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Evaluation of Cadmium Absorption by Pepper *Capsicum Annuum* involving Microwave Induced Plasma Atomic Emission Spectrometry (MIP-AES)

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ABSTRACT
Contamination by toxic metals in vegetables destined for consumption has been caused recurrent concerns due to the cumulative effect on the human body, resulting in risks to health. The aim of this work was to evaluate the absorption of cadmium by roots and fruits of green and red square peppers cultivated in solutions 0.00; 5.00; 10.00 and 15.00 mg $^{L-1}$ CdCl2.2H2O (cadmium chloride dihydrate p.a.). Microwave induced plasma atomic emission spectrometry (MIP-AES) was employed for the cadmium quantification in the samples. The results of cadmium absorption by the roots and fruits of the red square pepper were 0.00; 0.25; 1.50 and 1.99 and 0.00; 0.00; 0.08; 0.19 mg $^{L-1}$ and for green pepper, 0.00; 0.33; 1.67 and 1.99 mg $^{L-1}$ and 0.00; 0.00; 0.09 and 0.17 mg $^{L-1}$, respectively. The increase in the concentration of contaminant solutions applied to the soil resulted in higher rates of cadmium absorption by the roots in relation to the fruits of both pepper varieties. The results were promising, according to the proposed curves and adjustments (0.87 > R > 1.0), generating predictability in the ranges of concentrations evaluated.

Keywords: Toxic metals, Pepper.

1 INTRODUCTION

Plants of the solanaceae family are among the most cultivated vegetables in the Southeast, Northeast and Midwest regions of Brazil because they encompass several species of vegetables, such as peppers, peppers, soybeans, eggplant, tomatoes, potatoes, corn, among others. It is estimated that the average Brazilian production is 555 thousand tons/year for peppers [1], which belong to the genus Capsicum, whose name is derived from the Greek Kapso, meaning to sting or burning [2], and are classified according to size, color, and odor. They have important nutritional parameters, containing the minerals potassium, calcium, sodium, iron and phosphorus and vitamins A, B1, B2, B5 and C in their composition [3].

The incessant search for improving the quality of life has resulted in a considerable increase in the consumption of vegetables, driving the development of agricultural and industrial practices that ensure rapid productivity, food quality and food security. In this context, the use of agricultural inputs is increasing, since fertilizers provide essential nutrients for plant development, and pesticides prevent exposure to pests and diseases in crops, improving production [4]. However, pesticides may contain toxic metals in their composition, increasing the risk of contamination of soils, crops, water, and vegetables intended for consumption [5]. Among the toxic metals present in pesticides, copper, lead, cadmium and chromium are considered dangerous due to their cumulative effect. In a study carried out with the application of pesticides in wineries, it was shown that the cultivation soil accumulated about 3.2 g kg-1 copper, 12.8 g kg-1 zinc and 1.6 g kg-1 In the case of lead, however, the accumulation of chromium and cadmium was eight times higher than the values cited for zinc and lead [6].

According to the Program for the Analysis of Pesticide Residues in Food (PARA), the peppers were analyzed for the first time in 2008, and about 60% of the samples showed concentrations of various pesticides above those stipulated by the MRL - Maximum Residue Limit, established by ANVISA. In 2011, chemical analyses showed that 84% of pepper samples were contaminated by pesticide residues not authorized for use in agriculture and 0.9% of them contained pesticide concentrations above the MRL. The total number of contaminated samples was 90%, and twenty different types of pesticides were detected, including 17 insecticides and 3 fungicides, which contained toxic metals in their composition [7].

The accumulation and translocation of toxic metals in soils can result in the contamination of leafy vegetables and legumes by absorption by the roots, followed by their transport to the stem, leaves and fruits, thus reaching the food chain. The uptake of these metals by plants is selective and transport to the upper regions of the plant occurs via xylem and ionic form, and via phloem to the leaf and fruit regions [8]. The main physicochemical parameters related to the absorption of toxic metals by plants are mainly pH, temperature and concentrations of calcium, zinc, iron, manganese and magnesium [9]. The transfer of toxic metals from the soil to the plant is evaluated by the transfer factor (F), and the decreasing order of this index is cadmium > cobalt > lead > nickel > copper > zinc > iron for most plant species [10]. As the bivalent form of cadmium is very soluble in soil solution and has higher F, it can then be easily transferred to plants and accumulate in specific regions [11]. In the process of transferring the roots to the upper regions of the plant, there are stages of immobilization of toxic metals in the cell walls of roots, stems, leaves and fruits, functioning as a detoxification mechanism [9]. Thus, in the regions of the plant that are closer to the soil, there is a greater probability of accumulating cadmium, compared to the others, due to the retention mechanisms in the walls of the plant cells that make up the roots. In the stem and leaves, these mechanisms vary according to the concentration of the metal present in the soil [8].

Characterized as bioaccumulative and toxic to humans, plants and animals, cadmium was considered to be a threat to human health, according to the "Agency for Toxic Substances and Disease Registry", after the selection of 275 toxic substances [12]. In Brazil, Resolution - RDC No. 42 of 2013 provides for maximum limits of inorganic contaminants in food and establishes a limit of 0.20 mg kg-1 of cadmium for leafy vegetables and fresh herbs. Thus, the objective of the present study was to evaluate the cadmium uptake by the roots and fruits of Capsicum Annum peppers belonging to the green and red square pepper varieties, grown in soils contaminated with solutions of CdCl2.2H2O (cadmium chloride dihydrate p.a.) with different concentrations. Microwave-Induced Plasma Atomic Emission Spectrometry (MIP-AES) was used to quantify cadmium in samples of pepper roots and fruits.

2 DEVELOPMENT

The study was carried out between April 2020 and February 2021, on the campus of the Federal University of Triângulo Mineiro (UFTM), located in Uberaba (MG).

The experiment was carried out from seeds packed in polyethylene trays with a capacity of 36 tubes (1 plant/tube) that contained 30 mL of commercial product consisting of pine bark, natural fibers and enriched with mineral fertilizer. The seedlings obtained after 77 days of cultivation were transplanted into seedling bags with dimensions of 20 cm in diameter and 30 cm in height, filled with a commercial product consisting of a mixture of vegetable soil and substrate.

The information regarding the physicochemical parameters of the soil used for the cultivation of peppers was obtained from analyses of the commercial product.

The experimental design took into account the triplicate of the plants for each concentration of contaminating solution (0.00, 5.00, 10.00 and 15.00 mg $^{L-1}$ Cd2+), totaling twelve plants of each variety of pepper. The contaminating solutions were prepared by diluting the stock solution 1000 mg $^{L-1}$ in CdCl2.2H2O (cadmium chloride dihydrate p.a.) [13].

Contamination occurred 87 days after sowing, in which 300 mL of contaminant solutions selected for each seedling, as previously described [14]. During the cultivation period (255 days), the soil was irrigated with urban water supply in order to maintain humidity to favor the development of the plants. After harvesting, the roots and fruits of the plants were sanitized with running tap water to remove soil residues and organic matter. Then, they were dipped in beakers containing 200 mL of deionized water, dried on absorbent paper and placed in properly labeled plastic bags. Following this step, the root and fruit samples were sent to drying in a previously heated oven, at a temperature of 105ºC, for a period of 24 h [15]. The dried samples were removed from the oven and placed in a desiccator until they reached room temperature, being weighed at 4h intervals and placed back in the oven until the mass was constant [16]. Subsequently, they were ground with the aid of a mortar and pistil before weighing. The mass of 0.50 g of the samples was submitted to wet preparation in a digester block, in the presence of nitric and perchloric acids (2:1), until it reached 210ºC and a colorless extract was obtained [17]. Cadmium quantification was performed using Microwave-Induced Plasma Atomic Emission Spectrometry (MIP-AES) [18].

In order to clarify which regression model best characterizes cadmium uptake by the varieties of peppers under study, analytical curves were elaborated with linear and polynomial analyses, and the respective R2 adjustment values were determined in order to evaluate the predictability of the results.

3 RESULTS AND DISCUSSION

In order to confirm whether the cadmium contents absorbed by the roots and fruits of the pepper varieties would be derived from the application of the contaminant solutions of CdCl2.2H2O under study, and not from the soil used for cultivation, analyses of the physicochemical parameters of the commercial product were carried out and the contents corresponding to potential acidity, aluminum, sodium, potassium, calcium, magnesium, phosphorus and cadmium, organic matter (M.O), clay, silt, sand and the pH value are shown in Table 1.

Table 1: Physicochemical characterization of the soil

Physicochemical parameter	Value
pH	5,90
H + Al (cmolc dm^{-3})	2,40
Al^{3+} (cmolc dm^{-3})	0,92
Na^{+} (cmolc dm^{-3})	0,17
K^{+} (cmolc dm^{-3})	0,08
Ca^{2+} (cmolc dm^{-3})	0,97
Mg^{2+} (cmolc dm^{-3})	0,08
P (mg dm^{-3})	9,00
CO (g kg^{-1})	2,45
Cd^{2+} (mg dm^{-3})	0,00
Clay (g $^{kg-1}$)	340,0
Labels (g $^{kg-1}$)	32,0
Sand (g $^{kg-1}$)	628,0
M.O. (%)	3,80

The values mentioned in table 1 ensured that cadmium uptake did not come from the cultivation soil, therefore, it did not occur during the seed development period until transplanting (77 days). As the application of the contaminating solutions was made after 87 days of sowing (Figure 1), it is confirmed that the availability of cadmium in the soil and,

Consequently, the levels absorbed by the pepper varieties are due, in fact, to the application of contaminating solutions.

Figure 1: Green and red square bell pepper seedlings after transplanting.

None of the concentrations of the contaminant solutions that were applied to the soil interfered in the development, flowering and fruiting of the pepper varieties, since the plants contaminated with the analytical white solution (0.00 mg $^{L-1}$ Cd2+) had fruit production periods approximately equal to those that received the solutions of concentrations 5.00, 10.00 and 15.00 mg $^{L-1}$ Cd2+. The green and red square peppers flowered after 158 days of sowing and the collection of fruits and roots occurred after 255 days.

Prior to cadmium quantification, fruit and root samples were placed in Petri dishes organized in trays (Figure 2) and taken to the greenhouse for the dehydration stage.

Figure 2: Packaging of fruit and root samples for dehydration.

Note: a - Samples of "red square" and "green" peppers. b - Root samples of "red square" and "green" bell peppers.

Once the dehydrated and dry matter was in possession, the samples were sent to wet preparation [17] and to the quantification stage, performed by means of Microwave-Induced Plasma Atomic Emission Spectrometry (MIP-AES).

The transport of cadmium to the upper regions of green and red square peppers is due to mechanisms that occur in the roots via xylem and processes of immobilization of the metal in the cell walls [8].

The cadmium levels obtained by the roots of both varieties of peppers were similar. However, as the concentrations of the contaminant solutions increased, higher levels of the toxic metal were absorbed by both. The evaluation of cadmium uptake by the roots of the peppers showed that the two varieties absorbed 1.99 mg $^{L-1}$ Cd2+, when the contaminating solution 15.00 mg $^{L-1}$ Cd2+ was applied. The highest levels of cadmium were absorbed by the roots in relation to the fruits (Table 2), characterizing a significant reduction in the transport of the metal to the upper regions of the plants. In a study carried out with peppers of the genus Capsicum involving the metal cadmium, Xin et al. [19] observed the occurrence of higher concentrations of the metal accumulated in the roots in relation to the leaves and fruits. The results described in this study agree with those obtained in the present study. According to Lombi et al. [20], the immobilization of cadmium on the outside and inside of the roots is one of the first barriers of the plant's defense against toxicity from metals. In general, cadmium levels are higher in the roots than in the fruits, accumulating, mainly in the vacuole or cell wall, making it difficult to transport to the upper regions [21].

Comparing the cadmium uptake by green and red square peppers, it was observed that green peppers absorbed higher levels in fruits and roots, compared to red square, when lower concentrations of contaminating solutions were applied (Table 2).

Table 2: Cadmium contents absorbed by green and red square peppers.

Concentration of cadmium applied to soil (mg $^{L-1}$)	Cadmium concentration absorbed (mg $^{L-1}$)			
	Red square bell pepper		Green pepper	
	Root	Fruit	Root	Fruit
0,00	0,00	0,00	0,00	0,00
5,00	0,25	0,00	0,33	0,00
10,00	1,50	0,08	1,67	0,09
15,00	1,99	0,19	1,99	0,17

According to Araújo [22], the differences between the cadmium levels absorbed by peppers can be explained by variations in the composition of toxic minerals and metals present in the soil, type of fertilization, cultivation period and irrigation. However, these facts do not justify the difference in the contents obtained in this study, since we used a commercial product, whose physicochemical parameters were previously analyzed, and the cultivation and irrigation periods were identical for both varieties. Thus, the differences in the contents can be explained by the specific nutrient requirements of each plant, which varies according to its physiological maturity. In a study carried out with peppers, Albuquerque [23] discusses the rates of germination speed, development and fruit production, and associates the metabolic processes of the plant with different rates of absorption of nutrients and toxic metals.

The low concentrations of cadmium absorbed by the roots and fruits can be explained by the shallow depth of the cultivation soil (ca of 20 cm), so that the planting was carried out in seedling bags [24]. This depth usually results in a decrease in the concentration of toxic metals and nutrients that would probably be absorbed by plants, due to leaching losses when metals are carried away by the addition of water, and/or soil erosion when preferential flow paths are formed [25]. Leaching and erosion processes affect water retention in the soil, altering its characteristics and causing the aforementioned effects [26]. However, the results obtained by the absorptions mentioned above may be associated with the subcellular distribution of cadmium and its different chemical forms in the soil (Cd2+, CdSO4, CdCl42+, CdCl+, CdHCO+), since interactions occur with the other ions present in this environment, justifying the existence of these forms [27].

Comparing the cadmium concentrations absorbed by the roots of the two varieties of peppers by means of the graphs shown in Figures 3 and 4, an upward behavior is observed for the linear and polynomial adjustment curves, as the concentrations of the contaminant solutions applied to the cultivated soil increase.

Figure 3: Linear and polynomial fit curves (red square bell pepper roots).

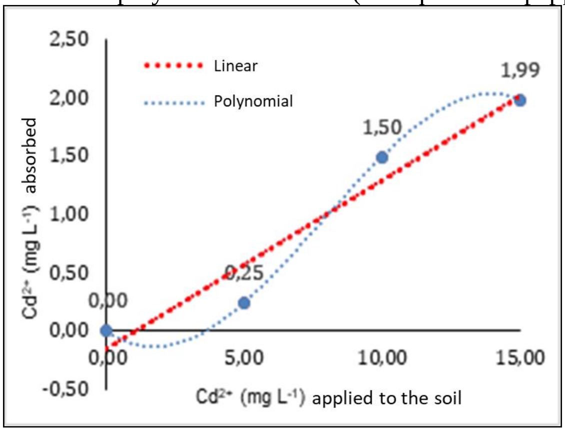
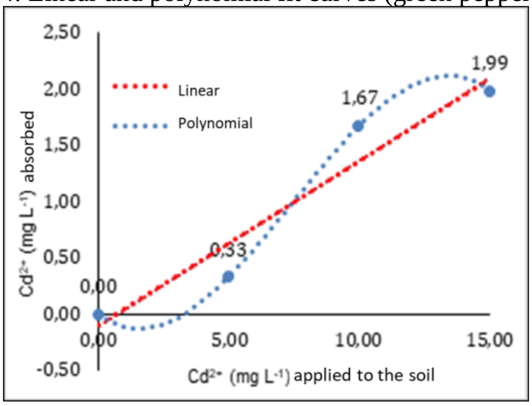


Figure 4: Linear and polynomial fit curves (green pepper roots).



By means of linear and polynomial analyses (Figures 3 and 4), a significant percentage increase of 600 and 506% in the cadmium absorption rate by the roots of the green and red square pepper varieties is observed, respectively, when the ratio between the results obtained from the concentrations corresponding to 10.00 and 5.00 mg $^{L-1}$ is calculated. However, when comparing the ratio between the results of the 15.00 and 10.00 mg $^{L-1}$ concentrations, a reduction in the cadmium absorption rate is observed, affecting the slope of the linear curves. Considering these last concentration values, the percentage corresponded to 33% for the red square pepper and 19% for the green variety.

A similar behavior can be observed in relation to cadmium uptake by the fruits of both varieties of peppers, in which the linear and polynomial curves are shown in figures 5 and 6.

Figure 5: Linear and polynomial fit curves of the results of the analysis of red square pepper fruits.

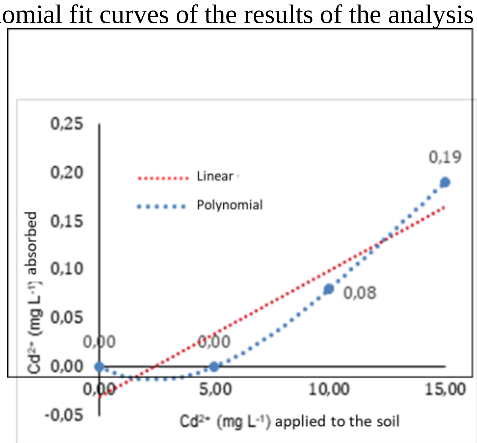
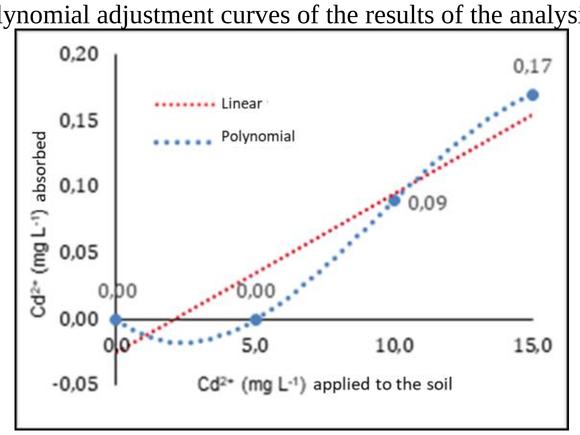


Figure 6: Linear and polynomial adjustment curves of the results of the analysis of green pepper fruits.



The transport of cadmium from the roots to the fruits of the two varieties of peppers was evidenced from the solution of concentration of 10.00 mg $^{L-1}$ (Figures 5 and 6). The selectivity of the plant, the ability to tolerate and/or not accumulate toxic metals, preventing high concentrations of cadmium from being absorbed and transported to the fruits may explain this fact [8]. The analysis of polynomial and linear curves showed that possible cadmium levels could be absorbed when the concentration range of the contaminating solutions varied between 5.00 and 10.00 mg $^{L-1}$, however, these values are unknown, since concentrations in this range have not been evaluated, however, by means of the developed models, mainly by the Polynomial model for roots and fruits (R2 = 1.0) these values can be predicted up to the concentration of 15.00 mg $^{L-1}$. Studies involving concentrations of contaminating solutions with values intermediate to those mentioned above are recommended, in order to know the exact value of the concentration at which the absorption of cadmium by the fruits of peppers of both varieties began.

The equations obtained by means of regression adjustments and the respective estimated R2 values are described in Tables 3 and 4.

Table 3: Regression Adjustment Equations (Red Square Bell Pepper Root and Fruit)

Regression model	Regression equation	R2
Lineara	y = 0.013x - 0.03	0,87
Linearb	y = 0.1444x - 0.148	0,94
Polynomial	y = -7-05x3 + 0.0026x2 - 0.0113x - 8-15	1,00
Polynomialb	y = -0.0023x3 + 0.0552x^2 - 0.1673x - 1-13	1,00

Note: y = absorbed cadmium concentration (mg $^{L-1}$), x = cadmium concentration applied to the soil (mg $^{L-1}$). a = fruit, b = root.

Table 4: Regression Adjustment Equations (Green Bell Root and Fruit)

Regression model	Regression equation	R2
Linear	y = 0.012x - 0.025	0,90
Linearb	y = 0.1462x - 0.099	0,93
Polynomial	y = -0.0001x3 + 0.0038x 2 - 0.0157x - 7-15	1,00
Polynomialb	y = -0.0027x3 + 0.0608x 2 - 0.1703x - 1-13	1,00

NOTE: y = absorbed cadmium concentration (mg $^{L-1}$), x = cadmium concentration applied to soil (mg $^{L-1}$). A = fruit, b = root.

The linear regression equations described in Tables 3 and 4 provided a predictability of results both for the range of concentrations of cadmium solutions added to the soil, as well as for higher values when calculated, respecting the increasing absorption behavior noted, however, it does not provide precise results by means of adjustment (0.87 < R2 < 0.95).

Considering the regression equations referring to the polynomial model (Tables 3 and 4), there was a perfect fit of the results in relation to the cubic equations (R2 = 1), although they provided a better predictability in the range of concentrations between 0.00 and 15.00 mg $^{L-1}$. For cadmium concentrations above 15.00 mg $^{L-1}$, the cubic equations do not guarantee the accuracy of the data, resulting in low values of cadmium accumulated in roots and fruits, contradicting the behavior of the increasing curve, since a fixed/constant value was not identified regarding cadmium absorption, as the concentration of the solution added to the soil increased. In a study that aimed to evaluate the uptake of toxic metals by green peppers and tomatoes grown in soils with phosphogypsum residues, the authors observed that cadmium levels were increasing and varied proportionally as the phosphogypsum concentration increased. By means of linear regression adjustment, the authors obtained the equation: $y = 0.071x + 0.1172$, and R-value equal to 0.93 [28]. The results of this research are in agreement with those obtained in the present study, and the values of R and regression coefficients were similar and demonstrated a cadmium absorption behavior proportional to the increase in concentration of the toxic metal applied to the soil.

The percentage corresponding to the variation of cadmium transport from roots to fruits corresponded to 5.3% for the red square pepper variety and 5.4% for the green variety, when the contaminant solution 10.00 mg L was applied to the soi^{l-1} Cd2+. These values tended to be higher as the concentration of the solutions increased, reaching 8.5% for 15.00 mg $^{L-1}$ (Table 2). Thus, it is possible to infer that the susceptibility to cadmium concentration in red and green square pepper varieties depends on the level of soil contamination, the ability of the plants to tolerate the presence of the toxic metal, the capacity of absorption of the roots and mobility of the metal through the cellular tissue of the plant, until it accumulates in the region of the fruits. The translocation through the different regions of the plant is differentiated, as it moves away from contact with the cultivation soil.

4 CONCLUSIONS

Both green and red square bell pepper varieties have accumulated cadmium in their roots and fruits. The cadmium uptake rates were higher in the roots compared to the fruits, varying proportionally with the increase in the concentration of the contaminant solution applied to the cultivated soil.

The moderate use of pesticides is recommended, especially those containing cadmium in their composition, even if in low concentrations.

The Microwave-Induced Plasma Atomic Emission Spectrometry used for the quantification of cadmium in samples of roots and fruits of green and red square peppers proved to be effective, as it provides results that are in agreement and satisfactory with studies in the literature.

For future studies, concentrations of cadmium contaminants in the range between 0.00 and 15.00 mg L are indicated-1 are evaluated for the same varieties of peppers, in order to know the exact concentration value at which absorption began.

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