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Articles

Path analysis of the influence of cadmium on mahogany

Análise de trilha da influência do cádmio no mogno

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ABSTRACT

African mahogany (*Khaya grandifoliola*) is a tree species that has gained space in the forestry market, presenting utility in a wide range of uses, especially in Brazilian territory, where it is the main substitute for Brazilian mahogany wood. The objective of this work was to perform a path analysis between the response of nutrient solution to cadmium treatment and the other variables and attributes studied: amino acid, nitrate, protein, ammonium, reductase, IDM, cadmium. The experiment was carried out in a greenhouse. At first, seedlings were habituated to be later taken to the treatment, where the applicability was given by cadmium chloride monohydrate ($CdCl\ 2.H_2O$) according to the nutrient solution. The experimental design was completely randomized (DIC), mixed in five concentrations (0; 10; 20; 30 and 40 mg L^{-1}) with seven replications, totaling 35 experimental units. To perform the comparative examination, the data were exposed to the analysis of variance, followed by regression analysis. The path analysis allowed to directly verify that increasing doses of treatment with Cadmium (CAR) reflected in a negative correlation with the amino acid content (AAR) in the roots of mahogany plants. Abiotic stress, which was exposure to a toxic chemical (cadmium), decreased, in this case, the ability of plants to synthesize amino acids. Increasing doses of cadmium treatment (CAL) reflected in negative correlation with the protein content in the leaf (PRL) of mahogany plants. Evidently, the higher the cadmium concentration, the greater the damage to mahogany's metabolic systems. With this study, we showed that excess cadmium in the soil affects the development of seedlings of forest species such as mahogany.

Keywords: Forest species; Forestry; *Khaya grandifoliola*; Multivariate statistics

RESUMO

O mogno africano (*Khaya grandifoliola*) é uma espécie arbórea que vem ganhando espaço no mercado florestal, apresentando utilidade nas mais diversas utilizações, principalmente em território brasileiro, onde é o principal substituto da madeira do mogno brasileiro. O objetivo deste trabalho foi realizar uma análise de trilha entre a resposta da solução nutritiva ao tratamento com cádmio e as demais variáveis e atributos estudados: aminoácido, nitrato, proteína, amônio, redutase, IDM, cádmio. O experimento foi conduzido em casa de vegetação. A princípio, as mudas foram habituadas para posteriormente serem levadas ao tratamento, onde a aplicabilidade se deu pelo cloreto de cádmio monohidratado ($CdCl$ $2.H_2O$) de acordo com a solução nutritiva. O delineamento experimental foi inteiramente casualizado (DIC), misturado em cinco concentrações (0; 10; 20; 30 e 40 mg L^{-1}) com sete repetições, totalizando 35 unidades experimentais. Para realizar o exame comparativo, os dados foram submetidos à análise de variância, seguida de análise de regressão. A análise de trilha permitiu verificar diretamente que doses crescentes de tratamento com Cádmio (CAR) refletiram em correlação negativa com o teor de aminoácidos (TAA) nas raízes das plantas de mogno. O estresse abiótico, que foi a exposição a um produto químico tóxico (cádmio), diminuiu, neste caso, a capacidade das plantas de sintetizar aminoácidos. O aumento das doses de tratamento com cádmio (CAL) refletiu em correlação negativa com o teor de proteína na folha (PRL) das plantas de mogno. Evidentemente, quanto maior a concentração de cádmio, maiores serão os danos aos sistemas metabólicos do mogno. Com este estudo, evidenciamos que excesso de cádmio no solo afeta o desenvolvimento de mudas de espécies florestais como o mogno.

Palavras-chave: Espécies florestais; Silvicultura; *Khaya grandifoliola*; Estatística multivariada

1 INTRODUCTION

Mahogany is a forest species of the genus Khaya, belonging to the Kingdom Plantae, Phylum Tracheophyta, Class Magnoliopsida, Order Sapindales, and Family Meliaceae (IUCN, 2018). It is part of a group especially known for its high timber potential, and which had its origin designated in several regions of Africa, which led to it being popularly known as African mahogany. Currently, the cultivation of this species has grown significantly, and its native wood has been recognized worldwide as noble, being used in a wide range of uses, especially in Brazilian territory, since it is the main substitute for the wood of Brazilian mahogany (*Swietenia macrophylla*) (Reis; Oliveira; Santos, 2019).

Soon we also have an increase in the search for the development of silvicultural information on African mahogany (*Khaya* spp.), whether in the form of monocultures or integrated crop-livestock-forest systems (Aquino; Pinheiro; Aquino Júnior; Aquino;

Brito; Pinheiro; Couto, 2018). Aiming at this scenario, it is also necessary to develop research related to the nutritional requirements of forest species, especially the native forest essences of the Amazon, such as mahogany, it is urgent since the constant exploitation of species of high economic value is increasing.

The development of anthropic activities such as industry, mining, agriculture, urban waste disposal 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, especially in plant structures (Nogueira; Brito; Resende; Albuquerque; Amarantes; Oliveira; Oliveira Neto, 2022).

Cadmium (Cd) which is classified as a non-essential element, but which can act in plants as a potent enzyme inhibitor, thus causing cellular damage even at low concentrations (Haider; Liqun; Coulter; Cheema; Wu; Zhang; Farooq, 2021), which may allow transfer to the food chain. Soil microorganisms play a crucial role toward the Cd tolerance in plants by decreasing metal phytoavailability and increasing morphological and physiological parameters of plant (El Rasafi; Oukarroum; Haddioui; Song; Kwon; Bolan; Rinklebe, 2021).

To evaluate and quantificate these possible damages, a comparative examination was carried out through path analysis, which is a tool specially developed to work with variables, to make clear the direct and indirect actions of several analyzed factors, where the results will not depend on a single variable, but of comparing the relationship of all the data with each other.

In this sense, the present study aims to evaluate and quantificate, through path analysis, the influence of different doses of cadmium, applied to roots and leaves of Mahogany species, in addition to the behavior of other variables such as amino acid, nitrate, protein, ammonium, reductase, IDM, thereby understanding the pollution potential of excess cadmium in seedlings of forest species such as mahogany.

2 MATERIALS AND METHODS

The present study was carried out in a greenhouse of the Laboratory of Plant Physiology, of the Institute of Agrarian Sciences, Universidade Federal Rural da Amazônia-UFRA, Belém-Pa. Belonging to the analysis of variables carried out at the Laboratory for the Study of Biodiversity of Higher Plants (EBPS) and Museu Paraense Emílio Goeldi.

The analyses aimed to determine the cadmium concentrations in leaves and roots, also aiming to understand the possible variations given according to the other attributes present in the plant, namely: amino acid, nitrate, protein, ammonium, reductase, IDM, and cadmium itself.

The technique to analyze the composition of amino acids used was chromatography. The quantification of proteins, nitrate, ammonium and reductase in the samples was performed using spectrophotometry. Evaluations referring to plant physiology were obtained using a portable IRGA meter (Infra-red Gas Analyzer / ADC equipment - mod. LCi 6400, Hoddesdon, UK) being measured under favorable environmental conditions, between 9:00 am and 11:00 am. To determine the IDM, it was calculated according to Equation (1):

$$IDM\ (\%) = (L1\ /\ l2) \times 100 \quad (1)$$

where: L1 = first reading of the conductivity meter, dimensionless; L2 = second reading of the conductivity meter, dimensionless.

To carry out this work, African mahogany seedlings (*Khaya grandifoliola*) were used, from the São Francisco commercial nursery, located in the municipality of Castanhal-PA, with an age of approximately 90 days, the seedlings were in good phytosanitary condition and of good homogeneity. To avoid moisture loss, the seedlings were transported on moistened paper and then placed in polyethylene plastic pots, remaining in a greenhouse for 35 days (DAS) of acclimatization.

After acclimatization, the seedlings were transplanted to Leonard pots with a capacity of 4.6 L, which were adapted and wrapped with aluminum foil (to minimize the interference of solar radiation on root growth), containing sterilized washed sand. A.V.A paper in the form of circles was added to the edge of each pot, being attached to the seedlings, seeking not to damage them, thus avoiding the proliferation of algae. Leonard's vessels contained Sarruge's solution, with ¼ ionic strength, with a pH maintained between 5.8 ± 0.5, using NaOH or 1N HCl solutions, when necessary, and the solutions were changed weekly.

The exposure of the plants to Cadmium took place after the 16 day of acclimatization of African mahogany seedlings in a nutrient solution, where the concentration applications were given in the form of cadmium chloride monohydrate ($CdCl_2H_2O$). For its application, it 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 seven plants per treatment, which was renewed every seven days.

The collection and storage of the material were given as follows: the collection of plants took place at 60° DAS (period of exposure of plants to heavy metal) at 04:30 h pm. Completely expanded primary leaves of each replication were selected in a greenhouse, material *in vivo*. The plants were then washed with distilled water separated into leaves, stems, and roots wrapped in aluminum foil and taken to drying in a forced air ventilation oven at 65 ºC for 48 h, after drying, the material was crushed in a mill until obtaining a fine powder being properly stored in falcon tubes until its use in the biochemical analysis tests to determine the components of the present study.

Part of the dried material was taken for analysis to determine cadmium concentrations in leaves and roots. To determine the concentration of cadmium (Cd) in leaves and roots of young African mahogany plants, samples of the respective plant parts were sent to the Laboratory of Chemical Analysis of the Soil, a department belonging to the Museu Paraense Emílio Goeldi. The values obtained followed the guidelines of CONAMA nº 460/2013 regarding soils contaminated with heavy metals (cadmium).

For the experimental design and statistical analysis, a completely randomized design (DIC) was used, five concentrations of cadmium chloride monohydrate applied in the form of $CdCl_2.H_2O$ (0 mg L^{-1} (control); 10 mg L^{-1}; 20 mg L^{-1}; 30 mg L^{-1} and 40 mg L^{-1}) with seven replications (the seven replications were used to compose each of the treatments), totaling 35 experimental units (one plant/pot). The seven replicates of each treatment were submitted to the same type of acclimatization throughout the evaluation period. For comparative effect analysis, the data obtained were submitted to analysis of variance (ANOVA) followed by regression analysis (quantitative data) using the Sisvar program version 5.4 and the means compared by Tukey's test at a 5% probability level.

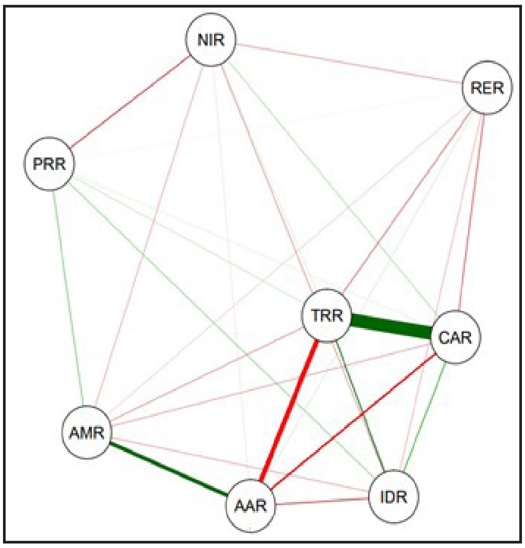
The path analysis performed in the present work sought to associate the relationship between treatment performed on roots (TRR) and treatment performed on leaves (TRL) with the other variables, so that multicollinearity is eliminated, seeking to achieve maximum effectiveness in the results. The most common problem found in research failures and/or errors in the "mask" created by the presence of multicollinearity, which can lead those who are analyzing certain data to have wrong interpretations (Pinheiro; Silva; Vieira; Aguiar; Nascimento; Vieira, 2021).

3 RESULTS AND DISCUSSIONS

Figure 1 shows the network of correlations between the variables studied, highlighting positive correlations in green and negative correlations in red. It is worth noting that the thickness of the line is influenced by the highest degree of correlation, that is, thicker lines represent higher correlations in attributes.

Analyzing Figure 1, it is observed that Cadmium about the treatment has a strong positive correlation, indicating that the increase in the treatment dose resulted in higher nutrient content in the plant root, symbolizing that the treatment was efficient. The amino acid has a close negative relationship with the treatment, which indicates an interaction of the amino acid on the accumulation of cadmium in the root.

Figure 1 – Correlation network of variables



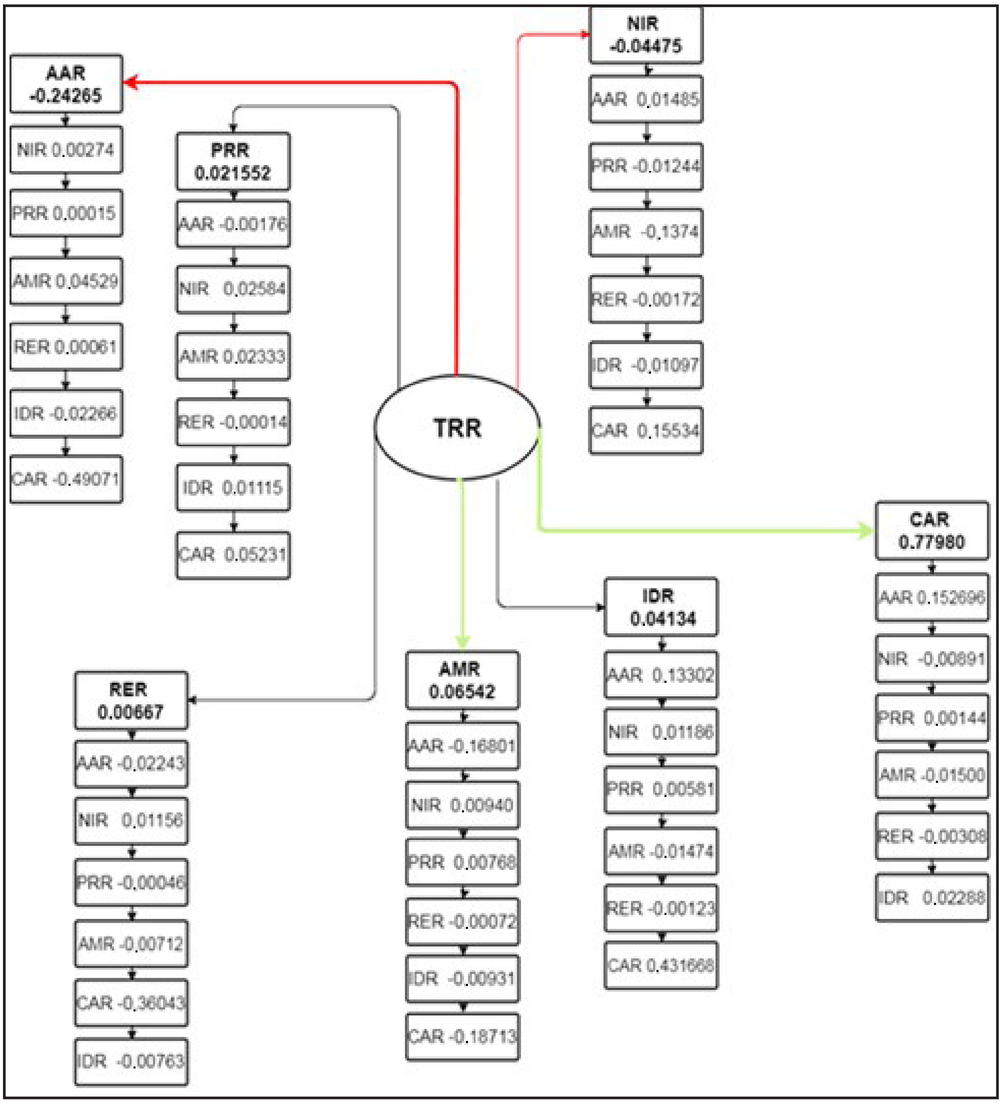
Source: Authors (2023)

In where: treatment with cadmium (TRR); amino acid in the roots (AAR); nitrate in the roots (NIR); protein in the roots (PRR); ammonium in the roots (AMR); reductase in the roots (RER); IDR in the roots (IDR); cadmium in the roots (CAR).

The phytochelatin complex can become a mobile form for cadmium to translocate from the roots to the aerial part. This same author highlights that the Cd-phytochelatin complex may represent a mobile form for the transport of cadmium from the roots to the aerial parts. Phytochelatin is a complex structure, forming peptides rich in cysteine, that is, rich in amino acids, which are electron donors, a necessary condition for the formation of complexes with transition metals (Lewis acids), such as Cd (Malavolta, 2018).

The path analysis is derived from a variable and synthesizes the direct and indirect correlations dynamized with the others. In this way, the path analysis aims to make these data unbiased and make it possible for the description of properties to be as equivalent as possible to the real situation. Figure 2 below shows the result of the path analysis performed on the roots of Mahogany plants.

Figure 2 – Path analysis between cadmium treatment index (TRR) and other study variables



Source: Authors (2023)

In where: amino acid in the roots (AAR); nitrate in the roots (NIR); protein in the roots (PRR); ammonium in the roots (AMR); reductase in the roots (RER); IDR in the roots (IDR); cadmium in the roots (CAR); Lines colored in green demonstrate positive correlations and in red negative correlations.

The path analysis allowed to directly verify that increasing doses of Cadmium treatment (CAR) reflected in a positive correlation with the content of the metal contained in the roots in mahogany plants. But on the other hand, increasing doses of Cadmium treatment (CAR) reflected in negative correlation with the Amino Acid content (AAR) in mahogany plants. The observed values are 0.7798 and -0.2426 respectively.

Abiotic stresses, such as exposure to toxic chemicals, can decrease the ability of plants to synthesize amino acids. Stress leads to the reallocation of resources and often to the disruption of metabolic pathways, including amino acid synthesis (Nogueira; Brito; Resende; Albuquerque; Amarantes; Oliveira; Oliveira Neto, 2022).

Plant species, in general, show great variation regarding the absorption of heavy metals, including Cd (Malavolta, 2018). The roots, generally, constitute the main organ of the plant involved in the absorption and, therefore, almost always, the highest concentrations of heavy metals are also found in this part of the plant, some analyzes have shown that the absorption of cadmium by the roots is related to the amount of metal concentration in the soil solution and root system morphology (Cogo; Lopes; Vielmo, 2020).

Nogueira, Brito, Resende, Albuquerque, Amarantes, Oliveira and Oliveira Neto (2022) found results that corroborate the present study, where they highlight that the sensitivity of Paricá seedlings to increasing concentrations of cadmium was evident. In addition, they point out that cadmium was transported to the shoot, however, it is concentrated mainly in the root system, characterizing it as a phytoextractor species.

Normally, cadmium has a different tendency for concentrations in plant organs, where most of the tested species accumulate a higher percentage of cadmium in the roots than in the shoot. This is justified due to the low translocation, this inefficiency is related to phytochelatin and glutathione synthesis. They function as a mechanism to complex Cadmium and thus prevent its transport to the aerial part of the plant since these compounds have catalytic, regulatory, and structural functions in plant metabolism (Capaldi; Gratão; Reis; Lima; Azevedo, 2015).

Based on the analysis indirectly, it is noticeable that the activity of the enzyme nitrate reductase is strongly influenced by cadmium. When under abiotic stresses, this enzyme can act as an important biomarker of plant tolerance towards phytotoxic metals, since when under cadmium stress, plants tend to decrease the production of this enzyme. This behavior has already been reported in several studies where other metals such as Cr, Cu, and Zn have been shown to directly affect the activity of the nitrate reductase enzyme, especially when found in high concentrations, thus affecting the main input of nitric nitrogen in plant metabolism. Another important interference of this metal seems to be related to the imbalance of homeostatic function and plant development (Paiva; Machado; Sousa; Moraes; Conceição; Nogueira; Oliveira; Okumura; Silva; Oliveira Neto, 2021).

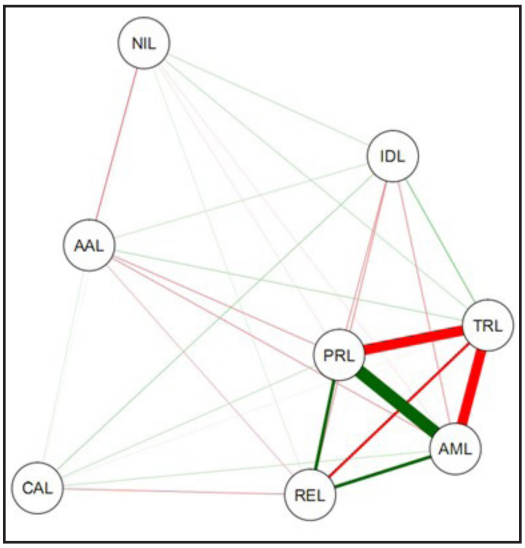
Summarizing, in Figure 2 we can see a high and positive correlation coefficient, under the direct effect between the treatment (TRR) with Cadmium (CAR) where it demonstrated the value of 0.7798, expressing that the results are reliable. Pinheiro, Vieira, Barata, Mota, Sousa, Vieira and Silva (2021) found a correlation coefficient of 0.7544 in a study with physical attributes in other species and considered it a high and significant coefficient. Directly, the path analysis made it possible to verify that the attribute CAR - Cadmium was the attribute that best reacted with the TRR - treatment with cadmium, which only reinforces the positive correlation that the CAR - Cadmium in the roots maintains with the other attributes that were positively correlated. On the other hand, indirectly, it is noted that Amino Acids - AAR has a significantly negative relationship with CAR - Cadmium in the roots.

We have that a high or low correlation coefficient between two variables can also be the result of the effect that a third variable and/or that a group of variables have on these two factors, which on the other hand can cause ambiguity or mistakes in the conclusions, not giving the exact relative importance of the direct and indirect effects of these factors (Cruz; Regazzi; Carneiro, 2012).

Figure 3 shows the network of correlations between the variables studied in relation to treatment with cadmium (TRL).

Pondering Figure 3, where we have a network of correlations of the reactions of the variables under the effect of the treatment on the leaf, we can see that the treatment obtained a strong negative correlation with the protein and with the ammonium, having a negative effect also on the reductase. The constant positive correlation between the protein and ammonium variables was similarly noticeable, where both showed a positive correlation with reductase.

Figure 3 – Correlation network of variables: treatment with cadmium (TRL)



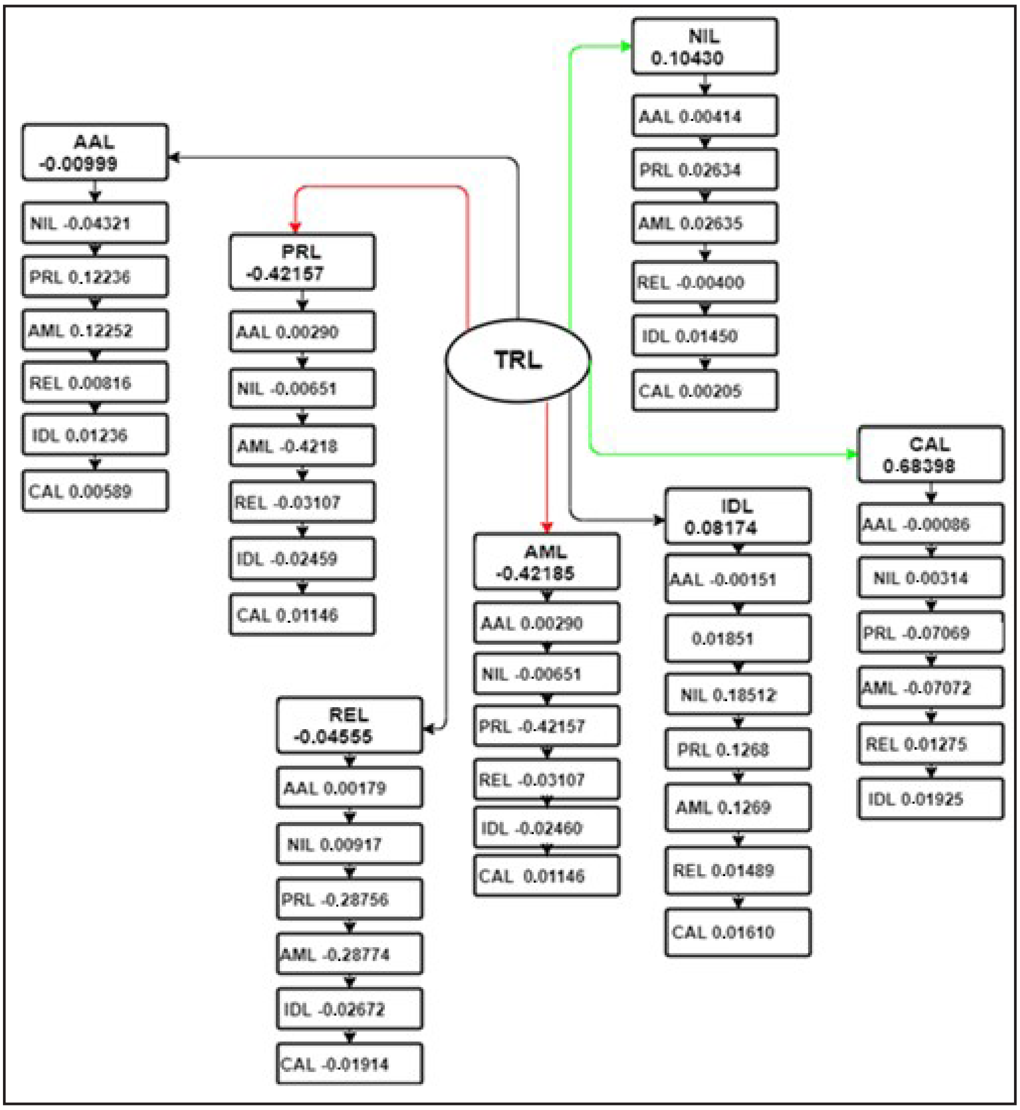
Source: Authors (2023)

In where: amino acid in the leaf (AAL); nitrate in the leaf (NIL); protein in the leaf (PRL); ammonium in the leaf (AML); reductase in the leaf (REL); IDM in the leaf (IDL); cadmium in the leaf (CAL).

Through several studies, it has been shown that the action of cadmium in plants can cause a decrease in transpiration and photosynthesis, an increase in respiratory rate, leaf chlorosis, inhibition of root and shoot growth, and even a reduction in accumulation. dry matter (Vecchia; La Rocca; Moro; Faveri; Andreoli; Rascio, 2005). Concentrations, essential and non-essential heavy metals can lead to symptoms of toxicity in plants, symptoms that become increasingly visible as excessive concentrations of heavy metals are being attributed to an extensive interaction at a cellular and molecular levels.

Knowing the correlations is essential when measuring the degree of association between variables, in order to make it possible to assess how much the change in one attribute can affect the others. Variables that are positively correlated indicate that both are benefited or harmed for the same reasons or fundamentals, and correlations with negative values indicate that there is one character to the detriment of the other. Soon understanding the results of a correlation becomes important, because it is through the knowledge of the magnitude of the performance of a variable, that we will be able to evaluate the influence on another. Figure 4 presents the results of the path analysis.

Figure 4 – Path analysis between cadmium treatment index (TRL) and other study variables



Source: Authors (2023)

In where: amino acid (AAL); nitrate (NIL); protein (PRL); ammonium (AML); reductase (REL); MDI (IDL); cadmium in the sheet (CAL); Lines colored in green demonstrate positive correlations and in red negative correlations.

The uptake of heavy metals by leaves is so far little known and, even less, the influence this would have on the uptake by the roots and the subsequent translocation to the aerial part of the plants (Cakmak; Welch; Hart; Norvell; Ozturk; Kochian, 2000), the path analysis carried out and demonstrated, aims to add elucidations and references on the subject, evidencing the action of cadmium on mahogany leaves and roots.

Directly speaking, the path analysis of Figure 4 clarified that the attributes Cadmium in the sheet (CAL) and nitrate (NIL) have a strong positive correlation with the cadmium treatment, but on the other hand the harmful action of the treatment on the variables ammonium (AML) and protein (PRL) occurs significantly, this negative effect was already expected due to the toxicity of cadmium. The values observed in positive correlations were respectively 0.6839 for cadmium and 0.1043 for nitrate; already in negative correlations, we notice mainly the ammonium with -0.4218 and the protein with -0.4215 mutually.

Evidently, the higher the concentration of cadmium, the greater the damage to the metabolic systems of mahogany, since this element was shown to be harmful in several analyzed aspects, including its negative correlation with the reductase enzyme and the strong correlation it showed with nitrate, which together is one of the main responsible for the nitrogen assimilation of plants.

4 CONCLUSIONS

The path analysis allowed to directly verify that increasing doses of treatment with Cadmium (CAR) reflected in a negative correlation with the amino acid content (AAR) in the roots of mahogany plants. Abiotic stress, which was exposure to a toxic chemical (cadmium), decreased, in this case, the ability of plants to synthesize amino acids.

Increasing doses of cadmium treatment (CAL) reflected in negative correlation with the protein content in the leaf (PRL) of mahogany plants. Evidently, the higher the cadmium concentration, the greater the damage to mahogany’s metabolic systems.

With this study, we showed that excess cadmium in the soil affects the development of seedlings of forest species such as mahogany.

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