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Article

Winter Species Promote Phytoremediation of Soil Contaminated with Protox-Inhibiting Herbicides

Espécies de Inverno Promovem Fitorremediação de Solo Contaminado com Herbicidas Inibidores da Protox

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ABSTRACT - Phytoremediation comprises one of the main forms of decontamination of organic and inorganic substances in the soil, being economically viable and with a low environmental impact. The aim of this study was to verify the efficiency of winter plant species in the phytoremediation of soil contaminated with fomesafen and sulfentrazone using cucumber as an indicator species to the presence of residue. The experimental design was a completely randomized design arranged in a 6 x 4 factorial scheme with four replications for each herbicide. Factor A consisted of the phytoremediator species black oats, garden vetch, radish, bird's-foot trefoil, white lupine, and a treatment without prior cultivation. Factor B, on the other hand, consisted of the doses of fomesafen (0.000, 0.125, 0.250, and 0.500 kg h^{-1}) or sulfentrazone (0.000, 0.300, 0.600 and 1,200 kg ha^{-1}) applied in crop pre-emergence. At 45 days after sowing, the phytoremediator species were cut close to the soil. Subsequently, the bioindicator species of herbicide residues in the soil (cucumber) was sown in the pot. Phytotoxicity of herbicides to cucumber plants was assessed at 7, 14, 21, and 28 days after emergence (DAE). At 28 DAE, leaf area, height, and dry matter were determined in the bioindicator plant. Fomesafen and sulfentrazone doses interfered negatively with the assessed variables of cucumber when cultivated in succession to phytoremediator species. Cucumber phytotoxicity increased for all potential phytoremediator species as fomesafen and sulfentrazone doses increased. Sulfentrazone residues promoted the highest toxic effects on the bioindicator plant when compared to fomesafen. In general, black oats, radish, and white lupine were the species with the highest capacity to phytoremediate soil contaminated with fomesafen and sulfentrazone when applying the dose and twice the recommended doses of the herbicides.

Keywords: *Cucumis sativus*, carryover, soil remediation.

RESUMO - A fitorremediação compreende uma das principais formas de descontaminação de substâncias orgânicas e inorgânicas no solo, economicamente viável e de baixo impacto ambiental. Objetivou-se com este trabalho verificar a eficiência de espécies vegetais de inverno na fitorremediação de solo contaminado com fomesafen e sulfentrazone, utilizando pepino como espécie indicadora da presença de resíduo. O delineamento utilizado foi o inteiramente casualizado, arranjado em esquema fatorial 6 x 4, com quatro repetições para cada herbicida. No fator A foram alocadas as espécies fitorremediadoras: aveia-preta, ervilhaca, nabo, cornichão, tremoço e um tratamento sem cultivo prévio; e no fator B, as doses de fomesafen (0,000; 0,125; 0,250 e 0,500 kg ha^{-1}) ou sulfentrazone (0,000; 0,300; 0,600 e 1.200 kg ha^{-1}), aplicadas em pré-emergência das culturas. Aos 45 dias após a semeadura, as espécies fitorremediadoras foram seccionadas rente

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ao solo. Posteriormente, efetuou-se, no próprio vaso, a semeadura da espécie bioindicadora de resíduos de herbicidas no solo (pepino). A fitotoxicidade dos herbicidas às plantas de pepino foi avaliada aos 7, 14, 21 e 28 dias após a emergência (DAE). Aos 28 DAE, determinou-se a área foliar, altura e matéria seca da planta bioindicadora. As doses de fomesafen e sulfentrazone interferiram negativamente nas variáveis avaliadas do pepino, quando cultivado em sucessão às espécies fitorremediadoras. A fitotoxicidade ao pepino aumentou, para todas as espécies potencialmente fitorremediadoras, com o incremento das doses de fomesafen e sulfentrazone. Os resíduos de sulfentrazone promoveram os maiores efeitos tóxicos sobre a planta bioindicadora do que o fomesafen. De modo geral, a aveia-preta, o nabo e o tremoço foram as espécies que apresentaram a maior capacidade de fitorremediar solos contaminados com fomesafen e sulfentrazone ao se aplicar a dose e o dobro das doses recomendadas dos herbicidas.

Palavras-chave: *Cucumis sativus*, *carryover*, despoluição de solo.

INTRODUCTION

Some herbicides may remain in the soil for long periods, causing the so-called carryover in successor crops, as well as contaminating surface and groundwater due to leaching (Lavorenti et al., 2003; Martinez et al., 2008; Silva et al., 2014). Among them, fomesafen and sulfentrazone, widely used in Brazil for the control of weeds in bean, soybean, sugarcane, and forest crops, among others, stand out (Rodrigues and Almeida, 2011).

Fomesafen and sulfentrazone belong to the protoporphyrinogen oxidase (protox) inhibiting herbicides, with an average half-life from 34 to 160 days and 172.5 days, respectively (Martinez et al., 2008; Khorram et al., 2016). Soil type, a low organic matter content, a sandy texture, and a high soil pH increase fomesafen leaching in agricultural soils (Silva et al., 2014). Microorganisms influence sulfentrazone degradation and fungal and bacterial growth was not affected, unlike that observed for fomesafen, in which its application significantly decreased fungal biomass and the abundance of gram-positive and gram-negative bacteria (Martinez et al., 2008; Wu et al., 2018). Understanding soil-plant-herbicide interaction is determinant for an adequate recommendation of these products, guaranteeing the effectiveness and reducing environmental contamination (Oliveira Jr. et al., 2006).

Phytoremediation is one of the strategies for removing toxic compounds from the environment. Currently, 1% of the money spent on environmental cleanup in the world is through phytoremediation (Montpetit and Lachapelle, 2016; Dickinson et al., 2017). It is an economically viable technique that provides a low environmental impact (Ali et al., 2013). However, to achieve efficiency in the technique it is necessary to understand the rhizospheric processes of absorption, translocation, and degradation of pollutants (Dickinson et al., 2017).

Many studies have been carried out to identify plants able of decontaminating soils contaminated with herbicides (Pires et al., 2005; Santos et al., 2007; Madalão et al., 2012; Ferraço et al., 2017). Madalão et al. (2013) reported that the decontamination of soils treated with sulfentrazone was efficient with the species *Dolichos lablab*, *Crotalaria juncea*, and *Canavalia ensiformis*. The latter responds efficiently to sulfentrazone phytoremediation with an increased plant density (Ferraço et al., 2017). Recent evidences indicate that planning phytoremediation strategies and the selection of tolerant species are directly related to antioxidant responses induced by pesticide exposure (Mitton et al., 2018).

In addition to phytoremediation of soils contaminated with herbicides, plant species can become alternatives of economic interest, being cultivated in the fallow period of crops to improve soil attributes, as they promote the addition of organic matter, incorporate atmospheric N (legumes), decrease soil N leaching, increase water storage, improve soil hydraulic properties, and hence crop yield (Alvarez et al., 2017; Sun et al., 2018). Due to soil physicochemical changes, some species cultivated in the winter may have the capacity to phytoremediate the soil contaminated with the herbicides fomesafen and sulfentrazone and the potential use as forage, cover plants or even for seed or grain production.

This study aimed to verify the efficiency of the winter species black oats, garden vetch, radish, bird's-foot trefoil, and white lupine in the phytoremediation of soil contaminated with fomesafen and sulfentrazone using cucumber as an indicator species to the presence of residue.

MATERIAL AND METHODS

The study was conducted from July to December 2016 in a greenhouse at the Universidade Federal da Fronteira Sul (UFFS), Campus of Erechim, RS, Brazil. Two experiments were installed in a completely randomized design arranged in a 6 x 4 factorial scheme with four replications. The first experiment was installed with the herbicide fomesafen and the second with sulfentrazone. Factor A consisted of potentially phytoremediator species chosen as alternatives to sowing in the off-season: black oats (*Avena strigosa*), garden vetch (*Vicia sativa*), radish (*Raphanus sativus*), bird's-foot trefoil (*Lotus corniculatus*), white lupine (*Lupinus albus*), and a treatment without prior cultivation. Factor B consisted of the doses of fomesafen (Flex®) (0.000, 0.125, 0.250, and 0.500 kg a.i. ha^{-1}) and sulfentrazone (Boral 500®) (0.000, 0.300, 0.600 and 1,200 kg a.i. ha^{-1}), applied in crop pre-emergence. The doses of 0.250 and 0.600 kg a.i. ha^{-1} correspond to the commercially recommended doses of fomesafen and sulfentrazone, respectively (Rodrigues and Almeida, 2011).

Sowing of the phytoremediator species was carried out in polyethylene pots with a capacity of 8 dm^3 filled with soil from an area with no herbicide application history. Soil chemical and physical characteristics were a pH in water of 4.8, OM of 35 g dm^{-3}, P of 4.0 mg dm^{-3}, K of 117.0 mg dm^{-3}, Al^{3+} of 0.6 $cmol_c$ dm^{-3}, Ca^{2+} of 4.7 $cmol_c$ dm^{-3}, Mg^{2+} of 1.8 $cmol_c$ dm^{-3}, CEC(t) of 7.4 $cmol_c$ dm^{-3}, CTC(T) of 16.5 $cmol_c$ dm^{-3}, H+Al of 9.7 $cmol_c$ dm^{-3}, SB of 6.8 $cmol_c$ dm^{-3}, V of 41%, and clay of 600 g dm^{-3}. Soil fertility was corrected based on its chemical analysis and in accordance with the technical recommendations for the crops used in the experiments (SBCS, 2004). Immediately after pot filling with soil and sowing the phytoremediator species, the herbicides were applied with a precision backpack CO_2-pressurized sprayer equipped with two spray tips TT 110.02, set to spray a volume of 150 L ha^{-1}.

After the germination of the species with phytoremediation potential, thinning was carried out, remaining 10 plants per pot. Irrigation was controlled daily in the experimental units, maintaining a soil moisture of approximately 80% of the field capacity, through the visualization of soil surface moisture. At 45 days after sowing (DAS), plants were cut close to the soil and removed from the pots. Immediately after harvesting the phytoremediator plants, the soil was sieved and fertilized according to the recommendation for cucumber (*Cucumis sativus* cv. Pioneiro) (SBCS, 2004). The bioindicator species (cucumber) was sown in the previously phytoremediated soil, with a density of 10 plants per pot.

At 7, 14, 21, and 28 days after emergence (DAE) of cucumber plants, the herbicide phytotoxicity (%) was assessed visually by two evaluators, who assigned scores from zero (no injury) to 100% (plant death), according to the methodology of the Brazilian Society of Weed Science (SBCPD, 1995). At 28 DAE, the average height of 10 plants (cm), leaf area (cm^2 pot^{-1}), and shoot dry matter (g pot^{-1}) of cucumber plants were determined. Plant height was measured with a ruler graduated in centimeters by measuring the distance from the base close to the soil to the apex of the last leaves. Leaf area was measured in five plants of each treatment with a portable CI-203 BioScence leaf meter. After leaf area determination, the plant shoot was packed in kraft paper bags and dried in a forced air circulation oven at 60 ± 5 °C until constant weight in order to obtain shoot dry matter.

The data were submitted to analysis of variance by the F test. When significant, linear or non-linear regressions were applied for the quantitative factor and the Tukey's test for the qualitative factor. All tests were performed at $p \leq 0.05$.

RESULTS AND DISCUSSION

An interaction was observed between the tested factors (potentially phytoremediator species x doses) in all assessed variables for both herbicides (fomesafen and sulfentrazone). Phytotoxicity assessed at 7 days after emergence (DAE) of cucumber plants increased as fomesafen doses increased only when black oats and bird's-foot trefoil were cultivated. For the other species and tested doses, the data were not fitted to the tested models (Figure 1A).

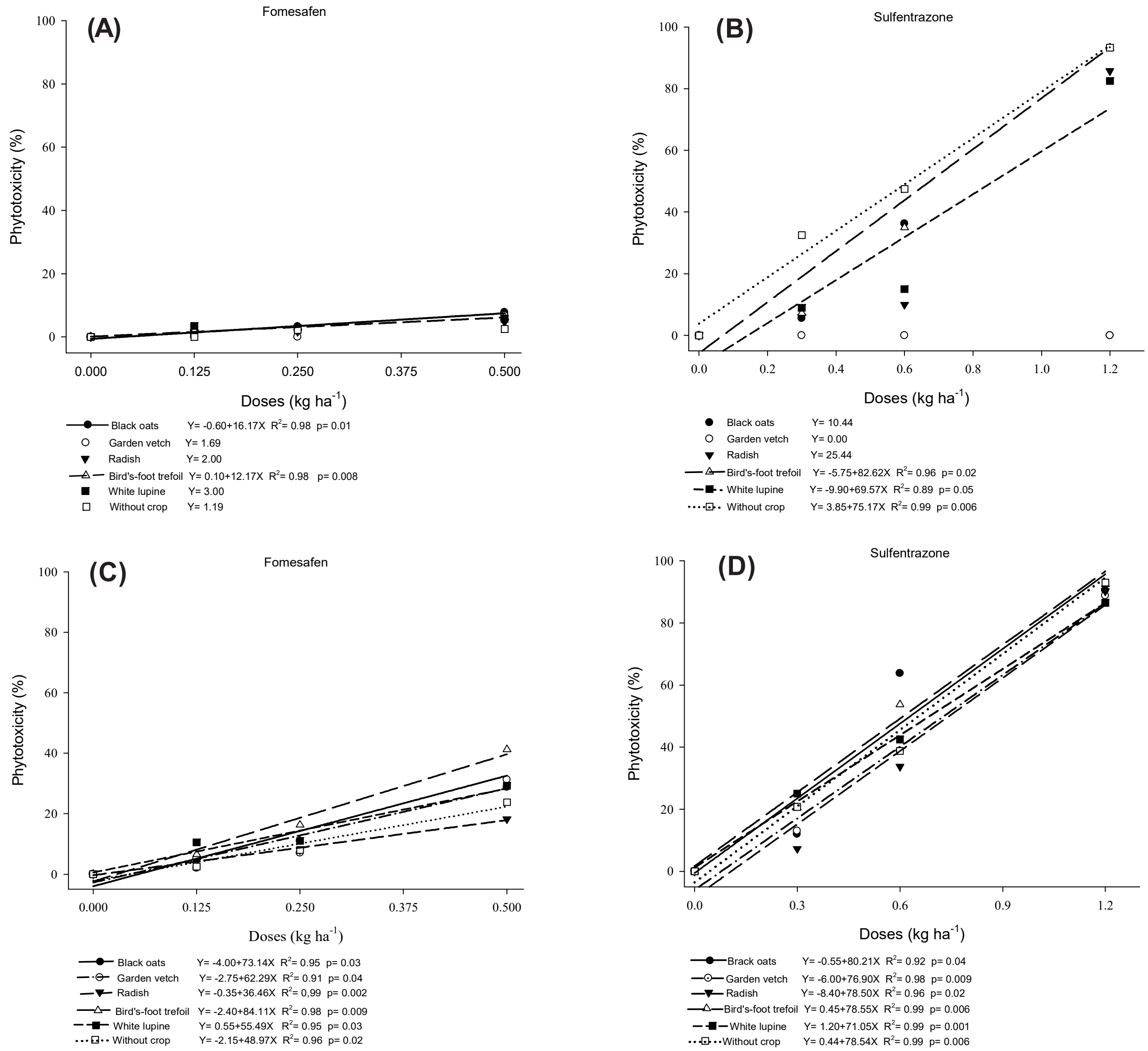

Figure 1 **-** Phytotoxicity (%) in cucumber plants at 7 (A and B) and 14 (C and D) days after emergence as a function of the prior cultivation of black oats, garden vetch, radish, bird's-foot trefoil, white lupine, and treatment without crop after fomesafen and sulfentrazone application.

Cucumber plants under sulfentrazone application also at 7 DAE showed a significant increase in phytotoxicity as the herbicide dose increased when sown after the cultivation of bird's-foot trefoil, white lupine, and soil without cultivation (Figure 1B). The same phytotoxic effect of sulfentrazone was observed for millet in a study carried out by Madalão et al. (2012). This indicates the high half-life and control efficiency in both mono and dicotyledonous (Martinez et al., 2008; Rodrigues and Almeida, 2011).

Fomesafen caused a phytotoxicity lower than 42% in cucumber with the cultivation of phytoremediator species or without prior cultivation at 14 DAE by applying twice the recommended dose (0.500 kg ha^{-1}). Except for the treatment without cultivation at 21 and 28 DAE and at the recommended fomesafen dose (0.250 L ha^{-1}), phytotoxicity was lower than 20% at all analyzed times (Figures 1A, 1C, 2A, and 2C). At 21 and 28 DAE, the treatment without prior cultivation was the most damaging, with phytotoxicity values of 93.5 and 91.5%, respectively, to cucumber plants when using twice the dose of fomesafen (Figure 2A, C). Sulfentrazone with the application twice the recommended dose (1,200 kg ha^{-1}) showed a phytotoxicity above 80% at 14, 21, and 28 DAE, except for the treatment without cultivation at 28 DAE, which reduced phytotoxicity drastically (Figure 1D, Figure 2B, D). This result may be associated with very specific conditions,

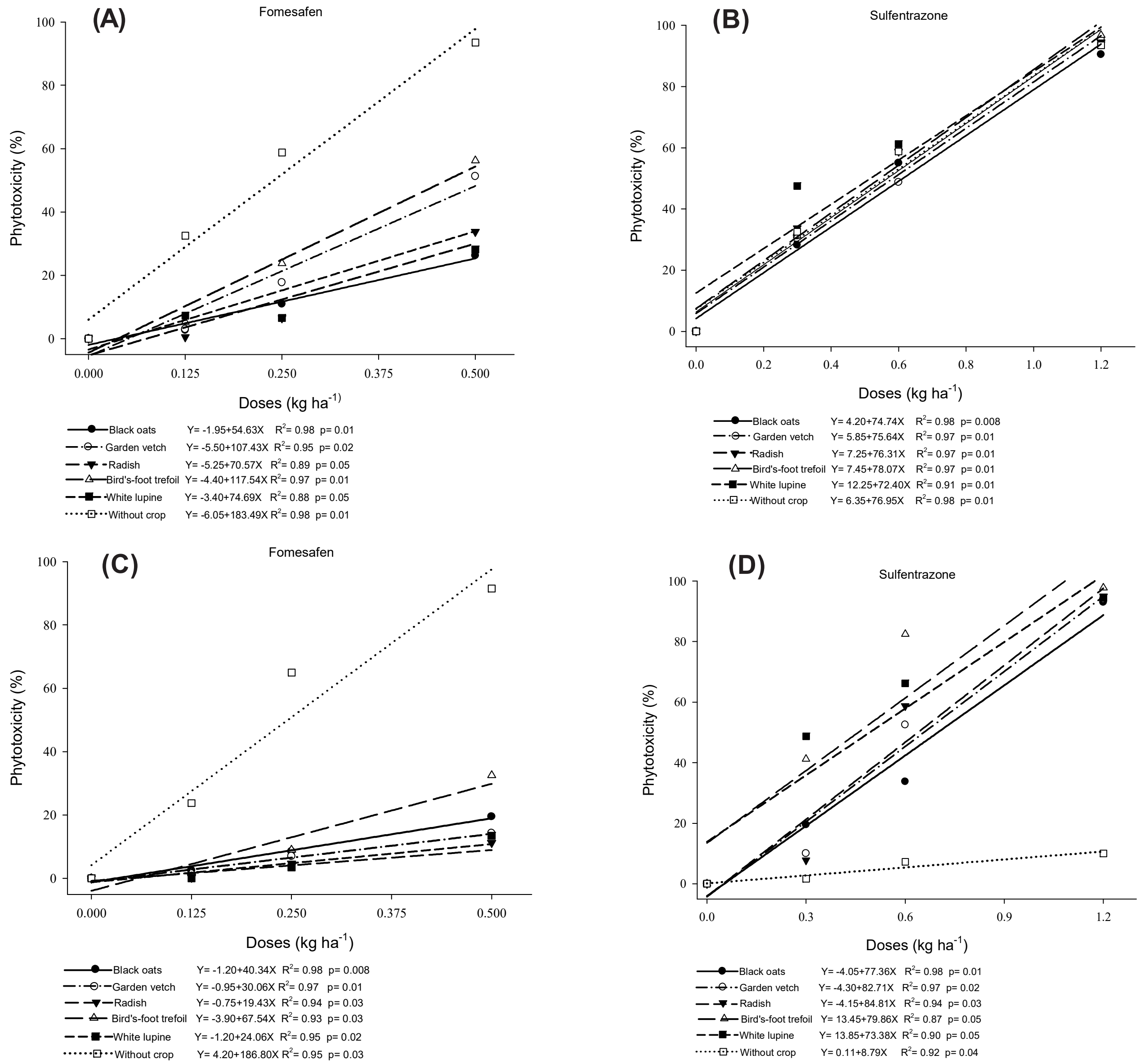

Figure 2 - Phytotoxicity (%) in cucumber plants at 21 (A and B) and 28 (C and D) days after emergence as a function of the prior cultivation of black oats, garden vetch, radish, bird's-foot trefoil, white lupine, and treatment without crop after fomesafen and sulfentrazone application.

indicating that may have occurred the degradation of part of the herbicide due to a higher variation in temperature and higher availability of light in the uncovered soil, i.e. in the treatment without cultivation (Martinez et al., 2008; Huang et al., 2017).

When comparing all species with phytoremediator potential within each dose, treated cucumber plants at 7 DAE showed phytotoxicity levels below 8% even at twice the recommended fomesafen dose (Table 1). During this period, cucumber plants grown in succession to black oats, radish, bird's-foot trefoil, white lupine, and treatment without cultivation did not present significant differences from each other when applying the recommended fomesafen dose. In addition, in the assessment at 7 DAE, when the dose of 1.200 kg ha^{-1} of sulfentrazone (higher dose) was applied, cucumber plants grown in succession to bird's-foot trefoil and in the treatment without prior cultivation showed the highest phytotoxicity levels. On the other hand, cucumber plants cultivated in succession to black oats and garden vetch did not present phytotoxicity to fomesafen and sulfentrazone at 7 DAE.

At 14 DAE, an increase in phytotoxicity was observed in cucumber plants when compared to the assessment at 7 DAE in all crops within each applied dose. The use of 0.500 kg ha^{-1} of fomesafen

Table 1 **-** Phytotoxicity (%) of cucumber plants cultivated in succession to species with phytoremediation potential (black oats, garden vetch, radish, bird's-foot trefoil, white lupine, and treatment without crop) assessed at 7, 14, 21, and 28 days after emergence (DAE) after fomesafen and sulfentrazone application

Phytotoxicity	Dose (kg ha^{-1})	Black oats	Garden vetch	Radish	Bird's-foot trefoil	White lupine	Treatment without crop	CV (%)
Fomesafen								
7 DAE	0.000	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	42.21
	0.125	0.75 bc	0.00 c	1.50 bc	2.00 ab	3.50 a	0.00 c	
	0.250	3.25 a	0.00 b	1.75 ab	2.75 a	2.75 a	2.25 a	
	0.500	7.75 a	6.75 ab	4.75 c	6.25 abc	5.75 bc	2.50 d	
14 DAE	0.000	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	19.12
	0.125	2.00 c	5.25 bc	4.25 bc	6.50 ab	10.50a	2.50 bc	
	0.250	11.00 b	7.00 b	8.00 b	16.25 a	11.00 b	8.00 b	
	0.500	28.75 b	31.25 b	18.25 d	41.25 a	29.25 b	23.75 c	
21 DAE	0.000	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	24.32
	0.125	2.75 ab	3.00 ab	0.50 b	5.25 ab	7.25 a	0.00 b	
	0.250	11.00 b	17.75 a	6.50 b	23.75 a	6.50 b	7.00 b	
	0.500	26.25 c	51.25 a	33.75 b	56.25 a	28.25 bc	25 c	
28 DAE	0.000	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	18.03
	0.125	2.5. b	1.25 b	0.25 b	2.00 b	0.00 b	23.75 a	
	0.250	8.50 b	7.00 bc	4.75 bc	9.00 b	3.50 c	65.00 a	
	0.500	19.50 c	14.25 d	11.25 d	32.5 b	13.50 d	91.5 a	
Sulfentrazone								
7 DAE	0.000	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	10.52
	0.300	5.50 b	0.00 c	6.00 b	7.25 b	9.00 b	32.50 a	
	0.600	36.25 b	0.00 d	10.00 c	35.0 b	15.00 c	47.50 a	
	1.200	0.00 c	0.00 c	85.75 b	93.25 a	82.50 b	93.25 a	
14 DAE	0.000	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	8.43
	0.300	12.00 b	13.00 b	7.25 b	20.50 a	25.00 a	20.75 a	
	0.600	63.75 a	38.75 cd	33.75 e	53.75 b	42.50 c	38.75 cd	
	1.200	90.25 a	88.75 a	90.25 a	92.50 a	86.50 a	93.00 a	
21 DAE	0.000	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	12.57
	0.300	28.25 b	31.25 b	33.75 b	31.25 b	47.50 a	32.50 b	
	0.600	55.00 ab	48.75 b	58.75 ab	60.00 ab	61.25 a	58.75 ab	
	1.200	90.50 a	95.50 a	94.50 a	96.75 a	93.50 a	93.50 a	
28 DAE	0.000	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	11.66
	0.300	19.50 b	10.00 c	7.75 c	41.25 a	48.75 a	1.65 c	
	0.600	33.75 d	52.50 c	58.75 bc	82.50 a	66.25 b	7.25 e	
	1.200	93.00 a	94.00 a	95.00 a	97.75 a	94.50 a	10.00 b	

Means followed by the same letter in the row do not differ from each other by the Tukey's test at p≤0.05.

caused the greatest injuries in relation to the other doses (Table 1). On the same day of assessment (14 DAE), cucumber plants cultivated in succession to black oats, garden vetch, and white lupine did not statistically differ from each other but presented the second highest phytotoxicity level when applied at a dose of 0.500 kg ha^{-1} of fomesafen. However, when using the highest dose, the lowest phytotoxicity was observed in cucumber plants grown succession to radish.

When using twice the sulfentrazone dose, cucumber plants presented a phytotoxicity above 86% for all the assessed species (Table 1). At 14 and 21 DAE, a lower phytotoxic effect of sulfentrazone was observed on cucumber in succession to black oats, garden vetch, and radish when using half the dose (0.300 kg ha^{-1}) and on garden vetch at the recommended herbicide dose (0.600 kg ha^{-1}).

In the assessment of fomesafen phytotoxicity of cucumber plants at 21 DAE, no statistical difference was observed when cultivated in succession to garden vetch and bird's-foot trefoil (51.25 and 56.25%, respectively) with twice the herbicide dose (Table 1). Black oats, white lupine, and the control without prior cultivation presented the highest phytoremediation effect in the soil when the highest fomesafen dose was applied (0.500 kg ha^{-1}) (Table 1).

The application of 0.300 kg ha^{-1} of sulfentrazone caused a higher phytotoxicity to cucumber plants after white lupine cultivation. At the recommended dose, white lupine presented a higher phytotoxicity only when compared to garden vetch at 21 DAE (Table 1). The application of twice the sulfentrazone dose, assessed at 21 DAE, did not present significant phytotoxicity differences when using the phytoremediation treatments with injury indices above 90% (Table 1).

The data show that cucumber plants grown in succession to black oats, garden vetch, radish, and white lupine showed the lowest phytotoxicity levels in the last assessment performed at 28 DAE for all fomesafen doses (Table 1). The treatment with the worst performance as a phytoremediator of fomesafen was that without cultivation in all applied doses.

The use of the treatment without cultivation caused the lowest phytotoxicity cucumber plants at all sulfentrazone doses at 28 DAE (Table 1). The treatment without cultivation possibly increased soil temperature and moisture, factors that together increase the biological activity and reduce sulfentrazone half-life (Martinez et al., 2008; Brum et al., 2013). The worst performance among the phytoremediator crops of sulfentrazone within all the applied doses was that of bird's-foot trefoil, whose phytotoxicity was higher than 41% at 28 DAE.

For plant height, leaf area, and dry matter, the bioindicator plant (cucumber) presented, in general, a similar behavior when cultivated in succession to black oats, garden vetch, radish, and white lupine for both assessed herbicides (Figures 3, 4, and 5).

The data did not fit the models tested for plant height and leaf area of cucumber in all treatments used as soil cover when applying fomesafen doses (Figures 3A and 4A). For sulfentrazone, a linear decrease in plant height was observed when cucumber plants were grown in succession to garden vetch, radish, bird's-foot trefoil, white lupine, and the treatment without cultivation (Figure 3B). Santos et al. (2007) observed similar results with sorghum as a bioindicator plant in succession to *Stizolobium aterrimum* and applications of trifloxysulfuron-sodium doses. The bioindicator plant was negatively affected regarding plant height and dry matter in the presence of the herbicide.

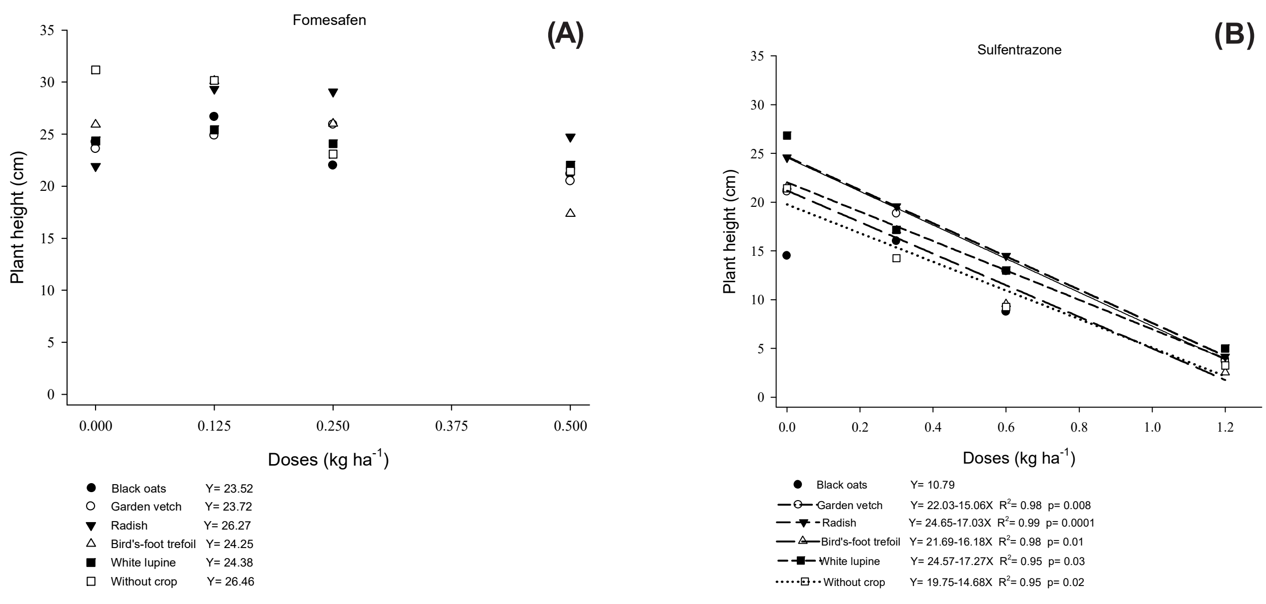

Figure 3 - Height (cm) of cucumber plants at 28 days after emergence as a function of the prior cultivation of black oats, garden vetch, radish, bird's-foot trefoil, white lupine, and treatment without crop after fomesafen (A) and sulfentrazone (B) application.

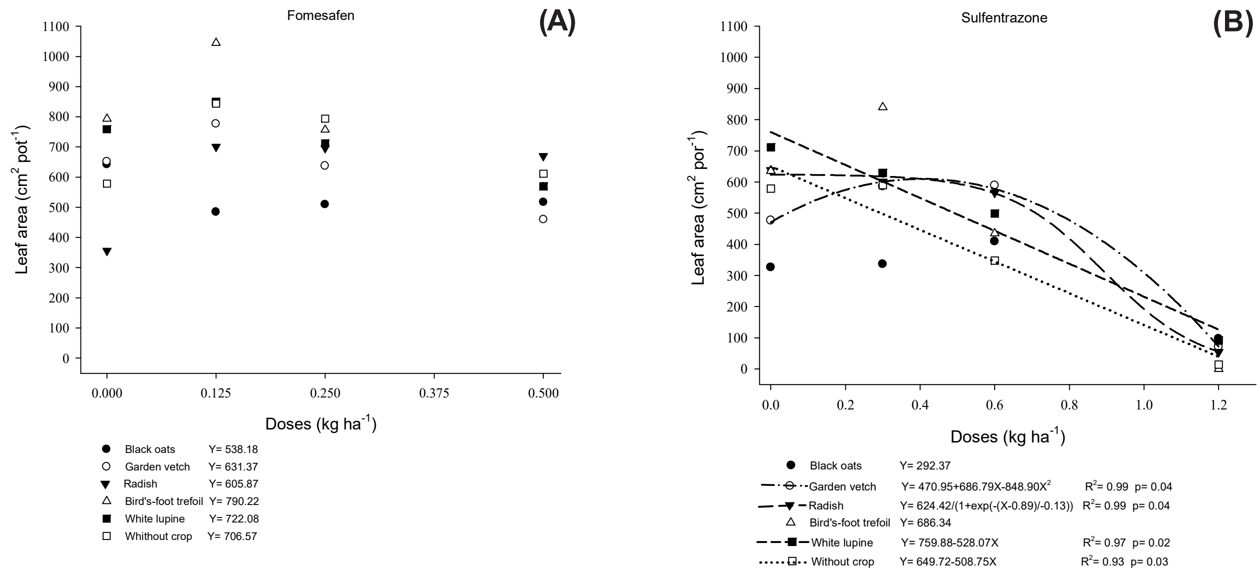

Figure 4 **-** Leaf area (cm² pot⁻¹) of cucumber plants at 28 days after emergence as a function of the prior cultivation of black oats, garden vetch, radish, bird's-foot trefoil, white lupine, and treatment without crop after fomesafen (A) and sulfentrazone (B) application.

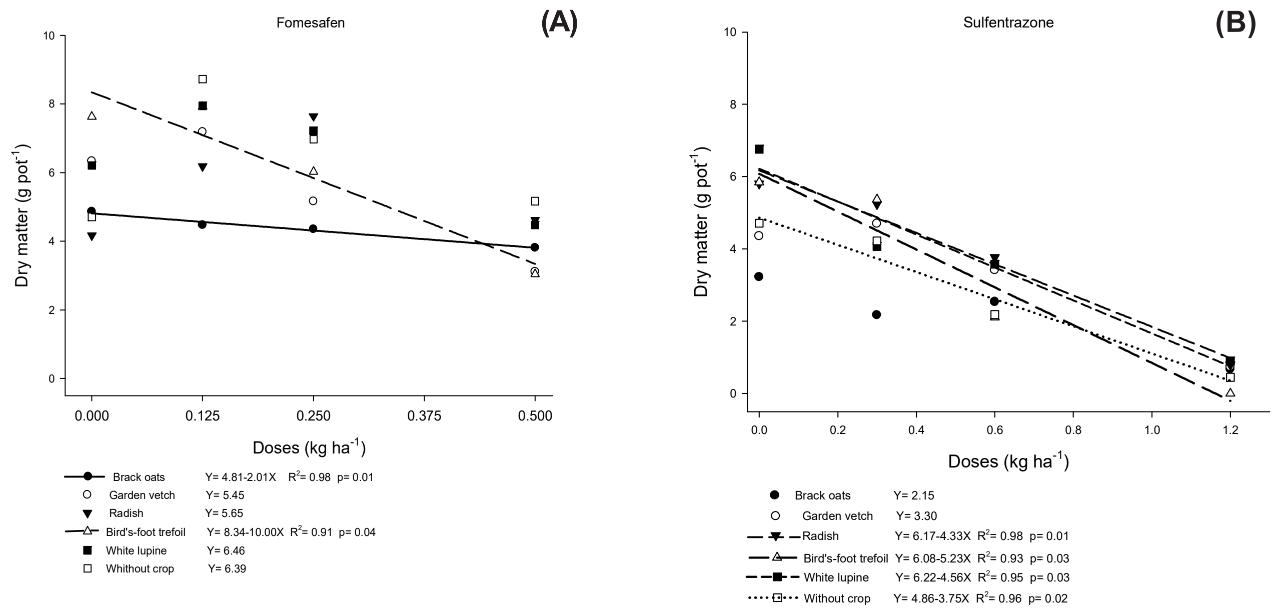

Figure 5 **-** Dry matter (g pot⁻¹) of cucumber plants at 28 days after emergence as a function of the prior cultivation of black oats, garden vetch, radish, bird's-foot trefoil, white lupine, and treatment without crop after fomesafen (A) and sulfentrazone (B) application.

Treatments without prior cultivation and radish presented the highest heights of cucumber plants when compared to cover crops within each tested fomesafen dose (Table 2). The worst performance of cucumber regarding plant height was observed when applying 0.125, 0.250, and 0.500 kg ha⁻¹ of fomesafen in white lupine, black oats, and bird's-foot trefoil, respectively. The increased fomesafen dose impaired both the height and fresh mass of corn plants, thus demonstrating that residues of this herbicide can negatively affect the growth of susceptible species (Khorram et al., 2016).

Cucumber cultivation after the soil covers garden vetch, radish, and white lupine showed the highest plant height when the recommended dose of sulfentrazone was applied (Table 2). When submitted to the dose of 1.200 kg ha⁻¹, no significant difference was observed in the height of cucumber plants in all phytoremediator treatments. Plant species with phytoremediator

Table 2 **-** Height (cm) of cucumber plants cultivated during 28 days in succession to species with phytoremediation potential (black oats, garden vetch, radish, bird's-foot trefoil, white lupine, and treatment without crop) after fomesafen and sulfentrazone application

Dose (kg ha^{-1})	Black oats	Garden vetch	Radish	Bird's-foot trefoil	White lupine	Treatment without crop	CV (%)
Fomesafen							
0.000	24.25 a	23.58 a	21.92 a	25.92 a	24.33 a	21.42 a	9.43
0.125	26.67 ab	24.89 b	29.34 ab	27.42 ab	25.42 b	31.17 a	
0.250	22.00 b	25.92 ab	29.09 a	26.00 ab	25.78 ab	30.17 a	
0.500	21.17 ab	20.50 ab	24.75 a	17.33 b	22.00 ab	23.08 a	
Sulfentrazone							
0.0	14.50 d	21.08 c	24.58 ab	21.59 bc	26.83 a	21.42 c	11.19
0.3	16.00 bc	18.84 ab	19.58 a	17.08 abc	17.17 abc	14.25 c	
0.6	8.75 b	12.92 a	14.50 a	9.55 b	13.00 a	9.25 b	
1.2	3.92 a	3.67 a	4.17 a	2.54 a	5.00 a	3.25 a	

Means followed by the same letter in the row do not differ from each other by the Tukey's test at 5%.

potential may present morphological changes, such as height, leaf area, and dry matter, which are important with increasing sulfentrazone dose, drastically reducing plant height (Madalão et al., 2013).

A significant reduction was observed in the leaf area of cucumber plants due to the increased sulfentrazone doses and when cultivated in succession to garden vetch, radish, white lupine, and the treatment without cultivation, mainly when 1.200 g ha^{-1} of the herbicide was applied (Figure 4B). Black oats and bird's-foot trefoil had the leaf area drastically reduced when twice the dose was applied and did not fit the data to the tested models (Figure 4B). This result was also observed when phytoremediator species were compared at the same dose (1,200 g ha^{-1}), with no significant difference (Table 3). The application of 0.000, 0.125, and 0.250 kg ha^{-1} of fomesafen using the black oats as phytoremediator species provided the highest reduction of leaf area in cucumber plants. This high decrease in the leaf area of cucumber plants can be associated not only with the low phytoremediation of black oat but also with the negative effect on the bioindicator growth since it is a non-fixing species of atmospheric N that demands high amounts of this nutrient.

Phytoremediator species showed a variation for each fomesafen dose (Table 3). Bird's-foot trefoil provided a leaf area of cucumber plants 19 to 54% higher when compared to the other species in all tested doses. The leaf area of cucumber cultivated in succession to black oats, radish, bird's-foot trefoil, white lupine, and the treatment without cultivation did not present significant differences when treated with 0.500 kg ha^{-1} of fomesafen, with differentiation between

Table 3 **-** Leaf area (cm^2/pot) of cucumber plants cultivated during 28 days in succession to species with phytoremediation potential (black oats, garden vetch, radish, bird's-foot trefoil, white lupine, and treatment without crop) after fomesafen and sulfentrazone application

Dose (kg ha^{-1})	Black oats	Garden vetch	Radish	Bird's-foot trefoil	White lupine	Treatment without crop	CV (%)
Fomesafen							
0.000	641.80 ab	651.79 ab	355.61 c	793.20 a	758.61 a	578.53 b	11.96
0.125	484.55 c	776.71 b	700.90 b	1044.67 a	849.12 b	842.98 b	
0.250	509.54 b	637.44 ab	696.65 a	756.83 a	711.43 a	793.88 a	
0.500	516.84 ab	459.53 b	670.31 a	597.07 ab	569.18 ab	610.88 ab	
Sulfentrazone							
0.0	326.29 c	476.55 b	638.03 a	635.79 a	711.22 a	578.53 ab	16.65
0.3	336.41 c	585.64 b	600.67 b	839.68 a	629.05 b	589.55 b	
0.6	409.22 cd	588.63 a	566.25 ab	435.06 bcd	498.98 abc	348.17 d	
1.2	97.57 a	70.81 a	54.59 a	0.0 c	91.33 a	14.25 b	

Means followed by the same letter in the row do not differ from each other by the Tukey's test at 5%.

treatments only between radish and garden vetch. The application of the recommended fomesafen dose caused a smaller leaf area only for black oats, with the other species and the treatment without cultivation statistically equal.

Leaf area of cucumber plants was higher when using bird's-foot trefoil as a phytoremediator species at a dose of 0.300 kg ha^{-1}. When applying 0.600 kg ha^{-1} of sulfentrazone, the species garden vetch, radish, bird's-foot trefoil, and white lupine stood out (Table 3). The treatment without cultivation, whenever the sulfentrazone dose was applied, had a smaller leaf area than at least one phytoremediator species.

Cucumber plants cultivated in succession to black oats and/or bird's-foot trefoil presented a linear reduction of shoot dry matter as fomesafen doses increased (Figure 5A). However, garden vetch, radish, and the treatment without cultivation did not fit the tested models with the use of fomesafen doses. When comparing the recommended dose (0.250 kg ha^{-1}) and the dose of 0.000 kg ha^{-1}, a reduction of 11.50 and 23.15% was observed in the dry matter of black oats and bird's-foot trefoil, respectively. Therefore, black oats showed a higher capacity to phytoremediate the soil when compared to bird's-foot trefoil with the use of the recommended fomesafen dose. Pires et al. (2005) observed that black oats cultivated in succession to *C. cajan*, *C. ensiformis*, *D. lablab*, *P. glaucum*, *E. deeringianum*, *E. aterrimum*, and *L. albus* showed a reduction in height and dry matter from the application of 0.500 kg ha^{-1} of tebuthiuron, not developing with the use of a dose of 1,500 kg ha^{-1}.

When cultivated in soil treated with sulfentrazone, a linear reduction was observed in the dry matter of cucumber plants cultivated in succession to radish, bird's-foot trefoil, white lupine, and the treatment without cultivation as herbicide doses increased (Figure 5B). When comparing a dose of 0.000 kg ha^{-1} of sulfentrazone (absence of herbicide application) with the recommended dose (0.600 kg ha^{-1}), a reduction of 38, 50, 48, and 44% was observed in the dry matter accumulation of cucumber plants when using radish, bird's-foot trefoil, white lupine, and the treatment without cultivation, respectively (Figure 5B). In this case, radish had the highest capacity to remediate the soil treated with this herbicide. Madalão et al. (2012) observed similar results when cultivating *C. juncea* previously to *P. glaucum* up to a dose of 0.200 kg ha^{-1} of sulfentrazone.

When comparing the plant covers at each applied fomesafen dose, white lupine and the treatment without cultivation allowed a higher dry matter accumulation in cucumber plants within each used herbicide dose (Table 4). The use of the recommended dose allowed the cucumber to demonstrate a higher dry matter accumulation when grown in succession to radish, bird's-foot trefoil, white lupine, and the treatment without cultivation. Cucumber plants showed differences in the dry matter with the use of 0.500 kg ha^{-1} of fomesafen (twice the dose) when grown after the phytoremediator species garden vetch and bird's-foot trefoil and the treatment without cultivation. At doses of 0.125, 0.250, and 0.500 kg ha^{-1}, the treatment without cultivation

Table 4 - Dry matter (g pot^{-1}) of cucumber plants cultivated during 28 days in succession to species with phytoremediation potential (black oats, garden vetch, radish, bird's-foot trefoil, white lupine, and treatment without crop) after fomesafen and sulfentrazone application

Dose (kg ha^{-1})	Black oats	Garden vetch	Radish	Bird's-foot trefoil	White lupine	Treatment without crop	CV (%)
Fomesafen							
0.000	4.86 bc	6.33 ab	4.17 c	7.63 a	6.21 ab	4.71 bc	15.91
0.125	4.47 c	7.16 ab	6.18 bc	7.92 ab	7.95 ab	8.73 a	
0.250	4.35 c	5.16 bc	7.64 a	6.02 abc	7.22 a	6.97 ab	
0.500	3.81 ab	3.11 b	4.61 ab	3.06 b	4.47 ab	5.17 a	
Sulfentrazone							
0.0	3.22 d	4.36 cd	5.81 ab	5.85 ab	6.76 a	4.71 bc	18.82
0.3	2.17 c	4.70 ab	5.24 ab	5.37 a	4.07 b	4.22 ab	
0.6	2.54 abc	3.42 ab	3.77 a	2.11 c	3.58 a	2.18 bc	
1.2	0.68 a	0.74 a	0.80 a	0.0 b	0.90 a	0.45 a	

Means followed by the same letter in the row do not differ from each other by the Tukey's test at p≤0.05

showed a superiority in degrading the herbicide fomesafen. This fact is probably due to a higher photodegradation rate of the herbicide provided to the absence of soil cover (Rodrigues and Almeida, 2011). At the recommended dose of fomesafen (0.250 kg ha^{-1}), the species with the lowest dry matter accumulation in cucumber plants were black oats and garden vetch.

Sulfentrazone doses of 0.600 and 1,200 kg ha^{-1} applied in the phytoremediator species garden vetch, radish, and white lupine provided the highest dry matter accumulation in cucumber plants (Table 4). The use of black oats as a predecessor cover to cucumber at a dose of 0.300 kg ha^{-1} of sulfentrazone, as well as the use of bird's-foot trefoil and the treatment without cultivation at a dose of 0.600 kg ha^{-1}, provided the worse dry matter accumulations in cucumber plants. These results corroborate in part those reported by Belo et al. (2011), who used a sulfentrazone dose of 0.600 kg ha^{-1} and observed that only the treatment without prior cultivation showed the lowest dry matter accumulation in sorghum (used as a bioindicator plant) when compared to the phytoremediation by other plant species.

Cucumber dry matter did not present significant differences when applying a dose of 1,200 kg ha^{-1} of sulfentrazone in all the species used for phytoremediation, including the treatment without cultivation, i.e. they caused an equal dry matter accumulation to cucumber plants, except for the bird's-foot trefoil, in which this variable was not determined (Table 4). A high reduction (above 80%) of cucumber dry matter occurred at this sulfentrazone dose when compared to the zero dose. Madalão et al. (2013) observed this same effect and doses up to 1.6 kg ha^{-1} reduced 100% of the bioindicator plant dry matter in the majority of the tested phytoremediator species.

These results allow concluding that fomesafen and sulfentrazone doses interfered negatively with the variables assessed in cucumber plants when cultivated in succession to phytoremediator species. Phytotoxicity to cucumber increased in all potential phytoremediator species as fomesafen and sulfentrazone doses increased. Sulfentrazone residues promoted higher toxic effects on the bioindicator plant when compared to fomesafen. In general, the species black oats, radish, and white lupine showed the highest capacity to phytoremediate soils contaminated with fomesafen and sulfentrazone when the dose and twice the recommended doses of herbicides were applied. However, in order to confirm these preliminary tests, in which cucumber was used as a bioindicator plant, it is necessary to implement experiments in the field using the species with phytoremediator potential to fomesafen and sulfentrazone.

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