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RESEARCH ARTICLE OPEN ACCESS

MORPHOPHYSIOLOGICAL EFFECTS ON JAMBU PLANTS EXPOSED TO CADMIUM: A MULTIVARIATE APPROACH

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ABSTRACT

The study has as objective an analysis of the cadmium (Cd) effects in *Acmella Oleracea* (L.) K. Jansen (jambu), a widely consumed vegetable in the Amazon region, used and distributed in many parts of the world. It was performed a systematic analysis of the plant, with creation of indicators composed by biometric variables, gas exchange and cadmium content. The results showed that low doses of Cd promoted significant increase in growth, mass gain, net assimilation of CO_2, stomatal conductance and transpiration on jambu. On the other hand, high doses of Cd induce toxicity in the vegetable. The Cd content in the aerial part (edible) exceeds the maximum limit allowed by Brazilian legislation to leafy vegetables. The multivariate indicators allowed the determination of the optimal dose of 1.05 mg.L^{-1}, where there was increase in growth, mass gain and gas exchange, and the threshold dose of 2.1 mg.L^{-1}, where the jambu plants don't present toxic signs. As the fact that many cultivation areas are near to great urban centers, the exposition of the plants to Cd is maximized, needing to intensify the soil monitoring of cultivation areas.

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INTRODUCTION

The increase in heavy metal concentration in the environment is a consequence of an increase in production activities and the use of natural resources. Among the potential sources of these metals, geological/geogenic areas with elevated heavy metal concentrations, the excessive use of fertilizers, the use of sewage sludge in agriculture, metal-based pesticides, atmospheric deposition, inadequate wastewater treatment, and anthropogenic contamination of soils may be highlighted (Alloway, 2013; Hanaham, 2013; Rai *et al.* 2019). Whether essential or not, heavy metals can be considered toxic to humans depending on their concentrations, specially, when they are part of the diet (Marschner, 2012). Cadmium (Cd) deserves special attention due to its effects in plants and humans. Its concentration in earth crust ranges from 0.1 to 0.2 mg□kg^{-1}; it is found in diverse minerals, such as greenockite and monteponite; and may also be found in association with sulfides of zinc (Zn) and lead (Pb). In the soils, the average contents vary between 0.2 and 1.1 mg·kg^{-1}, but in areas of great anthropic activity, such as those of metal processing, it can reach 1781 mg·kg^{-1} (Kabata-Pendias, 2010). Thus, Cd may become available to plants and animals, thereby posing risks to the population (Kabata-Pendias, 2010; Shanmugaraj *et al.* 2019; Hussain *et al.* 2019). Cd is considered one of the most toxic metals because of its adverse effects on humans, animals, and plants (Kabata-Pendias, 2010; Alloway, 2013). Exposure of plants to Cd can cause growth retardation, chlorosis, necrosis, and damage to the photosynthetic apparatus, among other effects (Shanmugaraj *et al.* 2019; Qin *et al.* 2020). However, in small doses, Cd has an inductive effect at some stages of growth in some plant species. This phenomenon is known as hormesis and is characterized by a binary effect, with both stimulatory and inhibitory responses on organisms (Ray *et al.* 2014; Pincelli-Souza *et al.* 2020). According to Calabrese & Baldwin (2003a, 2003b), hormesis is characterized by stimulation of an organism at low doses and inhibition at high doses of a stress agent. According to Calabrese and Blain (2009), this response has been commonly identified in different plant species, regardless of the chemical class of the stress agent. Many plants that are tolerant to heavy metals belong to families of plants that accumulate and hyperaccumulate metals, including sunflower from the *Asteraceae* family, and Indian mustard and canola from the *Brassicaceae* family (Andrade *et al.* 2014; Sytar *et al.* 2016). In this context, Hungria *et al.* (2019) evaluated the phytoremediation potential of a typical Amazonian *Asteraceae* called *Acmella oleracea* (L.) R. K. Jansen or, simply, jambu. According to the authors, jamb grown in soils contaminated with 9 mg□kg^{-1} of Cd showed low sensitivity to the metal, with no evidence of toxicity. Since jambu is used in cooking (either raw or cooked), in the cosmetics industry, and even in medicine, because it has analgesic, bactericidal, and antioxidant properties, the ability to tolerate and accumulate Cd, coupled with its distribution in Central America, Asia, and Africa, as well as the increase of its cultivation in various regions of the world, brings concerns and the need to investigate the ability of the plant to absorb and accumulate the element (Dubey *et al*. 2013; Andrade *et al*. 2014; Lalthanpuii *et al*. 2017; Uthpala and Navaratne, 2021). This study aimed to analyze the effects of Cd on jambu, as well as to propose a systemic and integrated evaluation method of the data, through multivariate indicators, in order to facilitate the understanding of the dose/response relationship and the limits between the maximum stimulus and phytotoxicity that may be caused by this metal in jambu plants.

MATERIALS AND METHODS

Experimental procedures: The experiment was conducted between February and March 2020 at the greenhouse of the Agrarian Science Institute (ASI), from the Rural Federal University of the Amazon — UFRA (1º 27' 13,70" S and 48º 26' 32,65" W). At an approximate altitude of 14 m. According to the Af category of Koppen, the weather in this area is equatorial hot and humid (Kottek *et al.*2006). The temperature and air humidity, during the experiment, were measured using a thermo-hygrometer installed in the greenhouse. The medium temperature values oscillated between 28 and 35 °C. The average relative humidity during the experimental period was approximately 70%.

Crop system: Hydroponics were used as crop system; plants were grown in 2 Lvases with an inert substrate, and Hoagland and Arnon (1950) formulation 2 was used as nutritive solution. The nutritive solution was composed of 1 mL·L^{-1} NH_4NO_3, 6 mL·L^{-1} KNO_3, 4 mL·L^{-1}$Ca(NO_3)_2$, 2 mL·L^{-1} $MgSO_4$, 1 mL·L^{-1} Fe-EDDHA, and 1 mL·L^{-1} micronutrient solution. Vases were illed with pre-washed ground silica, coated with aluminum foil to avoid the incidence of solar radiation, and drilled at the base; a silicone tube was placed in the system to facilitate the recirculation of the nutritive solution. Throughout the experiment, the nutritive solution was oxygenated by draining it at the end of the afternoon, andreplacing it at the beginning of the morning (Netoand Barreto, 2011; Sampaio *et al*. 2020).

Production of seedlings: The jambu seeds was obtained from the germplasm bank in the horticultural sector from UFRA, were sown in the second week of February 2020 in 128-cell polystyrene trays filled with coconut fiber. In each cell, 10 to 15 seeds of Yellow Flower jambu, a regional variety, were deposited. After germination, the seedlings were watered with Hoagland and Arnon (1950) nutritive solution in a 25% ionic force (Sampaio *et al*. 2020). Thinning of the smallest plants was performed 10 days after germination, leavingonly one seedling per cell. After 21 days, those seedlings with four expanded leaves and in good phytosanitary conditions were removed from the polystyrene trays, washed with deionized water, and transplanted to vases with inert silica and nutritive solution at 50% of ionic force; to ensure the correct acclimatization ofthe system, this conditions were kept unchanged for seven days. Both the pH and electrical conductivity of the nutritive solution were monitored daily during the experiment. The pH was maintained within a 5.5 to 6.5 range, and the electric conductivity within a 1.0 – 1.8 µs□cm^{-1} range.

Experimental design: The experimental design was fully randomized, with five treatments and six repetitions, totaling 30 plants with one plant per vase. Treatments included four different doses of cadmium (1, 3, 6, and 9 mg□L^{-1} cadmium chloride, $CdCl_2$) and the control with nutrient solution only (Hungria *et al*. 2019). Every seven days, the nutrient solution was renewed, and the same was done with $CdCl_2$solutions to ensure that the correct Cd concentration was supplied to plants until the end of the experiment. Water that was evapotranspirated during the day was replenished with deionized water. To measure the plant capacity for Cd absorption, accumulation potential, translocation, and tolerance, as well as Cd effects on jambu, the plants were harvested at the end of the growth cycle, 40 days after transplantation.

Laboratory Analytical Procedures

Biometric Analysis: After harvesting, the plants were washed with distilled water. Plant height (PH), stem diameter (SD), and inflorescence number (IN) were measured. The plant parts were divided into leaves, stems, inflorescences, and roots, and separately washed with deionized water, dried with paper towels, and oven-dried for 72 hours at 70º C until a constant weight was achieved. Plant parts were then weighed using a precision analytical balance to calculate leaf (LDM), stem (SDM), inflorescence (IDM), and root (RDM) dry masses according to Costa *et al*. (2020) and Sampaio *et al*. (2020).

Leaf gas exchange: Gas exchange parameters were measured in the first physiologically mature expanded leaf after verifying that it was in good phytosanitary conditions. Measurements were done from the apex to the base. The plants were evaluated 15 d after the first contact with Cd. The net assimilation rate of CO_2 (A), stomatal conductance to water vapor (gs), intercellular CO_2 concentration (Ci), and transpiration rate (E) were measured between 11:00 and 13:00 h, according to the diurnal curve of gas exchange performed previously using a portable open-flow gas exchange system(LI -6400XT, LI-COR, Lincoln, NE) under an external concentration of CO_2 of 400 µmol□mol^{-1} of air and an artificial photosynthetic active radiation (PAR) of 1,000 µmol photons□m^{-2}□s^{-1}.

Chemical Analysis: After drying, the leaves, stems, inflorescences, and roots of jambu plants were milled in a porcelain bowl. Subsequently, 0.25 g aliquots of each part of the plant placed in Teflon tubes with 4.0 mL HNO_3(7 mol L^{-1}), 2 mL H_2O_2 (30% mm^{-1}), and 2 mL ultrapure water, and left standing for about 1 h. Samples were then allocated for complete digestion in a microwave digester. The digestion ramp consisted of three steps: a temperature rise from 0 to 180 °C in 10 min at 800 W, left stand at a constant temperature of 180 °C for 20 min, and ventilation for 50 min (Pereira and Dantas, 2016). After digestion, the samples were filtered and adjusted to 50 mL with deionized water. The Cd content was determined by atomic absorption spectrophotometry using a Varian AA 240 Z graphite oven at the Laboratory of Water Quality of the Amazon of the State University of Pará.

Accumulation translocation, and tolerance indexes: To quantify the growth ability of *Acmella oleracea* in the presence of Cd, the accumulation of Cd in the plant (Ac; equation 1) and the translocation (Tr; equation 2) and tolerance (It; equation 3) indexes were determined according to the following equations:

$$Ac\,(CCd * DM)/1000 \qquad (1)$$

whereCCd is the concentration of Cd in the plant organs (leaves, stem, inflorescences, and roots) in mg□kg^{-1}, and DM, the plant dry mass in g (Siddiqui and Glass, 1981; Swiader*et al*. 1994).

$$Tr = (Ac/Cct) * 100 \qquad (2)$$

Where Ac represents Cd accumulation in plant organs (leaves, stems, inflorescences, and roots), and Cct, Cd accumulation in the roots (Mattina *et al*. 2003; Jia *et al*. 2013).

$$It = (Mi/Mc) * 100 \qquad (3)$$

where Mi is the dry mass of the plants treated with Cd (from the same dose treatment) and Mc in the dry mass of control (untreated) plants (Piotto*et al*. 2018).

Creation of representative growth dimension, mass, and physiology indicators: The Principal Component Analysis (PCA) method was used for creating representative indicators of the plant parameters analyzed. The principal components (PCs) were extracted and hierarchically organized according to their capacity to describe the variance, from the highest to the lowest ($PC_1 > PC_2 > \dots > PC_p$). The relative importance of each PC is represented by the variance relation explained for the PC itself and the total variance. The variance of the extracted components represents the eigen value or its contribution to explaining the total variance. After generating the eigenvalue vector of the components, it was combined with the absolute value matrix of the component axle magnitudes to generate the weight vector used in the construction of each indicator. The representative indicators of the dimension defining variables included the plant growth indicator (gI;tocapture the effects of plant height, stem diameter, and inflorescence number), plant mass accumulation indicator (mI;to capture the effects of leaf, stem, inflorescence, and root dry masses), physiological indicator (pI;combining CO_2 net assimilation rate, stomatal conductance, and transpiration), and general indicator (gnI) of the survey (contemplating the whole set of explanatory variables). During the development of these indicators, all the components were considered to be able to contemplate 100% of the total variance of the data mass. The coefficient vectors associated with each principal component were estimated using SPSS software. In contrast to the traditional experimental analysis, which works with individual models, this methodological technique expanded the scope of the analysis to incorporate all the captured effects for the set of variables that represent each analyzed dimension. For example, instead of partially observing the influence of Cd dose in “plant height, stem diameter, and inflorescence number” the gI was designed to represent the behavior of these three variables(related to plant growth)as one set. Therefore, the effects on the plant were captured in a complete form, considering the influence of each variable. Following the model proposed by Santana and Santana (2004), the weights associated to the components of each set of parameters belonging to the physiological, growth of mass accumulation, and general dimensions were estimated using SPSS software.The process involved the following steps (Santana and Santana, 2004; Santana, 2005): *i*) estimating the vector with a relative participation of the eigenvalues λ ($\lambda_{kj}/\sum \lambda_k$); *ii*) estimatingthe matrix absolute values (a_{kj}) of the eigenvectors of each transformed component; *iii*) estimating the relative coefficient matrix of the eigenvectors ($a_{kj}/\Sigma a_k$), and; *iv*) defining the linear combination of the descriptor variables and performinga matrix multiplication to estimate the weights Θ using equations 4 and 5.

$$\Theta_{j(kx1)} = \left(\frac{a_{kj}}{\sum a_k}\right)_{(kxk)} \cdot \left(\frac{\lambda_k}{\sum \lambda_k}\right)_{(kx1)} \quad (4), \text{ With } \Theta_1 + \Theta_2 + \dots + \Theta_p = 1 \ (j = pI, gI, mI, gnI) \quad (5)$$

where k is the number of components of each j dimension.

This mathematical model was used to estimate the weights associated with the descriptor variables of the plant, in reference to the physiology, growth, mass accumulation, and general behavior of the plant itself.

Regression Analysis: The specifications of the multiple regression systems used to demonstrate the phenomena studied in this survey, involving the effects of different Cd doses on growth, physiology, and mass accumulation in jambu plants and the general behavior of the vegetable were defined by the equations below:

$$pI = b_{10} + b_{11}D_s + b_{12}D_s^2 + u_2 \quad (6)$$

$$gI = b_{20} + b_{21}D_s + b_{22}D_s^2 + u_3 \quad (7)$$

$$mI = b_{30} + b_{31}D_s + b_{32}D_s^2 + u_4 \quad (8)$$

$$gnI = b_{40} + b_{41}D_s + b_{42}D_s^2 + u_5 \quad (9)$$

Where D_s represents the Cd doses resulting in a linear answer (including the control, and 1, 3, 6, and 9 mg Cd□L^{-1}); $D_s^2$is the dose of Cd resulting in a quadratic answer; b_{i0} are the equations intercepts (i = 10, 11, 12, 13); b_{ij} are the parameters associated with the equation variables; u_i are the random error terms of the equations. All biometric, physiological, accumulation, Cd content (in leaves, stems, inflorescences, roots, aerial parts, and whole plants), translocation, tolerance, and indicator data were subjected to variance analysis (ANOVA) and the assumptions of homogeneity of the data, homoscedasticity, and independence of the errors were met. The averages were compared using the Scott-Knot test ($p < 0.05$) using R software version 3.5.2 (R Core Team, 2018).

RESULTS

Accumulation and Cd content in jambu: The accumulation and content of Cd in the leaves, stems, inflorescences, and roots of jambu were influenced by the doses of Cd included in the nutritive solution ($p<0.05$). The plants concentrated the majority of the Cd in their roots, especially thosewitha 9 mg□L^{-1} dose (Table 1). In the leaves, the dose that promoted the major content of Cd was 3 mg□L^{-1}, followed by 1mg□L^{-1}. Likewise, the stems concentrated more Cd when exposed to doses of3 mg□L^{-1}. In contrast, the Cd concentration that induced the lowest metal content in the stems was 1 mg□L^{-1}. The inflorescences, similar to the roots, presented their minor Cd content when watered with the solution with the lowest Cd concentration, and the major Cd content when watered with the most concentrated solution, that is, with the 9 mg□L^{-1} dose. The 9 mg□L^{-1}Cd dose treatment led to more metal content in the roots, which was successively less abundant in inflorescences, stems, and leaves. A similar behavior was observed at concentrations of 6 mg□L^{-1} and 3 mg□L^{-1}. However, at a dose of 1 mg□L^{-1}, the Cd content was higher in the root and successively less abundant in leaves, inflorescences, and stems. These results suggest that metal distribution at this concentration differs from that at other concentrations (Table 1). In relation to the aerial part (leaves, stems, and inflorescences), treating plants with a 1 mg□L^{-1} Cd dose resulted in a 1.36 mg□kg^{-1} content. As the element concentration increased in the solution, the uptake and distribution of Cd was higher, reaching 3.10 mg□kg^{-1} in the aerial part of jambu plants treated with the 9 mg□L^{-1} Cd dose.

Table 1. Cd content and accumulation in jambu plants cultivated in hydroponic systems

Cd dose (mg□L^{-1})	Cd content in plant organs (mg□kg^{-1})					
	Leaf	Stem	Inflorescence	Root	Aerial Part	Total
1	0.52 ± 0.06 B	0.39 ± 0.04 cC	0.44 ± 0.02 dC	0.60 ± 0.04 dA	1.36 ± 0.05 c	1.97 ± 0.09 c
3	0.68 ± 0.06 aB	0.76 ± 0.07 aB	0.62 ± 0.06 cB	2.21 ± 0.17 cA	2.06 ± 0.14 b	4.27 ± 0.25 b
6	0.43 ± 0.05 cC	0.70 ± 0.02 bC	1.06 ± 0.09 bB	2.77 ± 0.6 bA	2.19 ± 0.08 b	4.97 ± 0.71 b
9	0.36 ± 0.03 dD	0.66 ± 0.04 bC	2.07 ± 0.22 aB	4.02 ± 0.30 aA	3.10 ± 0.18 a	7.13 ± 0.47 a
Cd dose (mg□L^{-1})	**Cd accumulation in plant organs (µg□plant^{-1})**					
	Leaf	Stem	Inflorescence	Root	Aerial Part	Total
1	1.08 ± 0.17 bA	0.66 ± 0.08 bB	0.14 ± 0.007 bC	0.67 ± 0.07 cB	1.90 ± 0.17 b	2.57 ± 0.18 c
3	1.23 ± 0.09 aB	1.10 ± 0.08 aB	0.18 ± 0.01 aC	2.19 ± 0.26 aA	2.52 ± 0.13 a	4.71 ± 0.32 a
6	0.52 ± 0.07 cB	0.65 ± 0.05 bB	0.18 ± 0.04 aC	1.85 ± 0.42 bA	1.36 ± 0.06 c	3.22 ± 0.45 b
9	0.14 ± 0.05 dC	0.25 ± 0.03 cB	0.07 ± 0.02 cC	0.65 ± 0.19 cA	0.47 ± 0.06 d	1.12 ± 0.17 d

Different lowercase letters indicate significant differences between Cd doses and different uppercase letters indicate significant differences between plant organs, according to the Scott-Knot test ($p < 0.05$).

Altogether, the plant absorbed 1.97 mg·kg^{-1} Cd when exposed to 1 mg·L^{-1} of Cd, and 7.13 mg·L^{-1} when grown with a 9 mg·L^{-1} Cd dose (Table 1). The maximum Cd concentration tolerated in leafy vegetables by the Normative Instruction N°88 from March 26,2021 of the AGENCIA NACIONAL DE VIGILÂNCIASANITÁRIA (ANVISA) is of 0.05 mg·kg^{-1} (Ministério da Saúde, 2021). Here, it was observed that jambu plants absorbed 27 times more Cd than the maximum allowed by ANVISA when exposed to Cd concentrations as low as 1 mg·L^{-1}. Cd accumulation in jambu leaves increased significantly between plants treated with 1 mg·L^{-1} and 3 mg·L^{-1}Cd doses; however, there was a tendency to fall between the 6 mg·L^{-1} and 9 mg·L^{-1} dose treatments, showing a biphasic behavior of the plant in the presence of this metal (Table 1).

part and 46.43% retained in the root. At doses of 6 and 9 mg·L^{-1}, Cd was retained in the radicular system at 57.06% and 56.95%, respectively, showing that more than half of the Cd available in solution was fixed in the radicular system of jambu plants (Figure 1A). In the analysis of the Tolerance Index results (Ti), significant differences were observed between the plants contaminated with Cd and the control plants. It is important to highlight the results from the 1 mg·L^{-1}treatment, where treated plants showed a greater tolerance to Cd than the control plants. In contrast, Ti gradually decreased as the Cd doses in the nutritive solution increased, showing a quadratic behavior. Jambu showed a minor tolerance capacity in the presence of Cd at a metal dose of 9 mg·L^{-1} (Figure 1B).

Table 2. Cd concentration effects on variables related to growth and mass of jambu plants cultivated in hydroponic systems

Cd dose (mg·L^{-1})	Mass and growth variables						
	PH	IN	SD	LDM	SDM	IDM	RDM
Control	17.96 ± 1.06 c	8.00 ± 0.89 c	4.98 ± 0.51 b	1.82 ± 0.11 b	1.31 ± 0.14 c	0.26 ± 0.03 c	1.02 ± 0.10 b
1	23.87 ± 1.26 a	11.50 ± 0.83 a	6.06 ± 0.24 a	2.05 ± 0.11 a	1.68 ± 0.05 a	0.33 ± 0.02 a	1.12 ± 0.05 a
3	19.77 ± 1.66 b	9.33 ± 0.51 b	5.11 ± 0.35 b	1.81 ± 0.06 b	1.45 ± 0.08 b	0.30 ± 0.01 b	0.99 ± 0.06 b
6	13.53 ± 0.97 d	5.67 ± 0.81 d	3.60 ± 0.25 c	1.20 ± 0.09 c	0.94 ± 0.08 d	0.18 ± 0.03 d	0.68 ± 0.07 c
9	6.59 ± 0.89 e	2.50 ± 0.54 e	1.73 ± 0.30 d	0.40 ± 0.11 d	0.38 ± 0.05 e	0.04 ± 0.007 e	0.16 ± 0.04 d

LN, leaf number; IN, inflorescence number; SD, stem diameter (mm); PH, plant height (cm); LDM, leaf dry mass (g·plant^{-1}); RDM, root dry mass (g·plant^{-1}); SDM, stem dry mass (g·plant^{-1}); IDM, inflorescence dry mass (g·plant^{-1}). Lowercase letters indicate significant differences between the treatments according to the Scott-Knot test ($p < 0.05$).

Table 3. Effect of Cd concentration in jambu physiological variables

Cd doses (mg·L^{-1})	Physiological Parameters			
	A	*gs*	*Ci*	*E*
Control	13.73 ± 0.42 b	0.106 ± 0.011 c	364.08 ± 2.91 b	3.46 ± 0.63 c
1	17.19 ± 0.64 a	0.166 ± 0.014 a	366.30 ± 2.48 b	4.89 ± 0.41 a
3	13.64 ± 0.43 b	0.121 ± 0.005 b	367.52 ± 3.98 b	3.91 ± 0.33 b
6	10.52 ± 0.97 c	0.079 ± 0.006 d	370.92 ± 5.58 b	2.52 ± 0.24 d
9	3.78 ± 1.04 d	0.021 ± 0.004 e	378.27 ± 7.39 a	0.45 ± 0.21 e

A, CO_2 assimilation rate (μmol m^{-2}·s^{-1}); *gs*, stomatal conductance (mol·m^{-2}·s^{-1}); *E*, transpiration (mmol·m^{-2}·s^{-1}); *Ci*, intercellular CO_2 concentration (mmol H_2O·m^{-2}·s^{-1}). Lowercase letters indicate significant differences between the treatments according to the Scott-Knot test ($p < 0.05$).

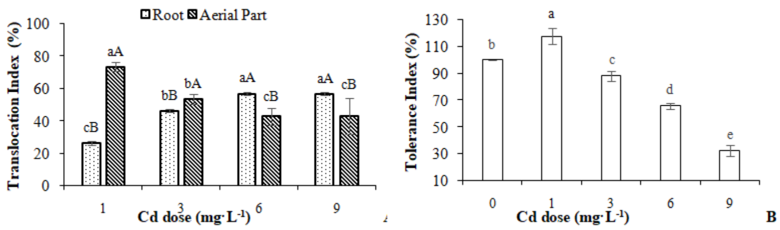

Figure 1. Translocation index (A) andtolerance index (B) of jambu exposedto Cd. Different lowercase and uppercase letters above bars indicate significant differences between the Cd doses and between the aerial part and the roots of the plant, respectively, according to Scott-Knot tests (p < 0.05). Bars represent the mean ± S.D

When the metal behavior in isolated doses was analyzed, it was observed that, with the 1 mg·L^{-1} dose, the accumulation in leaves was larger, whereby that in the roots and stems did not differ. At 3 mg·L^{-1}, a major part of the metal was kept in the root, showing low distribution of Cd along the plantas the dose in the nutritive solution increased, indicating a possible metal fixation in the radicular plant system.

Tr and Ti indexes: The Cd Tr index measures the relationship between metal accumulation in plant organs, and is presented in figure 1A. For this survey, comparisons were made between the aerial parts of jambu plants and their radicular systems Figure 1. Translocation index (A) and tolerance index (B) of jambu exposed to Cd. Different lowercase and uppercase letters above bars indicate significant differences between the Cd doses and between the aerial part and the roots of the plant, respectively, according to Scott-Knot tests ($p < 0.05$). Bars represent the mean ± S.D. At 1 mg·L^{-1} doses, the jambu plants translocated about 73.67% of absorbed Cd to the aerial parts and only 26.32% was retained in the roots. At 3 mg·L^{-1} doses, there was a significant reduction in the Cd translocation capacity, with 53.57% of the metal being translocated to the aerial

Plant mass and growth: The effects of Cd in jambu plants varied with the increase in Cd dose. After 28 days of exposure to 1 and 3 mg·L^{-1}Cd doses, different plant variables involving growth, such as PH, IN, and SD, and mass, such as LDM, SDM, IDM, and RDM, showed an increase in relation to those of the control plants. In contrast, these variable showed a significant reduction in plants exposed to 6 and 9 mg·L^{-1} doses of Cd (Table 2). The behavior of the biometric variables can be better understood by using the gI, which bundles LDM, SDM, IDM, and RDM variables. The gI showed that there was a significant improvement in plant growth in those plants exposed to 1 and 3 mg·L^{-1}Cd doses, in relation to that of the control plants, suggesting that Cd promoted growth in jambu when exposed to low metal doses. However, when facing higher Cd doses, the plant began to show toxicity signals, resulting in loss of growth performance, and peaking with the smallest gI value at the 9 mg·L^{-1} dose. The gI was capable of demonstrating that the dose/response relationship had a J-shaped form, with a stimulant effect of the metal all the way to 3 mg·L^{-1} and inhibitory effects at 6 mg·L^{-1}or higher doses. The 1 and 3 mg·L^{-1} doses provided growth gains in jambu of 34.46% and 16.7%, respectively, where as the 9 mg·L^{-1}dose decreased plant growth by 65.02% (Figure 2A). The mI demonstrated that jambu obtained significant gain of mass,in relation to the control, when subjected to 1 and 3 mg□L^{-1} doses of Cd, corroborating the observations for gI.

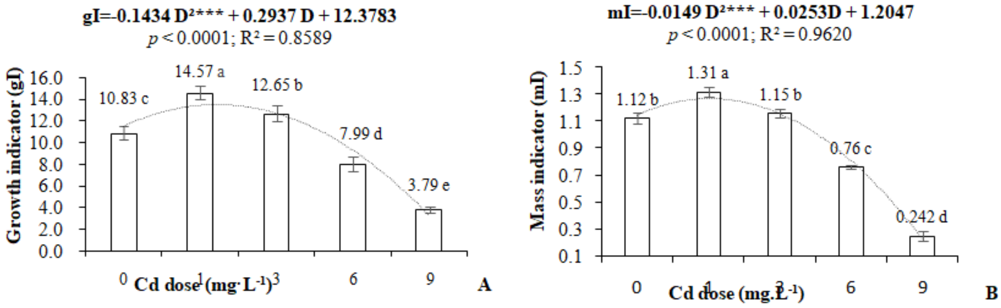

Figure 2. Multivariate models of growth (A) and mass (B) dimensions. Different lowercase letters above bars indicate significant differences between treatments, according to the Scott-Knott test (p< 0.05). *** p<0.0001(F test); D = linear response dose; D² = squared response dose. Bars represent the mean ± S.D.

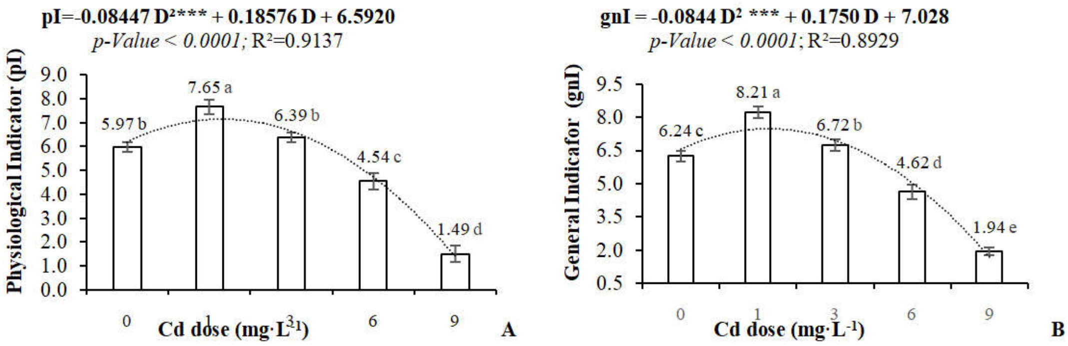

Figure 3. Multivariate models of the physiological (A) and general (B) dimensions. Different lowercase letters indicate significant differences between the treatments, according to the Scott-Knott test (p< 0.05). *** p<0.0001(F test); D = linear response dose; D² = squared response dose. Bars represent the mean ± S.D

This gain corresponded to increases of 17.16% and 3.09% at doses of 1 and 3 mg□L^{-1}, respectively. With the increase in Cd concentration in solution to 6 mg□L^{-1}, there was a reduction of 32.25% in the capacity of jambu plants to accumulate mass. Moreover, at doses of 9 mg□L^{-1}, this reduction was of 77.91%, showing an inhibitory effect of the metal at high doses, with a quadratic behavior (Figure 2B).

Physiological dimension: The behavior of the jambu photosynthetic characteristics was similar to those of the variables of growth and mass dimensions, and presented a biphasic response with a significant increase in *A*, *gs*, and *E* when plants were in the presence of Cd at 1 and 3 mg□L^{-1}, compared to the characteristics shown by the control plants. On the contrary, in the presence of 6 and 9 mg□L^{-1}Cd doses, a reduction in the photosynthetic capacity of the plant occured, showing an increased inhibitory effect at higher Cd concentrations. Interestingly, *Ci* presented a divergent behavior in relation to that of any other variable, with a significant increase only ata 9 mg□L^{-1}Cd dose, suggesting that, among the treatments, this was the most toxic Cd dose for jambu plants (Table 3). As the pI groups those variables regarding CO_2 assimilation rate, stomatal conductance, and transpiration, it integrally demonstrates how Cd and photosynthesis relate in the plant (Figure 3A). The pI showed that Cd doses of 1 and 3 mg□L^{-1} promoted a significant increase in the physiological characteristics of the plants, compared to those of the control, with 28.1% and 6.9% increments in the photosynthetic activity in the presence of 1 and 3 mg□L^{-1}Cd doses, respectively. As seen for other indicators, higher Cd doses provoked a reduction in photosynthetic activity, with a 74.93% reduction in the presence of 9 mg□L^{-1}Cd.

gnI: To have an integral view of the behavior of all the variables studied, that is, to analyze the general response of jambu to different concentrations of Cd, we created gnI. In a similar way to all other indicators, gnI showed that plant performance increased at low Cd concentrations compared to that of the controls, but decreased as Cd concentration increased in the nutritive solution used for watering, showing its lowest value at a 9 mg□L^{-1}Cd dose (Figure 3B). Figure 3. Multivariate models of the physiological (A) and general (B) Different lowercase letters indicate significant differences between the treatments, according to the Scott-Knott test (p< 0.05). *** p<0.0001(F test); D = linear response dose; D^2 = squared response dose. Bars represent the mean ± S.D. To establish the limit dose between the stimulant and inhibitory effects of Cd onjambu, the maximum stimulatory Cd doses were calculated for each multivariate indicator, using the regression models shown in Figures 2 and 3. As observed using the gI equation, the Cd dose that showed the highest stimulatory effects on jambu growth variables was 1.0 mg□L^{-1} of nutritive solution, and the limit concentration (where no effects were observed as compared to the control plants) was 2.05 mg□L^{-1}. Regarding gain mass variables, evaluated by the mI, the maximum stimulatory Cd dose was of 0.85 mg□L^{-1}, and the limit dose showing no adverse and no beneficial effects on jambu was of 1.7 mg□L^{-1}. In relation to the physiological performance of the plant, the maximum beneficial dose was of 1.1 mg□L^{-1}, and the limit concentration of 2.2 mg□L^{-1}. As the plant responds in a systematic form and is not zoned, the gnI shows the integrated behavior of all the studied variables. This indicator allowed the visualization of the binary behavior of the plant in a clean form. The maximum stimulatory concentration (at which the plants reached their maximum performance) was close to 1.05 mg□L^{-1}. At increasing Cd concentrations, jambu plants showed developmental deficiencies; it was determined that 2.1 mg□L^{-1} of Cd was the maximum dose at which the plant did not showany toxicity symptoms. Thus, according to the gnI, from this threshold dose on, the plant performance should worsen compared to that of plants not exposed to Cd (Figure 3B).

DISCUSSION

The present study clearly shows that Cd had positive effects on jambu plants, including gains in mass, height, stem diameter, and leaf number, at a dose of 3 mg□Lg^{-1}, but had an inhibitory effect at higher doses (Table 2). Similar to these results, Jia *et al*. (2013) demonstrated that *Lonicera japonica* plants exposed to 10 mg□kg-1 of Cd for 90 days increased their biomass. Piotto *et al*. (2018) demonstrated that tomato plants from the *Calabash Rouge* genotype, in the presence of Cd, had higher dry weights from both the aerial part and root compared to other varieties studied. Małkowski *et al*. (2020) studied coleoptile sections of corn seedlings and showed that there was an increase in the growth of Cd-treated seedlings. The maximum Cd content allowed by ANVISA in leaf vegetables is 0.05 mg□kg^{-1}. Here, we observed that jambu plants exposed to 1 mg□L^{-1}Cd concentrated in their aerial (edible) parts up to 27 times more Cd than the permitted concentration, and about 62 times above this maximum limit when exposed to Cd at 9 mg□L^{-1}.The Tr index showed that, in the presence of 1 mg□L^{-1}Cd doses, jambu had a Cd-translocation capacity of 42.05% to the leaves, 25.96% to the stems, and 5.66% to the inflorescences, whereas only 26.32% remained in the radicular system. When the aerial part was analyzed, 73.68% of the Cd in the solution was partitioned by the plant, especially to the leaves. The observed behavior in this survey gainsay most of the studies on Cd effects on plants, especially agronomical ones, because they report that in the presence of the metal, plants activate their defense systems, producing phytochelatins and retaining a major part of the Cd in their radicular systems or in the nutritive solution; this does not occur in jambu, mainly when exposed to low metal doses. Nascimento *et al*. (2020) pointed out that plants have many components with antioxidant properties such as flavonoids (quercetin), amino acids (tryptophan), and polyphenolic amides, that act as growth regulators, pathogenic defenses, and inhibitors of the production of reactive oxygen species. These compounds may reduce the negative effects of Cd and promote a positive physiological response of the plant, thereby increasing its growth.

Nevertheless, with the increase in the concentration of Cd doses, the capacity of jambu to translocate the metal was reduced, possibly due to the activation of the plant defense system, unleashing compounds that neutralize the metal (Ahmad *et al*. 2019; Jia *et al*. 2015).The gI clearly demonstrated this biphasic behavior, showing that the plants exposed to Cd at1 and 3 mg□L^{-1}had growth gains of 34.46% and 16.6%, respectively, compared to the plants grown in the absence of Cd (Figure 2A). In relation to mass gain, the behavior was similar, with a mass increase of 17.16% with Cd at 1 mg□L^{-1} and of 3.09% at 3 mg□L^{-1}, as shown by the mI (Figure 2B). The photosynthetic performance of plants is extremely important for mass production and growth. Some studies have revealed that Cd affects the photosynthesis, production, quality, and performance of biomass due to its high toxicity in plants (Khan, *et al*. 2016; Rizwan *et al*. 2017). Hussain *et al*. (2019) reported that the metal decreased the root length and aerial parts of peppers cultivated in hydroponic systems. Kurdizel *et al*. (2004) showed that in some plants, Cd substituted Mg in Rubisco, modifying its structure. In addition, Grajek *et al*. (2020) demonstrated the effect of Cd on the degradation of chlorophyll molecules through spontaneous substitution of Mg, which links with porphyrin, resulting in the inhibition of the photosynthetic activity. This survey, in contrast with the referenced studies, shows that Cd improvedCO_2 assimilation rates, stomatal conductance, and transpiration in jambu plants at doses of 1 and 3 mg□L^{-1}, and decreased their photosynthetic activity at higher doses.

Notably, this behavior reflects the hormetic effect of Cd towards jambu, as evidenced by the results of the biometric variables involving growth and mass, similar to the phenomena seen using the gI and mI. These results can be corroborated with other Cd-plant studies. For example, Jia *et al*. (2015) reported the beneficial effect of low Cd doses in the photosynthetic apparatus, chlorophyll, and carotenoids of *L. japonica* Thunb. Liu *et al*. (2015) demonstrated that Cd doses below 10 mg□L^{-1}resulted in higher chlorophyll contents in *L. japonica* Thunb. Furthermore, Jia *et al*. (2013) showed that, at low doses, Cd in *L. japonica* Thunb. did not produce any defense system response against reactive oxygen species, indicating that the metal did not induce any toxicity in the plant. Małkowski *et al*. (2020) showed that, when treated with 0.25 μM Cd, corn seedling growth was stimulated. The same result was demonstrated by Ying *et al*. (2010) in a study on Cd stress induced in *Picris divaricate*; the results showed an increase in CO_2 assimilation rate, conductance, and transpiration, with an inverted U-shaped response pattern indicating the hormetic effect provoked by the metal over the plant. In addition, according to Ying *et al*. (2010), at low doses of Cd, no significant difference in the activity of Rubisco of treated plants compared to that of control plants could be found, indicating that Cd did not damage the photosynthetic apparatus. In this study, *Ci* only showed a significant increase at a Cd dose of 9 mg□L^{-1}, indicating that this dose caused a toxic effect on the plant with possible modifications in Rubisco activity. The pI, as a function of the multivariate model of analysis, was able to broadly capture the responses to Cd of the photosynthetic apparatus of jambu, demonstrating the biphasic effect that characterizes hormesis in plants.

Noteworthy, at a dose of 1 mg□L^{-1}, the photosynthetic performance was 28% higher than that of plants not exposed to the metal, showing an improvement in plant conditions or an adaptation to the presence of Cd. On the other hand at a dose of 9 mg□L^{-1}, there was a reduction of more than 70% in the photosynthetic performance of jambu. Using pI, it was possible to determine that 1.1 mg□L^{-1} was the maximum Cd dose for achieving a stimulatory effect on jambu plants. In a more comprehensive way than other studies that approached the subject, the multivariate analysis done here allowed a more robust evaluation of the behavior of jambu exposed to Cd. This analysis clearly demonstrated the biphasic behavior of jambu responses and thus the hormetic effect of the metal on the plant. gnI showed that, at the lower doses used, there was an increase in plant performance, whereas there was a performance inhibition at higher doses. According to Calabrese (2008, 2015), the hormetic effect model includes a hormetic zone composed of a maximum dose for achieving the stimulatory response and a dose with no observed adverse effects. In this research, through the use of multivariate indicators, it was possible to identify the characteristics described by Calabrese (2008). Based on the gnI model, the maximum stimulatory response dose was 1.05 mg□L^{-1} of Cd and the dose with no adverse effect observed was 2.1 mg□L^{-1}, this is considered as the threshold dose between the stimulatory and toxic effects of Cd in jambu. This research introduces a new way to analyze experimental data in a more integrated manner, enabling the understanding of the phenomena that occur when a plant is affected by an external agent, whether in the form of stimulation or inhibition. The models proposed here can be widely used in the areas of agronomic sciences, especially in studies that prioritize the inductive effect of some agents, whether inorganic or not.

CONCLUSION

Low doses of Cd in nutritive solutions promoted stimulant effects in jambu, increasing its biomass and growth, without increasing the risk of leaf gas exchange. The multivariate indicator presented in this survey provides a new methodological systemic approach to measure the total effects of heavy metals in plants, allowing us to reveal the biphasic behavior and effect dose/response in jambu, which is characterized as a hormesis-presenting plant. The gnI was useful to determine that 1.05 mg□L^{-1} of Cd in nutritive solution was the ideal concentration to improve plant performance and that 2.1 mg□L^{-1}of Cd was the threshold concentration between the stimulant and toxic effects. This survey demonstrated that the plant concentrates Cd in its aerial parts at levels above the maximum limits allowed by Brazilian legislation for leafy (edible) vegetables. Many crop areas are located near great urban centers, as in the case of Belém, in the Amazon. These areas are potentially exposed to many pollutant sources that could make heavy metals available in the form of particles, untreated effluent, and technological waste such as batteries. Because of the phytoextraction and accumulation capacity of Cd, it is necessary to establish environmental policies with the aim of monitoring heavy metals in soils used for crops, reducing particulate matter in the areas around the crops, and paying more attention to phosphate fertilization in plant management. Moreover, to support further research to identify other toxic species to which jambu is tolerant to.

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