UFSM

Ciência Florestal

ISSN 1980-5098
OPEN ACCESS

Ci. Fl., Santa Maria, v. 35, e89275, p. 1-20, 2025 • https://doi.org/10.5902/1980509889275
Submitted: 8th/10/2024 • Approved: 28th/02/2025 • Published: 31st/07/2025

Articles

Nitrogen metabolism in young plants of African mahogany submitted to different concentrations of cadmium

Metabolismo do nitrogênio em plantas jovens de mogno africano submetidas a diferentes concentrações de cádmio

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ABSTRACT

The present study aimed to evaluate the biochemical changes in nitrogen metabolism promoted by the toxic action of cadmium (Cd) in young African mahogany plants (*Khaya grandifoliola*). The concentrations of cadmium were applied in the form of cadmium chloride monohydrate ($CdCl_2.H_2O$), using a completely randomized design (DIC), with five concentrations of $CdCl_2.H_2O$ (0, 10, 20, 30 and 40 mg L^{-1}) and seven replicates for each treatment. Analyzing the higher dosage of $CdCl_2.H_2O$ in the present study, it was verified that the accumulation of cadmium in leaves and roots were equivalent to 228.5% and 743.75%, respectively, followed by reductions in the activity of the nitrate reductase enzyme. For the nitrate concentration, it was verified that the highest contents occurred in the leaves, reducing in the roots, the ammonium on the other hand, presented reduced contents in the foliar tissues. Already for the amino acids there was increase in the leaves. However, it can be inferred that young African mahogany plants (*Khaya grandifoliola*) it presented possible defense mechanisms that were able to prolong its biochemical activities.

Key words: Concentrations; Defense; Mechanisms; Toxic action

RESUMO

O presente estudo teve como objetivo avaliar as alterações bioquímicas no metabolismo do nitrogênio promovidas pela ação tóxica do cádmio (Cd) em plantas jovens de mogno africano (Khaya grandifoliola). As concentrações de cádmio foram aplicadas na forma de cloreto de cádmio monoidratado ($CdCl_2.H_2O$), utilizando-se delineamento inteiramente casualizado (DIC), com cinco concentrações de $CdCl_2.H_2O$ (0, 10, 20, 30 e 40 mg L^{-1}) e sete repetições para cada tratamento. Analisando a maior dosagem de $CdCl_2.H_2O$ no presente estudo, verificou-se que o acúmulo de cádmio nas folhas e raízes foram equivalentes a 228,5% e 743,75%, respectivamente, seguido de reduções na atividade da enzima nitrato redutase. Para a concentração de nitrato, verificou-se que os maiores teores ocorreram nas folhas, reduzindo nas raízes, já o amônio, apresentou teores reduzidos nos tecidos foliares. Já para os aminoácidos houve aumento nas folhas. No entanto, pode-se inferir que plantas jovens de mogno africano (*Khaya grandifoliola*) apresentaram possíveis mecanismos de defesa que foram capazes de prolongar suas atividades bioquímicas.

Palavras-chave: Concentrações; Defesa; Mecanismos; Ação tóxica

1 INTRODUCTION

In 2023, the total area dedicated to tree planting in Brazil exceeded 10 million hectares for the first time, a 3% increase compared to the previous year (Ibá, 2024). The African mahogany belongs to the *Meliaceae* family, originates from the African continent, specifically from Ivory Coast, Cameroon, Angola and Ghana (Alves; Chaves; Bastos, 2020). These authors report that the African mahogany is one of the timber species most sought after by reforesters, considered of moderate density with high economic value, besides being able to produce seedlings and moderate initial growth, reaching an average height of 40 to 50 meters with up to 3, 5 meters in diameter; under favorable conditions can present increments up to 40 m³ ha^{-1} $year^{-1}$ and the value of the cubic meter of sawn wood can exceed U$ 2,000.00.

Among the mineral elements, nitrogen (N) is the mineral element of greatest demand for plants, acting as a constituent component of the vitality of the plant cell, including amino acids, proteins and nucleic acids. it is inferred that its deficiency rapidly inhibits plant growth (Taiz; Zeiger 2013; Conceição; Cruz; Lima; Lima; Silva Teixeira; Sousa; Oliveira Neto, 2020).

Metals are among the most common environmental contaminants because they are non-degradable, remaining for long periods in the environment, cycling in the environmental compartments; these metals may originate from lithogenic and anthropogenic processes, being related to geological sources such as rock residues and weathering processes, as well as to human activities by the use of fertilizers in agricultural zones and mining activities (Ali; Khan; Ilahi, 2019).

In this context, cadmium (Cd) is classified as a non-essential element can act in plants as a potent enzyme inhibitor, causing in this way vegetable cell damage even at low concentrations (Machado; Paiva; Sousa; Costa; Martins; Nascimento; Oliveira Neto, 2024). The exposure of plants to this trace element results in the degeneration of mitochondria and mitosis, as well as damaging the photosynthetic apparatus (Ismael; Elyamine; Moussa; Cai; Zhao; Hu, 2019). Agricultural soils may also show accumulation of Cd due to the application of phosphate fertilizers, fertilizers, metallurgical waste and untreated wastewater (Conceição; Cruz; Lima; Lima; Silva Teixeira J. S.; Sousa; Oliveira Neto, 2020).

The authors Ratke, Aguilera, Zuffo, Baio, Teodoro, Yokota, Oliveira, (2024), highlight that the low-carbon production system brings productive and environmental benefits, necessary for the sustainability of the agricultural system.

Considering the importance of mahogany in the Amazon and the impacts that contamination by heavy metals can cause for the crop, this work aimed to evaluate the influence of cadmium on the nitrogen metabolism in young plants of African mahogany.

2 MATERIALS AND METHODS

2.1 Plant material and growing conditions

The experiment was carried out in a greenhouse of the Federal Rural University of Amazonia-UFRA, Belém-Pa, with geographic coordinates of 01° 27 ‘21 “S, 48° 30’ 16”

W and average altitude of 10 meters, being the biochemical analyzes carried out in the Laboratory of Biodiversity Study of Upper Plants (EBPS), established in the same place. The climate classification is Af according to Köppen and Geiger with average temperature of 26.8 ° C and relative humidity of 95%.

For the present study, mahogany seedlings. From the São Francisco commercial nursery, located in the municipality of Castanhal-PA, were used, presenting an age of approximately 90 days, the seedlings were in good phytosanitary status and homogeneous. The 35th day after the acclimatization (in the greenhouse), the transplant was transplantated for Leoanard pots with capacity of 4.6 L adapted and wrapped with aluminum foil (to minimize the interference of radiated solar radiation) containing washed, sterilized and autoclaved sand. At the edge of each vessel were added e.v.a paper in the form of circles, being coupled to the seedlings in a way not to damage them, avoiding with this method the proliferation of algae. Leonard's vessels contained a solution of Sarruge (1975) with ¼ ionic strength with pH maintained between 5.8 +/- 0.5 (as recommended by the methodology used), using 1N NaOH, or HCl solutions, when necessary, and the solutions were changed weekly.

After the 16th day of acclimatization (in the greenhouse) of African mahogany seedlings in nutrient solution, the concentrations of cadmium in the form of cadmium chloride monohydrate ($CdCl_2H_2O$) were started. For application, the same was added to the nutrient solution at concentrations of 0 (control) mg L^{-1}; 10 mg L^{-1}; 20 mg L^{-1}; 30 mg L^{-1} and 40 mg L^{-1}, in which, each concentration corresponded to a treatment, and these were renewed every seven days. The plants were collected at 60° day (plant exposed to heavy metal) at 04:30 h. In addition, a completely expanded primary leaf of each replicate was used in the greenhouse, in which part of this material was reserved for the determination of nitrate reductase activity (NRA). The other analyses used virgin parts of this same sheet.

The plants were then washed with distilled water and separated into leaf, stem and root and wrapped in foil and stored in a freezer at -80 °C. To ensure optimal preservation of the material. In order to determine the biochemical analyzes, all the material was taken to the forced air ventilation oven at 65 ºC for 48 h, dry material was obtained from the aerial part of the plant and the root system. The dried material was milled until a fine powder was obtained and stored in falcon tubes until its use in biochemical tests.

Cadmium Analyse followed the methodology used was the one described in the "Manual of Chemical Analysis of Plants, Soils and Fertilizers", adapted from Embrapa, where humid digestion with HNO_3 + $HClO_4$ (3:1) was used the digester block. The mineral composition was determined from this solution in the Laboratory of Chemical Analysis of the Museu Paraense Emílio Goeldi using a flame atomic absorption spectrometer of Thermo brand, model ICE3000.

2.2 Calculation of Biomass Factor (BF) and Translocation Factor (TF)

The measurement of the bioaccumulation factor (BF) and translocation factor (TF) are were performed according to Shah, Ahmad, Masood, Zahid, (2008).

2.3 Biochemical assessments

The Nitrate Reductase (RN), with the help of a punch leaf discs was taken, in vivo, according to the method described by Hageman and Reed (1980). The contents of Nitrate (NO_3^-) were determined according to Cataldo, Haroon, Yougs, (2008). The contents of Ammonium (NH_4^+) was determined utilizing the method according to Weatherburn (1967). For determination Total Soluble Aminoacids (AA), the method utilized was according to Peoples, Faizah, Rerkasem, Herridge, (1989).

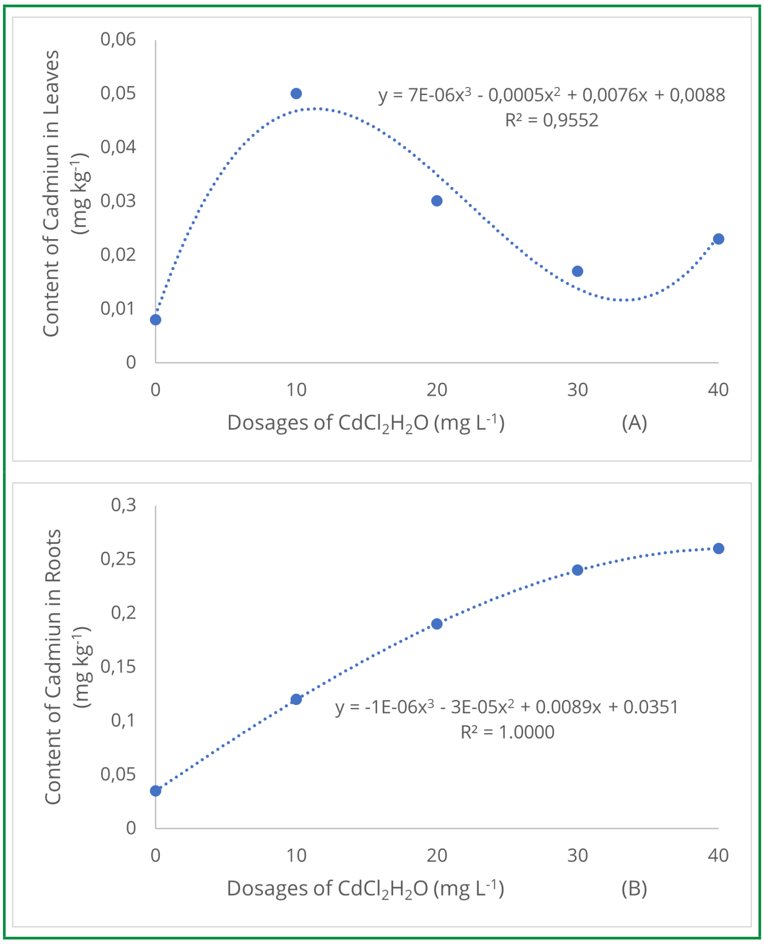
For comparative effect analysis, the data were subjected to analysis of variance, followed by regression analysis using the Sisvar 5.4 program. Means were compared using the Tukey test at a 5% probability level.

3 RESULTS AND DISCUSSION

3.1 Cadmium concentration

The absorption occurs mainly by the roots, and can be soon transported through the xylem vessels to the aerial part of the plants, allowing their movement throughout the plant (Ketehouli; Idrice Carther; Noman; Wang; Li; Li, 2019). Such absorption followed by the translocation can be justified for the African mahogany, which had in its roots high concentrations of Cd, obtaining also the accumulation of the same in the leaves, being inferred, therefore, the transport of the metal of the roots to the leaves. However, the translocation was higher in the dosage of 10 mg L^{-1} of cadmium, according to Figure 1 A, B.

Figure 1 – (A) Cadmium concentrations in leaf; (B) root in young plants of African mahogany submitted to different concentrations of cadmium chloride ($CdCl_2.H_2O$)



Source: Authors (2024)

The cadmium concentrations observed in African mahogany plants, especially those that did not receive doses of cadmium chloride (control plants) during the study time, are possibly related to the formation of seedlings, since they came from a commercial nursery and when they were already present with a certain stage of development, the vegetative phase, therefore, due to the environmental, nutritional conditions or different forms of fertilization coming from the nursery the seedlings may have absorbed small amounts of this metal (Conceição; Cruz; Lima; Lima; Silva Teixeira; Sousa; Oliveira Neto, 2020).

The roots serve as a barrier against the translocation of Cd to the aerial part by the immobilization of toxic ions in the cell wall (Bali; Sidhu; Kumar, 2020). In the leaves, the toxic ions can be incorporated into proteins or translocated through the phloem, along with the photo-assimilators, thus causing a series of phytotoxic effects (Andresen; Peiter; Küpper, 2018). For Shi, Zhang, Chen, Polle, Rennenberg, Luo, (2019) the distribution of cadmium in the plant is also closely associated with the phytochelatins, where the cadmium complex formed together with the phytochelatins may have a mobile form for transporting the heavy metal from the roots to the aerial part through peptides rich in amino acid donors of electrons.

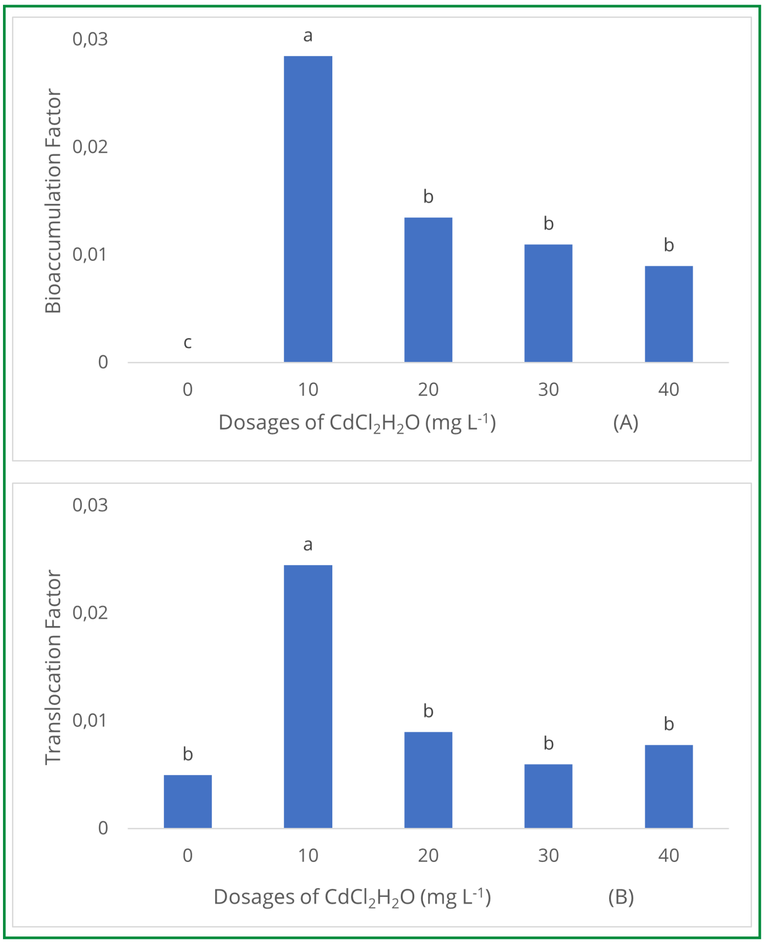
3.2 Bioaccumulation Factor (BF), Translocation Factor (TF)

The results showed that African mahogany showed similar behavior between TF and BT at different concentrations of Cd (Figure 2 A, B). The TF was higher only in the treatment with 10 mg L^{-1} dosage, for the other treatments with dosages, this factor was low, that is, there was a restriction in the translocation of the metal to the aerial part of the plant.

Tolerant species are not always hyperaccumulating, so the translocation factor demonstrates the efficiency of the plant in transporting a root element to the aerial part, while the bioaccumulation factor evaluates the efficiency of the plant in accumulating the metal in relation to the soil concentration (Usman; Al-Ghouti; Abu-Dieyeh, 2019).

A study by Bali, Sidhu, Kumar, (2020) also found higher Cd accumulations in the roots of plants, which may be related to the presence of organic acids in root exudates, and possibly the metal binds to these acids, limiting the translocation to the aerial part.

Figure 2 – (A) Mean values for Bioaccumulation Factor; (B) Translocation Factor, in plants of African mahogany submitted to different dosages of cadmium chloride



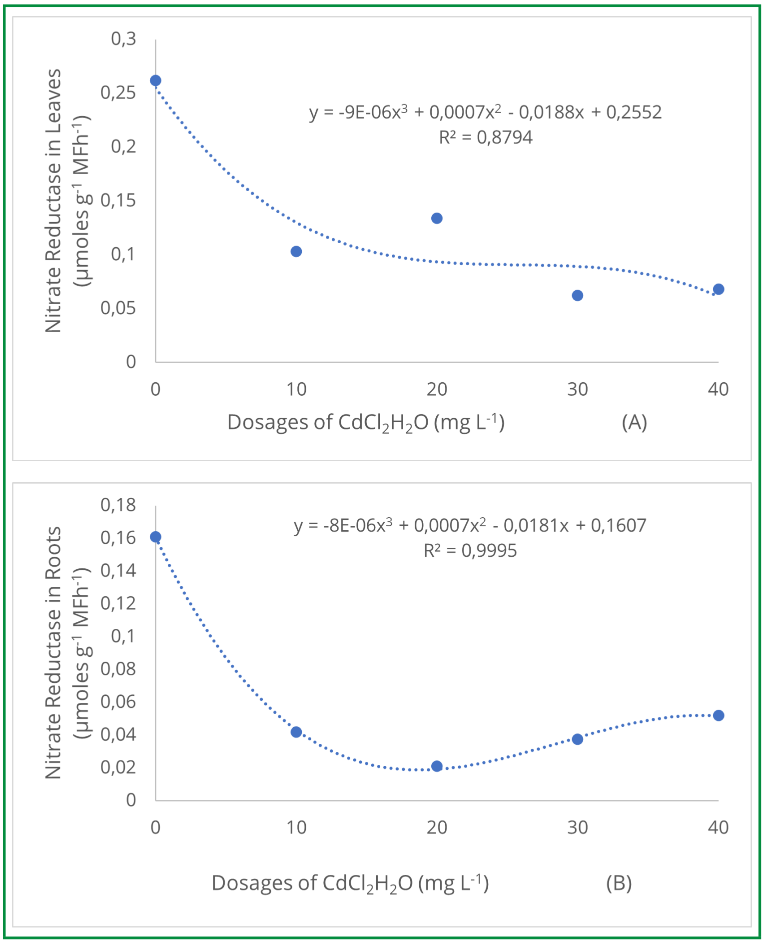
Source: Authors (2024)

3.3 Nitrate reductase activity

It was also verified that the activity of the RN enzyme in leaves presented reductions in its concentration with averages of 0.262; 0.103; 0.134; 0; 062; 0.068 µmoles g^{-1} FM h^{-1} corresponding to the control treatments, 10, 20, 30 and 40 mg L^{-1}, respectively (Figure 3A). And the treatment of higher concentration (40 mg L^{-1} of $CdCl_2.H_2O$) significantly reduced ($p < 0.05$) in 76.3% the activity of the enzyme, when compared to plants that did not receive cadmium chloride monohydrate dose.

For the roots, the means found were 0.161; 0.042; 0.021; 0.037; 0.052 μmoles g^{-1} FM h^{-1}, corresponding to the control treatments, 10, 20, 30 and 40 mg L^{-1} of Cd, respectively (Figure 3B). The treatment of 40 mg L^{-1} of cadmium chloride also showed a significant decrease ($p < 0.05$) with effect of 67.7% under the activity of the nitrate reductase enzyme in relation to the control plants. The obtained results were observed through the adjustment of the cubic regression model.

Figure 3 – (A) Nitrate reductase concentration in leaf; (B) Root of young plants of African mahogany submitted to different dosages of cadmium chloride ($CdCl_2.H_2O$)



Source: Authors (2024)

This reduction in NR activity may be due to the osmotic effects induced by $CdCl_2.H_2O$, which alter the rate of transportation of the NO_3^- (enzyme substrate), as well as the ionic effect, due to competition between NO_3^- and Cl^- (Gloser; Dvorackova; Mota; Petrovic; Gonzalez; Geilfus, 2020). In addition, the effects of stress on the activity of the enzyme may be due to the stimulus to its degradation and or inactivation, or even by the lesser expression of the RN synthesis gene.

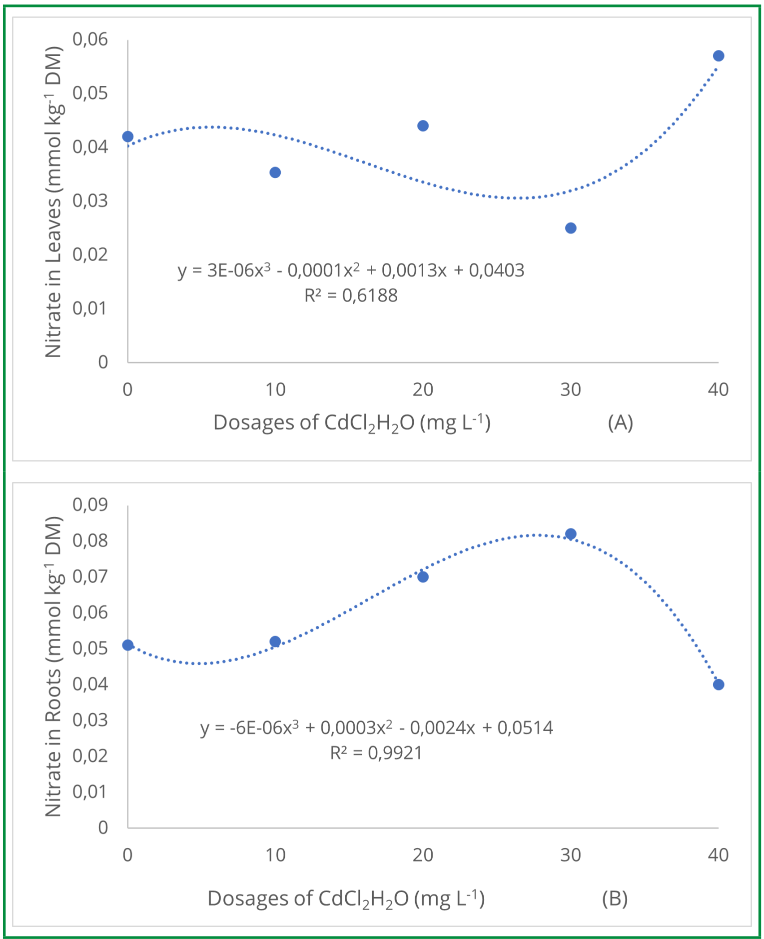
In the present study, the stress factor is the action of Cd in the form of $CdCl_2.H_2O$, which acts as a source of alteration of the enzyme activity synthesis, since the predominance of NH_4^+ in the soil makes the NO_3^- form unavailable for vegetables according to Hernández- Baranda, Rodríguez-Hernández, Peña-Icart, Meriño-Hernández, Cartya-Rubio, (2019). In which, due to stress, the activity of the enzyme is affected considerably, resulting from the accumulation of nitrate in the cytosol of the root tissues.

3.4 Nitrate concentration

She stress induced by $CdCl_2.H_2O$ affects the concentration of NO_3^- (Y) (Figures 4A, and 4B) the plant similar to the damages caused on nitrate reductase, and it is also attributed to the competition effect between Cl^- and NO_3^- by the same carrier, in the synthesis of the plasma membrane, (TAIZ AND ZEIGER 2013). Part of the absorbed NO_3 is assimilated in the roots, and the remainder follows the transpiratory flow, being translocated internally in the plants via xylem to the aerial tissues. Thus, the reduction of nitrate by the African mahogany roots occurs through specific transporters located on the cell surface, by the “sympathetic” process, where the H + ions are co-transported next to the NO_3^- (Sérvulo; Vellame; Casaroli; Alves; Souza, 2017).

In leaves, the increase of nitrate according to Liang and Zhang (2020) is due to the fact that NO_3^- and NH_4^+ are transported through the tonoplast and subsequently stored in the cell vacuole to be reduced in the cytosol of the same cell or translocated unchanged from the roots to the aerial part of the plant via xylem, in stems and leaves, nitrate is reduced to nitrite by the action of nitrate reductase enzyme, and ammonium by the enzyme RN.

Figure 4 – (A) Nitrate concentration in leaf; (B) Root of young plants of African mahogany submitted to different concentrations of cadmium chloride ($CdCl_2.H_2O$)



Source: Authors (2024)

Studies show that the nitrate reduction observed in the roots is also related to the result of the low flow of physiological and biochemical activities of the plant, aiding in the transport of nutrients with reflexes in the low activity of the enzyme in the roots and leaves, considering that it depends of the nitrate supply (Asim; Ullah; Xu; An; Aluko; Wang; Liu, 2020). According to Gloser, Dvorackova, Mota, Petrovic, Gonzalez, Geilfus, (2020) the activity of the RN enzyme does not totally reduce its activity due to the constant, albeit reduced, supply of nitrate from the vacuole reserve of the cells. Shi, Zhang, Chen, Polle, Rennenberg, Luo, (2019) report that plants use much more energy in the reduction of nitrate instead of ammonium as a source of nitrogen utilization, has as probable advantage the energetic cost of detoxification by the process that would be necessary in case of excessive ammonium absorption.

3.5 Ammonium concentration

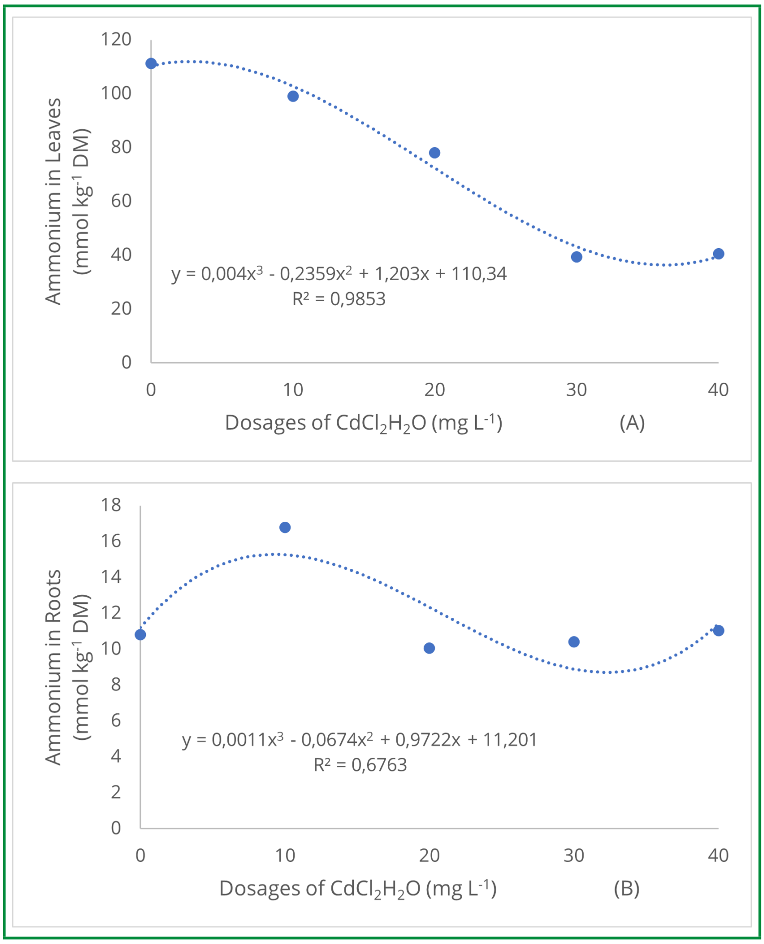
The free ammonium contents were found in leaves (Figure 5A) of young plants of African mahogany considerable decreases in their content, with averages equivalent to 111.3; 99.0; 78.1; 39.4, 40.6 (0, 10, 20, 30 and 40 mg L^{-1}), with a significant reduction ($p < 0.05$) of 67.1% at the maximum Cd dosage compared to the control plants.

In the root tissues, different from the leaves, low accumulations were observed in the NH_4^+ content (Figure 5B), obtaining as average results corresponding to 10.82; 16.79; 10.05; 10.42 and 11.04 mmol kg^{-1} DM respectively to the treatments of cadmium chloride monohydrate (0, 10, 20, 30 and 40 mg L^{-1}) exhibiting a significant increase of respective 2.03% at the dosages of Cd previously mentioned. Thus, a low expressive but significant accumulation ($p < 0.05$) was observed for the dosage of 40 mg L^{-1} of $CdCl_2.H_2O$ in 2.03% at the maximum Cd dosage compared to the control plants. of the adjustment of the cubic regression model.

The reduction of ammonium concentrations under the effect of $CdCl_2.H_2O$ on the leaf tissue of African mahogany plants may be associated with NO_3^-, which as it is present as a source of N is reduced in its biosynthesis to NH_4^+, however, this process occurred adversely in mahogany plants, evidencing its preference in assimilating nitrate uptake (Sérvulo; Vellame; Casaroli; Alves; Souza; 2017).

The accumulation of ammonium in root tissue can be attributed to its direct absorption, reduction of nitrate, deamination of nitrogen compounds, photorespiratory cycle or biological fixation. In this way, the plants of African mahogany. Can be observed that this one possibly presents displays low concentrations of NH_4^+ in the cytosol, as well as exudation of minimum contents in the plant according to Ataíde, Nogueira, Oliveira Neto, Brito, Costa, Martins, Sousa, (2020) that showed similar results in studies carried out with ammonium contents.

Figure 5 – (A) Ammonium concentration in leaf; (B) Root of young plants of African mahogany submitted to different concentrations of cadmium chloret ($CdCl_{2.}H_2O$)



Source: Authors (2024)

3.6 Total soluble aminoacids concentration

Gradual increases in the concentrations of total soluble amino acids (AA) present in the foliar tissues of African mahogany. Plants were observed, with subsequent reduction in the dosage of 40 mg L^{-1} of Cd, whereas for the root tissues, the occurrence of decreases in the contents of the same.

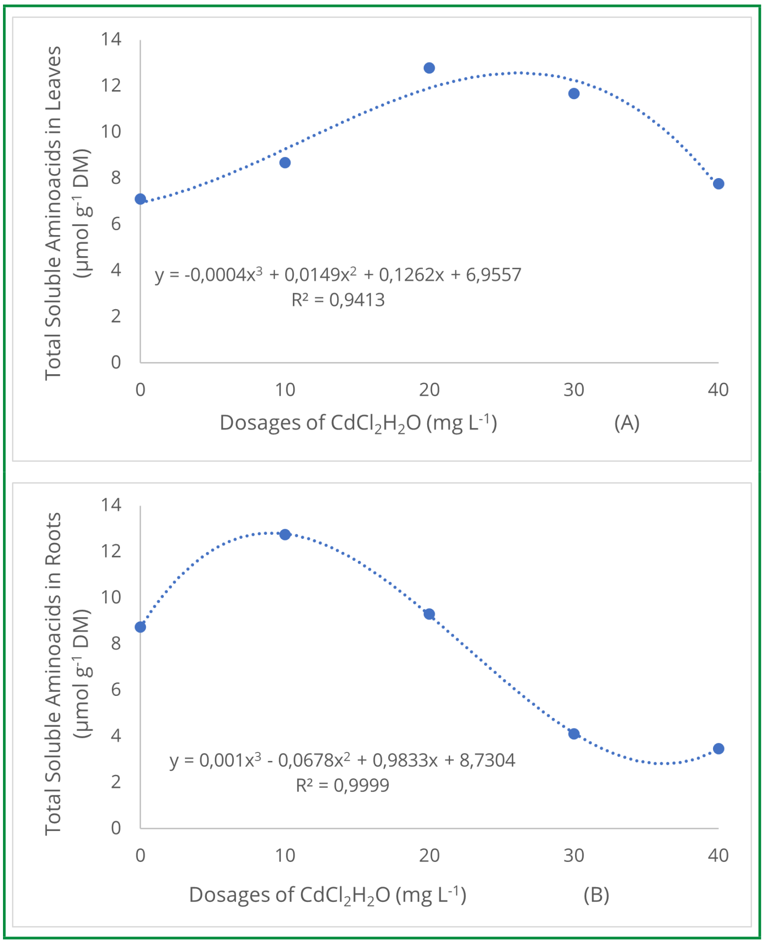
The results obtained in the leaves showed a significant statistical difference ($p < 0.05$) in the concentration of amino acids under influence of heavy metal stress and the averages obtained were equivalent to 7.10; 8.69; 12.78; 11.67 and 7.77 µmol of AA g^{-1} of DM according to the control treatments, 10, 20, 30 and 40 mg L^{-1} of Cd, respectively (Figure 6A). Concentrations, it was verified that the plants dosed with 40 mg L^{-1} of $CdCl_2.H_2O$ present an increase of 9.43% of amino acids in comparison to the control plants, inferring that the action of cadmium influenced the increase of this variable.

In radicular tissue the plants submitted to stress induced by $CdCl_2.H_2O$ presented averages of 8.74, 12.74; 9.31; 4.11 and 3.47 μmol of AA g^{-1} of DM according to the control treatments, 0, 10, 20, 30 and 40 mg L^{-1} of Cd, respectively (Figure 6B), while the control plants showed a corresponding average to 8.74 μmol g^{-1} amino acids of DM. Thus, it was observed reductions in the levels of AA in African mahogany roots, evidencing that the highest Cd dosage (40 mg L^{-1}) corresponded to the largest decrease, being this represented by 60.2% in relation to the control plants. The results were observed through the adjustment of the cubic regression model.

According to the results, the root tissues had little accumulation of amino acids under the Cd treatments, due to this response, according to Sharma, Shahzad, Kumar, Kohli, Sidhu, Bali, Zheng, (2019), the occurrence of decreases in the free amino acids contents may be due to the stress promoted to the plants, being associated to the synthesis of new proteins in the cellular mechanism, or also, can be justified by the absorption of the metal by the roots followed by transporting it to the aerial part of the African mahogany plants.

Plant species differ in their ability to absorb, accumulate or even tolerate concentrations of heavy metals (Cardoso; Palheta; Sousa; Nascimento; Nogueira; Machado; Santos Filho, 2017). According to the present study, the concentrations of amino acids in leaves tended to increase under the action of the stress promoted by $CdCl_2.H_2O$, accumulation of amino acids in leaves according to Bali at al. (2020) are due to the translocation of the heavy metal to the aerial part of the plants after the absorption of the same by the roots, where this transport will occur via xylem. For The reduction from 30 mg L^{-1} of Cd in leaf tissues may be attributed to the phytotoxic action of Cd concentrations in the activities present in nitrogen metabolism, according to Cardoso, Palheta, Sousa, Nascimento, Nogueira, Machado, Santos Filho, (2017).

Figure 6 – Total soluble aminoacids concentration in leaf (A) and root (B) in young plant of African mahogany submitted to different concentrations of cadmium chloret ($CdCl_2.H_2O$)



Source: Authors (2024)

The accumulation of amino acids up to the dosage of 30 mg L^{-1} of Cd in plants can be closely related to the characteristics of the species, developmental conditions or to the presence of other elements such as nitrogen compounds (Cardoso; Palheta; Sousa; Nascimento; Nogueira; Machado; Santos Filho, 2017). The important thing is that society mobilizes itself in order to keep our soils always rich. Chemically, the organic matter in the soil is the main source of macronutrients and micronutrients essential to plants, acting indirectly on the availability of the elements (Crespo; Piscoya; Moraes; França; Fernandes; Cunha Filho; Araújo Filho, 2024).

4 CONCLUSIONS

The biochemical processes of the nitrogen metabolism of the plants of African mahogany were negatively affected by the stress caused by cadmium, mainly in the concentration of 40 mg L^{-1}, by reducing the activity of nitrate reductase enzyme and nitrogen compounds. According to the literature, African mahogany is not a hyperaccumulator, but it is a species suitable for use in phytoremediation programs to stabilize areas contaminated with cadmium.

ACKNOWLEDGEMENTS

Thanks to Universidade Federal Rural da Amazônia (UFRA) for the possibility to execute this work. The Universidade Federal de Mato Grosso do Sul. The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) - Brazil. for the scholarship. FUNDECT: Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul. Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), processo 308769/2022-8.

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How to quote this article

MACHADO, L. C.; PAIVA, R. C.; AMARANTE, C. B.; OLIVEIRA, J. T.; CUNHA, F. F.; SILVA, P. A.; OLIVEIRA NETO, C. F. Nitrogen metabolism in young plants of African mahogany submitted to different concentrations of cadmium. **Ciência Florestal**, Santa Maria, v. 35, e89275, p. 1-20, 2025. DOI 10.5902/1980509889275. Available from: https://doi.org/10.5902/1980509889275. Accessed in: day month abbr. year.

Data Availability Statement:

Datasets related to this article will be available upon request to the corresponding author.