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CADMIUM STRESS-INDUCED SENSITIVITY RESPONSES IN *Peltophorum dubium*

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Resumo

Respostas de sensibilidade induzidas por estresse de cádmio em Peltophorum dubium. O cádmio (Cd) ocupa posição de destaque entre os problemas observados mundialmente devido à sua maior biodisponibilidade decorrente de diversas atividades poluidoras. Assim, faz-se necessário a realização de estudos voltados para a investigação da associação desse metal com a flora, pois espécies capazes de tolerar níveis tóxicos de Cd podem ser indicadas para a fitorremediação de áreas contaminadas. O objetivo do presente estudo é investigar a tolerância da espécie *Peltophorum dubium* ao excesso de Cd, com base na avaliação de variáveis morfofisiológicas e bioquímicas. Mudas de *P. dubium* foram cultivadas em solução nutritiva com cinco diferentes concentrações de Cd (0, 25, 50, 75 e 100 µM) e quatro repetições. Cada unidade amostral compreendeu uma bandeja com 16 plantas, totalizando 64 plantas por tratamento. Características morfofisiológicas como biomassa seca, variáveis morfológicas das raízes e variáveis fotossintéticas foram analisadas. Além disso, as concentrações de pigmento fotossintético, enzimas antioxidantes, peróxido de hidrogênio (H_2O_2) e peroxidação lipídica (MDA) foram investigadas. Mudas de *P. dubium* apresentaram sintomas de toxicidade, como redução da concentração de pigmentos e da taxa fotossintética, bem como inibição do crescimento radicular e da produção de biomassa. Com base nesses achados, mudas de *P. dubium* mostraram sensibilidade ao Cd. Portanto, esta espécie pode ser utilizada como bioindicadora de áreas contaminadas por cádmio.
Palavras-chave: áreas degradadas; canafístula; enzimas antioxidantes; excesso de cádmio; metais pesados. toxicologia ambiental

Abstract

Cadmium (Cd) ranks prominent position among issues observed worldwide due to its increased bioavailability that results from several polluting activities. Thus, it is necessary conducting studies focused on investigating the association between this metal and the flora, since species capable of tolerating toxic Cd levels can be indicated for the phytoremediation of contaminated areas. The aim of the present study is to investigate the tolerance of species *Peltophorum dubium* to excess Cd, based on the assessment of morphophysiological and biochemical variables. *P. dubium* seedlings were cultivated in nutrient solution with five different Cd concentrations (0, 25, 50, 75 and 100 µM) and four repetitions. Each sampling unit comprised a tray with 16 plants, and it totaled 64 plants per treatment. Morphophysiological features such as dry biomass, roots' morphological variables, and photosynthetic variables were analyzed. In addition, photosynthetic pigment concentration, antioxidant enzymes, hydrogen peroxide (H_2O_2), and lipid peroxidation (MDA) concentrations were investigated. *P. dubium* seedlings showed toxicity symptoms, such as reduced pigment concentration and photosynthetic rate, as well as root growth and biomass production inhibition. Based on these findings, *P. dubium* seedlings have shown sensitivity to Cd. Therefore, this species can be used as bioindicators of cadmium-contaminated areas.
Keywords: antioxidant enzymes; canafistula; Cadmium excess; degraded areas; environmental toxicology; heavy metals.

INTRODUCTION

Increased concentration of heavy metals in the atmosphere, water and in the soil have been the object of global concern. Among these metals, Cadmium (Cd) plays important role in contamination processes, due to its high toxicity and mobility in the soil (KICIŃSKA *et al.*, 2022). The main activities contributing to increase Cd concentrations in the environment comprise fossil fuel burnings, metal ore exploitations, waste incineration, urban dumps and sewage sludge deposition, and excessive use of fertilizers. In addition, soil acidity and Cd adsorption by organic matter and clay are other factors collaborating to increase the bioavailability of this metal (WANG *et al.*, 2022).

Thus, Cd can be easily available and absorbed by plant cultures and, consequently, it can enter both the human and the animal food chain, besides having several negative effects on the health of living beings

(HAIDER *et al.*, 2021). Cd taken up by plants can cause root growth inhibition, leaf chlorosis, as well as reduce photosynthetic activity and affect plant biomass production (ZHAO *et al.*, 2021). Furthermore, Cd toxicity induces changes in membrane permeability and, subsequently, reactive oxygen species (ROS) production at organelle level. Different ROS resulting from Cd stress encompass hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet-}$), superoxide anion ($O_2^{\bullet-}$) and singlet oxygen ($^1O_2$) (BAMAGOOS *et al.*, 2022). These ROSs are the main causes of membrane protein and lipid oxidation, which is associated with cell death (KUINCHTNER *et al.*, 2021).

However, plants' natural defense system comprises enzymatic and non-enzymatic antioxidants capable of protecting them from oxidative damage. Superoxide dismutase (SOD) and guaiacol peroxidase (POD) stand out among enzymatic antioxidants, since they act as effective defensive mechanism to protect plants from stress conditions (HASSAN *et al.*, 2021). Thus, certain plants may be tolerant to toxic metals due to mechanisms triggered by them upon their exposure to high levels of heavy metals (SILVA *et al.*, 2019).

Phytoremediation is a technique that uses plants to reduce the amount of heavy metals in contaminated soils (AGUILAR *et al.*, 2023). Thus, in addition to conducting studies focused on investigating the growth potential of plant species subjected to such conditions, it is also necessary assessing their physiological effects on plants, such as oxidative damage and antioxidant enzyme activity (TRENTIN *et al.*, 2022). The aforementioned variables can be used to help better understanding the limit concentrations tolerated by, or causing toxicity to, these species, in order to recommend their use in revegetation projects implemented in areas contaminated with Cd (KUINCHTNER *et al.*, 2021). It is plausible because plants only show visual symptoms resulting from metal toxicity, such as growth inhibition and reduced biomass production, after oxidative damage takes place within their cells.

Although woody species have lower tolerance to heavy metals than some herbaceous plants, using tree species in phytoremediation processes has emerged as viable technique, since woody crops can immobilize metals absorbed in their plant tissues for longer periods-of-time and, consequently, delay their return to the soil (AGUILAR *et al.*, 2023). Other benefits associated with tree species encompass their fast growth, the production of large biomass amounts, carbon sequestration and the potential bioenergetic use of its by-products.

Species *Peltophorum dubium* (Spreng.) Taub, which belongs to Fabaceae family, naturally grows in degraded areas, a fact that gives it intrinsic features associated with tolerance to altered soils (MARQUES *et al.*, 2019). *P. dubium* is a large tree species that grows fast and plays important ecological role in the environment. Studies have reported *P. dubium*'s potential tolerance to places contaminated with other metals and organic pollutants (MARQUES *et al.*, 2018; 2019). However, no studies focused on investigating the biochemical / physiological behavior of this species upon its exposure to high Cd concentrations were found in the literature. Therefore, the aim of the present research was to investigate *P. dubium* species' tolerance to excess Cd concentrations, based on morphological, physiological and biochemical variables. Our hypothesis is that *P. dubium* seedlings show tolerance to Cd, as well as the potential to be used in areas contaminated with this metal.

MATERIALS AND METHODS

Study site and plant material

The experiment performed in the current study was conducted in greenhouse environment, whereas the analyses were carried out in the Plant Physiology and Nutrition Laboratories of the Biology Department at Federal University of Santa Maria, Santa Maria County, RS. The greenhouse remained under controlled temperature of approximately 25 ºC, at 60% air humidity, on average.

Peltophorum dubium seeds were obtained at the Forest Research Center (DDPA), Santa Maria, RS. The seeds were subjected to dormancy-overcoming process, based on criteria described in the Instructions for Forest Species Seed Analysis, before they were sown in substrate (BRASIL, 2013). To do so, the seed coat on the side of the upper third of the seeds was manually cut (although superficially, without reaching the cotyledon) on the opposite side of the micropyle. Then, seeds were subjected to asepsis and placed on Germitest® paper inside Petri dishes to germinate. Seedlings were sown in polyethylene trays (55x34x15 cm) added with Bioplant® commercial substrate (composition: pine bark, ash, coconut fiber, rice husk, and vermiculite), after radicle emission. The seedlings were irrigated manually with the aid of a watering can, adding 100 mL of common water per plant daily. Thus, the sown seedlings were irrigated daily, until they reached approximately 15 cm in height (this process lasted approximately 30 days).

P. dubium exposure to Cd

Seedlings were transferred to hydroponic system and left to acclimate in nutrient solution for seven days. The nutrient solution by Hoagland encompassed (in mg L^{-1}): NO_3^- = 196; NH4 = 14; P = 31; K = 234; Ca = 160; Mg = 48.6; S = 70; Fe-EDTA = 5; Cu = 0.02; Zn = 0.15; Mn = 0.5; B = 0.5; and Mo = 0.01.

Based on this system, seedlings were fixed to the trays with the aid of sponges inserted in small holes in the polystyrene plates. These plates were placed on plastic trays added with 16 L of nutrient solution, and PVC microtubes were connected to an air compressor to enable root aeration. The trays were defined as experimental units and comprised 16 seedlings each.

The trays were distributed by following a completely randomized design, with four repetitions and five Cd concentrations, and it totaled 20 experimental units. Cadmium concentrations ($CdCl_2.H_2O$) added to the nutrient solution comprised 0, 25, 50, 75 and 100 µM Cd. These Cd levels were defined after preliminary tests were conducted with other woody species and based on the scientific literature.

The nutrient solution in each tray was replaced every seven days, and its pH was adjusted to 4.5±0.1, daily, with 1.0 mol L^{-1} HCl or 1.0 mol L^{-1} NaOH. Plants were collected as soon as they started showing visual symptoms of toxicity, mainly at the highest Cd concentration (100 µM) (14-day exposure to Cd).

Assessed morphological variables

Taproot length and shoot height of six plants per tray were measured with millimeter ruler, one day before their exposure to Cd. These traits were measured again after 14 day-exposure to Cd (end of experiment). Taproot and shoot increase were determined by subtracting measurement values recorded after Cd addition from the ones recorded before it. Three plants from each repetition were collected to determine root and shoot dry biomass, as well as to assess root morphology. The other seedlings in the tray were used to analyze the biochemical variables.

Seedlings were separated into shoots and roots, placed in Kraft paper bags, dried in forced air circulation oven at 65ºC, and weighed on precision scale (0.0001g) until they reached constant weight, to determine the dry biomass.

Root morphology, which was measured based on total root length, surface area and volume, was determined as follows: plants' root system was carefully cleaned in beaker filled with distilled water; then, roots were arranged on filter paper sheets, placed in plastic bags, and taken to the refrigerator (4 ºC). Roots were scanned with Epson 11000XL scanner, equipped with additional light (TPU), at 600-DPI resolution - images were generated and analyzed in Winrhizo Pro software.

Assessed physiological variables

Photosynthetic apparatus analyses were performed in two plants per repetition, at the 13th day of exposure to Cd. Assessments took place from 8:00 am to 11:00 am, based on using a portable infrared CO_2 meter, LICOR brand, model LI-6400XT. The following variables were assessed: net CO_2 assimilation rate (photosynthetic rate) (A), stomatal conductance of water vapors (Gs), internal CO_2 concentration (Ci), transpiration rate (E), as well as water use (WUE) and Rubisco carboxylation efficiency (A/Ci), at ambient CO_2 concentration of 400 µmol mol^{-1}, 20-25 °C, 50 ± 5% relative humidity and photon flux density of 1.500 µmol m^{-2} s^{-1}.

Biochemical variables

Fresh leaf and root samples were collected at the 14th day of exposure to Cd, frozen (-80 ºC) and, subsequently, macerated with liquid nitrogen to determine photosynthetic pigments, hydrogen peroxide (H_2O_2) and membrane lipid peroxidation concentration, as well as the activity of the antioxidant enzymes guaiacol peroxidase (POD) and superoxide dismutase (SOD).

Chlorophylls *a* and *b* and carotenoids were extracted according to the method of Hiscox and Israelstan (1979) and estimated using the Lichtenthaler equation (1987). The absorbance of the solution was measured in a spectrophotometer at 663, 645, and 470 nm for chlorophyll *a*, chlorophyll *b*, and carotenoids, respectively. Total chlorophyll is the sum of chlorophyll *a* + chlorophyll *b*.

Hydrogen peroxide concentration was determined according to Loreto and Velikova (2001). The H_2O_2 concentration of the supernatant was evaluated by comparing its readings with a standard calibration curve at 390 nm. The concentration of H_2O_2 was expressed as µmol g^{-1} fresh weight.

Lipid peroxidation was determined by the concentration of malondialdehyde (MDA) following the method of El-Moshaty *et al.* (1993). The absorbance of the supernatant was read at 532 and 600 nm (to correct non-specific turbidity). Lipid peroxidation was expressed as nmol of MDA mg^{-1} protein.

The activity of the guaiacol peroxidase enzyme (POD) was determined according to Zeraik *et al.* (2008), using guaiacol as substrate. The reaction mixture contained 1.0 mL of potassium phosphate buffer (100 mM, pH 6.5), 1.0 mL of guaiacol (15 mM) and 1.0 mL of H_2O_2 (3 mM) in the quartz cuvette. After homogenization, 50 µL of the plant extract was added to this solution. The enzyme activity was measured by oxidation of guaiacol to tetraguaiacol with that of the increase in absorbance at 470 nm, at 15-second reading intervals. The results were expressed as enzyme units per mg protein (U mg^{-1} protein). For calculation, the molar extinction coefficient of 26.6 mM^{-1} cm^{-1} was used.

The activity of enzyme superoxide dismutase (SOD) was analyzed based on the method by Beauchamp and Fridovich (1971), which uses a mix comprising potassium phosphate buffer (pH 7.8), 13 mM methionine, 0.1 µM EDTA, 75 µM NBT, and 2 µM riboflavin. Then, 300 µL of sample and 2.7 mL of the mix were incubated under 15-watt fluorescent lamp, for 3 minutes. After the incubation procedure was over, samples were subjected to readings in spectrophotometer, at 560 nm. SOD activity results were expressed as U mg^{-1} of protein.

Statistical data analysis

Error distribution normality was investigated through Shapiro-Wilk test, whereas error variance homogeneity was investigated through Bartlett test; both tests were applied to all experimental variables. Analysis of variance and Tukey test were applied to all treatments, at 5% probability of error, in the Sisvar statistical software, whenever these assumptions were met (FERREIRA, 2019).

RESULTS

Morphological variables

Analysis of variance has evidenced the significant effect ($p \leq 0.05$) of the assessed factor (different Cd concentrations) on morphological growth variables.

Thus, reduced total root length, surface area and volume were observed in the presence of Cd (Table 1). Thus, the highest means recorded for the variables were only evidenced in the control group (Table 1). However, the lowest shoot (SDW) and root (RDW) dry mass values were observed at 75 and 100 µM Cd.Nonetheless SDW values recorded at the Cd concentrations did not significantly differ from the one recorded at 50 µM Cd (Table 1).

Table 1. Mean values recorded for root length (cm) root surface area (cm^2), root volume (cm^3), of increment in taproot length (cm) shoot dry weight (SDW) (g $plant^{-1}$), root dry weight (RDW) (g $plant^{-1}$) in *Peltophorum dubium* seedlings grown under different Cd concentrations.

Tabela 1. Valores médios registrados para comprimento da raiz (cm) área de superfície da raiz (cm^2), volume da raiz (cm^3), de incremento no comprimento da raiz principal (cm) peso seco da parte aérea (SDW) (g $planta^{-1}$), peso seco da raiz (RDW) (g $planta^{-1}$) em mudas de *Peltophorum dubium* cultivadas sob diferentes concentrações de Cd.

Variables	Cd concentrations (µM)				
	0	25	50	75	100
Root length	1767±429 a	1117±56 b	954±94 bc	477±42 cd	340±24 d
Root surface	215.7±39 a	145.7±9.1 b	125.9±12 b	71.7±5.8 c	53.4±2.9 c
Root volume	2.2±0.3 a	1.53±0.11 b	1333±0.17 b	0.857±0.06 c	0.67±0.034 c
Taproot length	9.6±1.7 a	4.27±0.8 b	1.85±0.83 bc	0.89±0.37 c	1.2±0.66 c
Shoot dry weight	1.35±0.04 a	1.30±0.14 a	1.18±0.13 ab	0.86±0.034 b	0.84±0.1 b
Root dry weight	0.26±0.0 a	0.22±0.024 a	0.20±0.02 a	0.11±0.01 b	0.11±0.012 b

[1]Different letters on the lines represent statistical difference in the Tukey test, at 5% probability of error ($p \leq 0.05$). Data represent the mean ± standard deviation

Physiological variables

There was significant effect ($p \leq 0.05$) of different Cd concentrations on most of the assessed physiological variables, except for water use efficiency (WUE) (Table 2). The control group accounted for the highest values recorded for stomatal conductance (Gs) and transpiration rate (E), and they significantly differed from values recorded for the other treatments (Table 2). Photosynthetic rate (A), intercellular CO_2 concentration (Ci) and Rubisco instantaneous carboxylation efficiency (A/Ci) also recorded the highest mean values in the control group (Table 2). However, the photosynthetic rate recorded at 25 µM Cd was statistically equal to that of the control, whereas no significant difference in Ci and A/Ci was observed between the control and the 25 µM Cd condition (Table 2).

Table 2. Mean values recorded for stomatal conductance (Gs) (mol H_2O m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}), net CO_2 assimilation rate (A) (µmol CO_2 m^{-2} s^{-}), intercellular CO_2 concentration (Ci) (µmol CO_2 ar mol^{-1}), instantaneous carboxylation efficiency (by Rubisco) (A/Ci) (µmol CO_2 ar mol^{-1}) and water use efficiency (WUE) (WUE (mol CO_2 mol H_2O^{-1}) in *Peltophorum dubium* seedlings grown under different Cd concentrations.

Tabela 2. Valores médios registrados para condutância estomática (Gs) (mol H_2O m^{-2} s^{-1}), taxa de transpiração (mmol H_2O m^{-2} s^{-1}), taxa líquida de assimilação de CO2 (A) (µmol CO_2 m^{-2} s^{-}), concentração intercelular de CO2 (Ci) (µmol CO_2 ar mol^{-1}), eficiência de carboxilação instantânea (por Rubisco) (A/Ci) (µmol CO_2 ar mol^{-1})) e eficiência de uso da água (WUE) (WUE (mol CO_2 mol H_2O^{-1}) em mudas de *Peltophorum dubium* cultivadas sob diferentes concentrações de Cd.

Variables	Cd concentrations (µM)				
	0	25	50	75	100
Gs	0.24±0.07 a	0.15±0.014 b	0.09±0.01 c	0.048±0.01 c	0.036±0.01 c
E	4.83±1.7 a	3.92±0.5 b	2.2±0.58 b	1.166±0.31 b	0.93±0.18 b
A	10.48±1.58 a	9.3±0.46 a	6.64±0.97 b	3.40±0.31 c	3.14±0.3 c
Ci	289.95±1.3 a	267.15±9.2 ab	247.9±579 bc	230.8±148 c	210.5±27 c
A/Ci	0,038±0.01 a	0.034±0.0 ab	0.028±0.0 b	0.01±0 c	0.012±0.0 c
WUE	2.39±0.75 a	2.41±0.44 a	3.0±0.47 a	3.03±0.55 a	3.45±0.7 a

[1]Different letters on the lines represent statistical difference in the Tukey test, at 5% probability of error (p≤0.05). Data represent the mean ± standard deviation

Biochemical variables

There was significant effect ($p \leq 0.05$) of the tested Cd concentrations on most biochemical variables assessed in the present study, except for hydrogen peroxide concentration in the shoot (Table 4). Decrease in total chlorophyll and carotenoid concentration was observed after Cd addition to the nutrient solution, but there was no significant difference in carotenoid concentration between the control and 100 µM Cd (Table 3). SOD activity in the shoots and roots, and POD activity in the roots, recorded the highest mean values at concentration of 50 µM Cd (Table 3). However, there was no difference between the concentrations of 50 and 75 µM Cd for SOD activity in the roots (Table 3). On the other hand, decrease in POD activity in the shoot was observed at 25 µM Cd, whereas the activity of this enzyme increased in plants subjected to the subsequent Cd concentrations, in comparison to the control (Table 3).

Table 3. Mean total chlorophyll (a), carotenoids (b), superoxide dismutase (SOD) enzyme activity in shoot (c) and roots (d) and guaiacol peroxidase enzyme (POD) in shoot (e) and roots (f) in *Peltophorum dubium* seedlings grown under different Cd concentrations.

Tabela 3. Valores médios de clorofilas totais (a), carotenóides (b), superóxido dismutase (SOD) na parte aérea (c) e raízes (d) e enzima guaiacol peroxidase (POD) na parte aérea (e) e raízes (f) em mudas de *Peltophorum dubium* cultivadas sob diferentes concentrações de Cd.

Variables	Cd concentrations (µM)				
	0	25	50	75	100
Chl total	3.85±0.16 a	3.4±0.04 b	3.55±0.01 b	3.19±0.309 c	3.45±0.102 b
Carotenoides	0.46±0.05 a	0.43±0.01 b	0.44±0.03 b	0.43±0.02 b	0.45±0.02 ab
SOD Shoot	5930±138 b	4923±160 b	12767±256 a	3553±74 b	6610±5.3 b
SOD Root	499±47 bc	4872±144 bc	6877±255 a	6283±80 ab	4494±70 c
POD Shoot	269.9±0.7 d	125.2±2.5 e	439.7±26 c	557±27 b	758±27 a
POD Root	278.5±23 b	252.3±13 b	3854.1±20 a	267.3±28 b	2993.5±9.5 b

[1]Different letters on the lines represent statistical difference in the Tukey test, at 5% probability of error (p≤0.05). Data represent the mean ± standard deviation

There was no significant difference in hydrogen peroxide (H_2O_2) concentration in the shoot, regardless of the applied Cd concentrations (Table 4). However, the highest means recorded for H_2O_2 concentration in the roots and for MDA concentration in the shoot were observed from 75 µM Cd, onwards (Table 4). However, the 50 µM Cd condition recorded the highest MDA concentration in the roots, and it significantly differed from MDA concentration recorded for plants subjected to the other Cd concentrations (Table 4).

Table 4. Mean values recorded for hydrogen peroxide (H_2O_2) concentration in shoot (a) and roots (b), and membrane lipid peroxidation (MDA) in shoot (c) and roots (d) in *Peltophorum dubium* seedlings grown under different Cd concentrations.

Tabela 4. Valores médios registrados para concentração de peróxido de hidrogênio (H_2O_2) na parte aérea (a) e raízes (b) e peroxidação lipídica da membrane (MDA) na parte aérea (c) e raízes (d) em mudas de *Peltophorum dubium* cultivadas sob diferentes concentrações de Cd.

Variables	Cd concentrations (µM)				
	0	25	50	75	100
H_2O_2 Shoot	0.56±0.05 a	0.62±0.08 a	0.62±0.08 a	0.64±0.06 a	0.81±0.18 a
H_2O_2 Root	0.03±0.01 b	0.036±0.02 b	0.037±0.01 b	0.046±0.01 ab	0.076±0.02 a
MDA Shoot	0.175±0.03 b	0.122±0.09 b	0.183±0.02 b	0.663±0.16 a	0.864±0.13 a
MDA Root	0.0015±0 bc	0.0026±0.01 b	0.006±0.0 a	0.0005±0 c	0.0005±0 c

[1]Different letters on the lines represent statistical difference in the Tukey test, at 5% probability of error ($p \leq 0.05$). Data represent the mean ± standard deviation

DISCUSSION

The lowest values recorded for total root length, surface area and volume, as well as taproot increase were observed after Cd addition to the nutrient solution (Table 1). It is so because Cd toxicity has negative effect on the mitotic division of root meristematic cells and causes abnormal changes in cortical cell layers and in the apical region of the epidermis. These changes decrease water and nutrient absorption, as well as imply reduction in these plants' root length and biomass production (HAIDER *et al.*, 2021).

Thus, the lowest values recorded for shoot and root dry mass were also observed when Cd was added to the growth medium (Table 1). This response may have happened because excess Cd likely damaged the plasma membrane of root cells and changed their permeability, a fact that has affected nutrient uptake by roots (Hassan *et al.*, 2021). This biomass reduction can also be explained by the fact that Cd stress limits and/or inhibits plant photosynthesis, and it decreases their photosynthetic ability and ultimately reduces their biomass production (ZHAO *et al.*, 2021).

Excess Cd has severely affected the photosynthetic variables assessed in the present study (Table 2). It happened because Cd stress can decrease stomatal conductance, which implies lower transpiration rate and, subsequently, results in reduced photosynthetic rate (ZHANG *et al.*, 2020). In addition, Cd^{2+} ions may have bound to the functional groups of some enzymes (for example, to sulfhydryl–SH groups), and replaced essential metal elements in these proteins. This replacement type can change biological macromolecules' conformation, inhibit their activity and, consequently, decrease plants' photosynthetic ability (HUIHUI *et al.*, 2020).

The lowest A/Ci values were observed after plants were exposed to Cd (Table 2). It happened because Cd ions decreased the activity of enzyme ribulose 1,5-bisphosphate carboxylase oxygenase (Rubisco), and directly damaged its structure by replacing Mg^{2+} ions, which are essential cofactors in carboxylation reactions. This process altered Rubisco activity in oxygenation reactions (CONCEIÇÃO *et al.*, 2020) and, consequently, Cd may have resulted in irreversible Rubisco subunits' dissociation and fully impaired enzyme activity (HAIDER *et al.*, 2021). Thus, high Cd stress has likely reduced photosynthetic efficiency by downregulating Rubisco synthesis and activity.

Thus, the lowest total Chl and carotenoid values were also observed after Cd addition to the growth medium (Table 3). The likely reasons for decline in photosynthetic pigments under Cd stress comprise oxidative stress, direct metal ions' influence on pigment biosynthesis pathways, and Mg^{2+} replacement by metal ions within the chlorophyll molecule (BAMAGOOS *et al.*, 2022).

Heavy metals can stimulate plants to increase reactive oxygen species (ROS) levels, which react to lipids, proteins, and nucleic acids, among other substances, and cause lipid peroxidation, enzyme inactivation, as well as affect cell performance and viability (KUINCHTNER *et al.*, 2021). However, plants are endowed with a natural defense system, which is composed of enzymatic and non-enzymatic antioxidants and protects them from oxidative damage induced by several environmental stress types (ZHAO *et al.*, 2021). SOD and POD, which play crucial role in eliminating ROS and in maintaining homeostasis in plant cells, stand out among antioxidant enzymes (HASSAN *et al.*, 2021).

SOD plays the most important role in the antioxidant defense mechanism since it is the most effective enzyme in stress resistance. In addition, SOD plays key role in $O_2^{\bullet -}$ dismutation into H_2O_2 and molecular oxygens in plants subjected to stress conditions (ZHANG *et al.*, 2020). POD enzyme, in its turn, accounts for

converting H_2O_2 into H_2O and O_2 by dissociating H_2O_2. Therefore, POD also plays key role in providing tolerance in plants under unfavorable conditions (LIU *et al.*, 2021).

However, SOD activity in the shoot and roots, and POD activity in the roots, increased at moderate Cd levels, although their values decreased at higher Cd concentrations (Table 3). It happens because some plants subjected to heavy metal stress tend to gradually increase antioxidant enzymes' activity at increasing concentrations of these toxic metals (GUTIÉRREZ-MARTÍNEZ *et al.*, 2020), to mitigate oxidative stress caused by them. However, plants' protective enzyme system can be damaged or inhibited, and enzyme activity can decrease, when the concentration of heavy metals gets too high.

Thus, the highest H_2O_2 concentrations were observed in the roots after Cd addition (Table 4). It happeend because antioxidant enzymes failed to sufficiently protect the exposed plants from Cd stress, and it resulted in increased H_2O_2 levels and, likely, in other ROS production, which potentiated oxidative damage (KUINCHTNER *et al.*, 2021). Thus, antioxidant enzymes' resistance to heavy metal-related stress is a complex physiological process influenced by plant species, as well as by heavy metal concentrations and/or properties (ZHAO *et al.*, 2021).

POD activity in the shoot has increased from 50 µM Cd, onwards (Table 3). This POD activity increase has indicated enzymatic response triggering to prevent oxidative damage caused by excessive metal toxicity (AGUILAR *et al.*, 2023). Thus, H_2O_2 concentration in the shoot did not show significant difference after Cd application, and it indicated that H_2O_2 produced by SOD activity was dismuted by POD. Thus, there was positive correlation between POD activity and H_2O_2 concentration in the shoot (Table 4). However, although there was no H_2O_2 concentration increase in the shoot, there was increase in malondialdehyde (MDA) levels in it (Table 4). Thus, the action of other ROS may have damaged membrane lipids since it did not have significant effect on H_2O_2 concentrations in the shoot but led to increase in MDA concentration.

MDA is an end product of lipid peroxidation that has been widely used to assess metal-induced oxidative stress. MDA concentration can be used as indicator of stress severity, since MDA accumulation can damage both the membrane and the cells, a fact that affects plant growth and development (LIU *et al.*, 2021).

Thus, species *P. dubium* was overall sensitive to high Cd levels, since it has shown increased ROS-induced oxidative stress, and SOD and POD enzymes failed to fully eliminate the excess of harmful substances, such as $O_2^{\bullet-}$ and H_2O_2, to maintain normal free radical metabolism in plants. Therefore, oxidative stress-induced changes in ROS accumulation had negative affect on plant growth, as indicated by the observed increased H_2O_2 and MDA (lipid peroxidation) levels. Thus, *P. dubium* seedlings showed toxicity symptoms, such as reduced pigment concentration and photosynthetic rate, as well as root growth and biomass production inhibition. Considering these features, it is possible saying that *P. dubium* seedlings did not show tolerance to Cd, and they can only be used as bioindicators of Cd-contaminated areas. This finding rejected our initial hypothesis. Thus, it is possible assuming that *P. dubium* seedlings did not show and / or did not trigger Cd tolerance mechanisms, such as Cd compartmentalization in cell vacuoles, as well as Cd complexation / chelation to decrease Cd bioavailability and, consequently, to lessen the toxic effects caused by the excess of this metal.

CONCLUSION

- Cadmium concentrations had negative effect on biochemical and morphophysiological variables assessed in *Peltophorum dubium* seedlings.
- Cadmium was harmful to seedling growth and this outcome has evidenced sensitive behavior by the species. Considering these features, *P. dubium* seedlings can be used as bioindicators of cadmium-contaminated areas.

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