



Article

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BIOSOLIDS IN LEACHING OF HERBICIDES MIMICKING AUXIN IN TROPICAL SOILS

Biossólido na Lixiviação de Herbicidas Mimetizadores de Auxina em Solos Tropicais

ABSTRACT - Biosolids are residues from the treatment of urban fluids used as a source of nutrients for agricultural and forestry crops. The organic matter contained in this residue and its chemical characteristics may interfere with the behavior of herbicides in the soil. The objective of this study was to evaluate the influence of biosolids on the potential for leaching herbicides mimicking auxin. Two simultaneous experiments were performed: a leaching test of picloram + 2,4-D in soil column with addition of thermally treated biosolids or solarized biosolids and another one to evaluate the effect of leachate application from the leaching tests under inert material. Each type of biosolid was incorporated in sandy soil in the proportions of 0%, 50%, 100% and 150% of the maximum recommended dose for subsurface fertilization for eucalyptus. The soil was conditioned in PVC columns and the herbicide columns based on picloram + 2,4-D (Turuna® Commercial Formulation), corresponding to 240 g L⁻¹ of 2,4-D + 64 g L⁻¹ of picloram at a dose of 3.5 L ha⁻¹ of the commercial product. The columns were submitted to rain simulation and the resulting leachate was collected, followed by its application in sand-filled pots. The *Cucumis sativus* was sown along the profile of the soil columns and in the pots. The incorporation of the biosolid, independently of the type and dose tested did not interfere in the leaching potential of picloram + 2,4-D. Symptoms of intoxication were observed along all soil columns and pots. Therefore this residue is not very effective for the resolution of environmental problems caused by the leaching of auxin-mimicking herbicides in the soil.

Keywords: picloram + 2,4-D, sewage sludge, Bioassay, leaching herbicides.

RESUMO - O biossólido é um resíduo proveniente do tratamento de fluidos urbanos utilizado como fonte de nutrientes para culturas agrícolas e florestais. A matéria orgânica contida nesse resíduo e as características químicas dele podem interferir no comportamento de herbicidas no solo. Objetivou-se neste estudo avaliar a influência do biossólido sobre o potencial de lixiviação de herbicidas mimetizadores de auxina. Implantaram-se dois experimentos simultâneos, sendo um teste de lixiviação do picloram + 2,4-D em coluna de solo com adição de biossólido termicamente tratado ou de biossólido solarizado e outro para a avaliação do efeito da aplicação do lixiviado proveniente dos testes de lixiviação sob material inerte. Cada tipo de biossólido foi incorporado em solo arenoso nas proporções de 0% 50%, 100% e 150% da dose máxima recomendada para adubação subsuperficial para o eucalipto. O solo foi acondicionado em colunas de PVC e aplicou-se, na superfície das colunas, herbicida à base de picloram + 2,4-D (formulação comercial Turuna®), correspondente a 240 g L⁻¹ de 2,4-D + 64 g L⁻¹ de picloram, na dose de 3,5 L ha⁻¹ do produto comercial. As colunas foram

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*submetidas à simulação de chuva e fez-se a coleta do lixiviado resultante, seguida de sua aplicação em vasos preenchidos com areia. Foi semeado *Cucumis sativus* ao longo do perfil das colunas de solo e nos vasos. A incorporação do biossólido, independentemente do tipo e da dose testada, não interferiu no potencial de lixiviação do picloram + 2,4-D. Observaram-se sintomas de intoxicação ao longo de todas as colunas de solo e nos vasos. Portanto, esse resíduo é pouco eficaz para a resolução de problemas ambientais causados pela lixiviação de herbicidas mimetizadores de auxina no solo.*

Palavras-chave: picloram + 2,4-D, lodo de esgoto, bioensaio, lixiviação de herbicida.

INTRODUCTION

Application of herbicides for weed control is an important practice for management of agricultural and forestry crops. However, the indiscriminate use of such inputs can contribute to aggravation of environmental problems, such as soil and water contamination. Such risks increase when there is use of herbicides that have high persistence in the environment and low sorption in soils, as they are subject to leaching (Melo et al., 2010).

Auxin-mimicking herbicides are selective for grasses and widely used in cultivation of pastures, sugarcane and wheat (Oliveira Jr. et al., 2013). Some herbicides of this group, such as picloram, are poorly adsorbed by soil colloids and exhibit long persistence and high mobility, which can lead to groundwater contamination (Assis et al., 2011). Dynamics of these herbicides are influenced by organic matter content available in the environment, which contributes to increasing their sorptive potential and consequently reduces inherent risks of leaching these inputs in the environment (Gaultier et al., 2008; D'Antonino et al., 2009). Thus, the use of organic residues in agriculture can minimize problems arising from herbicide movement and permanence in the soil (Kah et al., 2007; Chirukuri and Atmakuru, 2015).

Implementation of sewage treatment plants (STPs) in Brazil, experienced in recent years, has generated an enormous amount of biosolids, whose adequate destination needs to be developed in the country (Lobo et al., 2012). Biosolids are waste from urban fluids treatment in STPs. Due to their chemical and physical characteristics, their use as some source of plant nutrients has been an alternative in agriculture (Bettiol and Camargo, 2006; Maria et al., 2010; Bhatt et al., 2015). However, it should be noted that for this purpose biosolids must comply with certain specifications as a guarantee of their correct handling. However, the application of biosolids in the soil can still be efficient to increase herbicides sorptive potential in the soil, because the organic matter in its composition can make the herbicide complex and increase the amount of material not available in the soil solution. However, little is known about the use of this residue for this purpose.

Generation of techniques minimizing environmental problems caused by auxin-mimicking herbicides and, at the same time, the creation of adequate urban waste practices in agriculture are alternatives that are relevant for environmental sustainability in agroecosystems and deserve to be elucidated. In light of the above, the objective of this study was to evaluate biosolids influence on the leaching potential of auxin-mimicking herbicides in sandy soil.

MATERIALS AND METHODS

Materials description

The study was conducted in a greenhouse at the Institute of Agrarian Sciences at Brazilian federal university *Universidade Federal de Minas Gerais*, located in the municipality of Montes Claros, MG. Two simultaneous experiments were installed: one to simulate picloram + 2,4-D leaching dynamics in a sandy soil column with addition of STP biosolids from Montes Claros, MG, or STP biosolids from Juramento, MG, and another one for elucidation of some leachate application effect from these soil columns on a bioindicator.

For assembling the experiments, the Montes Claros' biosolids or the Juramento's bed biosolids were incorporated to the soil in proportions of 0%, 50%, 100% and 150% of their maximum subsurface application recommendation for eucalyptus cultivation. These recommendations were determined in accordance with Resolution no. 375 of August 29, 2006, of Brazilian government National Council of Environment (CONAMA, in the Portuguese abbreviation) and have dealt with the relationship between available N in the biosolid and the N need for cultivation of eucalyptus (Berton and Nogueira, 2010). Recommendations were of 4.6 and 6.0 t ha⁻¹ for the Montes Claros' treated biosolid and the Juramento's biosolid, respectively.

Biosolids used in the present research resulted from the sewage treatment system in the USBR reactor. In the Montes Claros' STP the biosolid was mechanically dried by a drying filter. And in the Juramento's STP drying was natural by deposition in a drying bed. Both STPs are managed by the Basic Sanitation Company of the Brazilian State of Minas Gerais (COPASA, in the Portuguese abbreviation) and for both residues there was a physicochemical characterization (Table 1).

The soil used was of sandy texture, collected in the community of Abóboras, rural area of Montes Claros, MG. For it a physicochemical characterization was also carried out (Table 1).

Table 1 - Soil attributes used in leaching tests

	pH	P-Mehlich	K	Ca	Mg	Al	H+Al	SB	t	T	m	V	OM	CS	FS	Sil.	Cl.
	(H ₂ O)	(mg dm ⁻³)			(mmol dm ⁻³)						(%)			(g kg ⁻¹)			
Soil	4.7	0.47	35	0.2	0.1	0.6	0.6	0.39	0.99	3.29	61	12	2.78	25.2	50.8	12	12
	pH	C.O.	N	P	K	Ca	Mg	S	Zn	Fe	Mn	Cu	Cd	Pb	Cr	Ni	Den.
	(H ₂ O)	(g kg ⁻¹)						(mg kg ⁻¹)									
JB	4.5	62.1	21.0	2.8	5.7	5.4	1.8	10.5	28	35	132	64	0	4.3	0	9.8	0.9
MCB	6.4	11.62	28.1	0.8	2.6	2.7	0.4	1.7	531	42	218	131.2	0	6.3	0	8.1	0.6

CS = coarse sand; FS = fine sand; Sil. = silt; Cl. = clay; OM = organic matter; SB = sum of bases; JB = Juramento's biosolid; MCB = Montes Claros' biosolid; Den. = density.

Water used for assembling and conducting the experiments was from a tubular well and classified as hard water. It presented the following physicochemical attributes: electrical conductivity (0.36 mS cm⁻¹), salinity (0.132% o), total alkalinity (452.88 mg L⁻¹), bicarbonate (452.8 mg L⁻¹), carbonate (< 1.0 mg L⁻¹), P (< 0.01 mg L⁻¹), nitrate (0.088 mg N L⁻¹), ammonium (< 0.28 mg L⁻¹), sulfate (9.159 mg L⁻¹), chlorides (3.107 mg L⁻¹), B (< 0.003 mg L⁻¹), Ca (65.219 mg L⁻¹), Mg (11.232 mg L⁻¹), K (1.138) and Na (14.3 mg L⁻¹).

Picloram + 2.4-D leaching test

Experimental units consisted of combining sandy soil samples and four doses of Montes Claros' or Juramento's biosolids stored in PVC pipes measuring 10 cm in diameter and 45 cm in length, graduated in 5 cm by 5 cm, submitted to application of picloram + 2.4-D.

The PVC pipes were previously coated with paraffin on the inner surface to avoid water percolation at the column edges. After filling with the soil plus the doses of biosolid, the columns were placed in the upright position and saturated with water. After 24 hours, a herbicide based on picloram + 2.4-D (commercial formulation Turuna[®]) was applied on the columns surfaces, corresponding to 240 g L⁻¹ of 2.4-D + 64 g L⁻¹ of picloram at the dose of 3.5 L ha⁻¹ of the commercial product. Application was provided via knapsack sprayer with a Teejet11002 fan-type spraying nozzle regulated at a working pressure of 200 KPa. A 40 mm rainfall was then simulated with an intensity of 1 mm s⁻¹.

Columns remained vertically standing for 36 hours under a greenhouse where the leachate resulting from herbicide application and rain simulation was collected in plastic bags previously

tied at the base of the soil columns. Subsequently, the soil columns were horizontally arranged and part of the 5 cm-wide pipe wall was extracted along the column longitudinal profile where the bioindicator *Cucumis sativus* (cucumber) was seeded with planting density of one plant per centimeter.

At 21 days after application (DAA) of picloram + 2,4-D and of water depth in the soil columns, visual evaluations of intoxication in the bioindicator were carried out. For this, percentage marks were assigned from 0 to 100, from visual aspects of control, where 0 corresponds to plant without injury and 100 to dead plant (ALAM, 1974). To serve as a parameter for attributing intoxication marks, plants were grown in the same experimental conditions but without herbicide application. The bioindicator aerial part was also collected, which was submitted to drying in a forced air circulation oven at 65 °C for a period of 72 hours to obtain the plants dry matter, which was measured in an analytical balance with precision of 4 decimal places.

The experiment was conducted in a randomized block design (RBD) with four replicates. For the statistical analysis, a 2 x 4 factorial design (two types of biosolids in four doses) with a subdivided plot was considered, with 9 depths (0-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40 and 40-45) being allocated in the subplots along the soil column. Therefore, the following statistical model was considered:

$$y_{ijk} = m + B_i + a_j + b_k + (ab)_{jk} + \delta_{ijk} + c_l + (ac)_{jl} + (bc)_{kl} + (abc)_{jkl} + e_{ijkl} \quad (\text{eq. 1})$$

where: y_{ijk} = amount observed in the experimental unit; m = overall average; B_i = effect of the i^{th} ($i = 1, 2, \dots, 4$) block; a_j = effect of the j^{th} ($j = 1, 2$) biosolid; b_k = effect of the k^{th} ($k = 1, 2, \dots, 4$) dose of biosolid; $(ab)_{jk}$ = effect of the interaction between the composed j^{th} and the k^{th} dose of biosolid; δ_{ijk} = effect of error a in the plot that received the i^{th} block, j^{th} biosolid and k^{th} dose (error a); c_l = effect of the l^{th} ($l = 1, 2, \dots, 9$) depth; $(ac)_{jl}$ = effect of the interaction between the j^{th} biosolid and the l^{th} depth; $(abc)_{jkl}$ = effect of the interaction between the j^{th} biosolid, the k^{th} dose and the l^{th} depth; e_{ijkl} = experimental error in the i^{th} block, j^{th} biosolid, k^{th} dose in the l^{th} depth.

Statistical analyzes were performed in the (open source programming language and software environment for statistical computing and graphics) R (R Development Core Team, 2014). In order to verify the variation sources effects significance by the F-test, the *aov* function of the *stats* package was used. For adjustment of characteristics intoxication and dry matter of the bioindicator regarding the biosolid dose and column depth, 13 multiple linear regression models were tested (Table 2). In order to verify the models adjustment quality, the Akaike information criterion (AIC) was considered. Regarding the multiple regression models adjustment, the least squares method was used with the aid of the *lm* function of the *stats* package. From the values predicted by the regression models, surface and response graphs were obtained using (proprietary software package for scientific graphing and data analysis) SigmaPlot 11.0.

Table 2 - Multiple regression models tested to describe intoxication caused by picloram + 2,4-D em *Cucumis sativus* (zi) due to doses of Montes Claros' or Juramento's biosolids (xi) and the soil column depth (yi)

Model	Function
1	$Z_i = a + bx_i + e_i$
2	$Z_i = a + by_i + e_i$
3	$Z_i = a + bx_i + cy_i + e_i$
4	$Z_i = a + bx_i + cx_i^2 + dy_i + e_i$
5	$Z_i = a + bx_i + cy_i + dy_i^2 + e_i$
6	$Z_i = a + bx_i + cx_i^2 + dy_i + fy_i^2 + e_i$
7	$Z_i = a + bx_i + cy_i + dx_i y_i + e_i$
8	$Z_i = a + bx_i + cx_i^2 + dy_i + fx_i y_i + e_i$
9	$Z_i = a + bx_i + cy_i + dy_i^2 + fx_i y_i + e_i$
10	$Z_i = a + bx_i + cx_i^2 + dy_i + fy_i^2 + gx_i y_i + e_i$
11	$Z_i = a + bx_i + cx_i^2 + dy_i + fy_i^2 + gx_i y_i + hx_i^2 y_i + e_i$
12	$Z_i = a + bx_i + cx_i^2 + dy_i + fy_i^2 + gx_i y_i + hx_i y_i^2 + e_i$
13	$Z_i = a + bx_i + cx_i^2 + dy_i + fy_i^2 + gx_i y_i + hx_i^2 y_i + jx_i y_i^2 + e_i$

Application of the leachate from the leaching tests on the inert material

For the soil system leached solution effect study on inert material, 350 mL pots filled with inert material (sand) were considered as experimental units. Five mL of the leachate resulting from herbicide application and the water depth were applied to the soil column, diluted in 10 mL of water. The pots did not have the base perforated to avoid loss of leachate present in the system.

For detection of the herbicide in the leachate, the cucumber was also sown in the experimental units. The same evaluation procedures were used in the leaching tests, that is, visual evaluations of intoxication and obtaining the bioindicator dry matter at 21 DAA (days after the application of the leached solution).

In the statistical analysis, a complete randomized block design (RBD) with four replications was used, adopting the 2 x 4 factorial design (two types of biosolids and four doses of biosolids incorporated in the soil). Therefore, the following statistical model was considered:

$$y_{ijk} = m + B_i + a_j + b_k + (ab)_{jk} + e_{ijkl} \quad (\text{eq. 2})$$

where: y_{ijk} = value observed in the experimental unit; m = overall average; B_i = effect of the i^{th} ($i = 1, 2, \dots, 4$) block; a_j = effect of the j^{th} ($j = 1, 2$) biosolid; b_k = effect of the k^{th} ($k = 1, 2, \dots, 4$) dose of biosolid; $(ab)_{jk}$ = effect of the interaction between the composed j^{th} and the k^{th} dose of biosolid; e_{ijk} = effect of the error a in the plot that received the i^{th} block, j^{th} biosolid and k^{th} dose (error a).

Regarding adjustments of the intoxication quantitative characters and the bioindicator dry matter according to the biosolid type and dose used in the leaching tests, linear and quadratic simple regression adjustments were also tested, also performed by the *lm* function of (open source programming language and software environment for statistical computing and graphics) R (R Development Core Team, 2014). In order to verify the variation sources effects significance by the F-test, the *aov* function of the *stats* package was used. Regarding the linear and quadratic regression models adjustment, the least squares method was used with the aid of the *lm* function of the *stats* package. From the values predicted by the regressions, graphs were obtained using (proprietary software package for scientific graphing and data analysis) SigmaPlot 11.0.

RESULTS AND DISCUSSION

Picloram + 2.4-D leaching test

For this test, evidence of picloram + 2.4-D leaching was observed regardless of the type and dose of biosolid incorporated into the soil. Symptoms of intoxication in the bioindicator cultivated along the soil column were diagnosed in all treatments, characterized as leaf epithelia and stem elongation in plants. The symptoms observed are common in sensitive plants poisoned by auxin mimics (Silva et al., 2007).

From the F-test ($p < 0.05$) in the intoxication behavior, significance was observed for the interaction between biosolid type and each depth level along the soil column (Table 3). Regarding the dry matter mass yield, only the depth level was significant.

Based on the Akaike information criterion (AIC), in relation to intoxication in the bioindicator a better adjustment of models 6 and 5 was verified, respectively, for the biosolids leaching tests

Table 3 - Summary of the average squares for intoxication by picloram + 2.4-D and *Cucumis sativus* dry matter yield cultivated in soil incorporated with different doses of Montes Claros' or Juramento's biosolids

Source of variation	GL	Intoxication	Dry matter yield
Block	3	1907.3 ***	0.001377 ^{ns}
Type of biosolid (A)	1	2205.6 ***	0.000231 ^{ns}
Dose (B)	3	119.9 ^{ns}	0.007083 ^{ns}
A X B	3	404.7 ^{ns}	0.000555 ^{ns}
Error A	21	141.4	0.0009450
Depth (C)	8	2840.1 ***	0.013061 ***
A X C	8	169.9 **	0.001064 ^{ns}
B X C	24	37.5 ^{ns}	0.001021 ^{ns}
A X B X C	24	54.1 ^{ns}	0.001021 ^{ns}
Residue B	192	61.4	0.000935

** significant at $p < 0.01$ and *** significant at $p < 0.001$ probability; ns: nonsignificant at $p < 0.05$.

from Montes Claros and Juramento incorporated to the soil. For the dry matter yield variable, models that best fit the observations were 9 and 10 for the respective incorporations of the Montes Claros' treated biosolids and the Juramento's biosolids (Table 4).

Table 4 - Akaike information criterion (AIC) and coefficient of determination (R^2) for multiple linear regression models adjusted to describe intoxication and dry matter mass yield of *Cucumis sativus* cultivated in soil with application of picloram + 2,4-D due to thermally treated biosolid (TTB) or solarized biosolid (SB) doses and the soil column depth

Model	TTB		SB	
	AIC	R^2	AIC	R^2
Intoxication				
1	282.42	00.47	259.04	0.34
2	224.37	80.15	208.51	75.51
3	225.51	80.62	210.01	75.85
4	223.58	82.63	211.95	75.89
5	223.63	82.60	202.46	81.48
6	221.21	84.61	204.39	81.51
7	227.36	80.70	211.82	75.98
8	225.41	82.71	213.76	76.02
9	225.46	82.68	204.22	81.60
10	223.03	84.69	206.14	81.64
11	225.00	84.70	207.52	81.95
12	222.16	85.86	208.00	81.71
13	224.13	85.87	209.39	82.02
Dry matter yield				
1	-148.92	0.18	-164.74	0.02
2	-161.09	28.81	-182.65	39.20
3	-159.18	28.98	-180.66	39.22
4	-157.71	30.02	-179.30	40.30
5	-160.55	35.34	-207.48	72.71
6	-159.13	36.37	-206.93	73.79
7	-160.28	34.84	-184.04	47.65
8	-158.86	35.88	-182.78	48.73
9	-161.97	41.19	-218.78	81.14
10	-160.61	42.23	-218.90	82.21
11	-158.61	42.23	-217.39	82.46
12	-160.64	45.39	-216.92	82.22
13	-158.64	45.40	-215.41	82.47

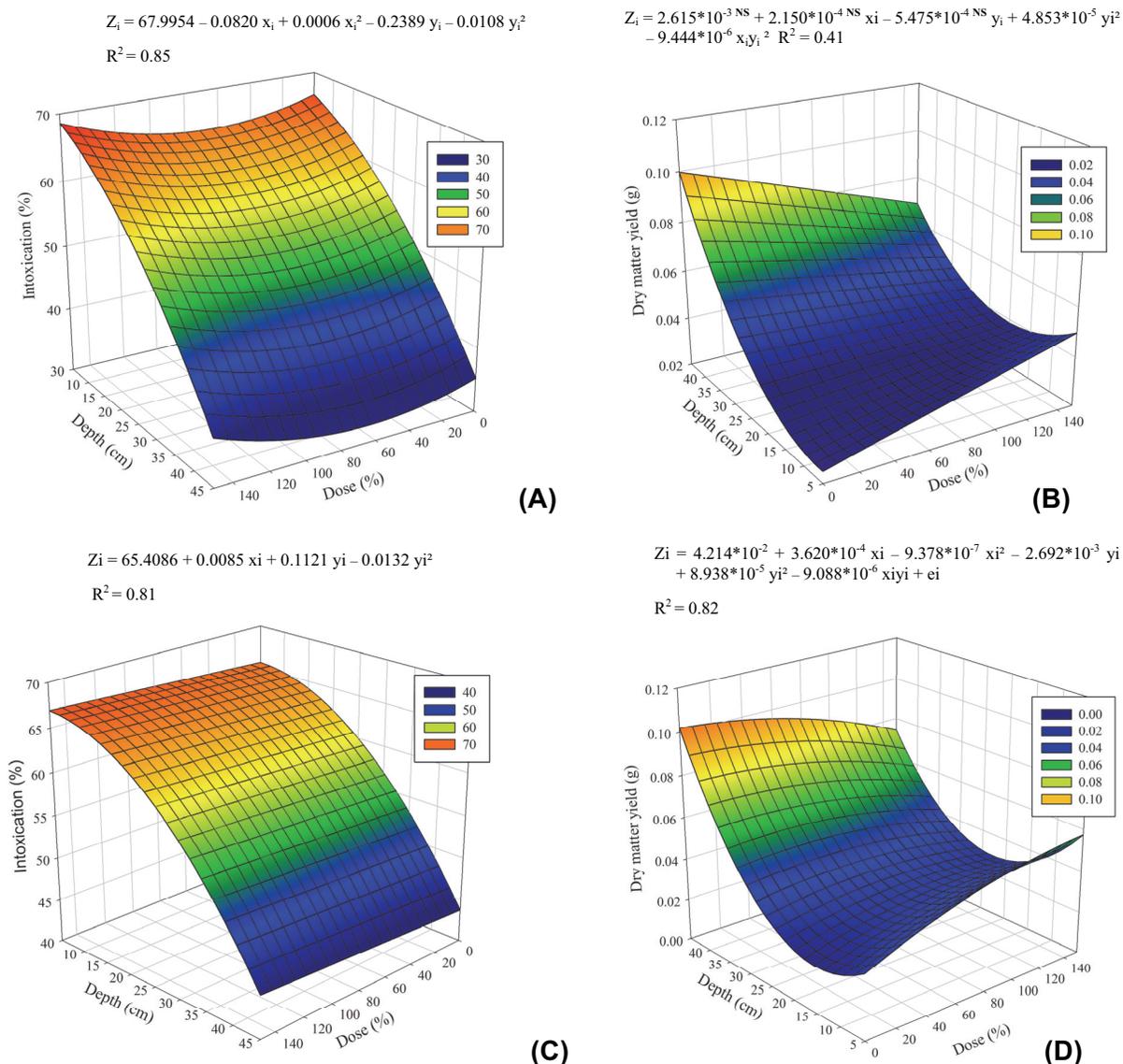
soil, which could increase the sorptive potential of auxin mimics.

After adding biosolid in the soil, Oliveira et al. (2002) and Albuquerque et al. (2015) have observed a pH increase, as well as increase in available organic carbon content. Such effects act in an antagonistic way in relation to herbicide sorption but soil characteristics in their original state, that is, before incorporation of residues, may have been preponderant so that the pH effect had greater relevance on the herbicide sorptive potential. This, together with the fact that the herbicide has an anionic character, may have contributed to its molecules leaching process. Marco-Brown et al. (2014) reinforce this assumption by emphasizing that picloram exhibits an anionic character in most soils because it has an acid nature ($pK_a \approx 2.3$), which reduces its adsorption capacity to reactive materials, such as clay and organic matter (Vieira et al., 1998). Results obtained in the leaching tests corroborate these authors since the soil used presents

In the case of incorporation tests of Montes Claros' biosolids, increasing the dose of incorporated biosolids promoted a slight quadratic trend in intoxication behavior caused by the herbicide. The model explains that the intoxication decreased up to 80% of the dose used and this trend was smoothed after increasing this variable. As for the depth level increase in the soil column, intoxication tended to diminish (Figure 1A).

In the assays with addition of Juramento's biosolids, trend was observed as almost constant in the bioindicator intoxication symptoms behavior from increasing the biosolid dose incorporated in the soil (Figure 1C). Regarding depth, behavior obtained was similar to the results from the leaching tests where there was addition of Montes Claros' biosolids. There was a trend towards bioindicator higher dry matter yield from the increase of the biosolid dose. This may be justified by the fact that biosolids appear as a source of plant essential nutrients (Nascimento et al., 2014). Depth increase in the test column also provided higher dry matter yield (Figure 1D).

Auxin-mimicking herbicides sorption such as picloram and 2,4-D is positively correlated with the soil organic matter content (Spadotto et al., 2003). Among the biosolids main characteristics that may influence soil sorption potential are (solid or dissolved) organic matter content, ionic composition and pH value. As the soil used does not have high organic matter content (Table 1), incorporation of the Montes Claros' or Juramento's biosolids at the doses tested may have been insufficient to significantly alter its organic matter content. This would be justified by the fact that biosolids present slow degradation of organic C (Andrade et al., 2013; Moretti et al., 2015). Applications frequency over a given period of time could have a considerable effect on organic C in the



(A) intoxication with incorporation of the Montes Claros' biosolid; (B) dry matter yield with incorporation of the Montes Claros biosolid; (C) intoxication in *C. sativus* with incorporation of the Juramento's biosolid, (D) dry matter yield of *C. sativus* with incorporation of the Juramento's biosolid.

Figure 1 - Surface and response of quantitative characters (z) of *Cucumis sativus* subjected to application of picloram + 2,4-D due to the biosolid dose (x) and depth along the soil column (y).

low organic matter content and pH higher than 6.5. In addition, both incorporated biosolids have a pH value higher than the herbicide pKa value (Table 1).

D'Antonino et al. (2009) have observed that pH was determinant for the picloram leaching potential, becoming less significant in soils with high content of organic matter. According to Marchese (2007), the fact that the addition of a certain residue promotes pH elevation, together with increase of the soil organic matter content, gives to this one characteristics that are antagonistic as to the influence on the acid herbicides sorptive potential. This author has also observed that the addition of biosolids in tropical soils has provided a decrease in ametrine sorption and this was correlated with soil characteristics such as textural degree and source material.

Some studies corroborate the present study results when they found picloram adsorption in very acidic systems and non-adsorption when in pH higher than 3 (Grover et al., 1971;

Biggar et al., 1978; Celis et al., 2002; Braga et al., 2016). As for Marco-Brown et al. (2014), they have observed considerable adsorption of picloram on clay material in medium with pH higher than 3.

Although the use of tubular well water has simulated a real field situation, this may have favored the possible elevation of the system pH due to its high total alkalinity (452.8 mg L⁻¹ of bicarbonate). In soils with pH higher than 5, more than 90% of picloram molecules are in the anionic phase, that is, they are less sorbed by organic matter and clays of the soil negative charge, being likely to be leached (Cheung and Biggar, 1974). Maia (2013) has observed an increase in soil pH in an agricultural area irrigated with water rich in calcium carbonate.

Results for dry matter yield by the bioindicator were incoherent with the biological phenomenon, which leads this variable to appear as a conclusion parameter for picloram + 2,4-D leaching dynamics in the present study. D'Antonino et al. (2009) corroborate these results in elucidating that the bioindicator dry matter is no variable indicated to elucidate the intoxication effect by auxin mimics in plants, since the absorption of a same amount of these herbicides can be reflected in a variable way in plants morphology. This shows that this variable does not have a good correlation with the intoxication caused by herbicides with this principle of action (Santos et al., 2007).

Effect of application of leachate from leaching tests on the bioindicator cultivated in inert material

From the F-test ($p < 0.05$), the type of biosolid used did not interfere with the bioindicator intoxication but the dose factor was significant. Regarding the dry matter mass yield, no significance was observed for any of the sources of variation (Table 5).

At 21 DAA of the leachate from the picloram + 2,4-D application and simulation of 40 mm of rain in the pots, severe symptoms of intoxication in the bioindicator were observed in all treatments, regardless of the biosolid dose incorporated into the soil (Figure 2). However, it was not possible to obtain adjustments for the regression models tested due to the non-explanation of the biological phenomenon and the regression parameters insignificance.

These results reinforce the hypothesis that the herbicide leached along the soil columns independently of the biosolid type and dose incorporated in the soil. Thus, in both assays, possibly the herbicide content present in the leach solution from the soil columns was similar. This may be related to the possibility that the organic matter contained in the biosolids in the doses tested was irrelevant to interfere with the herbicide sorptive potential or also to the possible increase of the soil pH, depending on the biosolids or the irrigation used.

Picloram and 2,4-D present, respectively, pKa values equal to 2.3 and 2.8 (Rodrigues and Almeida, 2011). Thus, the increase in pH, either by incorporation of biosolids or the water used in the system irrigation, may have negatively interfered in these molecules sorption. Assis et al. (2011) confirm this by observing that picloram sorption is higher in soil conditions with high organic matter content and low pH levels.

Thermally treated biosolids and solarized biosolids, despite having organic matter in their composition, are not efficient to raise the sorptive potential of auxin-mimicking herbicides.

Table 5 - F values calculated in a factorial design for intoxication by picloram + 2,4-D and dry matter yield of *Cucumis sativus* cultivated in pots with application of leached material from the soil columns

Source of variation	GL	Intoxication	Dry matter yield
Block	3	46.8 ^{ns}	0.0014 ^{ns}
Type of biosolid (A)	1	60.5 ^{ns}	0.0002 ^{ns}
Dose (B)	3	316.3 ^{***}	0.0071 ^{ns}
A X B	3	108.8 ^{ns}	0.0006 ^{ns}
Error A	21	35.8	0.0095 ^{ns}

***: significant at $p < 0.001$ probability; ns: nonsignificant at $p < 0.05$.

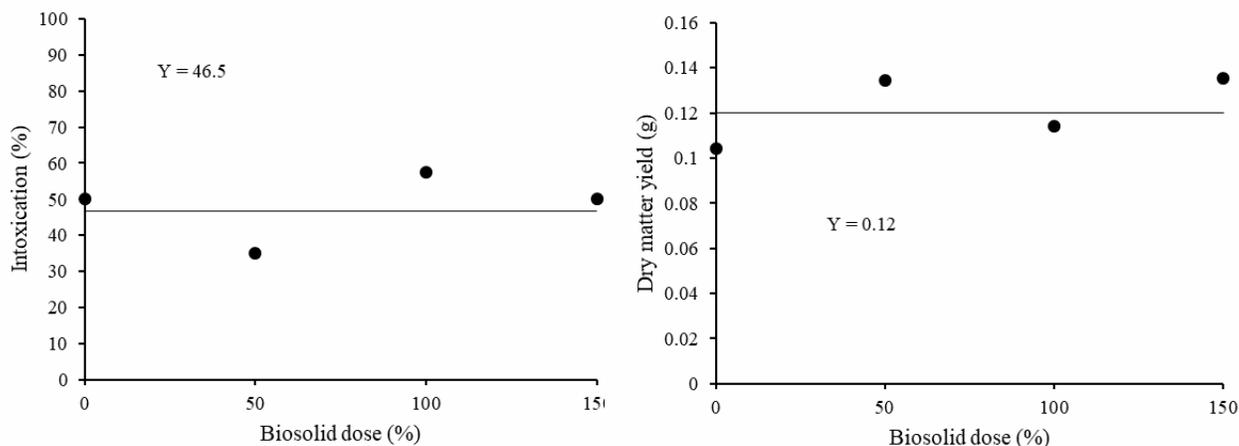


Figure 2 - Intoxication and dry matter of *Cucumis sativus* subject to application of leached solution of picloram + 2,4-D from the leaching test due to the biosolid dose incorporated in the soil column.

Thus, these residues are not very effective for resolution of environmental problems caused by the leaching of auxin-mimicking herbicides in sandy soils.

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REFERENCES

- Albuquerque H.C. et al. Residual effect of sewage sludge fertilization on sunflower yield and nutrition. **Rev Bras Eng Agric Amb.** 2015;19:1005-11.
- Andrade C.A. et al. Mineralização do carbono e do nitrogênio no solo após sucessivas aplicações de biossólido. **Pesq Agropec Bras.** 2013;536:44-5.
- Asociacion Latinoamericana de Malezas – ALAM. Recomendaciones sobre unificación de los sistemas de evaluación en ensayos de control de malezas. **ALAM.** 1974:01:35-8.
- Assis E.C. et al. Leaching of picloram in ultisol under different rainfall volumes. **Planta Daninha.** 2011;29:36-29.
- Berton R.S., Nogueira T.A.R. Uso de lodo de esgoto na agricultura. In: Coscione A.R., Nogueira T.A.R., Pires A.M.M., editores. **Uso agrícola de lodo de esgoto: avaliação após a resolução n. 375 do CONAMA.** Botucatu: FEPAF, 2010. p.31-50.
- Bettiol W., Camargo O.A.A. Disposição de lodo de esgoto em solo agrícola. In: Bettiol W., Camargo O.A., editores. **Lodo de esgoto: impactos ambientais na agricultura.** Jaguariúna: Embrapa Meio Ambiente, 2006. p.350.
- Bhatt P. et al. Recycled sewage sludge: a step to sustainable agriculture. **Int J Chem Sci.** 2015;13:1611-20
- Biggar J. et al. Equilibrium and kinetics of adsorption of picloram and parathion with soils. **J Agric Food Chem.** 1978;26:1306-12.
- Braga R.R et al. Effect of growing *Brachiria brizantha* on phytoremediation of picloram under different pH environments. **Ecol Eng.** 2016;94:102-6.
- Celis R. et al. Clay-herbicide com-plexes to retard picloram leaching in soil. **Int J Environ Anal Chem.** 2002;82:503-17.

- Cheung M.W., Biggar J.W. Soolubity and molecular structure of 4-amino-3,5,6-trichloropicolinic acid in reation to pH in temperature. **J Agric Food Chem.** 1974;22:202-6.
- Chirukuri R., Atmakuru R. Sorption characteristics and persistence of herbicide bispyribac sodium in different global soils. **Chemosphere.** 2015;138:932-9.
- D'Antonino L. et al. Lixiviação do picloram em argissolo vermelho-amarelo e latossolo vermelho-amarelo com diferentes valores de pH. **Planta Daninha.** 2009;589:600-27.
- Gaultier J. et al. Degradation of [carboxyl-14C] 2,4-D and [ring-U-14C] 2,4-D in 114 agricultural soils as affected by soil organic carbon content. **Soil Biol Biochem.** 2008;40:217-27.
- Grover R. Adsorption of picloram by soil colloids and various other adsorbents. **Weed Sci.** 1971;19:417-18.
- Kah M., Beulke S., Brown C.D. Factors influencing degradation of pesticides in soil. **J Agric Food Chem.** 2007;55:4487-92.
- Lobo T.F., Grassi Filho H., Bull L.T. Efeito do nitrogênio e do lodo de esgoto nos fatores produtivos do feijoeiro. **Rev Ceres.** 2012;59:118-24.
- Maia C.E. Qualidade ambiental em solo com diferentes ciclos de cultivo do meloeiro irrigado. **Ci Rural.** 2013;43:603-9.
- Marchese L. **Sorção/dessorção e lixiviação de herbicida ametrina em solos canavieiros tratados com lodo de esgoto** [dissertação]. Piracicaba: Escola Superior de Agricultura "Luiz de Queiroz", 2007.
- Marco-Brown J.L. et al. Adsorption of picloram herbicide on montmorillonite: Kinetic and equilibrium studies **Colloids Surfaces A: Physicocheml Eng Aspects.** 2014;449:121-28.
- Maria I.C. et al. Sewage sludge application to agricultural land as soil physical conditioner. **Rev Bras Cienc Solo.** 2010;34:967-74.
- Melo C.A.D. et al. Lixiviação de sulfentrazone, isoxaflutole e oxyfluorfen no perfil de três solos. **Planta Daninha.** 2010;28:385-92.
- Moretti S.M.L. et al. Decomposição de Lodo de Esgoto e Composto de Lodo de Esgoto em Nitossolo Háptico. **Rev Bras Cienc Solo.** 2015;39:1796-805.
- Nascimento A.L. et al. Metais pesados em girassol adubado com lodo de esgoto submetido a diferentes processos de estabilização. **Rev Bras Eng Agríc Amb.** 2014;694:99-18.
- Oliveira JR R.S. et al. Comparative sorption, desorption and leaching potential of aminocyclopyrachlor and picloram. **J Environ Sci Health.** 2013;1049:57-48.
- Oliveira F.C. et al. Efeitos de aplicações sucessivas de biossólido em um latossolo amarelo distrófico cultivado com cana-de-açúcar: carbono orgânico, condutividade elétrica, ph e CTC. **Rev Bras Cien Solo.** 2002;505:20-9.
- R Core Team. R: A language and environment for statistical computing. Vienna: Foundation for Statistical Computing. 2014.
- Rodrigues B.N., Almeida F.S. **Guia de herbicidas.** 6th. ed. Londrina: IPAR, 2011. 591 p.
- Santos J.B. et al. Fitorremediação de áreas contaminadas por herbicidas. In: Silva A.A., Silva J.F. **Tópicos em manejo de plantas daninhas.** Viçosa, MG: UFV, 2007. p.210-329.
- Silva A.A. et al. Herbicidas: Classificação e mecanismos de ação. In: Silva A.A., Silva J.F., editores. **Tópicos em manejo de plantas daninhas.** Viçosa, MG: UFV, 2007. p.156-209.
- Spadotto C.A., Hornsby A.G. Soil sorption of acidic pesticides: modeling pH effects. **J Environ Qual.** 2003;949:56-32.
- Vieira E.M. et al. Estudo da adsorção/dessorção do ácido 2, 4 diclorofenoxiacético (2, 4-D) em solo na ausência e presença de matéria orgânica. **Quím Nova.** 1998;305:8-22.