observed, which confirms that the greater the accumulated rainfall, the greater is the probability of dragging the S-metolachlor molecules in the profile of sandy or medium-textured soils, as observed in this study.

For daily rainfall accumulation of 131 mm at the 11 DAS, the symptoms of damage in cucumber plants were higher in the deeper layers of soil than on the surface layer (0-0.03 m), independently of the no significance between the results (Table 2). This fact demonstrated that more intense rainfall can carry more significant amounts of herbicide in the soil profile down to the 0.12-0.15 m deep layer.

It is known that the leaching of S-metolachlor depends on factors such as occurrence and intensity of rainfall or irrigation, mean temperature and soil temperature, humic acid content, and clay and organic matter of the soil (Marín-Benito et al., 2018). In Table 1, it is observed that the level of organic matter of the medium-textured soil studied can be considered low in the layers of 0-0.10 (2.2%), 0.10-0.20 (1.6%), and 0.20-0.30 m (1.3%). This fact infers that the organic matter content may have contributed to the leaching of the herbicide S-metolachlor in greater depths.

It is highlighted that at the 5 and 7 DAS, the visual damage in cucumber plants was more significant when there was an accumulation of 91 mm of rainfall, with values that decreased in subsequent evaluations (9 and 11 DAS) (Table 2). Because of that, it is assumed that even though there were no symptoms of phytotoxicity in the plants, less leaching of the herbicide S-metolachlor may have occurred up to layers greater than 0.06 m depth for this quantity of accumulated water.

It should also be noted that there was an accentuated accumulation of the herbicide in the 0-0.03 m deep layer for the 50 mm rainfall due to the intense increase in the percentage of visual damage during evaluations, with values that varied between 30 and 72.5% of phytotoxicity between the period of 5 to 11 DAS (Table 2).

Generally, the greater precipitation caused greater leaching of S-metolachlor molecules, increasing groundwater contamination potential. In this case, the potential estimation of leaching calculated by the GUS index classified the S-metolachlor as having high leaching potential once the sorption is intermediate (K_{oc} = 200 mL g^{-1} of soil) and it presents high water solubility (480 mg L^{-1} at 25 °C) (Inoue et al., 2010; Nunes & Vidal, 2016), which corroborates the results found in this study for cucumber.

Based on the phytotoxicity data observed for the lettuce plants used as bioindicator species, it was verified that at 5 DAS, all plants presented visual symptoms of damage induced by the herbicide S-metolachlor, with expressive symptoms down to the 0.03-0.06 m deep layer, regardless of the accumulated rainfall. Moreover, it is observed that at the 5 DAS, there was a greater significance of damage values with the accumulation of 91 mm of rainfall for the 0-0.03 m depth layer than the rainfalls of 51 and 131mm (Table 3).

Table 3. Visual symptoms of phytotoxicity in lettuce plants (*Lactuca sativa* L.) after spraying the herbicide S-metolachlor in medium-textured soil with different accumulated rainfall levels at 5, 7, 9, and 11 days after sowing (DAS)

Depth (m)	Accumulated precipitation - mm					
	50	91	131	50	91	131
		5 DAS			7 DAS	
0-0.03	13.8 aB	68.8 aA	6.0 aC	88.7 aA	70.0 aB	13.0 aC
0.03-0.06	13.8 aA	11.3 bAB	6.3 aB	15.0 bA	12.5 bA	11.2 aA
0.06-0.09	0.0 bA	0.0 cA	0.0 aA	0.0 cA	0.0 cA	0.0 bA
0.09-0.12	0.0 bA	0.0 cA	0.0 aA	0.0 cA	0.0 cA	0.0 bA
0.12-0.15	0.0 bA	0.0 cA	0.0 aA	0.0 cA	0.0 cA	0.0 bA
0.15-0.20	0.0 bA	0.0 cA	0.0 aA	0.0 cA	0.0 cA	0.0 bA
0.20-0.25	0.0 bA	0.0 cA	0.0 aA	0.0 cA	0.0 cA	0.0 bA
0.25-0.30	0.0 bA	0.0 cA	0.0 aA	0.0 cA	0.0 cA	0.0 bA
F Precipitation (P)		55.10**			44.18**	
F Depth (D)		123.53**			252.84**	
F (P x D)		50.75**			40.18**	
LSD (P)		5.3			7.4	
LSD (D)		6.9			9.7	
CV (%)		65.2			49.92	
		9 DAS			11 DAS	
0-0.03	93.8 aA	71.3 aB	26.3 aA	95.0 aA	70.0 aB	31.2 aC
0.03-0.06	31.3 bA	11.2 bB	18.8 aB	29.25 bA	8.25 bC	20.0 aB
0.06-0.09	0.0 cA	0.0 bA	0.0 bA	0.0 cA	0.0 bA	0.0 bA
0.09-0.12	0.0 cA	0.0 bA	0.0 bA	0.0 cA	0.0 bA	0.0 bA
0.12-0.15	0.0 cA	0.0 bA	0.0 bA	0.0 cA	0.0 bA	0.0 bA
0.15-0.20	0.0 cA	0.0 bA	0.0 bA	0.0 cA	0.0 bA	0.0 bA
0.20-0.25	0.0 cA	0.0 bA	0.0 bA	0.0 cA	0.0 bA	0.0 bA
0.25-0.30	0.0 cA	0.0 bA	0.0 bA	0.0 cA	0.0 bA	0.0 bA
F Precipitation (P)		30.03**			23.55**	
F Depth (D)		231.07**			222.43**	
F (P x D)		23.2**			19.21**	
LSD (P)		8.7			9.1	
LSD (D)		11.4			11.9	
CV (%)		49.08			50.86	

Means of visual symptoms of phytotoxicity followed by the same lowercase letter in the column and uppercase letter in the row do not differ statistically from each other according to the Tukey test ($p > 0.05$). ** - Significant at 0.01 level of probability

At the 7, 9, and 11 DAS, it was also possible to observe visual damage in the lettuce plants only down to 0.03-0.06 m deep layer; however, with a gradual increase of plant injury values during evaluations, which showed an increase in the toxic effect of S-metolachlor on this bioindicator plant (Table 3). These data reinforce the finding presented by Silva Junior et al. (2015b), which verified visual intoxication symptoms in lettuce plants caused by the leaching of the herbicide S-metolachlor (Dual Gold®) in the first layers of clay soil (448 g kg^{-1} of clay), being the higher percentages of visual damage in the plants in the 0-0.03 m layer.

Based on the comparison with the evaluations carried out with the cucumber bioindicator species (Table 2), it is observed that, for lettuce, there was also an expressive accumulation of herbicide in the layer 0-0.03 m for the lowest rainfall (50 mm). This was due to an intense increase of the phytotoxicity percentage during the evaluations, with values that varied between 13,8 and 95% of phytotoxicity between 5 to 11 DAS (Table 3).

The findings of the present study support results reported by Papiernik et al. (2009), who observed higher concentrations of the herbicide S-metolachlor in the surface soil (0-0.10 m). However, these authors observed that when the organic matter content of the soil is low, deeper leaching can occur, contaminating groundwater.

It is emphasized that the increase of leaching losses of S-metolachlor by decreasing the organic matter content occur since in soils with less organic matter, S-metolachlor has a higher movement, especially when the content of organic matter is close to 3.0 g dm^{-3}. Furthermore, the leaching of this herbicide can be inhibited in soils with high clay or silt content and increased in sandy soil because S-metolachlor has some mobility in light-textured soil (Zemolin et al., 2014).

The visual symptoms of intoxication observed in Alexander grass plants caused by the herbicide S-metolachlor at the 5 and 7 DAS occurred down to 0.03-0.06 m soil layer in the three rainfall levels (Table 4).

In the evaluations at 9 and 11 DAS, the action of the herbicide was verified in the same layers of the soil profile of the previous evaluations (5 and 7 DAS). However, it is noticed that the visual damage symptoms presented an intense increase in both evaluations. At 11 DAS, for the rainfall of 131 mm, it was possible to observe values of 100% phytotoxicity in Alexander grass plants in the superficial layer (0-0.03 m) (Table 4).

Rain or artificial irrigation interferes with the action of the herbicide, mainly on its intensity and movement in the soil profile, because herbicides are inefficient when spraying on dry soils. It is necessary to highlight that the water molecules have high polarity and can compete with herbicide molecules for particle adsorption sites in the soil. In dry soils, the herbicides are strongly adsorbed, while in moist soils, the herbicide molecules are dissolved in solution and can easily penetrate the soil (Schuhmann et al., 2019).

It is verified that there were no effects in the different studied rainfalls in any evaluated time when sorghum plants were used as bioindicator species. It is emphasized that an effect of depth in every analyzed period was observed, with

Table 4. Visual symptoms of phytotoxicity in Alexander grass (*Urochloa plantaginea* (Link) Hitchc.) plants after spraying the herbicide S-metolachlor in medium-textured soil, with different accumulated rainfall levels at 5, 7, 9, and 11 days after sowing (DAS)

Depth (m)	Accumulated precipitation - mm					
	50	91	131	50	91	131
		5 DAS			7 DAS	
0-0.03	71.3 aB	76.3 aAB	85.0 aA	83.7 aAB	75.0 aB	93.7 aA
0.03-0.06	67.5 aA	22.5 bC	34.3 bB	71.2 aA	28.2 bC	42.5 bB
0.06-0.09	0.0 bA	0.0 cA	0.0 aA	0.0 bA	0.0 cA	0.0 cA
0.09-0.12	0.0 bA	0.0 cA	0.0 cA	0.0 bA	0.0 cA	0.0 cA
0.12-0.15	0.0 bA	0.0 cA	0.0 cA	0.0 bA	0.0 cA	0.0 cA
0.15-0.20	0.0 bA	0.0 cA	0.0 cA	0.0 bA	0.0 cA	0.0 cA
0.20-0.25	0.0 bA	0.0 cA	0.0 cA	0.0 bA	0.0 cA	0.0 cA
0.25-0.30	0.0 bA	0.0 cA	0.0 cA	0.0 bA	0.0 cA	0.0 cA
F Precipitation (P)		4.21*			9.41**	
F Depth (D)		214.86**			336.68**	
F (P x D)		6.53**			7.55**	
LSD (P)		11.7			10.2	
LSD (D)		15.2			13.3	
CV (%)		46.3			36.7	
		9 DAS			11 DAS	
0-0.03	87.5 aB	75.0 aC	98.8 aA	93.0 aB	72.5 aB	100.0 aA
0.03-0.06	75.8 bA	27.5 bC	54.3 bB	78.5 bA	26.2 bC	66.2 bB
0.06-0.09	0.0 cA	0.0 cA	0.0 cA	0.0 cA	0.0 cA	0.0 cA
0.09-0.12	0.0 cA	0.0 cA	0.0 cA	0.0 cA	0.0 cA	0.0 cA
0.12-0.15	0.0 cA	0.0 cA	0.0 cA	0.0 cA	0.0 cA	0.0 cA
0.15-0.20	0.0 cA	0.0 cA	0.0 cA	0.0 cA	0.0 cA	0.0 cA
0.20-0.25	0.0 cA	0.0 cA	0.0 cA	0.0 cA	0.0 cA	0.0 cA
0.25-0.30	0.0 cA	0.0 cA	0.0 cA	0.0 cA	0.0 cA	0.0 cA
F Precipitation (P)		25.79**			24.9**	
F Depth (D)		660.88**			437.54**	
F (P x D)		16.53**			12.88**	
LSD (P)		7.7			9.7	
LSD (D)		10.0			12.7	
CV (%)		26.0			31.6	

Means of visual symptoms of phytotoxicity followed by the same lowercase letter in the column and uppercase letter in the row do not differ statistically from each other according to the Tukey test ($p > 0.05$). ** - Significant at 0.01 level of probability; * - Significant at 0.05 level of probability visual symptoms of damage that remained in the superficial soil layer (0-0.03 m) and gradually increased up to the 9 DAS evaluation, becoming constant in the subsequent evaluation (11 DAS) (Table 5).

Given this context, it can be observed that the phytotoxicity of the herbicide S-metolachlor varied according to the bioindicator species and the amount of rain. Independently of the bioindicator plant studied, the product concentration was probably reduced when the depth evaluated increased.

The findings of this study for the bioindicator plants analyzed in medium-textured soil demonstrate that among the four species studied, the most sensitive to the herbicide was cucumber, which permitted visualization of the herbicide in greater depths.

Table 5. Visual symptoms of phytotoxicity in sorghum plants (*Sorghum bicolor* L. Moench.) after spraying the herbicide S-metolachlor under different accumulated precipitations at 5, 7, 9, and 11 days after sowing (DAS)

Accumulated precipitation (mm)	Days after sowing (DAS)			
	5	7	9	11
50	3.9	8.75	9.53	9.68
91	3.18	4.06	4.53	4.37
131	3.9	4.37	4.37	4.37
Depth (m)				
0-0.03	29.33 a	45.83 a	49.16 a	49.16 a
0.03-0.06	0.0 b	0.0 b	0.0 b	0.0 b
0.06-0.09	0.0 b	0.0 b	0.0 b	0.0 b
0.09-0.12	0.0 b	0.0 b	0.0 b	0.0 b
0.12-0.15	0.0 b	0.0 b	0.0 b	0.0 b
0.15-0.20	0.0 b	0.0 b	0.0 b	0.0 b
0.20-0.25	0.0 b	0.0 b	0.0 b	0.0 b
0.25-0.30	0.0 b	0.0 b	0.0 b	0.0 b
F Precipitation (P)	0.03^{ns}	1.34^{ns}	1.77^{ns}	1.91^{ns}
F Depth (D)	7.69**	19.27**	23.34**	23.05**
F (P x D)	0.03^{ns}	1.34^{ns}	1.77^{ns}	1.71^{ns}
LSD (P)	16.51	16.29	15.88	15.98
LSD (D)	7.75	7.65	7.45	6.14
CV (%)	353.4	223.2	202.8	204.06

Means of visual symptoms of phytotoxicity followed by the same lowercase letter in the column do not differ statistically from each other according to the Tukey test ($p > 0.05$). ** - Significant at 0.01 level of probability; ns - Not significant

Conclusions

1. The responses of the bioindicator species to the residual effect of the herbicide S-metolachlor were variable and depended on the rainfall level.

2. Generally, in a medium-textured Oxisol, the higher values of leaching and concentration of S-metolachlor herbicide occur in depths ranging between 0 and 0.06 m.

3. The maximum leaching depth of S-metolachlor detected was in 0.12-0.15 m soil layer with 131 mm of precipitation.

4. Cucumber was the most sensitive species to the presence of the herbicide S-metolachlor in an Oxisol of medium texture since it presents symptoms of phytotoxicity at higher depths.

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