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Use of Vermicompost in Pepper Cultive in Cd Contaminated Soil

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Abstract

The recurring presence of heavy metals in the soil may contaminate vegetables that are subsequently consumed by humans. Depending on the concentration, the presence of substances such as cadmium (Cd) in the human body can lead to many diseases. Due to this, it is important to conduct studies that provide quantification and removal possibilities of these elements in contaminated soils. In this sense, the aim of this paper is to evaluate the use of vermicompost to reduce Cd uptake in pepper and study of the effects in the plants growing. To conduct the experiments, plastic worm reactors containing earthworms (*Eisenia andrei*) were prepared and filled with different organic matters. After 60 days with controlled temperature and humidity, the vermicompost was collected and mixed with Cd contaminated soil in different proportions, such 0:100; 25:75; 50:50; 75:25 and 100:0 of vermicompost:soil, respectively. After, plants of *Capsicum annuum L.* were cultivated and 70 days later, the plants were harvested, cleaned, measured, weighed and dried. Moisture, height, fresh and dry masses, fruit protein and Cd were analyzed in the vegetables.

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The results showed Cd presence in the plants affected the development of the peppers' shoots and caused a reduction fruits production. Using vermicompost mixed with the soil improved plant development and uptake Cd was reduced to the plants. The best results of plant development were reached in proportions 50% of vermicompost. In conclusion, it is possible to grow pepper on soil contaminated with Cd provided it has a certain amount of vermicompost in the substrate**.**

Keywords: Heavy metal; Contaminated soil; Vermicomposting; *Capsicum annuum* L.

1. Introduction

Cadmium (Cd) is a contaminant found in human foods because of its high rates of mobility in the soil. Depending on the concentration, this metal may cause damage to cells, critical renal and kidney problems, and increased cancer incidence in humans [1]. Cd can be taken up from soil by plants, which in turn accumulates in the food chain and human bodies [2,3]. Chilies are one of the earliest domesticated plants and they can be found in different forms in terms of size, thickness and colour. It is a fresh eaten vegetable and it has become a chief export product in agriculture, since it has many beneficial properties for consumers [4]. There are some studies about the effect of Cd in peppers, and it can produced a significant inhibition of growth, net photosynthesis, and water use efficiency and contamination dangerous for the consumers [5]. Thus, developing efficient techniques to reduce heavy metal accumulation of vegetable crops in metal-contaminated soils is urgently needed. There have been several studies that demonstrate the use of organic matter for the fertilization of soil to improve pepper cultivation [6], and there are studies in the literature that describe the application of this organic matter to reduce Cd uptake in some vegetables [7]. Thus, the aim of this paper is to evaluate the use of vermicompost to reduce Cd uptake in *Capsicum annuum L.* and study of the effects in the plants growing. The physical and chemical characteristics of the plant and soil, with and without vermicompost, were studied. Moisture, shoot height, shoot fresh mass, shoot dry mass, fruit production, fruit protein and Cd concentration were evaluated.

2. Material and Methods

2.1. Vermicomposting process and mix of soil and vermicompost

The vermicomposting process was performed using organic waste mixed with clayey soil in a proportion of 50% clay soil, 20% fruit peels, 10% dry leaves and 20% cow manure. All organics and the soil were put in a plastic vermireactor with dimensions of 0.20 x 0.40 x 0.58 m. 100 earthworms (*Eisenia andrei*) were incubated in each box. After 60 days of vermicomposting at 25 °C and with controlled humidity (60 a 70%), the vermicompost was ready to mix with the cultivation soil (with 30 $mg.kg^{-1}$ of Cd and without), composing the substrate. The substrate was composed of different proportions of vermicompost:soil (without and with Cd) using 0:100; 25:75; 50:50; 75:25 and 100:0, respectively.

2.2. Substrate characterization

Substrate characteristics (soil with vermicompost in different proportions) were analysed in accordance with Empresa Brasileira de Pesquisa Agropecuária [8]. The parameters evaluated were: wet density, dry density, apparent humidity, electric conductivity, total porosity, aeration space, easily available water, buffer water, water remaining and water retention capacity and Cd concentration.

2.3. Capsicum annuum L. growth

Capsicum annuum L. seedlings were produced in polypropylene trays expanded filled with organic substrate and taken to filed 30 day after sowing. For this, the seedlings of *Capsicum annuum L.* were transferred to a plastic vessel containing 5 kg of different proportions of vermicompost and soil (n = 25 for each test). The experiment was conducted in a greenhouse with controlled temperature and humidity. After a period of 70 days, the plants were harvested, cleaned, measured with a calliper, weighed (for the wet weight determination), and dried at 65 °C for 24 hours (for the dry weight determination). The fruits were separated and counted to evaluate the effects on fruit production. The roots, shoots and the fruits were separated for further Cd analysis.

2.4. Capsicum annuum L. physical and chemical characteristics

To determine the moisture content for all samples, each sample was dried in a hot air oven at 100 ± 5 °C to a constant weight according to AOAC method 950.46. The ash was determined by incineration at 550 °C according to AOAC method 930.30. The protein content in the fruit was analysed by the Kjeldahl method according to AOAC 991.20. These analyses were performed using methods described by authors in [9].

2.5. Sample preparation for cadmium determination

All chemicals used were analytical-grade reagents, and ultrapure water (Milli-Q, Millipore, USA) was used for solution preparation. For cadmium determination, 0.5 g of the dried sample was digested with 6 mL of 65 % HNO_3 (Merck) in a closed vessel in a microwave oven (Anton Paar, multiwave PRO). Each sample was analysed in triplicate. The samples were digested in a microwave digestion system, according to the following program: 150 °C, step 1 (time: 2 min, power: 250 w), step 2 (time: 2 min, power: 0 w), step 3 (time: 6 min, power: 250 w), step 4 (time: 5 min, power: 400 w) and step 5 (time: 5 min, power: 600 w). The digested samples were filtered using Whatman filter papers pore size 0.45 μm and then transferred to polypropylene tubes, with total volumes adjusted to 50 mL by adding ultrapure water. The samples were analysed using inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer, NexION 300x). The limit of detection (LOD) of the ICP-MS was 0.06 ng/L for Cd. A blank soil (0,2 mg/kg Cd) was used and the methodology for Cd determination was the same cited above. To evaluate the confiability of the methodology, reference material - NIST-SRM 695-Trace Elements in Multi-Nutrient Fertilizer (National Institute of Standards and Technology, Gaithersburg, MD, USA) was used. The samples were digested and Cd was determined by ICP-MS too. Standard stock solutions containing 10 mg/L of Cd (Perkin Elmer) were prepared and used for the calibration curve.

2.6. Statistical analysis

Completely randomized experimental design was used in the experiments, using two formulation criteria for a 2×5 factorial, with 3 repetitions by 3 plants, for each treatment. The first factor was the presence or absence of

Cd, and the second was based on the different proportions of vermicompost in the soil (0, 25, 50, 75 and 100%). The analysis of variance (ANOVA) with post-hoc DMS multiple comparisons and polynomial regression were obtained using Costat 6.4 and SigmaPlot 11.0 softwares. In addition, Pearson's coefficient was utilized to relate the influence of the Cd in the Zn absorption in fruits. Since the shoots fresh and dry masses and fruit moisture did not respond using ANOVA, these variables were transformed to Asen (√(x)).

3. Results and discussion

3.1. Characteristics of substrates with different proportions of vermicompost

For the recovery of Cd determination in the proportions of vermicompost and in the reference material was around 96%, showing the confiability of the methodology. The characteristics of all proportions of vermicompost and soil were evaluated (Table 1).

Table 1: Physical and chemical characterization of substrates with different ratios of vermicompost and soil used in the experiment

Parameters	**Proportions***				
	1	2	3	4	5
WD (Kg m^{-3})	1149.43	963.11	797.94	751.78	792.33
DD (Kg m^{-3})	379.39	274.71	176.76	129.62	61.67
AH (%)	67.79	71.75	77.95	82.64	92.04
pH (1:5 – in water)	5.80	5.30	6.05	6.18	8.62
EC (mS cm^{-1}) - 1:5 – in water)	0.08	1.62	2.01	1.86	2.01
TP (%)	62.15	65.64	79.15	87.83	97.72
AS (%)	6.52	15.04	16.45	21.40	26.55
EAW (%)	6.08	13.29	15.29	15.48	6.97
BW (%)	3.47	3.60	5.62	4.92	3.51
WR (%)	46.07	33.70	41.79	46.03	60.70
WRC 10 (%)	55.63	50.60	62.70	66.43	71.17
WRC 50 (%)	49.55	37.30	47.41	50.95	64.20
WRC 100 (%)	46.07	33.70	41.79	46.03	60.70
Total Cd (mg/kg)	2.90	2.90	2.91	2.92	2.90

***1:** 0% vermicompost; **2:** 25% vermicompost; **3:** 50% vermicompost; **4:** 75% vermicompost; e **5:** 100% vermicompost; at 10, 50 and 100 cm.

WD, wet density; DD, dry density; AH, apparent humidity; EC, electric conductivity; TP, total porosity; AS, aeration space; EAW, easily available water; BW, buffer water; WR, Water remaining; WRC, water retention capacity.

The increase in vermicompost proportion in the different treatments proportionally reduced the dry density of the substrate, from 379 $kg.m^{-3}$ with 100% soil to 62 $kg.m^{-3}$ with 100% vermicompost. It is important to note that the dry density is inversely proportional to the porosity. This indicates that the vermicompost has greater porosity than the cultivation soil used in the mixture to form the substrate, consequently, higher levels of vermicompost result in greater porosity and less dry density. About the results of Table 1, according to authors in [10], suitable values of dry basis density should be defined according to the size of the container, ranging from 100 to 300 $kg.m^{-3}$ for multicellular trays, from 200 to 400 $kg.m^{-3}$ for pots up to 15 cm in height, from 300 to 500 $kg.m^{-3}$ for vessels of 20 to 30 cm in height, and from 500 to 800 $kg.m^{-3}$ for vessels larger than 30 cm in height. In the present study, the use of vermicompost in the mixture, in any proportion, permitted obtaining substrates that can be used both for trays and pots up to 15 cm. The adequacy of the mixtures in terms of the total porosity variable was also verified. As with the aeration space results, the highest proportions of vermicompost in the mixture resulted in values closest to those considered adequate (80-90%), as given by authors in [10]. However, all mixtures presented percentages above ideal for water retention (>30%), resulting in poor drainage due to the high retention of water unavailable to the plants. The porosity of the substrate is responsible for gas exchange, movement and drainage of water, and must be carefully observed to obtain an adequate substrate. According to authors in [10], pH of the substrates should be between 5.2 to 5.5. The pH correction is relatively easy, just add acidic substrates, or sulphur to reduce the pH or calcium carbonate (agricultural limestone) to increase the pH, that are low cost products and are readily available in the market [11, 12]. In the present study, the higher proportions of vermicompost in the tested treatments caused an excessive increase in pH, but the plants developed normally, indicating that pepper is not sensitive to high pH values. With the addition of organic matter to soil improves its chemical, physical and biological qualities. For this reason, soil pH is the most influential factor affecting Cd uptake by plants grown on Cd contaminated soil [13]. The possible reason for this is increase pH due to mineralization of carbon, OH^{-1} ions production by ligands exchange and the release of basic cations such as Ca^{+2}, K^{+} and Mg^{+2}. In addition, reactive groups, such as phenoxyl, carboxyl and hydroxyl from the organic matter can react with Cd forming stable complexes [14]. Some papers show the decrease Cd uptake from vegetables using organic matter [15]. The results in the present paper are in accordance with the others. The Figures 1 shows the growing of peppers with and without Cd in the soil using different proportions of vermicompost in the soil. With the increase of vermicompost, it is possible to see the reduction of Cd effect in the plants. Probably, the vermicompost compounds (such as mineral and humic acids) reacted with Cd and reduced the uptake of this element in the pepper.

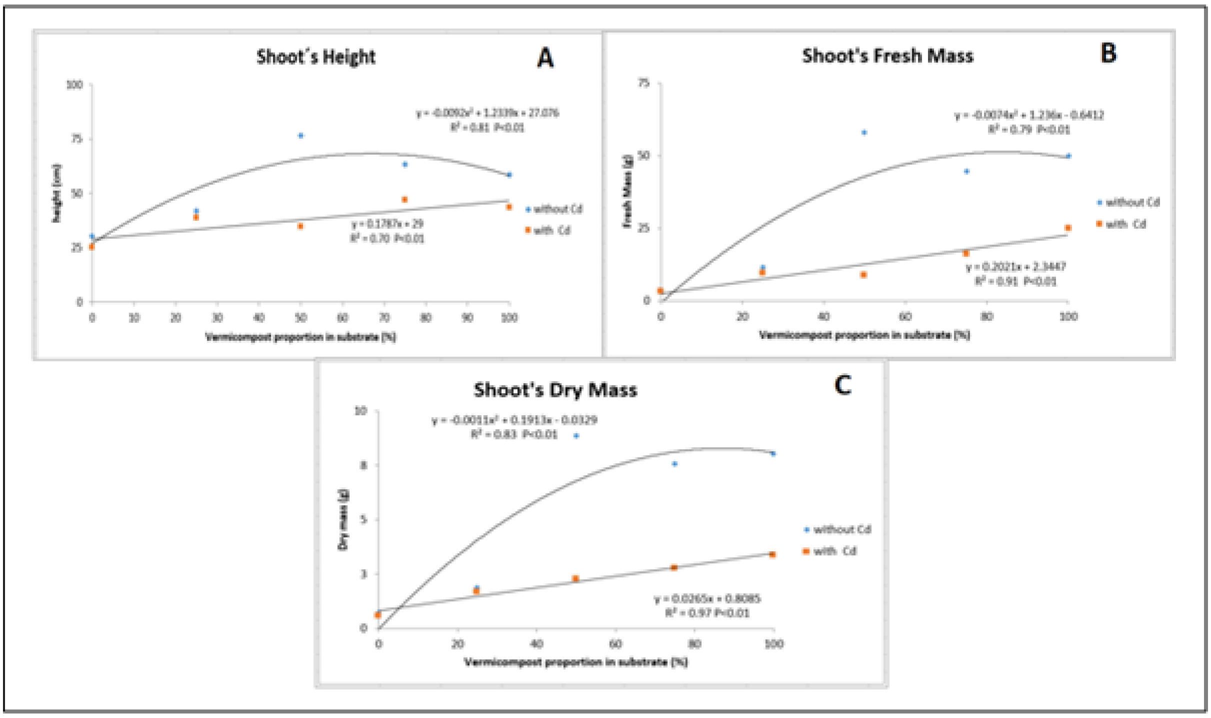

Figure 1: height (A), fresh mass (B) and dry mass of the shoot (C) of *Capsicum annuum* plants as a function of the presence of cadmium and vermicompost proportions in the substrate, at 70 days after the start of the experiment

Another remarkable characteristic of the vermicompost tested was its high electrical conductivity, corresponding to the salinity present in the material. According to authors in [16], the conductivity range suitable for most crops is between 0.36 and 0.65 $mS.cm^{-1}$, which is below all treatments in the present study, indicating the need for criteria for its use since there are species that do not tolerate higher rates of salinity [10]. In this case, materials with high electrical conductivity may be used but with restrictions. They should be avoided, especially in the stages of establishment of plants (sowing, rooting and establishment of crops) [17]. It was possible to see the reduction in fruit mass, height, fresh mass, and dry mass with vermicompost proportion greater than 50% in experiments without Cd (Figures 1 and 2). This indicates that amounts above 50% of vermicompost in the composition of a substrate can result in damages in the formation of the fruits.

3.2. Plant growth and fruit analysis of Capsicum annuum L.

For the variables height, fresh and dry masses of the plants shoots and protein content in the fruits, statistical analysis of the data showed that there was interaction between the different proportions of vermicompost and the presence of Cd in the substrate. Additionally, the different proportions of vermicompost in the substrate influenced all variables in fruit evaluation and plant growth, except for ash content (Table 2).

Table 2: Analysis of variance of fruits and shoots of *Capsicum annuum* plants as a function of the presence of cadmium and the vermicompost proportions in the substrate, at 70 days after the start of the experiment

	Fruit analysis				**Shoot's analysis**		
	Prod.	**H**	**A**	**P**	**HE**	**FM**	**DM**
Cadmium presence	0.417	0.125	0.856	<0.01*	<0.01*	<0.01*	<0.01*
Vermicompost proportion on substrate	0.036**	<0.01*	0.523	<0.01*	<0.01*	<0.01*	<0.01*
Interaction	0.965	0.141	0.754	<0.01*	<0.01*	<0.01*	<0.01*
DF Error	26	18	16	18	20	20	20
VC (%)	30.72	0.95	21.07	8.98	5.76	8.08	13.54

*Significant difference to 99% reliability; **Significant difference to 95% reliability.

DF, degree of freedom; VC, variation coefficient; Prod, productivity; H, humidity; A, ash; P, protein; HE, height; FM, fresh mass; DM, dry mass**.**

The variables related to plant development reached higher values in vermicompost proportions until 50% (Figure 1). According to authors in [18], an ideal substrate should contain 20 to 30% of aeration space. Only treatments with 75 and 100% of vermicompost reached these values, demonstrating the possibility of using vermicompost as a conditioner of this physical characteristic in substrates. Under cultivation conditions, this variable directly affects irrigation management, since the characteristics of the material in relation to its water retention capacity determines the need for irrigation. In relation to plant height, shoot's fresh mass and shoot's dry mass (Figure 1A, 1B and 1C, respectively) when vermicompost percentage increased without Cd in the soil, the increase presented a quadratic tendency and when Cd was present in the soil, there was a linear tendency increased, showing the negative influence of this metal in the pepper growing. This effect was related to other papers using pepper [19] and using different vegetables [20]. This fact is probably caused by the high concentrations of Cd may cause the reduction of other nutrients essential by uptake competitive effect and the bioaccumulation of Cd can cause the alteration of various physiological functions [20].

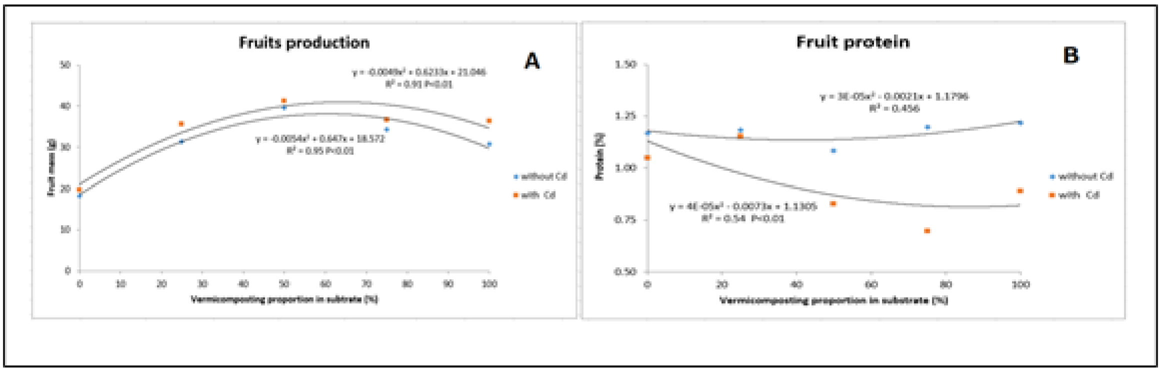

Figure 2: Fruit production (g) (A) and protein content in the fruits (%) (B) of *Capsicum annuum L* plants due to the presence of cadmium and the proportions of vermicompost in the substrate, at 70 days after the start of the experiment

In relation to fruit analysis, the yield and moisture were influenced only by the proportion of vermicompost present in the substrate, presenting a quadratic increase with increasing vermicompost percentage added to the soil for substrate composition (Figure 2A). The protein content of the fruits was influenced by both cadmium and vermicompost proportions (Figure 2B), with a higher concentration in the absence of Cd.

The presence of Cd in the substrate influenced the content of the fruits, shoot and root system of the pepper plants. The interaction between the factors tested and the effect of vermicompost proportions on the Cd content was checked in Table 3.

Table 3: Variance analysis of cadmium in the substrate, in the plants and fruits of *Capsicum annuum* as a function of the presence of this nutrient and the proportions of vermicompost in the substrate, at 70 days after the start of the experiment

	Cd in fruit	**Cd in the shoots**	**Cd in the root**	**Cd substrate w/ the plant**	**Cd substrate w/o the plant**
Cadmium presence	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*
vermicompost proportion on substrate	0.062	<0.01*	0.625	0.6149	0.357
Interaction	0.896	0.019*	0.423	0.725	0.601
VC (%)	51.91	43.24	29.24	83.52	94.22

*Significant difference at 1% probability of error; ** Significant difference at 5% probability of error.

Cd, cadmium; w/, with; w/o, without; VC, variation coefficient.

When the proportion of the vermicompost was increasing in the substrate, there was a decreasing of the Cd content in the fruit (Table 4). It was possible to see the importance of vermicompost in the substrate that reacts with this element and prevents its transfer to the fruits. Once, vermicomposting process can transform the bioavailable metal fractions to their recalcitrant forms [21].

Table 4: Cd in fruit in the substrates with different ratios of vermicompost and soil

Parameters	**Proportions***				
	1	2	3	4	5
Cd (mg/kg)	0.16	0.12	0.09	0.06	0.05

*Significant difference at 1% probability of error; ** Significant difference at 5% probability of error.

In relation to plant growing and fruit analysis, adding organic materials to the substrate for plant cultivation is favourable, because they improve the physical and chemical characteristics of the substrates, and therefore increase the supply of nutrients to the plant [22]. This fact can be observed in the production of *C. annuum*, because with the increase of vermicompost content in the substrate, an increase in height, fresh and dry masses of the shoots was noticed. The results showing superior development of the pepper plants with the use of vermicompost to condition the substrate agree with those found in studies with *Ilex paraguariensis* [22] and *Apium graveolens L. var. rapaceum* [23]. In relation to the yield and moisture of fruit analysis, the ash content was not influenced by any of the factors tested in the experiment. Similar results were verified by [24], who verified higher fruit yield ($kg.ha^{-1}$) of tomatoes when using vermicompost in the substrate composition, respectively. Cd can influenced fruit protein results. That is because Cd exerts its toxicity through membrane damage and the inactivation of enzymes, possibly through reaction with SH-groups of proteins. For the treatments with Cd in the substrate, its highest accumulation in the fruits is due to its translocation from the shoots to the fruits via phloem-mediated transport. This is because Cd is absorbed by the roots and small amounts of it are transported to the shoot and then accumulated in the leaves, which are the primary sources of sugars [25]. When there was an increasing of vermicompost in the substrate with or without Cd, up to 50%, there was an increasing of the yield. However, using a proportion above 50% of vermicompost, there was a decrease in the growing of the plants and on fruit production. This fact can be due to excessive organic matter and high salts concentration. Once salinity can be considered as one of the most serious plant nutrition problems and this may be damaging the fruit production. Hence, the use of these organic fertilizers in agriculture requires greater care about the composition and regarding the definition of doses to be applied in the crops [26].

3. Conclusions

The variables related to the development of the plants were superior when vermicompost proportions were up to 50%. The presence of Cd in the substrate reduced the production and protein in the fruits and influenced the shoot and root system of the pepper. In substrates containing Cd, when vermicompost was increasing, there was a reduction of Cd uptake in the plants. This study may support researches and farming of plant crops conducted in vermicompost-containing environments, where heavy metals are present. Additionally, this research shows the importance of the use of vermicompost in soil to improve the quality of nutrients for growing peppers. The greatest advantage in the use of vermicompost as a material for substrate composition is the ease of obtaining raw material to perform the composting process, since there is a high availability of such substances. In addition, vermicompost production procedures have relatively low costs and they offer a high-quality alternative substrate.

4. Limitations

This study described the relationship between the use of vermicompost in reducing the absorption of Cd by peppers and the influence on plant growth, using earthworms (*Eisenia andrei*) to obtain the vermicompost, consisting of 50% clay soil, 20% fruit peels, 10% dry leaves and 20% bovine manure, with temperature and humidity control. Thus, it is not possible to state that vermicomposites with different compositions or obtained by different methods have same effectiveness in reducing the absorption of Cd by peppers of the same species and in the growth of plants. Still, factors such as conditions of growth and development of seedlings and fruits, as well as the use of other plant species can provide different results from those obtained in the present study.

5. Recommendations

Our study will be a useful reference in future studies on the effect of vermicompounds on the absorption of Cd by peppers of the species *Capsicum anuum L*., being important the study of the absorption of heavy metals in food crops, due to possible damage caused to health when consumed. Additional research on the contents of humic and fulvic acids in order to verify the interaction with the absorption of Cd is necessary. Still, tests that assess the influence on the absorption of heavy metals using the vermicompost obtained in this study for other plant species are of great interest to society.

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