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NITROGEN AND CARBON METABOLISM EVALUATION IN PARICÁ PLANTS SUBJECTED TO DIFFERENT CADMIUM CONCENTRATIONS

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Abstract
The development of anthropogenic activities such as industry, mining, agriculture, urban waste discard has been, the main actions that result in increased contamination by heavy metals in soil, water and air. One of the most harmful metals made available by these activities is cadmium, and even at low concentrations it is very toxic mainly in plant structures. The objective of this work was to verify the biochemical behavior of nitrogen and carbon metabolism in young plants of paricá when submitted to increasing cadmium application. For this, a completely randomized experiment was carried out with five treatments (control, $CdCl_2$ 178 µM, $CdCl_2$ 356 µM, $CdCl_2$ 534 µM, $CdCl_2$ 712 µM), with seven replicates, totaling 35 experimental units. The sensitivity of this vegetable to the increasing concentrations of cadmium was evident. The root system it presents'' saw where the most toxic element accumulated, solutes such as carbohydrates, sucrose were affected in their concentrations, mainly in the leaves. The root system saw in its concentrations of glycine betaine a possibility of osmoprotection, but this did not reflect an increase in the concentration of nitrate in both leaf and roots. In the other hand, this fact not observed by the concentration of ammonium that increased in the root system. The results showed that the cadmium was transported to aerial part, however, concentrated mainly in the root system characterizing as a phytoextractor species.

Keywords: Biochemical. Cadmium chloride. Heavy metal. Phytoextractor. Phytoremediation.

1. Introduction

The development of human activities such as industry, mining, agriculture (fertilizers and pesticides), urban waste disposal inappropriately has been the main actions that result in increased contamination by heavy metals in soil, water and air (Ribeiro, 2013; Kumar et al. 2015). In the case of soils, their bioavailability and absorption by plants depend on several factors such as soil properties and types, pH, soil moisture, micronutrient levels (Hu et al. 2017).

One of the most harmful metals for plants is cadmium, it has a relatively high density and is very toxic in low concentrations (Zulfiqar et al. 2019). In the soil it does not suffer chemical and microbial degradation and its concentration persists for long periods (Kubier et al. 2019). Cadmium contamination generates disturbances in ecosystems and human health through direct ingestion of contaminated food, physical contact with polluted soil, ingestion of polluted water, thereby reducing food quality and reducing agricultural land and subsequent food insecurity (Hussain et al. 2021).

The toxic effects of cadmium on vegetables are diverse, such as a reduction in the relative water content in the leaves, transpiration and stomatal conductance, an increase in reactive oxygen species, a breakdown of cell membranes, destruction of biomolecules and organelles, as well as a reduction in the process of nutrient uptake (Abbas et al. 2017; Xu et al. 2017). As well as changes in the activity of various enzymes involved in nitrogen metabolism (Song et al. 2017), including those of nitrate assimilation (Nitrate reductase, NR and Nitrite reductase, NiR) (Singh et al. 2016).

The search for tree species that have characteristics and strategies that allow them to survive under conditions of stress by heavy metals such as Cd is not yet a consolidated reality in the scientific environment, requiring interdisciplinary studies (Usharani and Vasudevan 2017). Studies indicate that the species *Schizolobium amazonicum* Huber ex Ducke, known as paricá, has the potential to become a heavy metal with phytoremediation.

In view of this last information, the objective of this scientific work is to evaluate the biochemical behavior of this species in the face of increasing concentrations of cadmium in nutrient solution.

2. Material and Methods

The experiment was carried out in the greenhouse of the Institute of Agricultural Sciences (ICA), at Federal Rural University of Amazonia (UFRA), Belém, Brazil.

The paricá seedling production process comprised seed scarification with sandpaper number 80 and imbibition in cold water for 24 hours to help breaking dormancy. Next, seeds were placed in 4.6 L Leonard jars (adapted with polyethylene clamps) filled with washed and autoclaved sand (Staying for a period of 1 hour at 126 °C.); the jars were wrapped with aluminum foil to minimize solar radiation interference in root growth. Circular-shaped EVA paper was placed on the surface of each jar to avoid algae proliferation. Two-month-old seedlings were subjected to the application of cadmium chloride concentrations ($CdCl_2.H_2O$) in nutrient solution. Solutions were renewed on a weekly basis and pH was kept between 5.8 to 6.0.

Plants were collected for biochemical analysis as soon as signs of toxicity were observed after cadmium application; the symptoms observed were leaf chlorosis, leaf wilting and leaf epinasty, and they were separated into leaf, stem and root, which were stored in paper bags and taken to a forced-air ventilation oven at 65°C for 48 h. After the drying process was over, the aforementioned parts were ground in Wiley-type mill and stored in falcon tubes to be analyzed in the Laboratory of Studies on Superior Plants' Biodiversity (EBPS - Estudos da Biodiversidade em Plantas Superiores). Part of the dry material was sent to Emílio Goeldi Pára Museum for Cd^{+2} concentration analysis.

Cadmium Analysis

The methodology used was described in the "Manual of Chemical Analysis of Plants, Soils and Fertilizers" of the Cap. "Chemical Analysis of Plant Tissue", adapted from Embrapa, where wet digestion with HNO_3 + $HClO_4$ (3:1) in digester block. 0.5 g of the crushed material was weighed, transferred to a digester tube, added 8 ml of a solution HNO_3:$HClO_4$ (3:1) and left to digest cold overnight. Subsequently, the temperature of the digester block was increased to 120 °C until the brown steam was completely released and then to 200 °C until the white steam was completely released. Let it cool down. After digestion, the tube solution was filtered and measured up to the mark in a 50 mL volumetric flask with deionized water. The samples were digested in triplicate (1, 2 and 3) and the analytical blank was prepared by the same method without addition of the sample. The mineral composition was determined from this solution in the Chemical Analysis Laboratory of the Museu Paraense Emílio Goeldi, using a Thermo brand flame atomic absorption spectrometer, model ICE3000.

The biochemical analysis was carried out at the Laboratory of Studies on Superior Plants' Biodiversity of Federal Rural University of Amazonia (Belém, Pará, Brazil). The experiment followed a completely randomized design (CRD) with five treatments comprising control (cadmium-free plants) and plants subjected to cadmium doses ($CdCl_2$ 178 µM, $CdCl_2$ 356 µM, $CdCl_2$ 534 µM, $CdCl_2$ 712 µM), and seven repetitions, which totaled 35 experimental units (one plant per jar).

The biochemical analysis was carried out at the Laboratory of Studies on Superior Plants' Biodiversity (EBPS) of Federal Rural University of Amazonia (UFRA), Belém County, Pará State. The activity of the nitrate reductase enzyme was the only variable that was used fresh plant tissue. The experiment followed a completely randomized design (CRD) with five treatments comprising control plants (cadmium-free/ (T1)) and plants subjected to Cadmium doses ($CdCl_2$ 178 µM (T2), $CdCl_2$ 356 Mm (T3), $CdCl_2$ 534 µM (T4), $CdCl_2$ 712 µM) (T5), and seven repetitions (one plant/jar), which totaled 35 experimental units.

Data were subjected to analysis of variance; and evaluated for normality and homogeneity of variances by Shapiro-Wilk and Bartlett tests, respectively. Means were compared using the least significant difference in the Tukey test ($p < 0.05$) and adjusted using polynomial regression equations ($P < 0.01$ or 0.05) in Sisvar software (Ferreira 2019).

3. Results

Total Soluble Carbohydrates

The concentrations of carbohydrates in the leaves increased as the concentrations of cadmium were increased, the same did not happen with the roots, where the concentrations of carbohydrates decreased drastically reaching values close to zero. Even with this behavior of suddenly drop in carbohydrates in the roots, they showed the highest concentrations (Table 1). In terms of increased carbohydrates in the leaves, the best treatment that expressed this increase was T4 (534 µM) with approximately 47% when compared to the control treatment. Regarding the roots, the treatment that best expressed a marked decrease was T5 (712µM) with a percentage of 92% when compared to control plants.

Sucrose

The sucrose concentrations in the paricá leaves were lower when compared to the roots, in the latter organ as the cadmium concentrations were being increased, the sucrose concentration decreased dramatically in their structures (Table 1). But even with this decrease, the roots obtained high concentrations of sucrose. The treatment that was most affected by the concentration of cadmium in the roots was T4 (534 µM) with an approximate percentage of 53% decrease when compared to the control treatment. In leaves, T4 (534 µM) was also the treatment that was most affected by the toxic element with an approximate 25% decrease when compared to control plants. The averages of cadmium concentration, show that the roots accumulated this element in their vacuolar structures, not allowing the translocation in high concentrations of sucrose to the aerial part.

Reducing sugars

As shown in (Table 1), the concentrations of reducing sugars in the leaves increased along with the increase in cadmium until the T4 treatment. This increase in percentage terms was approximately 51% when compared to the control plants allowing a significant difference between these two treatments.

Nitrate concentrations (NO_3^-)

The concentrations of nitrate in the leaves and roots were very low close to zero, in the leaves the statistical differences when compared to the control treatment occurred only from treatment T3. There were no statistical differences in the roots, but there was an abrupt drop in this nitrogen compound as the cadmium concentrations were increased (Table 1). A higher concentration of nitrate is observed in the root system of paricá plants when compared to the aerial part.

Nitrate reductase

The nitrate reductase enzyme dramatically decreased its activity on leaves when subjected to increasing concentrations of cadmium. This negative influence of cadmium on the enzymes reductases reflected in a decrease of 95% of its activity, being readily observed in the highest concentration of cadmium T5 (712 µM). In the roots, it is observed a behavior opposite to that of the leaves, where there was an increase in this enzymatic activity, allowing significant differences when comparing all treatments with cadmium stress to the control plants (Table 1). In terms of the percentage of increased enzyme activity in this organ, it was 273%.

Free ammonium

Ammonium concentrations in paricá leaves decreased with increases in cadmium, showing statistical differences with the control plants from treatment T3 (356 µM). This decrease was more noticeable in this T3 treatment, with a 50% reduction in ammonia concentration (Table 1).

Total Soluble Aminoacids

The concentrations of amino acids in the leaves were increasing, however, only after treatment T3 (356 µM). Arriving to present values above the control. The statistical difference with the control plants occurred only in the last T5 stress treatment (712 µM). In percentage terms, a 68% increase in amino acids was observed in this organ. For the roots there was a behavior contrary to the leaf system, with a marked decrease in this amino acid as the cadmium concentrations were increased. The statistical differences occurred from the T3 treatment (356 µM) when compared to the control plants. In percentage terms of decrease, it was 67% (Table 1).

Table 1. Means, relative percentage decrease and increase (RPDI), coefficient of variation (CV) metabolism in aereal and root part in seedlings of *Schizolobium amazonicum* Huber ex Ducke submitted to dosages of cadmium, after 14 days.

Parameter	Cadmium doses (µM)					CV (%)	RPDI (%)	R^2 (%)
	0	178	356	534	712			
Sucrose in leaves	4.41^{a}	1.26^{b}	2.05^{ab}	1.13^{b}	1.56^{b}	0.38	-64.48	75.88
Sucrose in roots	5.27^{a}	4.26^{ab}	2.94^{bc}	2.49^{c}	2.74^{c}	27.10	-47.95	97.72
Total soluble carbohydrate in leaves	0.79^{b}	0.96^{ab}	1.04^{ab}	1.16^{a}	1.08^{a}	17.51	36.78	77.48
Total soluble carbohydrate in roots	0.39^{a}	0.33^{ab}	0.14^{cd}	0.24^{bc}	0.03^{d}	36.34	-92.63	78.44
Reducing sugars in leaves	0.75^{b}	0.86^{ab}	0.88^{ab}	1.13^{a}	1.00^{ab}	21.91	33.25	70.20
NO_3^- in leaves	0.02^{b}	0.02^{b}	0.03^{a}	0.04^{a}	0.03^{a}	1.76	38.22	76.22
NO_3^- in roots	0.06^{ab}	0.09^{a}	0.08^{ab}	0.07^{ab}	0.05^{b}	28.80	-17.97	76.97
Nitrate reductase in leaves	0.62^{a}	0.17^{b}	0.17^{b}	0.13^{b}	0.02^{c}	24.44	-96.20	98.82
Nitrate reductase in roots	0.74^{c}	1.92^{b}	2.76^{a}	2.61^{ab}	2.76^{a}	22.07	271.14	96.85
Ammonium in leaves	6.08^{a}	6.30^{a}	3.04^{b}	3.09^{b}	3.76^{b}	25.48	-38.20	87.68
Amino acids in leaves	4.47^{bc}	4.17^{c}	3.70^{c}	6.20^{ab}	7.52^{a}	22.10	68.46	91.61
Amino acids in roots	7.22^{a}	6.08^{a}	4.11^{b}	2.54^{b}	2.53^{b}	23.76	-64.89	93.92
Protein in leaves	0.32^{c}	0.47^{bc}	0.57^{ab}	0.59^{ab}	0.68^{a}	18.96	113.16	92.97
Protein in roots	0.47^{a}	0.39^{ab}	0.29^{ab}	0.24^{b}	0.40^{ab}	32.77	-15.04	84.73
Proline in roots	0.93^{a}	0.81^{ab}	0.68^{b}	0.66^{b}	0.68^{b}	17.62	-26.54	78.70
Glycine betaine in roots	15.60^{ab}	15.93^{ab}	18.20^{a}	13.93^{b}	14.64^{ab}	14.76	-6.13	37.10

Sucrose in leaves (mg sucrose g^{-1} DM); Sucrose in roots (mg sucrose g^{-1} DM); Total soluble carbohydrate in leaves (µmol carbohydrate g^{-1} DM); Total soluble carbohydrate in roots (µmol carbohydrate g^{-1} DM); Reducing sugars in leaves (µmol carbohydrate g^{-1} DM); NO3- in leaves (mmol $NO3^-$ kg^{-1} DM); $NO3^-$ in roots (mmol $NO3^-$ kg^{-1} DM); Nitrate reductase in leaves (µmoles $NO2^-.g.FM^{-1}.h^{-1}$); Nitrate reductase in roots (µmoles $NO2^-.g.FM^{-1}.h^{-1}$); Ammonium in leaves (mmol $NH4^+$ kg^{-1} of DM); Amino acids in leaves (µmol AA g^{-1} DM); Amino acids in roots (µmol AA g^{-1} DM); Protein in leaves (mg protein g^{-1} DM); Protein in roots (mg protein g^{-1} DM); Proline in roots (µmol.$g^{-1.}$ DM); Glycine betaine in roots (mg of glycine betaine per g DM).

Total Soluble Proteins

Protein concentrations in paricá leaves showed linear growth as cadmium concentrations were increased. Evidence of statistical difference when compared to the control plants from the T3 treatment (356 µM). This increase represented a percentage value of 112% when compared to the control plants. In the roots it was the inverse of the leaves, decreasing as the cadmium was increased, this behavior was observed until the T4 treatment (534 µM). This decrease in percentage represented 49% when compared to the control plants (Table 1).

Proline concentrations

The concentrations of proline amino acid in the roots of paricá decreased in the conditions of increase of cadmium, when compared to the control plants. This decrease was observed mainly in the T4 treatment, presenting a percentage value of 29% when compared to the controls (Table 1).

Concentrations of glycine betaine

The glycine concentrations in the roots were higher in the first cadmium stress treatments, decreasing from the T4 treatment, with this treatment being the lowest value of this amino acid, representing in percentage terms a 10% decrease (Table 1).

Table 2. Regression equations in aereal and root part in seedlings of Schizolobium amazonicum Huber ex Ducke submitted to dosages of cadmium, after 14 days.

Parameter	Regression equation	R^2 (%)
Sucrose in leaves	$y = 4.0229 - 0.012x + 1.2275E\text{-}5x^{2\,*}$	75.88
Sucrose in roots	$y = 5.3921 - 0.0093x + 7.6617E\text{-}6x^{2\,**}$	97.72
Total soluble carbohydrate in leaves	$y = 0.8512 + 0.0004x^{**}$	77.48
Total soluble carbohydrate in roots	$y = 0.3882 - 0.0005x^{**}$	78.44
Reducing sugars in leaves	$y = 0.7713 + 0.0004x^{**}$	70.20
NO_3^- in leaves	$y = 0.0242 + 1.5989E\text{-}5x^{**}$	76.22
NO_3^- in roots	$y = 0.0679 + 0.0001x - 1.9766E\text{-}7x^{2\,**}$	76.97
Nitrate reductase in leaves	$y = 0.6095 - 0.0038x + 9.5935E\text{-}6x^2 - 7.6344E\text{-}9x^{3\,**}$	98.82
Nitrate reductase in roots	$y = 0.7796 + 0.0075x - 6.8608E\text{-}6x^{2\,**}$	96.85
Ammonium in leaves	$y = 6.2157 + 0.0053x - 5.4986E\text{-}5x^2 + 6.0354E\text{-}8x^{3\,**}$	87.68
Amino acids in leaves	$y = 4.469 - 0.0054x + 1.401E\text{-}5x^{2\,**}$	91.61
Amino acids in roots	$y = 7.0757 - 0.0072x^{**}$	93.92
Protein in leaves	$y = 0.3585 + 0.0005x^{**}$	92.97
Protein in roots	$y = 0.4889 - 0.001x + 1.1678E\text{-}6x^{2\,**}$	84.73
Proline in roots	$y = 0.8809 - 0.0004x^{**}$	78.70
Glycine betaine in roots	$y = 15.6129 + 0.0071x - 1.3045E\text{-}5x^{2\,**}$	37.10

Sucrose in leaves (mg sucrose g^{-1} DM); Sucrose in roots (mg sucrose g^{-1} DM); Total soluble carbohydrate in leaves (µmol carbohydrate g^{-1} DM); Total soluble carbohydrate in roots (µmol carbohydrate g^{-1} DM); Reducing sugars in leaves (µmol carbohydrate g^{-1} DM); NO3- in leaves (mmol $NO3^-$ kg^{-1} DM); $NO3^-$ in roots (mmol $NO3^-$ kg^{-1} DM); Nitrate reductase in leaves (µmoles $NO2^-.g.FM^{-1}.h^{-1}$); Nitrate reductase in roots (µmoles $NO2^-.g.FM^{-1}.h^{-1}$); Ammonium in leaves (mmol $NH4^+$ kg^{-1} of DM); Amino acids in leaves (µmol AA g^{-1} DM); Amino acids in roots (µmol AA g^{-1} DM); Protein in leaves (mg protein g^{-1} DM); Protein in roots (mg protein g^{-1} DM); Proline in roots (µmol.$g^{-1.}$ DM); Glycine betaine in roots (mg of glycine betaine per g DM). * Equal letters do not differ from each other, in the lines, by the tukey test, at 5% probability. ** Equal letters do not differ from each other, in the lines, by the tukey test, at 1% probability.

Cadmium concentrations in paricá organs

The factorial statistical analysis of the cadmium concentrations in these three organs analyzed, showed that in the aerial part considering within each leaf and stem tissue, there were no statistically differences, regardless of the applied cadmium doses. In the case of roots there was an accumulation mainly in the highest concentrations of cadmium for treatments T4 (534 µM) and T5 (712 µM), differing statistically with the control and T2 plants (178 μM). When analyzed among plant tissues, it can be seen that from T3 (356 μM), there were statistical differences, accumulating mainly in the roots of plants with higher concentrations of cadmium (Table 2).

4. Discussion

Carbon balance in response to cadmium concentrations

Total Soluble Carbohydrates

The organ most affected by cadmium concentrations was the roots, and one of the consequences was the decrease in soluble carbohydrates in that organ and possibly the exudation of organic acids that influenced the solubilization of cadmium, allowing it to be more easily absorbed by the roots (Fu et al. 2017). The toxicity of cadmium in the roots was so relevant that substances such as soluble amino acids could support a certain tolerance (Yan et al. 2020), but this was not the case in this study, where the concentrations of amino acids in the roots decreased dramatically. A possible answer to this high concentration of cadmium in the root system and to have influenced carbohydrate concentrations is due to the root cell walls of vacuoles that have negative charges that adsorb metal ions, acting as an initial barrier for the transport of cadmium (Song et al. 2017). Once this element is present, it changes these soluble sugars, preventing their energetic activities in other biomolecules.

Sucrose

The low sucrose concentrations in the leaves can be possibly explained by the toxic action of cadmium, which drastically reduces the levels of chlorophyll a and b and carotenoids, characterizing leaf chlorosis, probably due to the inactivation of enzymes responsible for the synthesis of these pigments (Rodrigues et al. 2017). To explain the high sucrose concentrations in the root system when compared to the leaves is that in treatments with low cadmium concentrations, sucrose transport to the root system could have occurred (Table 3). Para Gangola e Ramadoss (2018), sugars such as sucrose can act as osmotic protectors during cell dehydration caused by abiotic stress, such as heavy metals in the root system. This would justify an action of tolerance towards the metal.

Table 3. Average values of cadmium concentrations in the three organs of the Schizolobium amazonicum.

Cadmium dosages in nutritive solution (μM)	Cadmium content in plant tissue (mg kg^{-1})		
	Leaves	Stem	Roots
0	0.00 (0.00)^{Aa}	0.00 (0.00)^{Aa}	0.00 (0.00)^{Ad}
178	28.66 (7.37)^{Aa}	51.03 (4.69)^{Aa}	1117.76 (104.36)^{Acd}
356	19.91 (4.97)^{Ba}	48.56 (5.19)^{Ba}	2564.30 (373.60)^{Abc}
534	23.91 (8.37)^{Ba}	55.33 (0.89)^{Ba}	3211.21 (203.15)^{Aab}
712	27.66 (5.54)^{Ba}	51.80 (12.64)^{Ba}	5017.80 (36.78)^{Aa}

Equal letters do not differ from each other, in the columns, by the tukey test, at 5% probability.

Reducing sugars

Reducing sugars belong to non-structural sugars whose levels are sensitive to environmental variations and damage to plants; these sugars are used to assess plant responses to stress conditions (Souza et al. 2013). As with the other sugars analyzed in this work, reducing sugars increased their concentrations in the leaf system of paricá plants. One of the possible responses to this behavior is the accumulation of reducing sugars as a result of the release of hexoses. These come from sucrose hydrolysis, a result presented in this work (Table 1), due to the action of the enzyme invertases, which could provide monosaccharides for the anabolic or catabolic processes of vegetables, in addition to providing reducing sugars for the osmotic adjustment in the leaf system. This accumulation of hexoses can contribute to osmotic adjustment, preventing further cell damage associated with cell dehydration caused by cadmium stress (Rahman et al. 2017).

Cadmium effects in nitrogen metabolism

Nitrate concentrations (NO_3^-)

The toxic effect of cadmium on paricá plants reflected directly in the nitrogen assimilation process (Rizwan et al. 2017), nitrate concentrations accumulated in paricá leaves, this must be related to the accelerated decrease in nitrate reductase activity in this organ, the nutritional dysfunction caused by cadmium in the roots partly explains this accumulation, mainly due to the deficiency of the potassium element (K), this element potentiates the activity of the NB, its inactivation in the health of the plant related to nitrate toxicity (Kyriacou and Rouphael 2018). Another fundamental point to explain this result is possibly the low absorption of magnesium (Mg), another fundamental mineral element in nitrogen metabolism. For NO_3^- to be absorbed, to a direct dependence on ATP hydrolysis by means of proton pumps located in the plasma membranes of the roots to be biologically active, ATP must be linked to a Mg atom. For, Each NO_3^- needs $2H^+$ to be absorbed. Therefore, Mg deficiency directly affects the pumping of H^+ to the external environment, impairing the absorption of NO_3^- (Pereira et al, 2020). This fact is found in the root system of the roots of paricá.

Nitrate reductase enzyme

Many scientific studies have pointed out that high concentrations of cadmium alter the functionality of the nitrate reductase enzyme (Irfan et al. 2014), and this result could be evaluated in this work due to the nutritional dysfunction of paricá under high concentrations of cadmium. The nitrate reductase enzyme has three groups of prosthetics, one of which is a molybdenum (Mo) cofactor linked to the pterine, forming the molybdopterin complex, both NO_3^- and Mo are substrates for enzyme activity, and deficiency of this micronutrient it can cause NO_3^- accumulation in plants (Kovács et al. 2015). And this result is visibly expressed in the leaf system (Table 1).

Free ammonium

Studies carried out with vegetables have shown that there is an accumulation of free ammonium under conditions of stress caused by the cadmium element (Khan et al. 2016). The results presented in this study showed that the higher concentration of cadmium provided an increase in ammonium in the leaves. This can be justified by the increased activity of the enzyme glutamate dehydrogenase (GDH), which is benefited by the element concentrations (Erdal and Turk 2016).

Total Soluble Aminoacids

Solutes compatible with amino acids contain nitrogen in their structures, a very important nutrient for plant biochemical functions (Park et al. 2016). For nitrate to be used in the biosynthesis of amino acids, other nitrogen compounds must be reduced to ammonium. This partly explains the reductions in nitrate and proteins in the root system of paricá plants (Table 1). In the leaves we can observe that the solute amino acid remained in high concentrations, not reducing it in other compatible compounds, this behavior is due to the action of cadmium interfering mainly in enzymes of nitrogen metabolism (Ma et al. 2017), such as nitrate reductase and nitrite reductase that catalyze nitrate and nitrite respectively (Balotf et al. 2016). However, this increase in paricá leaves showed a resistance to stress conditions due to maintaining concentrations of a nitrogenous solute (Zhu et al. 2018).

Total Soluble Proteins

The high concentrations of cadmium in the roots of paricá (Table 2), promoted a significant reduction of soluble proteins (Table 1). Although Cadmium's toxicity mechanisms are poorly understood. Skipper et al. (2016), speculated that this element interrupts protein synthesis in organs such as the roots. One of the consequences of this low protein concentration is the activation of reactive oxygen species (ROSs) (Seneviratne et al. 2019). On the other hand, protein concerts have increased in the leaves, these proteins partially justify the transport of this metal in the xylem even in low concentrations. Transport can take place by a concentration gradient according to Bouron et al. (2015).

Glycine betaine

Among the amino acids most produced in the condition of stress by cadmium was glycine, for Yan et al. (2020), this amino acid is associated as a protector of cell membranes, hijackers and inducer of tolerance to heavy metals. The high concentrations of glycine in the roots of paricá demonstrated this characteristic of protection and tolerance (Table 1). Amino acid metabolism has been identified as a key related to cadmium stress, mainly in the production of amino acids such as proline and glycine, substances that act as osmotic adjusters, allowing plants to resist osmotic imbalance caused by cadmium metal (Xie et al. 2019).

This behavior of concentration of glycine in the roots, demonstrated a very important role in the metabolism of nitrogen, when we observe the high concentrations of NO_3^- in the roots of paricá and proteins in the leaves (Table 1). This is justified, because these amino acid molecules participate in this nitrogen assimilation and protein synthesis (Zhu et al. 2018).

Proline

Even with a reduction in its concentrations in the roots, proline is an important amino acid in the adaptation and acclimatization process to cadmium stress (Ming et al. 2017). This is justified by the high concentrations of proteins in the paricá leaves (Table 1). Proline possibly functioned as a molecular chaperone capable of protecting the integrity of proteins and improving the activities of different enzymes (Mostek et al. 2015). In the case of enzymes, nitrate reductase (NR) was positively influenced by the amino acid proline, improving its activity mainly in the root system (Table 1). Another possible response to the action of this solute proline on the roots of paricá is the inhibition or elimination of reactive oxygen species (Hafsi et al. 2017).

5. Conclusions

The compatible osmolytes in leaves did not shown significance, and for roots only proline presented significative difference, however, decreased when the cadmium concentrations were increased, and it could be concluded that there were no tolerance characteristics.

The energy production in terms of carbohydrates was limited mainly in the roots due to the high concentrations of cadmium, in the leaves there was growth of the production of the reducing sugars and total soluble carbohydrates not interfering directly in the processes of assimilation of carbon and nitrogen.

The main enzyme affected in the route of nitrogen incorporation to nitrate reductase was affected for the cadmium doses, especially in the leaves. For the roots there was an increasing activity of this enzyme, but did not represent production in macromolecules like the amino acids.

The results showed that the cadmium was transported to aerial part, however, concentrated mainly in the root system characterizing as a phytoextractor species.

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