



MULTISPECTRAL IMAGING RELATED TO BIOCHEMICAL PARAMETERS OF BRAZILIAN MAHOGANY (*Swietenia macrophylla* King) EXPOSED TO NICKEL

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ABSTRACT

Objective: Due to this, this study aimed to evaluate the behavior of the normalized difference vegetation index (NDVI) correlating with leaf biochemical variables of Brazilian mahogany (*Swietenia macrophylla*) under the effect of increasing doses of nickel.

Theoretical Framework: Multispectral analyses are a way to assess the early, efficient, unbiased, and, most importantly, non-destructive responses of plants subjected to stress.

Method: The experimental design used was a randomized block design (RBD), with five treatments (0, 2, 4, 6 and 8 mg.L⁻¹ of NiCl₂.6H₂O), with six replicates. The data set obtained was subjected to Pearson's correlation (1892) and multivariate analysis by principal components using the R-Studio-1.3.1093 software.

Results and Discussion: The multispectral responses were quite distinct between control and stressed plants until the 30th day of exposure to nickel. The increase in chlorophylls was not accompanied by an increase in the production of photoassimilates, since although there was an increase in such pigments, they were possibly not at their potential operating capacity due to the nickel stress levels.

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Research Implications: In stress situations, mahogany plants tend to maintain NDVI with a relationship directly proportional to carbohydrates, on the other hand, variables such as leaf temperature, chlorophyll *a*, *b* and total tend to have an inversely proportional relationship.

Originality/Value: Even though a considerable number of studies have demonstrated the toxicological effects and stress mechanisms caused by nickel in forest plants, there are still unknown parameters, such as the biochemical response related to vegetative index parameters. Therefore, there is a need to clarify many aspects addressed in this study.

Keywords: Industrial Activities, Contamination, ROS, Phytoremediation.

IMAGENS MULTIESPECTRAIS RELACIONADA A PARÂMETROS BIOQUÍMICOS DE MOGNO BRASILEIRO (*Swietenia macrophylla* King) EM EXPOSIÇÃO AO NÍQUEL

RESUMO

Objetivo: Devido a isto, este trabalho teve objetivo de avaliar o comportamento do índice de vegetação de diferença normalizada (NDVI) correlacionando à variáveis bioquímicas foliares do mogno brasileiro (*Swietenia macrophylla*) sob efeito das dosagens crescentes de níquel.

Referencial Teórico: Análises multiespectrais são uma forma de avaliar respostas antecipada, eficiente, imparcial, e principalmente, não destrutiva de plantas submetidas a estresse.

Metodologia: O delineamento experimental utilizado foi blocos casualizados (DBC), com cinco tratamentos (0, 2, 4, 6 e 8 mg.L⁻¹ de NiCl₂·6H₂O), com seis repetições. O conjunto de dados obtidos foi submetido a correlação de Pearson (1892) e análise multivariada por componentes principais usando o software R-Studio-1.3.1093.

Resultados e Discussão: As respostas multiespectrais foram bastante distintas entre plantas controle e sob estresse até o 30º dia de exposição ao níquel. O aumento das clorofilas não foi acompanhado pelo aumento da produção de fotoassimilados, haja vista que embora houve o aumento de tais pigmentos, possivelmente estes não estavam em sua capacidade potencial de operação devido aos níveis de estresse pelo níquel.

Implicações da pesquisa: Em situações de estresses, as plantas de mogno tendem a manter NDVI com uma relação diretamente proporcional aos carboidratos, em contra partida, variáveis como temperatura foliar, clorofila *a*, *b* e totais tendem a ter uma relação inversamente proporcional.

Originalidade/Valor: Mesmo com um número considerável de estudos demonstrarem os efeitos toxicológicos e os mecanismos de estresse causados pelo níquel em plantas florestais, ainda há parâmetros desconhecidos, como a resposta bioquímica relacionada aos parâmetros dos índices vegetativos. Logo, há a necessidade de esclarecer muitos aspectos que foram abordados neste estudo.

Palavras-chave: Atividades Industriais, Contaminação, EROs, Fitorremediação.

IMÁGENES MULTIESPECTRALES RELACIONADAS CON PARÁMETROS BIOQUÍMICOS DE *Swietenia macrophylla* King BAJO EXPOSICIÓN AL NÍQUEL

RESUMEN

Objetivo: Debido a esto, este trabajo tuvo como objetivo evaluar el comportamiento del índice de vegetación de diferencia normalizada (NDVI) correlacionándolo con variables bioquímicas foliares del caoba brasileño (*Swietenia macrophylla*) bajo el efecto de dosis crecientes de níquel.

Marco Teórico: Los análisis multiespectrales son una forma de evaluar respuestas anticipadas, eficientes, imparciales y, principalmente, no destructivas de plantas sometidas a estrés.

Método: El diseño experimental utilizado fue de bloques al azar (DBC), con cinco tratamientos (0, 2, 4, 6 y 8 mg.L⁻¹ de NiCl₂·6H₂O), con seis repeticiones. El conjunto de datos obtenido fue sometido a correlación de Pearson (1892) y análisis multivariado por componentes principales utilizando el software R-Studio-1.3.1093.



Resultados y Discusión: Las respuestas multiespectrales fueron bastante distintas entre las plantas control y aquellas sometidas a estrés hasta el día 30 de exposición al níquel. El aumento de las clorofilas no estuvo acompañado por un incremento en la producción de fotoasimilados, dado que, aunque hubo un aumento de estos pigmentos, posiblemente no estaban operando a su capacidad potencial debido a los niveles de estrés causados por el níquel.

Implicaciones de la investigación: En situaciones de estrés, las plantas de caoba tienden a mantener el NDVI con una relación directamente proporcional a los carbohidratos. Por otro lado, variables como la temperatura foliar, la clorofila a, b y total tienden a mostrar una relación inversamente proporcional.

Originalidad/Valor: A pesar de que un número considerable de estudios ha demostrado los efectos toxicológicos y los mecanismos de estrés causados por el níquel en plantas forestales, aún existen parámetros desconocidos, como la respuesta bioquímica relacionada con los parámetros de los índices vegetativos. Por lo tanto, es necesario esclarecer muchos aspectos abordados en este estudio.

Palabras clave: Actividades Industriales, Contaminación, Eros, Fitorremediación.

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1 INTRODUCTION

Describe Geotechnological techniques, such as remote sensing, have been used and applied as important tools for monitoring degraded and contaminated areas, for study and analysis in specific areas with high detail. The use of sensors shows greater progress with more automation and integration, processing the huge amount of image data generated, such as data management, image analysis and visualization of results from datasets (Marin et al., 2019; Pradipta et al., 2022; Maresma et al., 2020).

One way to assess early, efficient, impartial, and most importantly, non-destructive responses of plants subjected to stress is through the use of spectral images. (Marin et al., 2019). Plants react to stress through physiological and biochemical adaptations (Júnior et al., 2021), This may be due to changes in the chlorophyll content and changes in the internal structures of the leaves, and therefore, they can be easily detected through differences in reflected energy in the visible and near infrared spectral regions (Kizilgeci et al., 2021; Marin et al., 2019).

Heavy metals have caused concern worldwide, as their presence in the soil tends to be more persistent when compared to organic pollutants, and can be introduced into the food chain through the consumption of contaminated food (Maftouh et al., 2023). Direct or indirect exposure to heavy metals poses a threat to human health, as they cause a series of dysfunctions, such as neurodegenerative and respiratory diseases, DNA degeneration and cancer (Maftouh et al., 2023; Sahoo et al., 2023).



It is common for there to be changes in the photosynthetic metabolism of the leaf due to heavy metal (Yildirim et al., 2019). Given this, NDVI is a technique for rapid detection of plant health, as it is associated with chlorophylls (Kizilgeci et al., 2021).

Vegetation indices were developed due to the difference in reflectance between the visible and near-infrared spectral regions in plants under stress (Marin et al., 2019). These are calculated as ratios or differences in the reflectance of wavelengths. These indices are highly correlated with changes in plant physiology and biochemistry (Kizilgeci et al., 2021; Marin et al., 2019).

Given the above, this study aimed to evaluate the behavior of the normalized difference vegetation index (NDVI) correlating with leaf biochemical variables of Brazilian mahogany (*Swietenia macrophylla*) under the effect of increasing nickel dosages.

2 METHODOLOGY

2.1 EXPERIMENTAL LOCATION

The experiment was carried out from August to October 2022, in the growth room of the Higher Plant Biodiversity Study Group (EBPS), located at the Institute of Agricultural Sciences of the Federal University of Amazonia (ICA/UFRA). Under controlled temperature, photoperiod and relative humidity.

2.2 GROWTH CONDITIONS AND EXPERIMENTAL DESIGN

Brazilian mahogany (*S. macrophylla*) seeds were collected from the Brazilian Agricultural Research Corporation (Embrapa Amazônia Oriental) in Belém, Pará State, Brazil. The seeds were sown in 5L polyethylene pots containing sand, previously washed and autoclaved, and kept at a constant temperature of 28°C, relative humidity (RH) of 90%, and a 12h photoperiod. The seedlings were grown in a growth room for 43 days, and were irrigated daily to replace water lost through evapotranspiration. They were monitored according to the rules and instructions for seed analysis.

To evaluate the toxic effect of nickel, the seeds were exposed to an aqueous solution of nickel chloride (OSO) P.A. ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$), prepared at concentrations corresponding to 0 mg.L⁻¹ (control), 2 mg.L⁻¹, 4 mg.L⁻¹, 6 mg.L⁻¹ and 8 mg.L⁻¹. The zero level corresponded to the control, where only deionized water was used. The work involves the three principles of



experimentation: repetition, randomization and local control, therefore the experimental design used was randomized blocks, with six replicates per treatment, totaling 30 experimental units. Each unit was composed of a pot with 7 seeds.

2.3 ANATOMICAL ANALYSIS

The semi-permanent slides were prepared with transversal histological sections, made with the aid of steel blades, sectioning the leaf mesophyll freehand, obtained by means of transversal cuts in the central region of the leaf to observe the parenchymatic and conductive tissues of the internal part of the leaf, according to the usual methods of plant anatomy. Stained with safrablau safrablau - a mixture of astra blue and safranin (Bukatsch, 1972; Kraus et al., 1998). Minutes after staining, the slide was removed and placed on another glass slide with 50% glycerin (Purvis et al., 1964) for better preservation of the botanical material, mounted between the slide and the coverslip.

Analysis of normalized difference vegetation index (NDVI) and thermal

Thermal images were obtained using a Flir Cx-Series camera (Compact Thermal Imaging Camera). To calculate the NDVI index (equation 1), images were captured in the morning at 9 am, RAW+JPG mode by the MAPIR Survey3 camera (Aerial mapping camera) and converted to TIFF to be processed in the QGIS 3.28.2 program using the formula shown below:

$$NDVI = \frac{NIR - Red}{NIR + Red} \quad (1)$$

Where

NIR is reflected light in the infrared range (band 3) and RED is reflected light in the red range (band 1).

2.4 PHOTOSYNTHETIC PIGMENTS DETERMINATION

100 mg of fresh leaves were ground in a mortar containing 3 mL of 80% acetone, followed by maceration and subsequent centrifugation. The supernatant was transferred to a 25 mL volumetric flask and the volume was adjusted with 80% acetone. The pigment contents in



the extracts were measured by absorbance readings in a spectrophotometer at wavelengths of 663 and 647 nm to calculate the contents of chlorophylls a and b. The wavelength of 537 nm was used to calculate the anthocyanin contents and 470 nm to calculate the carotenoid contents. Methodology described by Lichtenthaler (1987) and Sims et al. (2002).

2.5 CARBON METABOLISM ANALYSIS

The concentration of total soluble carbohydrates was determined by the colorimetric method described by Dubois et al. (1956), using phenol solutions (5%) and concentrated sulfuric acid. Readings were taken in a spectrophotometer at 490 nm.

The determination of sucrose concentration was carried out using the Van Handel method (1968), which was divided into three stages: extraction, phase separation by solvents, and purification and dosage of sucrose. The extraction was performed with MCW solution (methanol, chloroform, and water solution). The phase separation was performed by adding water to the extract, causing the solution to divide; the methanolic part separated from the chloroform. In the dosage of sucrose, potassium hydroxide was added to the methanolic aqueous phase to break down the non-reducing sugars so that they could join the anthrone and form a complex with a hue ranging from orange to green. The reading was taken on a spectrophotometer at 620 nm.

To determine the reducing sugars, 20 mg of dry matter powder was placed in test tubes with 2.5 mL of 80% ethanol. Afterwards, 500 µL of the extract was used with 500 µL of Nelson AB reagent, heated to 100°C and then cooled in an ice bath, adding 500 µL of the arsenitomolybdic solution and vortexing. Addition of 3.5 mL of deionized H₂O and vortexing. Readings were performed in a spectrophotometer at 540 nm, according to the methodology of Rinner et al. (2012).

2.6 STATISTICAL ANALYSIS OF DATA

Statistical analysis of the variables and generation of graphs was performed using R-Studio software, version 1.4.1717 (R Project for Statistical Computing, RRID:SCR_001905) (RStudio, 2021).

To identify statistical differences between nickel dosages, the ExpDes.pt package and the “dbc” function were used. This library performs normality analysis of residues using the Shapiro-Wilk test and equality of variances using the Oneillmathews test, also performing



ANOVA. Subsequently, the data were submitted to the Tukey Post-Hoc test, where a significance level of 5% was considered.

The data set obtained was subjected to Pearson correlation (1892), between NDVI and the other biochemical attributes evaluated, using the `corrplot` package and the “`cor.mtest`” function; the significance of the correlation was assessed by the t-student test, at a 5% significance level.

To group and identify the behaviors of the correlations between the variables of photosynthetic content and metabolism, a multivariate analysis by principal components was performed using the package `factoextra` and `FactoMineR`. Bartlett's sphericity test and the Kaiser-Meyer-Olkin (KMO) test were performed as assumptions to validate the analysis.

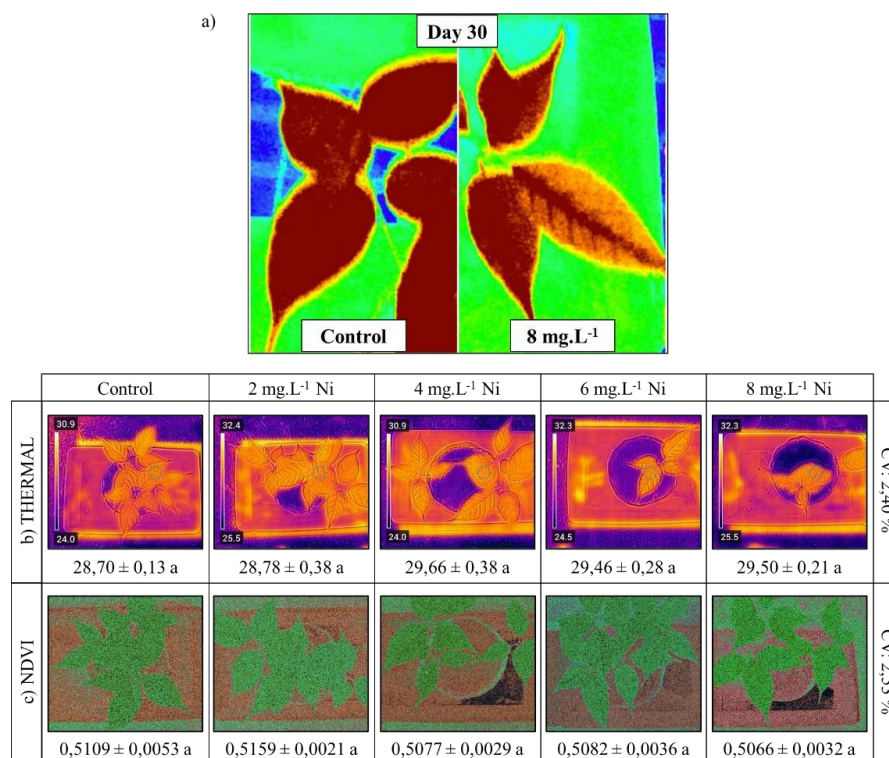
3 RESULTS AND DISCUSSIONS

The multispectral responses were quite distinct between control and stressed plants until the 30th day of exposure to nickel (figure 1A). However, at the end of the experiment, the vegetative indices of the mahogany seedlings did not show significant variations when in contact with the nickel doses (figure 1C). The thermal values also did not show a significant influence of the nickel dosages applied (figure 1B), that is, the mahogany plants, despite being under heavy metal stress, managed to maintain their plant temperature at normal levels, otherwise, the increase in temperature would result in more significant physiological and biochemical damage (Ventura et al., 2019), further reducing plant growth.



Figure 1

a normalized difference vegetation index (NDVI) at 30 days, *b* Leaf temperature, *c* NDVI at the end of the experiment (43 days) of Brazilian mahogany (*Swietenia macrophylla*) seedlings exposed to five nickel concentrations (0, 2, 4, 6 and 8 mg.L⁻¹). Different letters indicate significant differences in Tukey's test ($P < 0.05$). Mean $\pm$ SE, $n = 6$.



The generated NDVI values are real values contained between -1 and 1 (Krizek et al., 2021), these indices can be classified as: dead plants (-1 to 0), plants under stress or deficient (0 to 0.33), moderately healthy plants (0.33 to 0.66) and highly healthy plants (0.66 to 1). NDVI is an indicator of plant health and growth, as healthy plants have a lot of chlorophyll and cellular structures, consequently they actively absorb red light and reflect NIR when photosynthesis occurs. With a plant under stress, exactly the opposite happens, resulting in lower NDVI values (Kizilgeci et al., 2021; Li et al., 2021; Huang et al., 2021). Following this line of reasoning, it is acceptable for younger plants to have a relatively low NDVI value, as they do not have their entire photosynthetic apparatus fully operational, and the chlorophyll content and cellular structures are still in formation.

Regarding the characterization at the mesophyll level (figure 2), the leaf has an abaxial surface formed by tabular cells with interspersed stomata at the cell level. The adaxial surface does not present stomatal intercalations, characterizing a hypostomatic leaf. The palisade parenchyma cells make up a single layer of cells that account for $1/3$ of the mesophyll region,



while the spongy (lacunous) parenchyma occupies around 2/3. Photosynthetic activity requires radiation to occur, but it absorbs each frequency differently, according to the characteristics of the plant leaf (Pinto et al., 2022). The reflectance of solar radiation in vegetation, in general, presents low reflectance and radiation transmittance in the visible range of the spectrum (400 nm to 700 nm), especially in the blue (~450 nm) and red (~660 nm) regions that are absorbed by photosynthetic pigments (chlorophyll). In the near infrared region (~700 nm to 1,400 nm), there is greater reflectance influenced by the internal structure of the leaves, mainly due to the absence of absorption by pigments and dispersion of electromagnetic energy in the leaf mesophyll (Ribeiro et al., 2016). As a result, there is greater penetration of infrared in the leaf mesophyll to the spongy parenchyma, while other waves are absorbed in the palisade parenchyma, except for green, which is completely reflected by the leaf (figure 2A).

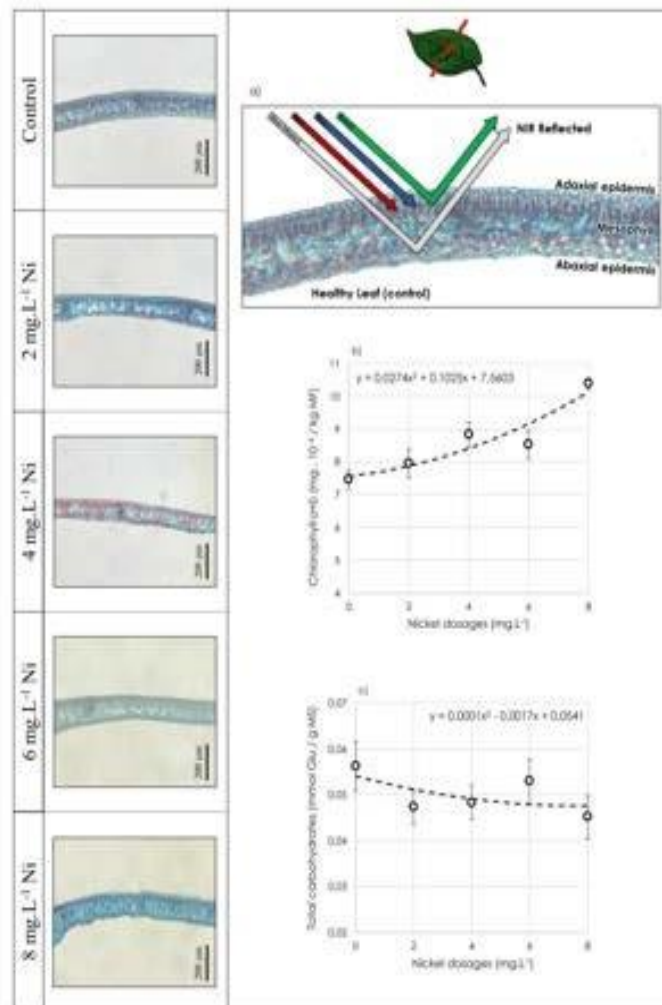
The increase in chlorophylls was not accompanied by an increase in the production of photoassimilates (figures 2B and 2C), given that although there was an increase in such pigments, they were possibly not at their potential operating capacity due to the levels of stress from nickel. One possible explanation is the lower abundance of proteins associated with the biochemical (functioning and structure of photosystems and ATP synthase) and biochemical phases of photosynthesis (RuBisCo and transketolases). RuBisCo, a key enzyme in carbon fixation during photosynthesis, can be inhibited or have its activity reduced by the excessive presence of nickel and other heavy metals (Khairy et al., 2016). This can lead to a decrease in the efficiency of carbon fixation, directly affecting the production of biomass and nutrients (Araujo et al., 2021). Furthermore, the functioning and structure of photosystems, which are vital for light absorption and conversion into usable energy, may be compromised (El-Khouly et al., 2017). Proteins and protein complexes associated with photosystems and ATP synthase, involved in the production of energy (ATP) from light, can be affected by nickel stress (Zhang et al., 2020), leading to a reduction in the efficiency of photosynthesis and energy production for the plant.

Transketolases, enzymes involved in the pentose phosphate pathway, which play a role in the generation of compounds necessary for plant growth and development, may also have their activity reduced under nickel and other metal stress conditions (Usman et al., 2022). In response, mahogany plants may have reduced production or efficiency of proteins associated with these affected biochemical pathways (Zhang et al., 2020). This may result in compromised growth and explains the lower efficiency of chlorophylls.



Figure 2

Leaf anatomical plate of Brazilian mahogany seedlings (*Swietenia macrophylla*) exposed to five nickel concentrations (0, 2, 4, 6 and 8 mg.L⁻¹).



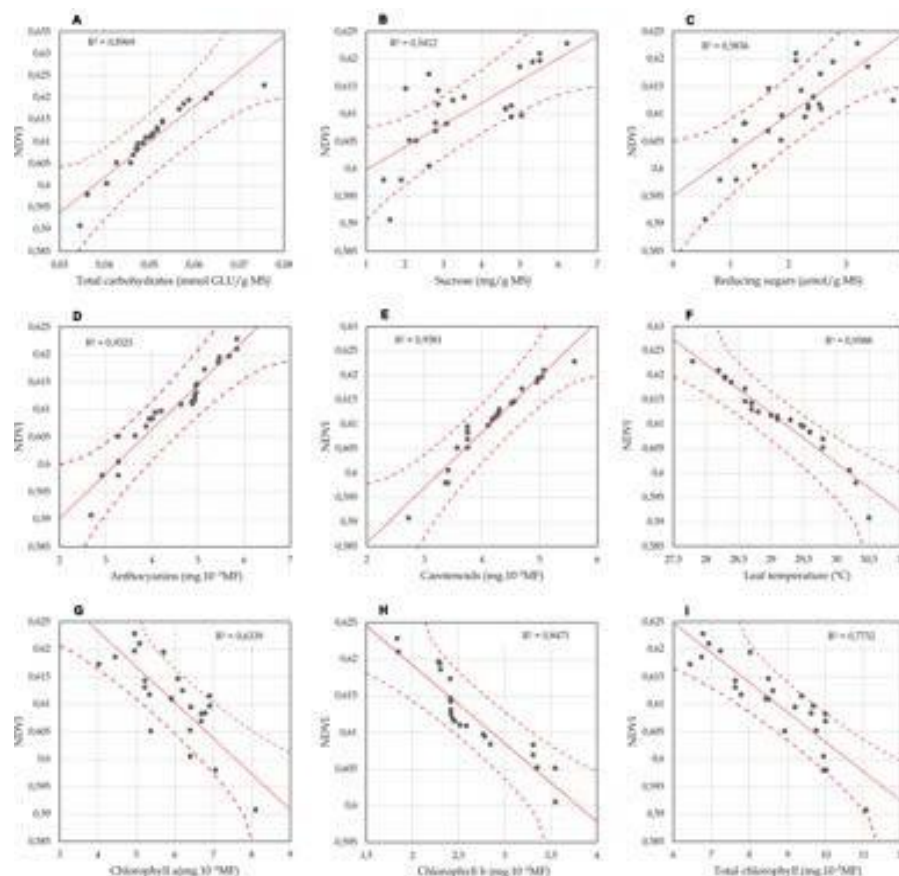
Figures 3 and 4 show the interactions of NDVI with pigment variables and photosynthetic metabolism. In stress situations, mahogany plants tend to maintain NDVI with a relationship directly proportional to total soluble carbohydrates, sucrose, reducing sugars, anthocyanins and carotenoids. On the other hand, variables such as leaf temperature, chlorophyll A, B and total tend to have an inversely proportional relationship.

NDVI is associated with plant health and vigor, as it reflects the quantity and quality of vegetation. Healthy, vigorous plants generally have higher levels of total soluble carbohydrates, sucrose, and reducing sugars, so NDVI may correlate positively with these sugars, as a healthier plant with greater photosynthetic activity tends to accumulate more sugars (Meyer et al., 2020; Wang et al., 2022).



Figure 3

Interactions of NDVI with the variables total carbohydrates (a), sucrose (b), reducing carbohydrates (c), anthocyanins (d), carotenoids (e), leaf temperature (f), chlorophyll a (g), chlorophyll b (h) and total chlorophylls (i) of Brazilian mahogany leaves (*Swietenia macrophylla*) subjected to nickel stress.

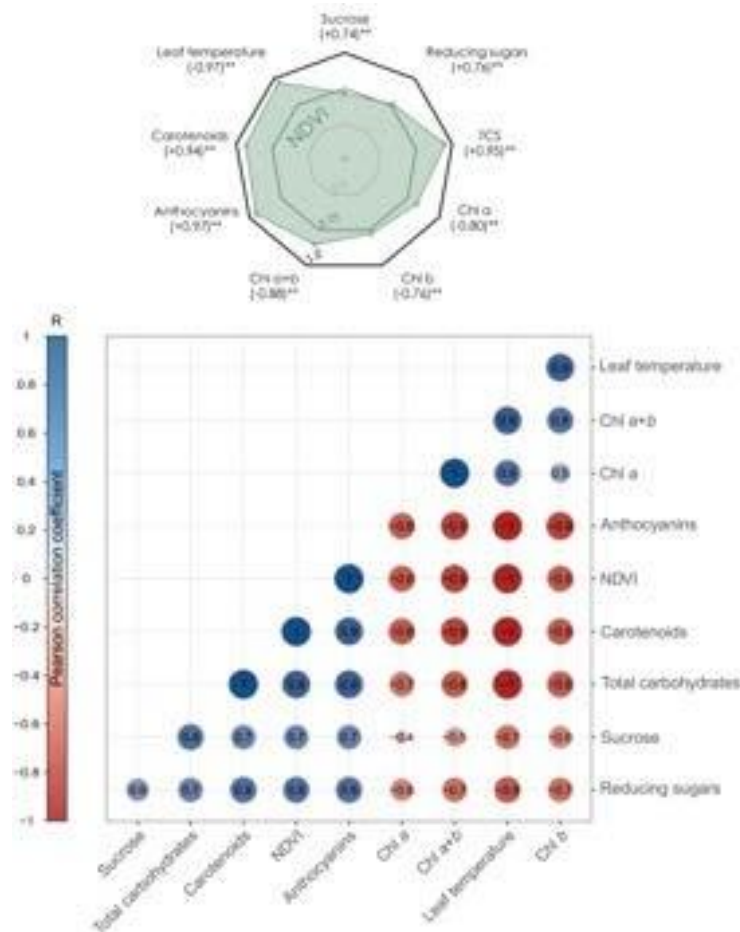


While anthocyanins and carotenoids, both are pigments associated with protection against oxidative stress and are produced in response to environmental stress. Stressed plants can increase the production of anthocyanins and carotenoids as a defense mechanism and adaptive response to the stressful condition, while leaf temperature, a higher NDVI may indicate greater photosynthetic activity, which in turn may lead to more effective thermal regulation (Zhang et al., 2020; Hlavinka et al., 2013; Kim et al., 2022).



Figure 4

*Pearson correlation coefficient of NDVI interaction with pigments and photosynthetic metabolism of Brazilian mahogany (*Swietenia macrophylla*) leaves subjected to nickel stress.*



NDVI is often positively associated with chlorophyll levels. Under ideal conditions, a higher NDVI is correlated with a higher density of plant biomass and, therefore, a higher amount of chlorophyll, since chlorophyll is a fundamental pigment for photosynthesis. However, situations of intense stress and possible degradation must be taken into account, which may have caused a reduction in chlorophyll activity, affecting the expected relationship between NDVI and the chlorophyll present in the plant. (Gan et al., 2019; Caruso et al., 2023; Kim et al., 2022).

These relationships between variables and NDVI are complex and can vary depending on the specific conditions of the plant, the environment, and the type of stress. In addition, a joint approach to NDVI with other biochemical parameters should be carried out, offering a more complete understanding of the health and condition of mahogany plants exposed to nickel. Therefore, in the multivariate analysis of principal components, the squared cosine (Cos^2) was evaluated, which was used to estimate the quality of the representation called commonality,



which is the sum of the squares of the correlations between each variable “i” and the principal component “j”. The larger the vector of a variable, the better its representation in the factor map, consequently the more important the interpretation of these components. On the other hand, the variables that are close to the center of the graph are less important for the first components, resulting in less representation in $\cos^2$. The variables that are correlated with PC1 and PC3 were the most important to explain the variability in the data set (Wold et al., 1987; Valladares et al., 2008; Liu et al., 2023; Oliveira et al, 2022).

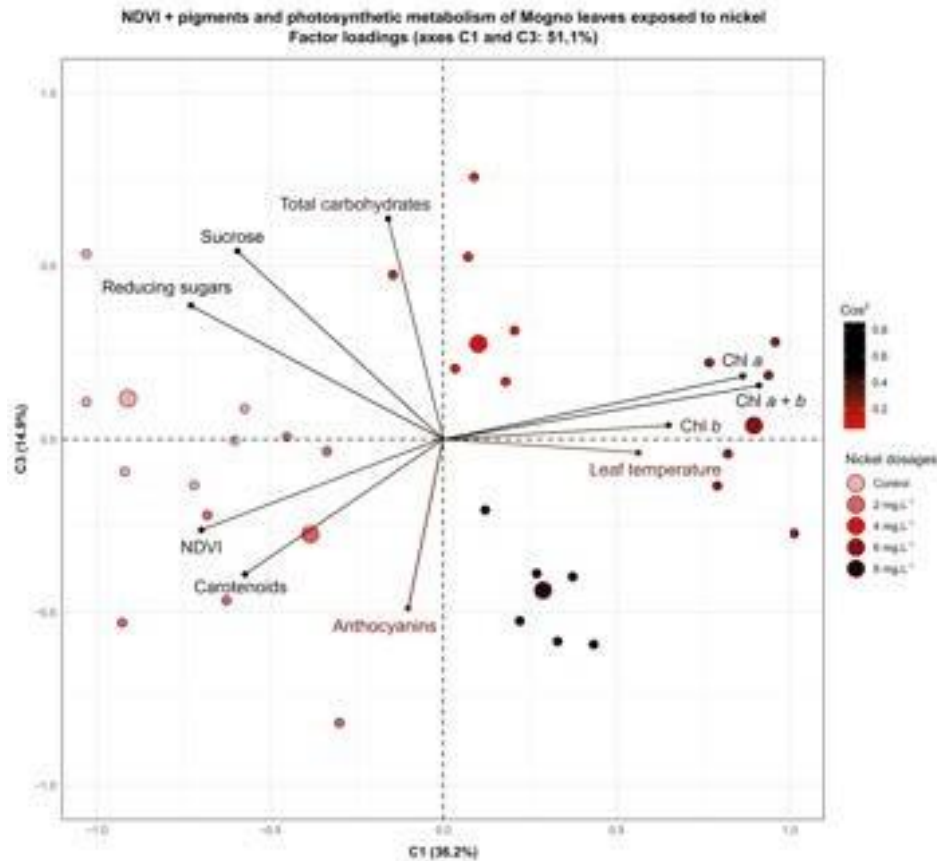
It was found that the first two PCs were responsible for 51.1% of the total variation of the photosynthetic pigment and metabolism variables, with principal component 1 (C1) responsible for 36.2% and principal component 3 (C3) for 14.9% of the data variance (Figure 5). The results showed a strong positive correlation of NDVI with carotenoids and anthocyanins, revealing that these variables showed a similar trend under different heavy metal treatments. However, the other parameters appeared dispersed in the PCA plot showing their different behaviors under strong metal stress.

One main explanation for this fact is that nickel may have promoted proteomic alterations. Proteomic alterations resulting from nickel stress in mahogany plants can trigger modifications in metabolic pathways and gene expression (Usman et al., 2022), directly affecting the production of proteins associated with photosynthesis and oxidative stress. These molecular changes influence leaf structure and function, possibly resulting in a reduction in photosynthetic efficiency (Zhou et al., 2015), which is reflected in variations in NDVI. Concomitantly, such changes can induce increased synthesis of carotenoids and anthocyanins, antioxidant pigments, as an adaptive plant response to stress (Zhang et al., 2020; Oliveira et al, 2024a), aiming to mitigate oxidative damage and protect the photosynthetic apparatus, providing defense against nickel stress and other environmental stresses, as evidenced by studies that correlate proteomic changes, expression of genes related to these pigments and plant adaptation to adverse conditions (Rodziewicz et al., 2014).



Figure 5

*Principal Component Analysis (PCA) C1 x C2, indicating the contribution of variables in the dimensions, referring to Brazilian mahogany leaves (*Swietenia macrophylla*) subjected to nickel stress.*



This may also have led to changes in the functioning of photorespiration, with different magnitudes depending on the severity of the stress. The functioning of photorespiration in mahogany plants subjected to nickel stress exhibits variations in its intensity according to the severity of the stress. In early and moderate stages of stress, photorespiration may increase as an adaptive response to compensate for the decrease in photosynthesis efficiency caused by the partial inhibition of the RuBisCo enzyme by nickel (Khairy et al., 2016). However, as the stress intensifies, photorespiration tends to decrease drastically due to the severe inhibition of RuBisCo, compromising the recycling of compounds and the effectiveness of carbon capture (Gan et al., 2019), resulting in detrimental impacts on plant metabolism and growth (Amaral et al., 2024, Oliveira et al, 2024a). This direct relationship between the severity of nickel stress and changes in photorespiration highlights the complex adaptation of mahogany plants to unfavorable environmental conditions (Sunil et al., 2019; Oliveira et al, 2024b).



4 CONCLUSION

The NDVI and thermal indices did not show statistical differences with the application of nickel. The increase in chlorophyll was not accompanied by an increase in the production of photoassimilates, given that although there was an increase in these pigments, they were probably not at their potential operating capacity due to the stress levels caused by nickel.

In stress situations, mahogany plants maintained NDVI with a relationship directly proportional to total soluble carbohydrates, sucrose, reducing sugars, anthocyanins and carotenoids. On the other hand, variables such as leaf temperature, chlorophyll a, b and total tend to have an inversely proportional relationship.

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