



Heavy metals in the soil and castor bean plants fertilized with sewage sludge stabilized by different processes

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ABSTRACT: This study evaluated the levels of heavy metals in the soil and castor bean plant after fertilization with sewage sludge (SS) subjected to different stabilization processes. The study was conducted in a Haplic Cambisol with the following treatments: control (CO), fertilization with solarized sewage sludge (SSS), composted sewage sludge (CSS), vermi composted sewage sludge (VSS), limed sewage sludge (LSS), and mineral fertilizer (MF). The experimental design included a subdivided plot scheme with fertilization being the primary factor and soil layers or leaf parts being the secondary factor. The data obtained were analyzed using analysis of variance and the Scott-Knott test with 5% significance. Fertilization with SSS, CSS, and LSS led to the highest increase in Zn in the soil, mainly in the surface layer. In the leaf tissues, Zn and Cu levels were higher with the CO and SSS treatments, whereas the Ni level was higher with SSS, CSS, VSS, and MF treatments. In general, Zn and Cu levels were higher in the leaf limb than in the petiole, whereas Ni, Pb, and Cr levels were higher in the petiole than in the leaf limb. In any case, no symptoms of toxicity in the plants and no risk of soil contamination were observed with any of the fertilization treatments.

Key words: biosolid, waste management, oilseeds, *Ricinus communis* L.

Metais pesados no solo e em mamoneira adubada com lodo de esgoto submetido a diferentes processos de estabilização

RESUMO: O objetivo deste trabalho foi avaliar os teores de metais pesados no solo e na planta de mamoneira em resposta à adubação com lodo de esgoto (LE) submetido a diferentes processos de estabilização. O experimento foi desenvolvido em Cambissolo Háplico com os tratamentos: testemunha, adubação com LE solarizado, LE compostado, LE vermicompostado, LE caleado e adubação mineral. O delineamento experimental foi em esquema de parcelas subdivididas, sendo as adubações o fator primário e as camadas do solo ou as partes da folha o fator secundário. Os dados obtidos foram submetidos à análise de variância e teste de Scott-Knott a 5% de probabilidade. No solo, as adubações com LE solarizado, compostado e caleado promoveram os maiores incrementos dos teores de Zn, principalmente na camada superficial. No tecido foliar, os teores de Zn e Cu foram mais elevados na testemunha e no LE solarizado, enquanto o Ni foi mais elevado nos LE solarizado, compostado, vermicompostado e na adubação mineral. De modo geral, os maiores teores de Zn e Cu ocorreram no limbo foliar em comparação ao pecíolo, enquanto os teores de Ni, Pb e Cr foram mais elevados no pecíolo do que no limbo foliar. Contudo, não foram constatados sintomas de toxidez nas plantas e nenhum risco de contaminação do solo com os diferentes tipos de adubações realizadas.

Palavras-chave: biossólido, gestão de resíduos, oleaginosas, *Ricinus communis* L.

INTRODUCTION

The progressive growth of the world population and urbanization has led to increased production of sewage sludge (SS), increasing the problems associated with its disposal (FEIZI et al., 2019). Although, there are several alternatives to its disposal, the agricultural use of SS is advantageous from an economic as well as ecological point of view

(LU et al., 2012) owing to the nutrients and organic matter in the sludge that can improve agricultural soil productivity (PREDA et al., 2017).

Despite the benefits of its agricultural use, higher than permitted concentrations of heavy metals in SS can lead to contamination of the food chain and serious consequences to plants and animals (SHARMA et al., 2017). The risk of increased levels of heavy metals in the environment

with the application of SS may arise because higher concentrations of these elements are present in the sludge than in natural soils; therefore, there is a need to evaluate their levels (NASCIMENTO et al., 2004; MELO et al., 2018).

Several studies have shown that the application of SS increases the levels of some heavy metals, with emphasis on copper (Cu) and zinc (Zn), in the soil and plants (LATARE et al., 2014; NASCIMENTO et al., 2015; MOTA et al., 2018; FEIZI et al., 2019; KOMINKO et al., 2022). Thus, to allow its agricultural use, SS must be stabilized to decrease the presence of biological contaminants and persistent organic pollutants and reduce the solubilization of heavy metals (NASCIMENTO et al., 2014; ZUBA JUNIO et al., 2019).

The castor bean (*Ricinus communis* L.) crop has good biomass production and displays potential for phytoremediation of heavy metals in contaminated soils (AZIERA; MAJID, 2015; BAUDDH et al., 2015; YASHIM et al., 2016; SU et al., 2018). The species has multiple purposes, including agricultural, energetic, environmental, and industrial purposes (KIRAN; PRASAD, 2017), and owing to its phytotechnical characteristics, Brazilian legislation permits its fertilization with SS (BRASIL, 2020).

A few studies have reported the effects of SS fertilization on the levels of heavy metals in the soil and castor bean plant, but only related to the applied doses of the sludge (CHIARADIA et al., 2009; CAVALCANTI et al., 2015; NASCIMENTO et al., 2015). However, it is also important to study the processes of SS stabilization and their respective effects on the levels of heavy metals in the soil and castor bean plant.

Thus, this study evaluated the levels of heavy metals in the soil and castor bean plant fertilized with SS stabilized by different processes.

MATERIALS AND METHODS

Description of the study area

The study was carried out from April to December 2010 at the Institute of Agricultural Sciences (ICA) of the Federal University of Minas Gerais (UFMG) located in Montes Claros, Minas Gerais (latitude 16° 40' 57.6" S, longitude 43° 50' 19.6" W) and at an altitude of 630 m in a Haplic Cambisol (Figure 1). The chemical and physical characteristics of soil are shown in table 1.

In this study, the area used had no agricultural use before the experiment was set up; it



Figure 1 - Satellite picture of the experimental area at the Institute of Agricultural Sciences (ICA) of the Universidade Federal de Minas Gerais (UFMG). Source: Adapted from "google Maps".

Table 1 - Chemical attributes of the HAPLIC CAMBISOL, solarized sewage sludge (SSS), composted (CSS), vermicomposted (VSS) and limed (LSS), and mineral fertilizer (MF).

		N _{disp}	Zn	Cu	Ni	Cd	Pb	Cr
		-----Concentration-----						
Soil		kg Mg ⁻¹	----- µg g ⁻¹ -----					
Depth	0-20	-	22.90	6.10	Nd	Nd	10.10	Nd
	20-40	-	2.20	1.30	Nd	Nd	11.80	Nd
Fertilizer		-----Applied quantity-----						
Fertilizer		Mg ha ⁻¹	----- g ha ⁻¹ -----					
SSS		4.09	2,151.34	327.20	190.14	4.95	379.06	2,863.00
CSS		24.84	10,010.50	1,564.92	1,154.81	34.78	2,908.02	22,356.00
VSS		12.78	4,779.72	907.38	549.54	15.46	1,527.34	10,863.00
LSS		33.61	15,258.94	2,386.31	1,050.99	84.36	9,591.29	27,728.25
SSP		0.00	12.78	1.46	5.60	0.56	10.44	29.97
Urea		0.04	0.1	0.065	0.00	0.028	0.32	0.00
KCl		0.00	0.075	0.139	0.45	0.072	2.49	0.00

N_{disp} = Nitrogen available and MAC = Maximum allowable concentration in Class 1 sewage sludge or derived product in mg kg⁻¹, according to CONAMA Resolution No. 498 of 2020 (BRASIL, 2020); Nd = below the limit of detection; SSP = single superphosphate.

was at an altitude of 647 m above sea level; it had average maximum temperatures ranging from 28.85 to 33.16 °C, average minimum temperatures ranging from 13.21 to 20.51 °C, and total rainfall of 390.8 mm during the cultivation period (Figure 2).

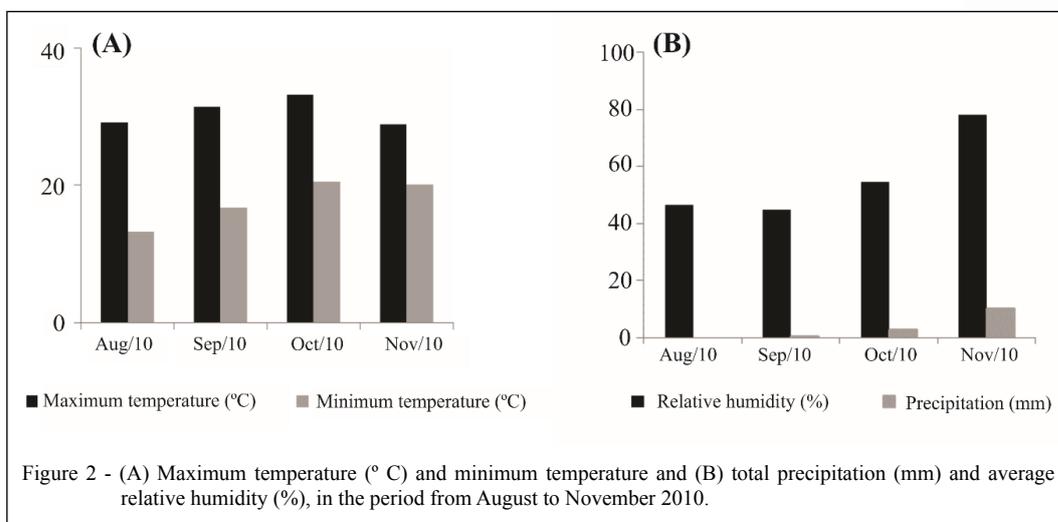
Experimental design and treatments

The experimental design included randomized blocks with four replicates in a split-plot scheme with fertilization being the primary factor and soil layers or leaf parts being the secondary factor. The following six treatments were used: (a) mineral fertilization (MF); (b) four treatments with SS stabilized in different ways: solarized sewage sludge (SSS) - (4.09 Mg ha⁻¹), composted sewage sludge (CSS) (24.84 Mg ha⁻¹), vermicomposted sewage sludge (VSS) (12.78 Mg ha⁻¹) and limed sewage

sludge (LSS) (33.61 Mg ha⁻¹); (c) control treatment without fertilization (CO).

The MF (90 kg ha⁻¹ of P₂O₅ and 30 kg ha⁻¹ of K₂O in planting; 40 kg ha⁻¹ of N, 45 days after emergence) was applied in doses following the recommendations for the use of correctors and fertilizers in Minas Gerais (RIBEIRO et al., 1999), using urea, simple superphosphate, and potassium chloride as a source of nitrogen (N), phosphorus (P), and potassium (K), respectively. The treatments with SS stabilized in different ways were applied based on the level of available N (Table 2), calculated as described in CONAMA Resolution 498 (BRASIL, 2020).

The BRS Energia castor bean cultivar (*Ricinus communis* L. 'BRS Energia') was used in the study. This cultivar has a height of approximately



1.40 m, growth cycle of 120 to 150 days, green waxy stem, conical clusters with an average size of 60 cm, waxy and indehiscent green fruits, and seeds with an average of 48% oil in a single harvest. It is cultivated at an altitude of 300 m above sea level at an average annual temperature between 20 and 30 °C (optimal temperature around 23 °C) and an annual rainfall of at least 500 mm (optimal rainfall between 650 and 800 mm) (GONÇALVES et al., 2005).

The SS was collected at the wastewater treatment plant (WWTP) in the municipality of Juramento, Minas Gerais, which estimated population

is 4,345 habitants (IBGE, 2020). The WWTP has a treatment line composed of preliminary treatment, a UASB anaerobic reactor interconnected in series to an optional post-treatment pond, and sewage treatment through solarization on drying beds for three months, without applying any products in the SS treatment process. The estimated production of wet SS is 54.2 Mg yr⁻¹.

SSS was collected from the drying beds at the wastewater treatment plant. To obtain CSS, SSS was mixed with potato grass pruning (*Paspalum notatum*) to obtain a C:N (carbon:nitrogen) ratio of

Table 2 - Metals content in the soil in response to the application of mineral fertilizer and sewage sludge stabilized in different ways.

Metal	Depth	CO	SSS	CSS	VSS	LSS	MF	Average
	-- cm --	-----mg dm ⁻³ -----						
Zn	0-20	1.50	7.62	8.95	4.92	10.55	4.45	6.33 A
	20-40	0.63	1.42	2.30	0.95	2.58	1.05	1.49 B
	Average	1.07 b	4.52 a	5.63 a	2.94 b	6.56 a	2.75 b	-
Cu	0-20	1.00	1.50	1.60	1.30	2.00	1.20	1.43 A
	20-40	0.60	0.83	0.95	0.60	0.98	0.63	0.77 B
	Average	0.80 a	1.17 a	1.28 a	0.95 a	1.49 a	0.92 a	-
Pb	0-20	10.37	9.23	10.70	12.45	11.83	11.57	11.02 A
	20-40	8.93	12.88	12.00	11.60	12.45	9.65	11.25 A
	Average	9.65 a	11.06 a	11.35 a	12.03 a	12.14 a	10.61 a	-

CO - Control; SSS - Solarized sewage sludge; CSS - Composted sewage sludge; VSS - vermicomposted sewage sludge; LSS - limed sewage sludge; MF - Mineral Fertilization. For each variable, means followed by the same lowercase letter in the row and uppercase in the column, do not differ statistically from each other, at 5% probability, using the Scott-Knott test.

30:1. Temperature and humidity were monitored and compost cells were systematically revolved.

To obtain VSS, precomposed SS mixed with grass pruning was used after the thermophilic phase (approximately one month after the beginning of the decomposition process) as a substrate for vermicomposting with California red earthworms (*Eisenia foetida*). LSS was obtained by adding lime to SSS, corresponding to 50% of the dry sludge mass. After adding and mixing, the humidity was raised to 70%.

Fertilization was performed by manual incorporation of the different types of SS into the soil up to a depth of 20 cm in the planting grooves. The experimental plots consisted of four 3-m-long rows spaced 1-m apart. The useful plots were the two central rows, disregarding 0.5 m from each end. The planting was carried out in grooves by placing three seeds in each sowing site at a distance of 0.5 m from each other. Fifteen days after emergence, we pruned the plants, leaving only one for further growth. We employed manual weeding and sprinkler irrigation for maintenance.

Evaluated parameters and chemical analysis

As the crops began to flower, approximately 45 days after emergence, the fourth leaf was collected from the apex of the plants in accordance with the methodologies of Malavolta et al. (1997). After harvest, approximately 150 days from planting, eight soil sub-samples per plot were collected from between plants in the rows from the depths of 0-20 cm (surface layer) and 20-40 cm (subsurface layer) to form composite samples. In the collected plant and soil samples were analyzed the heavy metals Zn, Cu, Pb, Cr, Cd, and Ni.

The analysis of heavy metals in leaf, soil, SS and SS products was carried out according to the methodologies of Tedesco et al. (1995), while in chemical fertilizers, they were in accordance with the methodologies of Alcarde (2009). Readings the heavy metals were performed using a Varian atomic absorption spectrophotometer, model AA 240, with the following quantification limits: Zn = 0.075 mg L⁻¹, Cu = 0.1 mg L⁻¹, Pb = 0.5 mg L⁻¹, Cr = 0.625 mg L⁻¹, Cd = 0.3 mg L⁻¹, and Ni = 0.5 mg L⁻¹.

Statistical analysis

The data obtained were tested for residual normality, by the Shapiro-Wilk test, and homogeneity of variances, by the Oneillmathews test, at 5% significance. Subsequently, data with normal distribution and homogeneous variances were submitted to analysis of variance and Scott-Knott test at 5% significance. The

calculations and the statistical analysis were performed using the gdata and ExpDes.pt packages of the R 3.3.0 Program (R CORE TEAM, 2019).

RESULTS AND DISCUSSION

The application of sewage sludge (SS) influenced the level of Zn in the soil mainly in the 0-20 cm surface layer (Table 2); the Zn level increased after treatments with solarized sewage sludge (SSS), composted sewage sludge (CSS), and limed sewage sludge (LSS) compared to the other treatments. As the treatments with SS were applied based on the level of available N, the amounts of organic fertilizer were different between treatments, ranging from 4.9 to 33.61 mg ha⁻¹, resulting in additions of different quantities of elements to the soil. Table 1 shows that fertilization with CSS and LSS resulted in the highest contributions of Zn to the soil, which justifies the highest availability of Zn in the soils of these treatments. VSS added to soil less than 50% of the amount of Zn added by the CSS and LSS treatments, which was not enough to change the concentrations compared to the Control (CO) and mineral fertilization (MF) treatments.

Regarding to LSS, with a soil pH of 7.6, the availability of Zn may have been overestimated as the extractor used was acidic and may have solubilized the forms bound to hydroxides and carbonates (NACHTIGALL et al., 2009; OLIVEIRA et al., 2018). Soil pH for the other treatments ranged from 5.8 to 6.0 in a narrow range, and may have led to greater availability of Zn for plants. The differences can be attributed to the quantities and effects of metal complexation by organic matter.

In the case of SSS treatment, the addition of Zn to the soil was lower than that with other SS treatments (Table 1), with higher availability possibly associated with a higher mineralization rate. SS digested anaerobically and incorporated into the soil under field conditions with favorable humidity and temperature (Figure 2) had mineralization rates of more than 90% (MATOS et al., 2017; SILVA et al., 2019).

Although, SS and mineral fertilizers (Table 1) contain Cu and Pb, the levels of these elements in the soil were not influenced by the treatments (Table 2). However, Cu level was higher in the surface layer (0-20 cm), and Pb level was equal in the two evaluated layers. There are positive correlations between concentration in 0-20 and 20-40 cm layers to Cu and Zn elements, indicating a possible migration of the elements in the soil profile, observed even after 150 days of organic fertilizer application.

The metals Cd, Cr and Ni were detected in SS and chemical fertilizers (Table 1); however, their levels in the soil (Table 2) were below the quantification levels by the analytical method used. Zn and Cu levels in the soil were lower than those before fertilization (Table 1), while the Pb level remained unchanged. This may be because of higher losses of Zn and Cu due to leaching and absorption by plants as well as changes to unavailable forms.

The slight increase in heavy metal contents in the soil with the application of SS (Table 2) was because these elements were also present at low levels in the sludge (Table 1), all below the maximum allowable concentration (MAC) in Class 1 and Class 2 SS or derived products established by CONAMA Resolution No. 498 (BRASIL, 2020). This resolution defines criteria and procedures for the production and application of biosolids in soils in Brazil. The Pb, whose addition to the soil was $9.59 \text{ kg ha}^{-1} \text{ yr}^{-1}$ with a dose of 33.61 Mg ha^{-1} of LSS, is the element that application is closest to the maximum annual load allowable ($15 \text{ kg ha}^{-1} \text{ yr}^{-1}$). Considering the concentration of the elements in the fertilizers and that the doses used were applied annually, as well as the maximum load allowable for non-degraded soils, according to the aforementioned resolution, Pb is also the element that first limits the application of these fertilizers, with 791, 103, 196 and 31 years of successive applications of the same doses of the SSS, CSS, VSS and LSS fertilizers, respectively.

Several studies have highlighted the risks of contamination with heavy metals from the agricultural use of SS (NASCIMENTO et al., 2015; SHARMA et al., 2017; EID et al., 2018; MOTA et al., 2018; FEIZI et al., 2019; ZUBA JUNIO et al., 2019). However, the degree of contamination depends on the composition of SS, rate of application of sludge, characteristics of the soil where it is applied, crop species planted, and type of management (LATARE et al., 2014; DUAN & FENG, 2022). In this study, we application of SS to the soil led to a low increase in available heavy metals in the soil similar to that observed with MF. This indicated that the use of stabilized sludge as an organic fertilizer in the castor bean crop has a low environmental impact and can lead to savings when used in place of mineral fertilizers.

With regard to the levels of heavy metals in the castor bean plant, the treatments only influenced the levels of Zn, Cu, and Ni in the leaf tissues (Table 3). The levels of Zn and Cu in the leaf tissues were higher with the CO and SSS treatments, while the level of Ni was generally high with all fertilization treatments (Table 3). The levels of Zn and Cu were

higher in the leaf limb than in the petiole, while the levels of Ni, Pb, and Cr were higher in the petiole than in the leaf limb.

Although, the contribution of heavy metals with the application of SS was reasonable, it was not enough to cause high levels in the plant. The lower levels of Zn and Cu observed in the leaf limb with the application of SS and MF may be associated with higher plant yields in these treatments, which were 1.94 Mg ha^{-1} in the control and 2.82 Mg ha^{-1} in the mean of the treatments, leading to dilution of these elements. The only exception was SSS, which had a yield similar to that of other fertilization treatments but led to higher levels of Zn and Cu in the leaf limb. Thus, despite dilution, the availability of Zn and Cu was higher with SSS because organic matter is barely humified and is subject to a higher rate of mineralization, thereby presenting the elements in more soluble forms, such as those that bind to fulvic acids (KANG et al., 2011; INGELMO et al., 2012).

From a nutritional point of view, Cu and Zn levels present in the leaf limb were generally within or near the ranges considered appropriate, i.e., from 4 to $10 \mu\text{g g}^{-1}$ and 15 to $40 \mu\text{g g}^{-1}$, respectively, according to the recommendations of Oliveira (2004). In this case, the maximum limits verified for Zn were detected even with treatments without fertilization.

Ni was below the limit of detection in the soil using spectrophotometry; however, its presence was verified in the leaf tissues of the castor bean plant (Table 3). Ni level in the leaf tissues was higher with SSS, CSS, VSS, and MF treatments. In addition, Ni was notably more concentrated in the petiole than in the limb or blade; in the leaf limb, it was within the range of 0.01 and 10 mg kg^{-1} of dry matter of the plant, which is considered appropriate from a nutritional point of view for plants, whereas the level of Ni in the petiole ranged from 147 to 168 mg kg^{-1} . This indicated that castor bean plant has efficient chemical mechanisms in the leaf that prevent the movement and excessive accumulation of Ni in the leaf limb (BROADLEY et al., 2012).

Cd and Cr were also below the limit of detection in the soil using spectrophotometry. However, there was an equal distribution of Cd in the petiole and leaf limb, while Cr was only concentrated in the petiole (Table 3). Pb was quantified in both the soil as well as the petiole and leaf limb of the castor bean plant. However, the level of Pb was higher in the petiole.

The level of Cd in the petiole and leaf limb was below the critical level of toxicity, which is 5 mg kg^{-1} dry matter, while the level of Pb was

Table 3 - Content of heavy metals in leaf blade (LB) and petiole (PE) of castor bean in response to the application of mineral fertilizer and differently stabilized sewage sludge.

Metal	Part of the plant	CO	SSS	CSS	VSS	LSS	MF	Average
		µg g ⁻¹						
Zn	LB	54.80 Aa	50.55 Aa	40.80 Ab	38.05 Ab	31.30 Ab	31.80 Ab	-
	PE	47.80 Aa	41.30 Ba	33.55 Ba	26.80 Ba	37.05 Aa	26.05 Aa	-
	Average	-	-	-	-	-	-	-
Cu	LB	5.00	5.50	4.25	4.50	4.75	4.75	4.79 A
	PE	4.00	3.50	3.00	3.25	3.25	2.75	3.29 B
	Average	4.50 a	4.50 a	3.63 b	3.88 b	4.00 b	3.75 b	-
Pb	LB	25.19	23.69	21.34	22.01	18.22	15.88	21.06 B
	PE	28.15	26.93	29.98	33.02	32.41	31.80	30.38 A
	Average	26.67 a	25.31 a	25.66 a	27.51 a	25.32 a	23.84 a	-
Ni	LB	6.03	5.95	6.43	6.19	6.19	7.62	6.40 B
	PE	147.68	168.90	168.09	168.09	157.02	160.27	121.68 A
	Average	76.86 b	87.02 a	87.26 a	87.14 a	81.61 b	83.95 a	-
Cd	LB	0.65	0.46	0.83	0.63	0.89	0.80	0.71 A
	PE	0.65	0.37	0.60	0.53	0.60	0.53	0.55 A
	Average	0.65 a	0.42 a	0.72 a	0.58 a	0.75 a	0.67 a	-
Cr	PE	66.70 a	75.00 a	68.80 a	62.50 a	62.50 a	62.50 a	-

CO - Control; SSS - Solarized sewage sludge; CSS - Composted sewage sludge; VSS - vermicomposted sewage sludge; LSS - limed sewage sludge; MF - Mineral Fertilization. For each variable, means followed by the same lowercase letter in the row and uppercase in the column, do not differ statistically from each other, at 5% probability, using the Scott-Knott test.

above 16 mg kg⁻¹, which is considered excessive in plants. The level of Cr was well above 1 mg kg⁻¹, which is considered the phytotoxic limit in many plant species (ZEITOUNI et al., 2007; KABATIA-PENDIAS, 2011).

It should be noted that the average Pb content was about twice in the petiole than in the leaf limb, while the average Ni content was about 19-fold higher in the petiole than in the leaf limb. Cr was detected only in the petiole. This can be attributed to active transpiration that leads to the mobilization of these elements via the xylem to the aerial parts of the plant with ion exchange and adsorption, with a chromatographic distribution of metals towards the leaf blade and the top of the plants. However, the formation of stable complexes of these elements with organic acids in the petiole may also be considered (ADHIKARI & KUMAR, 2012; BROADLEY et al., 2012).

With regard to the elements present at higher levels in the leaf limb than in the petiole, higher mobility can be attributed to the formation of chemical bonds with low-molecular weight organic

compounds present in the xylem fluid (BROADLEY et al., 2012; BURSZTYN F et al., 2018).

SS is an organic waste that can be used as a low-cost fertilizer with a low environmental impact. In general, the uptake of metals and organic contaminants does not appear to cause a significant hazard to the plants and the concentrations do not surpass the maximum values allowed in soil (SELEIMAN et al., 2020). In this study, no heavy metals were found to be available at high levels or at levels above the allowed limit that pose a risk to the sustainable production of castor bean crops.

CONCLUSION

Different sewage sludge (SS) stabilization processes result in different levels of heavy metals in the soil and in the plant. The main elements influenced are Zn in the soil and Zn, Cu and Ni in the leaf tissue of castor bean plants.

Therefore, a single SS application, no matter the stabilization treatment, does not present risk of contamination of soil or castor bean plants

with heavy metals. However, assessments of sequential applications in long-term experiments are highly recommend.

As the SS did not represent environmental risk and taking into consideration just the expense of time and energy, the application of SSS can be worthwhile, as it does not require additional treatment.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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