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Effect of growing *Brachiria brizantha* on phytoremediation of picloram under different pH environments

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ABSTRACT

Picloram is used in the management of *Brachiaria brizantha* (Poaceae) in pasture. The persistence of this herbicide varies with climate and soil characteristics, such as texture and pH. The aim was to determine the capacity of *B. brizantha* (Hochst.) Stapf. cv. Marandu to remediate soils of different pH levels contaminated with picloram. The experiment had a randomized block design with four replications. The remedial species was grown for 60 days after cucumber [*Cucumis sativus* L. (Cucurbitaceae)] was cultivated as an indicator of the presence of the herbicide in the soil. This plant reduced the picloram concentration in the soil layer surface, which can be attributed to its ability to degrade, to absorb, and/or to exude herbicides. The picloram has greater leaching potential in higher pH soils, in the absence of *B. brizantha*. Soils with lower pH tend to have higher sorption and concentration of this herbicide in the intermediate layers. *B. brizantha* can remediate soils contaminated with picloram and reduce leaching, which is higher in soils treated with limestone.

1. Introduction

Hormone herbicides have been used mainly for weed control in pasture, but they have longer periods and persistence in the environment (Santos et al., 2006; Seefeldt et al., 2014).

The main herbicides for weed control in pastures contain 2.4-D or picloram (MAPA, 2015). The latter one has longer residual activity period in soils that can reduce the cultivation in short and medium-terms (Santos et al., 2006). This molecule has a high retention in the soil and persistence in the environment (Santos et al., 2006; Seefeldt and Conn, 2011). Furthermore, it can have low sorption (Point-Brown et al., 2014), and its solubility in water facilitates reaching underground aquifers depending on soil characteristics, such as pH (Fast et al., 2010).

The excavation, incineration, solvent extraction, or oxidation–reduction can recover contaminated areas by herbicides, but they are costly methods and difficult to use (Oliveira et al., 2014). *In situ* methods, including phytoremediation is a strategy for the removal of toxic compounds from the environment using plants. This technique is recommended because it is economically viable and provides lower environmental impacts (Ali et al., 2013).

The objective of this study was two-fold: In soil contaminated to picloram, (1) determine the *Brachiaria brizantha* potential to prevent herbicide leaching and (2) evaluate the potential phytoremediator of *B. brizantha* for different soil pH and depth.

2. Material and methods

2.1. Experimental approach

Three assays were carried out in a greenhouse of the "Departmento de Pós-Graduação em Produção Vegetal" of the "Uni-

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versidade Federal dos Vales do Jequitinhonha e Mucuri" (UFVJM) in Diamantina, Minas Gerais State, Brazil. Red latosol (oxisol) samples from Diamantina, Minas Gerais State, Brazil, characterized as sandy clay loam with 23, 12 and 65% of clay, silt and sand, respectively, were used. Chemical analysis showed pH (in water) of 4.5; organic matter content of 8.7 dag kg^{-1}; P, K, Ca, Mg, Al, H + Al (potential acidity) and $CTC_{effective}$ of 3.0 and 39.0 mg dm^{-3}; 1.1, 0.3, 1.4, 15.7 and 2.9 cmolc dm^{-3}, respectively. The soil was not corrected in the first assay, but the equivalent to 2.6 and 5.2 t ha^{-1} of dolomitic limestone (PRNT = 80%) was applied in the second and third assays. The soil samples were moistened and incubated for 40 days for reaction with the corrective.

The substrates were fertilized with 2.7 g dm^{-3} formulated at 4-14-8 (N-P_2O_5-K_2O) and 100 mg dm^{-3} of urea 15 days after plant emergence. Watering was done daily to keep the soil moisture with 80% field capacity.

The container used for cultivation composed of four 100 mm high and 150 mm diameter rings each of polyvinyl chloride (PVC) tube internally lined with paraffin to reduce water seepage through the walls of the columns. These rings were mounted with the base closed by gauze and filter paper and supported on plates to prevent water and herbicide losses. After preparation, the columns were wetted, and the herbicide was applied with a sprayer with constant pressure coupled to a bar with nozzle-type range TT11002 with 3 bar pressure and volume sprayed of 150 L ha^{-1}.

2.2. Estimated phytoremediation

The assays were divided into two stages: in the first, the bioremediation was tested and in the second, the concentration of picloram was evaluated with a plant bioindicator. The experimental design was a randomized block with four replications. The treatments were arranged in a split plot design in 2 × 2 × 4 scheme; the first level with *B. brizantha* or not, the second with the herbicide doses (120 and 240 g ha^{-1} of picloram in Padron® formulation), and the third with the depths evaluated (0–10, 10–20, 20–30 and 30–40 cm).

Brachiaria brizantha (Hochst. ex. A. Rich) Stapf. belongs to the Poaceae family and it is native to the African savanna. *Brachiaria* is commonly used as forage grass in tropical rangelands worldwide. In addition, it is generally used as phytoremediator of contaminated soils. Six seeds of *B. brizantha* were sown per column 1 day after the herbicide application, later thinned and two plants left per plot. These plants were grown until the beginning of flowering at 60 days after their emergence. The rings were removed from the column when the soil layers and the roots were removed individually and the soil homogenized.

A total of 80 g of soil from each plot was weighed and placed in plastic cups to estimate the herbicide concentration in the soil. Three seeds of the plant bioindicator, *Cucumis sativus* L. (Cucurbitaceae) were placed per sample. The poisoning of the plants was evaluated at 30 days after sowing by Visual Intoxication Index. The degree of intoxication was set on a scale in percentage with 0% representing no injury and 100% plant death (EWRC, 1964).

2.3. Dose-response curve

Three dose-response curves were plotted per soil pH, estimating the relationship between the concentration of the herbicide in the soil and the effects on the plant bioindicator. Ten herbicide doses corresponding to 0, 0.5, 0.9, 1.9, 3.8, 7.5, 15.0, 30.0, 60.0 and 120.0 g ha^{-1} of picloram were applied. The design was a randomized block with four replications and evaluated as in the bioindicator test.

2.4. Statistical analyses

The variables were subjected to analysis of variance (ANOVA) when the significant interactions were deployed and the means compared by Tukey's test at 5% significance level. The depth factor data and the dose-response curve were subjected to linear and non-linear regression analysis ($p < 0.05$). Adjusted equations were compared by the model identity test to verify the null hypothesis. The models were grouped and represented by the same trend line in case of no differences (Regazzi and Silva, 1999).

3. Results

The triple interaction was significant only for soil with pH 4.5 and 5.6. *B. brizantha* reduced the concentration of the herbicide picloram at the surface layer of soil with pH 4.5 and increased it between 20 and 30 cm deep (Table 1).

The herbicide concentration was lower in the superficial and the deepest soil layers with the highest herbicide dose with remedial species cultivated in soil with pH 5.6 (Table 1).

The increase in picloram doses increased the cucumber plant's intoxication in soil with pH 4.5 from all depths with *B. brizantha* (Table 1). Plant poisoning increased in soils from 0 to 10 cm depth without previous cultivation of *B. brizantha*.

The intoxication of cucumber plants was higher with increasing picloram doses in the superficial soil layer with pH 5.6 with or without previous cultivation of *B. brizantha* and in those of greater depth without this plant (Table 1).

The regression for herbicide analysis throughout the soil depth is suited to a second degree with upward concavity (Fig. 1). The slope of the curve increased in soil with the highest herbicide doses without plant remedial, independently of the pH.

The value of "*b*" was lower in the equation with the lowest dose of the herbicide and with *B. brizantha* in soil with pH 5.6 (Fig. 1).

Table 1
Plant poisoning (%) of cucumber, *Cucumis sativus* (Cucurbitaceae) grown in soil pH 4.5 and 5.6 taken at different depths after cultivation (yes) or not (not) with *Brachiaria brizantha* (Poaceae) under two picloram doses.

pH	Picloram (g i.a. ha^{-1})	Depth (cm)							
		0–10		10–20		20–30		30–40	
		yes	not	yes	not	yes	not	yes	not
4.5	120	12.1Bb	16.3Ab	7.9Ab	10.2Aa	5.8Ab	9.5Aa	15.4Ab	14.0Aa
	240	23.2Ba	29.4Aa	15.2Aa	13.6Aa	20.7Aa	11.7Ba	19.9Aa	17.7Aa
	Coefficient of variation: CV^1 = 16.78, CV^2 = 28.67, CV^3 = 29.12								
5.6	120	25.6Bb	42.9Ab	6.3Aa	6.6Aa	5.8Aa	6.0Aa	13.3Aa	10.6Ab
	140	46.0Ba	51.9Aa	4.8Aa	6.8Aa	6.5Aa	5.8Aa	8.6Bb	16.8Aa
	Coefficient of variation: CV^1 = 23.87, CV^2 = 24.82, CV^3 = 19.91								

Means followed by the same uppercase per line or lowercase letters per column do not differ by F test at 5% significance. 1/presence or absence of *B. brizantha*; 2/picloram dose; 3/depths.