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Influence of organic matter on the phytoremediation of sulfentrazone in contaminated soil[1]

Influência da matéria orgânica na fitorremediação de solo contaminado com sulfentrazone

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Abstract - Organic matter exerts an influence on the chemical, physical, and biological properties of soil. It can also influence the rates of herbicide degradation and sorption into it. This study aimed at evaluating the effect of adding organic compounds to soil over the remediation ability of the plant species *Canavalia ensiformis* (L.) (jack bean) in sulfentrazone-contaminated soil. The study was conducted in two phases. In the first phase, treatments consisted in a combination of four concentrations of organic compounds and in the application or not of sulfentrazone herbicide. After soil preparation, the herbicide was applied on the surface. Subsequently, seeds of the remediating plant species *C. ensiformis* were sown. At 75 days after emergence (DAE), samples of the aerial portions and roots of plants and soil were collected. All the collected material was frozen at approximately -20°C and then the accumulation of the herbicide on plants and soil was determined by chromatography. In the second phase, *Pennisetum glaucum* (L.) (pearl millet) was sown. This species is used as an indicator of sulfentrazone residue in the soil; biometric assessments were performed at 30 and 60 DAE. No sulfentrazone residue was found in the aerial portions or roots of the evaluated remediating plant species; however, it was detected in the soil. The increase in organic compound levels contributed to the growth and accumulation of biomass in pearl millet, when grown after the remediating plant species *C. ensiformis*, although it did not help phytoremediation.

Keywords: *Canavalia ensiformis*; organic compound; chromatography; soil decontamination; herbicide

Resumo - A matéria orgânica exerce influência nos atributos químicos, físicos e biológicos do solo e também pode influenciar na taxa de degradação e sorção de herbicidas. Objetivou-se avaliar o efeito da adição de composto orgânico ao solo sobre a capacidade da *Canavalia ensiformis* (L.) (feijão-de-porco) em remediar solo contaminado com sulfentrazone. O trabalho foi conduzido em duas etapas, na primeira, os tratamentos foram compostos pela combinação de quatro níveis de composto orgânico e aplicação ou não do herbicida sulfentrazone. Após o

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preparo do solo aplicou-se à superfície o herbicida. Posteriormente, procedeu-se a semeadura da espécie fitorremediadora *C. ensiformis*. Aos 75 dias após a emergência (DAE) foram colhidas amostras da parte aérea, raiz e de solo. Todo material colhido foi congelado a aproximadamente -20ºC e, em seguida determinou-se a acumulação do herbicida na planta e no solo por meio da cromatografia. Na segunda etapa, foi semeado o *Pennisetum glaucum* (L.) (milheto), planta indicadora de resíduo de sulfentrazone no solo, e foram realizadas avaliações biométricas aos 30 e 60 DAE. Não foi encontrado resíduo de sulfentrazone na parte aérea e raízes da espécie fitorremediadora avaliada, todavia detectou-se no solo. O aumento nos níveis do composto orgânico contribuiu para o crescimento e acúmulo de biomassa do milheto, quando cultivado em sucessão a espécie fitorremediadora *C. ensiformis*, no entanto, não favoreceu a fitorremediação.

Palavras-chaves: *Canavalia ensiformis*; composto orgânico; cromatografia; descontaminação do solo; herbicida

Introduction

The use of herbicides is a common practice in mixed farming and constitutes an important tool to support high production in wide areas (Pires et al., 2008; Procópio et al., 2008). However, some herbicides may remain active in the soils for a longer period than the cycle of the culture onto which it was applied; thus, it may provoke intoxication in future cultures (Dan et al., 2011). Another important aspect is the negative environmental impact caused by the leaching of these molecules or their metabolites to deeper layers in the soil profile, being able to reach ground waters (Prata and Lavorenti, 2000).

Melo et al. (2010), while evaluating the residual effect of sulfentrazone, isoxaflutole and oxyfluorfen on three soils with different chemical and texture characteristics, observed that sulfentrazone herbicide presented an elevated residual effect on the three studied soils.

As mentioned before, sulfentrazone is persistent in the soil and, therefore, it may turn into a contamination source of groundwaters and extended areas, mainly as a result of sequential applications over the years. Among highly sulfentrazone-sensitive species, there is pearl millet (*Pennisetum glaucum* (L.) R. BR.), whose susceptibility was proved by Dan et al. (2011), when they analyzed the residual activity of this herbicide, applied during pre-emergence on soybean cultures, over pearl millet cultivated afterwards.

Actions aiming at the preservation of the ecosystem must be adopted primarily. However, in situations where the contamination already occurred, corrective or remedial measures must be taken. In the light of this, researches have been conducted in the last few years, with greater emphasis on the use of species that are able to remove and/or degrade xenobiotics from the soil (Madalão et al., 2013; Nascimento et al., 2015); they highlighted phytoremediation, which consists of the ability that some plant species have to accelerate the removal of toxic compounds, such as herbicides, from the environment (soil and water), promoting its decontamination (Cunningham et al., 1996).

Among the species of green manures with potential for sulfentrazone phytoremediation, sunn hemp (*Crotalaria juncea*) and jack bean (*Canavalia ensiformis* (L.) DC.) (Madalão et al., 2012) are among the most tolerant ones to this herbicide.

According to Procópio et al. (2007), the responsible mechanism for remediation in each one of the herbicide molecules is still not well elucidated, but as for herbicide phytoremediation (particularly sulfentrazone), phytodegradation is the most desirable mechanism, considering the possibility of complete degradation of the contaminant through it. With this, plants may be kept in the same area as the one where the phytoremediation occurred, resulting into improvements for the soil, such as in case of green manures and other groundcover plants.

Studies have been developed and they indicate that, in addition to improve soil quality, the organic matter may also influence the degradation rate (Duah-Yentumi and Kuwatsuka, 1982) and the sorption of herbicides into the soil (Pusino et al., 1992). Through its components, mainly humic acids, it adsorbs the herbicide (Sonon and Schwab, 1995). The presence of organic matters in the soil also contributes to the reduction of temperature and soil moisture variation amplitude, allowing favorable conditions to increase biomass and microbial activity, accelerating the biotransformation of herbicide molecules in the soil (Reddy et al., 1997) and thus broadening the beneficial effects in the processes of herbicide phytoremediation.

Considering the aforementioned, the goal was to evaluate the effects of adding organic compounds to soil on the remedial capacity of *Canavalia ensiformis* to decontaminate sulfentrazone treated soil.

Material and Methods

The work was conducted in a greenhouse, performing two steps. In the first one, planters were filled with soil that had been enriched with four levels of organic compound (0; 2.5; 5 and 10% soil volume), corresponding to 0.0; 1.1; 2.2 and 3.3 kg of organic matter; the second one was the application (400 g ha^{-1} a.i.) or not of sulfentrazone herbicide (Boral 500 SC, 500 g L^{-1} a.i., SC, FMC), with mixture volume equivalent to 200 L ha^{-1}, arranged in 4 x 2 factor scheme under completely randomized block design, with four replications.

As a substrate for the growth of the C*anavalia ensiformis* phytoremediation species, soil samples were used, sieved in a 4 mm mesh and collected in an area with no herbicide application history, at a 0-20 cm depth, classified as Yellow Argisol (Embrapa, 2013), whose chemical characterization presented: pH (H_2O) 4,6; P 7,6 mg dm^{-3}; K^+ 16,0 mg dm^{-3}; Ca^{2+} 1,0 cmol$_c$ dm^{-3}; Mg^{2+} 0,8 cmol$_c$ dm^{-3}; Al^{3+} 0,3 cmol$_c$ dm^{-3}; H+Al 3,8 cmol$_c$ dm^{-3} and CTC (t) 5,64 cmol$_c$ dm^{-3}. As for soil texture, the following values were obtained: sand = 69 g kg^{-1}, silt = 02 g kg^{-1} and clay = 29 g kg^{-1}, and the texture classification was loamy-clay-sandy. This characterization served as a basis to correct and fertilize the soil. The organic matter was analyzed, and its characteristics are presented in Table 1.

Table 1. Nutrient concentration observed in the organic compound used in the experiment.

Moisture (60 - 65°C) = 26.80%						Total organic Matter = 23.11%			
pH	Ca	Mg	K	P	N	Cu	Zn	Mn	Fe
$CaCl_2$	-----------------------g kg^{-1}------------------------					--------------------------mg kg^{-1}----------------------------			
6.0	8.1	3.9	6.8	6.7	9.4	14.5	70.8	21.9	1.36

Analysis performed in the Agronomic and Environmental Analysis Laboratory (Laboratório de Análise Agronômica e Ambiental).

Planters, with no perforations, were filled with a total of 10 kg substrate, considering the proportions of soil/organic matter for each treatment. After that, with the help of a 100 ml bottle with a spray valve, sulfentrazone herbicide was applied on the surface of each planter. One day after the application, remedial species were planted, distributing 12 seeds per pot, at a 2 cm depth. After plant emergence, thinning was performed, leaving six plants per pot. During the conduction of the experiment, daily irrigations were performed, in order to maintain soil moisture close the 80% of the field capacity. The value of the field capacity was determined during a preliminary test from the experiment implantation, using the methodology proposed by Casaroli and Lier (2008), considering a water content decrease rate of $|d\theta/dt| = 0.001$ d^{-1}.

At 75 days after emergence (DAE), plants were cut close to the soil, according to previous results obtained by Madalão et al. (2016) as for the minimum needed time for the effectiveness of sulfentrazone phytoremediation by *C. ensiformis*. In this occasion, soil samples were collected at two depths (0-5 and 5-10 cm), with a rig; root and plant aerial part samples were also collected. All collected material was frozen at approximately -20°C to determine the herbicide accumulation in the plant and soil. This determination was performed by high efficiency liquid chromatography (HELC), using a Waters 2695 equipment with UV/DAD detector, auto-injector and oven for columns. The chromatographic condition was optimized and validated according to what was described by Ohmes and Mueller (2007).

Sulfentrazone residues into the soil were determined by extraction with the use of 80 ml methanol for every 40 g of soil, after a 16-hour agitation in a 180 rpm agitator. After the agitation step, samples were centrifuged for 15 minutes at 3,200 (rpm) for separation. Subsequently, a part of the supernatant extract was removed with the help of a syringe and filtered at 0.45 µm in a PTFE Millipore® membrane for 1.5 ml “vials”, which were submitted to chromatographic analysis (Oliveira et al., 2014).

In order to determine sulfentrazone residues in the aerial part and roots of *C. ensiformis* plants, plant material was submitted to extraction by maceration. Previously frozen samples were removed from the freezer and, after reaching room temperature, were dried in paper towel and weighed on an analytic scale in the proportion of 1:10 (Barbosa, 2001; Serafim et al., 2007.); there were 5 g of plant material for 50 ml of methanol, macerated until complete dispersion of the sample. After this step, samples were filtered with filter paper and later centrifuged for 15 minutes at 3,200 (rpm) for separation. Subsequently, a part of the supernatant extract was removed with the help of a syringe and filtered at 0.45 µm in a PTFE Millipore® membrane for 1.5 ml “vials”, submitted to chromatographic analysis.

The qualitative analysis, with the identification of sulfentrazone in the extracts, was performed through the comparison of retention time obtained in the chromatographs of the standard solution and of each sample. The quantitative analysis was performed by comparing the areas of peaks between the standard solution and the sample solutions. The quantity of herbicide found for the soil and plant samples were calculated in sulfentrazone µg per g of soil or plant sample.

In the second step, after removing the phytoremediation species *C. ensiformis*, the sowing of the species indicating sulfentrazone residues into soil, pearl millet, was performed. Six seeds were planted for each pot, at a 2 cm depth, thus performing the bioassay in the pot itself. At 30 and 60 DAE of pearl millet, plant height was evaluated (cm), using the main growth zone as a basis for the measurement, and also phytotoxicity (visually evaluated in %), attributing grades in accordance to toxicity symptoms in the aerial part of plants, based on a 0 to 100 scale, for symptom absence and plant death, respectively. Moreover, at 60 DAE the green and dry biomass of the aerial part (g) was determined; the latter was determined after drying the matter in a forced air circulation oven (70°C until constant weight).

The obtained results were submitted to analysis of variance. The average comparison between herbicide doses, within each level of organic compound in the soil, was performed through Tukey’s test at 5% probability, and the effect of adding organic compounds to the soil for each herbicide dose, by regression at 5% significance. The choice of the model selected for each variable was based on the significance of coefficients and on R^2 values (Alvarez and Alvarez, 2006).

Results and Discussion

By analyzing sulfentrazone residues into soil cultivated with *C. ensiformis*, its presence was observed at 0-5 and 5-10 cm depths in treatments with and without the addition of organic compounds in a 400 g ha^{-1} a.i. dose(Table 2) However, the detected residue quantity was small, which indicates that part of the herbicide applied onto soil at the beginning may have been phytoremedied, degraded of strongly drained to the point of not being analytically extracted.

Table 2. Average sulfentrazone quantity found in two depths of soil enriched with four levels of organic compound and cultivated with *Canavalia ensiformis*.

Doses (g ha^{-1} a.i.)	Depth (cm)	Organic compound (% of soil volume)			
		0	2.5	5	10
		µg of sulfentrazone per soil g			
0	0 - 5	-	-	-	-
400		0.055	0.051	0.043	0.059
0	5 - 10	-	-	-	-
400		0.067	0.035	0.022	0.043

Martinez et al. (2008), while evaluating the effects of humidity and temperature over sulfentrazone degradation, found 0.62 herbicide µg per soil g, after 60 incubation days, at the depth of 0-10 cm under 70% humidity.

The presence of sulfentrazone was not verified in the aerial part and roots of the phytoremediation species *C. ensiformis*. These results possibly indicate that the herbicide is being degraded and/or the detection limit of the chromatography technique hinders the identification of sulfentrazone in low quantities (Oliveira et al., 2014). It may also be drained to the soil colloidal complex, making the absorption by remedial plants very difficult.

According to Freitas et al. (2014), sulfentrazone sorption is influenced by soil pH and organic matter content; these factors must be taken into consideration when recommending this herbicide with the goal of avoiding soil contamination and the phytotoxicity in subsequent cultures. Moreover, Ohmes and Mueller (2007), verified that under conditions of elevated organic matter content and clay and low pH, sulfentrazone sorption in the soil was higher. Due to the conditions of this work (soil pH equal to 4.6 and medium texture, with predominance of low-activity mineral colloids), it is possible to deduce that the contribution to the sorption of sulfentrazone in the soil derived from low pH and organic matter, supporting the information from the abovementioned authors.

Madalão et al. (2012) observed that, additionally, the phytoremediator species *C. juncea* may promote remediation by the rhizosphere, since flavonoids and other compounds released by roots may stimulate the growth and activity of degrading micro-organisms, or even react to the herbicide, immobilizing it (Chaudhry et al., 2005; Leigh et al., 2002). In addition, the growth and death of roots promoted soil aeration, which may increase the oxidative degradation of organic compounds (Kuiper et al., 2004; Leigh et al., 2002).

While evaluating biometric results, there was no statistic interaction between organic compound levels and sulfentrazone doses. However, it was possible to observe a significant effect of organic compound levels over the height and the green and dry biomass of the aerial part of the indicator plant - pearl millet, when cultivated after the phytoremediation species *C. ensiformis*.

The response to organic compound levels added to soil was increasing and linear for height, green and dry biomass of pearl millet aerial part, at 60 DAE, that is, it augmented with the increase of organic compound levels, in succession to the phytoremediation species *C. ensiformis* (Figure 1). Belo et al. (2007), when evaluating the phytoremediation of soil fertilized with organic compound and contaminated with trifloxysulfuron-sodium, verified that in the absence of the herbicide, the response to the increase of organic compound was linear and increasing, highlighting that the addition of organic compound to the soil helps the growth and development of the *C. ensiformis* species.

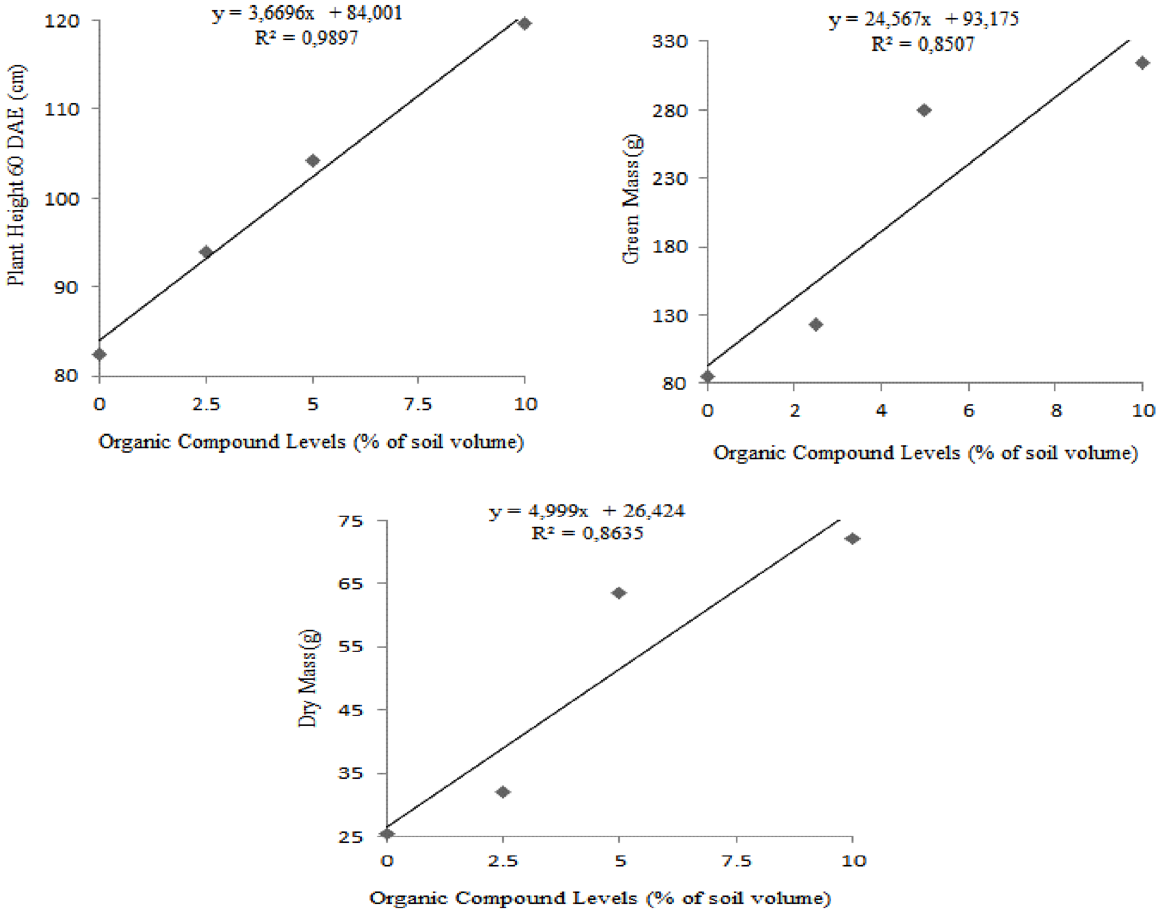

Figure 1. Height, green and dry biomass of pearl millet plants, at 60 DAE, cultivated after *Canavalia ensiformis* in soil enriched with four levels of organic compound.

As for phytotoxicity symptoms, there was no model adjustment to data. It was possible to observe a significant effect of sulfentrazone doses over the height and the green and dry biomass of the aerial part on the toxicity percentage of this herbicide on pearl millet, when following the phytoremediation species *C. ensiformis* (Table 3). In the 400 g ha^{-1} a.i. dose, however, even when cultivated after *C. ensiformis*, pearl millet presented a significant reduction in height, green and dry biomass of the aerial part.

Moreover, intoxication symptoms were evident in pearl millet plants cultivated in sulfentrazone-contaminated soil (400 g ha^{-1} a.i.). In the first evaluation, performed at 30 DAE, there was 87.37% phytotoxicity. In the second evaluation, at 60 DAE, the phytotoxicity percentage was slightly lower in absolute value (83.50%), when compared to the evaluation at 30 DAE (Table 3). However, visual symptoms of intoxication remained high in plants, highlighting that the effectiveness of phytoremediation, measured by the development of pearl millet, was not satisfactory. In spite of this, there was a reduction of sulfentrazone levels into soil, whose cause may be related to the herbicide sorption by organic colloids, promoted by the phytoremediation species (phytostabilization).

Similar results for the phytotoxicity of the bioindicator species were found by Madalão et al. (2012) when evaluating the use of legumes in the phytoremediation of sulfentrazone-contaminated soil, where there was a percentage of 78 and 86% phytotoxicity for pearl millet, in the 400 g ha^{-1} a.i. dose, in succession to the species *C. juncea* and *C. ensiformis*, respectively. This highlighted that the two species did not promote enough soil decontamination, indicating the high sensitivity of pearl millet to sulfentrazone (Dan et al., 2011) and reinforcing the attention with the sowing of this culture, in areas with history of this herbicide applications.

On the other hand, Belo et al. (2007) verified that the remediation of trifloxysulfuron-sodium contaminated by the species *Canavalia ensiformis* and *Stizolobium aterrimum* was effective, regardless of the addition of organic compound to soil.

Table 3. Height, phytotoxicity, green and dry shoot biomass of pearl millet plants cultivated after *Canavalia ensiformis,* according to the application or not of sulfentrazone.

Evaluations	Doses (g ha^{-1} a.i.)	
	0	400
Height 30 DAE (cm)	54.47 a	27.10 b
Height 60 DAE (cm)	110.65 a	89.46 b
Phytotoxicity 30 DAE (%)	0.00 b	87.37 a
Phytotoxicity 60 DAE (%)	0.00 b	83.50 a
Green Biomass (g)	245.00 a	156.31 b
Dry Biomass (g)	58.71 a	37.87 b

Averages followed by the same letter on the line do not differ among themselves by Tukey's test at 5% probability.

The evaluation of the effects of organic compounds over the phytoremediation process, in future works, shall test different sources of organic matter, since they present great variation in their constitution and, with that, they may also result in different behaviors as for the process of herbicide sorption and its lower bioavailability to be phytodegraded (rhyzodegraded).

Conclusions

The increase in levels of organic compounds contributes to the growth and accumulation of pearl millet biomass, when cultivated after the phytoremediation species *Canavalia ensiformis*; however, it does not help phytoremediation.

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