Research, Society and Development, v. 10, n. 4, e59110414372, 2021
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v10i4.14372

Fitorremediation of contaminated soil with 2,4-D + picloram in Eastern Amazonia

Fitorremediação de solo contaminado com 2,4-D + picloram na Amazônia Oriental

Fitorremediación de suelo contaminado con 2,4-D + picloram en Amazonia Oriental

Received: 03/28/2021 | Reviewed: 04/08/2021 | Accept: 04/13/2021 | Published: 04/24/2021

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Abstract

The objective of this work was to evaluate the potential of *Urochloa brizantha* cv. Marandu and *Panicum maximum* cv. Mombasa in phytoremediation of soil treated with 2,4-D + picloram herbicide, using *Raphanus sativus* Crimson Gigante as a bioindicator plant. The experiment was carried out in a greenhouse, in a completely randomized design in two stages. In the first stage the treatments were: cultivation of *U. brizantha* and *P. maximum* treated with and without the herbicide dose, with five replications. In the second stage, the treatments consisted of cultivating R. sativus in soil: free of herbicide residue; and soil contaminated with cultivation: prior to *U. brizantha; P. maximum*; and without previous cultivation of grass, with five replications. The units were treated with the herbicide, individually in pre-emergence, after 15 days the grasses were sown. After 50 days, forages were harvested and segregated in aerial and root parts, analyzing fresh and dry biomass (g) and height (cm). After removing phytoremediation plants, R. sativus was transplanted, evaluating visual phytotoxicity at 5, 10, 15 and 20 days after emergence (DAE) and at 20 DAE, the accumulation of green and dry matter (g), height (cm). The evaluated grasses have phytoremediation characteristics for auxinic herbicides; *R. sativus* can be used as a bioindicator of the herbicide 2,4-D + picloram; the evaluated period was not enough to fully remove the effects of the herbicide.
Keywords: Auxin; Bioindicator; Braquiarão; Herbicide; Mombasa.

Resumo

Objetivou-se neste trabalho avaliar o potencial da *Urochloa brizantha* cv. Marandu e *Panicum maximum* cv. Mombaça em fitorremediar solo tratado com herbicida 2,4-D+picloram, utilizando Raphanus sativus Crimson Gigante como planta bioindicadora. O experimento foi conduzido em casa-de-vegetação, em delineamento inteiramente casualizado em duas etapas. Na primeira etapa os tratamentos foram: cultivo da *U. brizantha* e *P. maximum* tratados com e sem a dose do herbicida, com cinco repetições. Na segunda etapa, os tratamentos consistiram no cultivo do *R. sativus* em solo: livre de resíduo de herbicida; e solo contaminado com cultivo: prévio da *U. brizantha*; da *P. maximum*; e sem o cultivo prévio de gramínea, com cinco repetições. As unidades foram tratadas com o herbicida, individualmente em pré-emergência, decorrido 15 dias procedeu-se a semeadura das gramíneas. Após 50 dias as forrageiras foram colhidas e segregadas em parte aérea e raiz, analisando-se a biomassa fresca e seca (g) e altura (cm). Retirada as plantas fitorremediadoras, transplantou-se a *R. sativus* avaliando a fitotoxidade visual aos 5, 10, 15 e 20 dias após a emergência (DAE) e aos 20 DAE determinou-se o acúmulo da matéria verde e seca (g), altura de planta (cm). As gramíneas avaliadas apresentam características fitorremediadora para herbicidas auxínicos; *R. sativus* pode

ser utilizada como bioindicadora do herbicida 2,4-D + picloram; o período avaliado não foi suficiente para a retirada total dos efeitos do herbicida.
Palavras-chave: Auxina; Bioindicadora; Braquiarão; Herbicida; Mombaça.

Resumen

El objetivo de este trabajo era evaluar el potencial de *Urochloa brizantha* cv. Marandu y *Panicum máximo* cv. Mombasa en suelo fitorretomía tratado con herbicida 2,4-D+picloram, utilizando *Raphanus sativus* Crimson Gigante como planta bioindicadora. El experimento se llevó a cabo en un invernadero, en un diseño completamente aleatorio en dos etapas. En la primera etapa, los tratamientos fueron: cultivo de *U. brizantha* y *P. máximo* tratado con y sin la dosis de herbicida, con cinco réplicas. En la segunda etapa, los tratamientos consistían en el cultivo de *R. sativus* en el suelo: libre de residuos de herbicidas; y suelo contaminado con cultivo: antes de *U. brizantha; P. máximo*; y sin el cultivo previo de hierba, con cinco réplicas. Las unidades fueron tratadas con el herbicida, individualmente en preemergencia, después de 15 días, las hierbas se estaban sembrando. Después de 50 días los forrajes fueron cosechados y segregados en brote y raíz, analizando biomasa fresca y seca (g) y altura (cm). Después de la eliminación de las plantas fitomediadoras, *R. sativus* fue trasplantado, evaluando la fitotoxina visual a los 5, 10, 15 y 20 días después de la aparición (DAE) y a las 20 DAE, se determinó la acumulación de materia verde y seca (g), altura de la planta (cm). Las hierbas evaluaron las características actuales del fitomediador para herbicidas auxinicos; *R. sativus* se puede utilizar como bioindicador del herbicida 2,4-D + picloram; el período evaluado no fue suficiente para la retirada total de los efectos del herbicida.
Palabras clave: Auxin; Bioindicador; Braquiarão; Herbicida; Mombasa.

1. Introduction

Herbicides in the synthetic auxin group have selective action and are often used to control the growth of weeds worldwide (Song, 2014). Of this group, picloram (4-amino 3,5,6 trichloro-2-pyridinecarboxylic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) make up the majority of registered products used in agriculture (Brazil, 2013).

2,4-D acid, from the group of phenoxyacetic acids, is a herbicide known as synthetic auxin and has a high tendency to leach into the soil, due to its low adsorption. Picloram, from the group of pyridinecarboxylic acids, is an auxin-mimicking herbicide, with a long residual effect on the soil, which characterizes a high risk of contamination of the groundwater (Brito et al., 2001; Oliveira Junior, 2011; Santos et al., 2006). The residual effects of the herbicides known as carryover, can be remedied or mitigated through phytoremediation (Andrade et al., 2007; Lambert et al., 2012). The technique consists of using plant species capable of tolerating, filtering, extracting, stabilizing or degrading certain contaminating compounds (Tavares et al., 2013; Vasconcellos et al., 2012).

The selection of plant species for the phytoremediation program depends on the plant's own characteristics, such as tolerance to heavy metals, high growth rate, high biomass production, abundant root system and good adaptability to local edaphoclimatic conditions (Oliveira et al., 2007). Based on the aforementioned attributes, forage species have the potential to apply the phytoremediation technique. Several studies have proven its effectiveness in the phytoremediation program, such as Belo et al. (2011) and Carmo et al. (2008). However, in the Northern region of Brazil, studies that determine the action of remedial plants for soil contaminants, with species adapted to regional edaphoclimatic conditions, are scarce.

The species *Urochoa brizantha* cv. Marandu (synonymous Brachiaria brizantha), known regionally as brachiarão or simply marandu grass and *Panicum maximum* cv. Mombasa, are among the most used forages in the Amazon production system, as they are a viable option for the local edaphoclimatic and management conditions (Camarão & Souza Filho, 2005). Due to the relevance of using these forages in the Amazon region, it is opportune to compare them in the phytoremediation program, for a better understanding of their response to the contaminant.

Thus, the present work aims to evaluate the potential U. *brizantha* cv. Marandu and P. *maximum* cv. Mombasa, in phytoremediation of soil treated with 2,4-D + picloram herbicide, using *Raphanus sativus* Crimson Gigante as a bioindicator plant.

2. Methodology

The scientific method of the research was through experimental field research, in order to find answers, proof or even new phenomena in relation to the problem questioned in the research, through the creation and production of specific, controlled situations and random samples (Koche, 2011).

The experiment was carried out in a greenhouse with a 25% shade screen, located at the Federal University of Pará / Altamira University Campus - Pará, under the coordinate 03 ° 11′40 ″ S 52 ° 12′33 ″ W, from June to September 2017, using a completely randomized design, carried out in two stages. In the first stage, the treatments were composed by the cultivation of *U. brizantha* cv. Marandu in soil free of herbicide residue (T1); *P. maximum* cv. Mombasa in soil free of herbicide residue (T2); soil contaminated with *U. brizantha* cv. Marandu (T3); contaminated soil with *P. maximum* cv. Mombasa (T4), with five repetitions. The second stage, carried out in order to confirm the potential of phytoremediation species, had as treatments the cultivation of the species *Raphanus sativus* Crimson Gigante in: soil free of herbicide residue (RT1); soil contaminated with previous cultivation of the species *U. brizantha* cv. Marandu (RT2); contaminated soil with previous cultivation of the species *P. maximum* cv. Mombasa (RT3); and contaminated soil without previous cultivation of grass (RT4), with five replicates each treatment. For the constitution of the RT1 treatment, the pots from the T1 and T2 treatments (with herbicide-free soil) from the first phase were used, in order to compose the control of the bioassay. The samples consisted of ravine soil, classified as Yellow Latosol, collected in the 0-20 cm layer and in a place with no history of herbicide application, whose chemical analysis is shown in Table 1.

Table 1. Chemical characteristics of the Yellow Latosol used in the experiment.

Análise química																	
pH	P	K	Ca^{+2}	Mg^{+2}	Al^{+3}	H+Al	SB	t	T	m	v	Zn	Fe	Mn	Cu	B	MO
H_2O	mg dm-3		$cmol_c$ dm-3							%		mg dm-3					dag kg^{-1}
5,3	13	39	1,3	0,2	0,3	2,6	1,7	2	4,3	16	40,1	1,5	21,6	0,2	0,6	0,23	1,6

* Analysis carried out by FULLIN - Laboratory of Agronomic and Environmental Analysis. (pH water: soil-water ratio 1: 2.5; P and K: HCl extractor 0.05 mol / L + H2SO4 0.025 mol / L; Ca, Mg and Al: KCl extractor 1 mol L-1; T: capacity of cation exchange at pH 7.0; m: saturation of aluminum; V: saturation by bases; MO: organic matter content determined by the Oxidation method: Na2Cr2O7 2H2O + 4 mol / L H2SO4 10 mol / L). Source: Authors (2017).

Such samples were submitted to the TFSA drying process (air-dried fine earth) and sieved in a 5 mm mesh. The experimental units consisted of pots with a capacity of 8 dm³ and without holes, in order to prevent the loss of the herbicide by leaching, filled with 9 kg of substrate, which were irrigated by adjusting the humidity to a value close to 80% of the field capacity and fertilized as recommended by Embrapa for forages (Dias Filho, 2012).

Then, the experimental units received treatment with the herbicide 2,4-D + picloram. The applications were carried out in pre-emergence, individually on the surface of each vessel, with the aid of a spray bottle. The solution was applied in twice the recommended dose, simulating the volume of 3.5 L of the herbicide product to 200 L of water, this being the volume of syrup used in the region per hectare. For the application it was calculated, using the simple three rule, the concentration of herbicide and syrup needed considering the circumference of the pot.

After fifteen days, the forage species were sown at a depth of 1 cm, with five plants per pot remaining after thinning. Irrigation was performed daily, according to the water needs of the plants. At 50 days after sowing (DAS), forages were harvested and their phytoremediation potential evaluated. These were segregated in aerial part and root, being analyzed the parameters of fresh biomass (g), dry biomass (g) and height (cm) of the parts segregated for each treatment, according to the methodology adopted by Franco et al. (2014).

For the second stage of the experiment, the species *Raphanus sativus* Crimson Gigante was sown in autoclaved sand, according to the manufacturer. After removal of phytoremediation plants, the bioindicator plant was transplanted for the presence of residues of the herbicide in the soil. At 5, 10, 15 and 20 after emergence (DAE) the visual phytotoxicity of the plants was evaluated, assigning scores according to the toxicity symptoms presented in the aerial part (Table 2), according to the methodology proposed by the Brazilian Society of Weed Science - BSWS (1995). At 20 DAE, the same analyzes used in the evaluation of forages were performed.

Table 2. Grade scale used for visual assessment of phytotoxicity of the herbicide 2,4-D + Picloram in the bioindicator species *Raphanus sativus* Crimson Gigante.

CONCEPT	NOTES	OBSERVATIONS
Light	0 – 1	Weak or barely evident symptoms. Zero score: no changes in the plants are observed
Acceptable	2 – 3	Pronounced symptoms, however, fully tolerated by the plant
Worrisome	4 – 5	Symptoms greater than in the previous category, but still subject to recovery, and without expectations of a reduction in economic performance.
High	5 – 7	Irreversible damage, with expected reduction in economic yield.
Very high	7 – 10	Very severe irreversible damage, with a forecast of drastic reduction in economic performance. Note 10 for death of the plant.

Source: Adapted SBCPD (1995).

The data obtained were subjected to analysis of variance. The analysis of the significant effects of the period of cultivation of the phytoremediation species and the bioindicator plant was carried out by the Dunnett test, while the phytotoxicity results of the bioindicator species were analyzed by the Kruskal-Wallis test, with the coefficients of the equations being tested at 5% of probability.

3. Results and Discussion

The species were affected differently by the contaminant as evidenced by the biometric data (Table 3). The effect of the application of 2,4-D + picloram in the treatments, reduced the accumulation of fresh biomass (aerial part and root) and production of root dry matter of the species *U. brizantha*, when compared with the control. Among the characteristics evaluated, in the species *P. maximum*, only aerial biomass (fresh and dry) showed a significant effect when compared to cultivation in soil without application of the herbicide.

Table 3. Biometric data obtained from *U. brizantha* cv. Marandu and *P. maximum* cv. Mombasa, under herbicide treatment (dose) and control (control). CA - air length; CR - root length; BFA - fresh aerial biomass; BFR - fresh root biomass; BSA - aerial dry biomass; BSR - dry root biomass.

TRATAMENTO	CA	CR	BFA	BFR	BSA	BSR
U. brizantha cv. Marandu (witness)	44,8a	30,0a	3,93a	2,95a	1,77a	1,85a
U. brizantha cv. Marandu (with dose)	33,8a	20,6a	2,80b	1,47b	1,30a	1,01b
C.V.	10,04%	8,75%	14,11%	10,17%	15,10%	13,49%
P. maximum cv. Mombasa (witness)	59,0a	30,8a	5,23a	2,20a	2,27a	1,51a
P. maximum cv. Mombasa (with dose)	51,8a	24,8a	3,43b	2,08a	1,56b	1,32a
C.V.	10,03%	9,42%	13,79%	10,79%	11,71%	15,82%

* Means followed by the same letter, in the column, within each characteristic evaluated, do not differ by Dunnett's test at the level of 5% probability.
Source: Authors (2017).

Similar results were found by D'Antonino et al. (2009) when evaluating the effect of auxinic herbicide, observed that the application of 2,4-D + picloram inhibited in 13.6% the accumulation of dry matter of corn, in relation to the control. Fulaneti et al. (2014), aiming to select plant species grown in consortium, with the purpose of phytoremediation of soils contaminated with the herbicide Picloram, realized that the height of Tanzania grass had a significant influence with the application of different doses of Picloram.

With a characteristic to control annual or perennial dicot weeds, the biometric results of forage species show sensitivity of species when subjected to treatment with higher than the recommended dose, even though the herbicide has selectivity for grasses in general, corroborating the need for the rational use of these products. In general, the tolerance of grasses to mimic herbicides is determined by a sum of factors, in which the penetration in these plants is low with limited translocation in the phloem, due to their anatomical structures (Oliveira Júnior *et al*., 2011).

Dados biométricos do Raphanus sativus Crimson Gigante utilizado como planta bioindicadora do resíduo do herbicida 2,4-D + picloram.

Table 4. Biometric data of *Raphanus sativus* Crimson Gigante used as a bioindicator plant for the residue of the herbicide 2,4-D + picloram.

TREATMENT	CA	CR	BFA	BFR	BSA	BSR
RT1	13,13a	8,38a	4,56a	6,11a	1,29a	1,18a
RT2	11,62a	7,64a	3,44b	4,95a	1,13b	1,28a
RT3	8,27b	5,48b	2,87b	2,91b	0,71c	0,75b
RT4	2,96c	1,80c	0,74c	0,69c	0,3c	0,01b
C.V.	10,27%	10,43%	13,34%	10,43%	12,22%	10,91%

* Averages followed by the same letter, in the column, within each evaluated characteristic, do not differ by Dunnett's test at the 5% probability level. (RT1- soil free of herbicide residue; RT2- soil contaminated with previous cultivation of *U. brizantha* cv. Marandu; RT3-soil contaminated with previous cultivation of *P. maximum* cv. Mombasa RT4 - contaminated soil without previous cultivation of grass. CA - air length; CR - root length; BFA - fresh aerial biomass; BFR - fresh root biomass; BSA - aerial dry biomass; BSR - dry root biomass)
Source: Authors (2017).

Observed that in the experimental units previously treated with the cultivation of *U. brizantha* and *P. maximum* in soil contaminated with 2,4-D + picloram, *R. sativus* used as a bioindicator culture, presented greater tolerance to the herbicide in all evaluated parameters, when compared to the cultivation in soil treated with herbicide without the previous treatment of grass, which presented an abrupt reduction in its growth and development.

For the characteristics of aerial and root length, fresh root biomass and dry biomass of the aerial part and root, the cultivation of the bioindicator in succession to *U. brizantha* provided better development when compared to the previous cultivation of *P. maximum*. When compared to the control treatment, less damage was observed to the morphological characteristics of *R. sativus*, when cultivated after *U. brizantha*, presenting similar behavior for the parameters plant height and accumulation of fresh and dry root biomass, indicating greater phytoremediation capacity of the species in the evaluated period.

Pires et al. (2005), aiming to evaluate seven species (*Cajanus cajan*, *Canavalia ensiformis*, *Dolichos lablab*, *Pannisetum glaucum*, *Estizolobium deeringianum*, *Estizolobium aterrimum* and *Lupinus albus*) of plants cultivated for green fertilization in soils contaminated with tebuthiuron, using black oat as an indicator plant, observed similar behavior of the species for the characteristics of plant height and dry biomass of the shoots.

The residues of the herbicide 2,4 D + picloram in the soil, caused an increase in poisoning in *R. sativus* throughout its cultivation, for all treatments that received the application of the herbicide, manifesting visual symptoms in the leaves (Table 5).

Table 5. Phytotoxicity of *Raphanus sativus* used as a bioindicator plant for the residue of the herbicide 2,4-D + picloram.

	DAYS AFTER EMERGENCY			
TREATMENT	**5**	**10**	**15**	**20**
RT1	5,5a	3,0a	3,0a	3,0a
RT2	5,5a	5,5a	8,0b	8,0b
RT3	5,5a	13,0b	13,0c	13,0c
RT4	13,0b	18,0c	18,0d	18,0d

* Averages followed by the same letter, in the column, within each evaluated characteristic, do not differ by the Kruskal-Wallis test at the 5% probability level. (RT1- soil free of herbicide residue; RT2- soil contaminated with previous cultivation of *U. brizantha* cv. Marandu; RT3- soil contaminated with previous cultivation of *P. maximum* cv. Mombasa; RT4- contaminated soil without previous cultivation of grass.)
Source: Authors (2017).

The previous cultivation of *U. brizantha*, promoted greater tolerance to the bioindicator plant, presenting mild symptoms of intoxication only after 15 days after emergence (DAE). When cultivated in soil contaminated in sequence to *P. maximum*, the worrying damages in the test plant originated from 10 DAE, presenting abnormal growth (reduction) of the plants in relation to the control.

The herbicide, caused greater poisoning to R. *sativus* when cultivated in soils without previous forage, showing pronounced symptoms at 5 DAE, observing yellowing of the leaves and twisting of the stem, evolving to irreversible damage promoting the death of 70% of the radishes grown to 20 DAE, with phytotoxicity symptoms being classified as high and very high.

In the test plant, the effects demonstrate the high sensitivity of *R. sativus* to the presence of picloram and 2,4-D and reinforces the care with the sowing of vegetables in areas with a history of herbicide applications that contain this active ingredient, indicating symptoms of intoxication at 5 days after emergency. Similar works such as those by Nascimento &

Yamashita (2009), Assis et al. (2010), Madalão et al. (2016) and Galon et al. (2017), observed similar intoxication behavior in the tested vegetables.

Determining the period of the intoxication effects of the herbicide compounds on the test plants, defines their persistence and qualifies their phytotoxic action, indicating the safe time for planting new crops in succession to the one that the herbicide was originally applied to. The probable cause of the effects of the herbicide 2,4-D + picloram observed in the decrease of the growth and development of the test plant, may be related to the mechanism of action in the plant and the long residual period of picloram in the soil, which induce metabolic and biochemical changes to species sensitive to the auxinic compound, causing senescence of the plants.

The initial action of these compounds involves the metabolism of nucleic acids and the plasticity of the cell wall, affecting the growth of plants in a similar way to natural auxin, causing damage to the chloroplast, causing chlorosis of the leaf structure and decreasing the chlorophyll rate, leading to desiccation and tissue necrosis. In high concentrations of herbicide, growth is inhibited due to the compound reaching the meristematic regions of the plant, which accumulate both assimilates from photosynthesis and the herbicide transported via phloem. Intoxication also causes the synthesis of abscisic acid (ABA), resulting in stomatal closure, limiting the assimilation of carbon and consequently the production of biomass, in addition to contributing to the inhibition of photosynthetic enzymatic activity, resulting in leaf senescence and plant death (Hansen & Grossmann, 2000; Oliveira Júnior et al., 2011), as observed in this work.

The relevance of the results in the test plant grown in sequence to *U. brizantha* cv. Marandu, may have an intrinsic relationship to the morphological characteristics of the species, since it has high production and root volume, providing a larger area occupied, facilitating the absorption of water and nutrients and, therefore, of the xenobiotic compounds present in the soil, in addition to exhibiting greater rusticity and adaptability inherent to edaphoclimatic conditions when compared to *P. maximum* cv. Mombasa (Kanno, 1999; Camarão & Souza Filho, 2005; Vilela, 2005).

4. Conclusion

The species *U. brizantha*, cv. Marandu and *P. maximum* cv. Mombasa, have phytoremediation characteristics for auxinic herbicides, with emphasis on *U. brizantha*, cv. Marandu. *Raphanus sativus* Crimson Gigante demonstrates potential as a biological indicator of the presence of the herbicide 2,4-D + picloram, presenting symptoms at 5 DAE. Its cultivation in succession to grasses, provided better conditions for the development of the vegetable garden, however it was not enough for the total removal of the xenobiotic compound from the soil.

Thus, the studied period proves that it is insufficient for the total removal of the effects of the herbicide, requiring continuity in the decontamination process, since as the plants grow, their phytoremediation action is increased by the greater absorption capacity, being able to present greater efficiency in the removal of xenobiotic compounds, which consequently cause less damage to the herbicide-sensitive culture.

Studies with a longer evaluation period are suggested, as well as the evaluation of *U. brizantha* cv species. Marandu, *P. maximum* cv. Mombasa in integrated cultivation system, so as to understand whether the simultaneous interaction of the species. It is also proposed to analyze nitroperchloric digestion by wet route in order to understand the mechanism(s)/process(s) active phytoreator(s) that result from the morphological characteristics of the studied species.

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