

Can species *Cedrela fissilis* Vell. be used in sites contaminated with toxic aluminum and cadmium metals?

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Toxic metals are among the main pollutants contributing to environmental degradation. Cadmium (Cd) and aluminum (Al) stand out among these metals as extremely toxic elements. The use of native species in reforestation programs can compensate for degradation and re-establish the ecological conditions of the affected environments. *Cedrela fissilis* Vell., popularly known as cedar, may be used as an alternative in phytoremediation, since it is a fast-growing native woody species widely distributed in tropical America. In this study we investigated the possibility of using *C. fissilis* in sites contaminated with Al and Cd by evaluating morphological, physiological, and biochemical variables of seedlings grown in hydroponic system. *C. fissilis* seedlings were subdivided into two experiments with a completely randomized design. The first experiment evaluated the effect of four Al concentrations, namely: 0 (complete nutrient solution without phosphorus), 25, 50 and 100 mg l⁻¹. The second experiment evaluated four Cd concentrations, namely: 0 (complete nutrient solution), 25, 50 and 100 µM. Each sample unit consisted in a pot with four plants. Morphological, physiological and biochemical variables of seedlings were evaluated after 15-day exposure to different treatments in the hydroponic system. Aluminum concentration of 100 mg l⁻¹ caused oxidative stress in *C. fissilis* seedlings, reduced shoot and root dry weight, and increased hydrogen peroxide contents, which led to lipid peroxidation. Cadmium concentration of 100 µM also damaged *C. fissilis* seedlings by significantly reducing root dry weight and involving the most severe effects on photosynthetic variables. Cadmium presence in the nutrient solution negatively affected morphophysiological and biochemical variables of *Cedrela fissilis* seedlings, and it was also harmful to their growth. Based on our results, the investigated species shows a sensitive behavior upon exposure to cadmium. On the other hand, *C. fissilis* tolerates high Al concentrations (up to 50 mg l⁻¹), which suggests a moderate tolerance to this metal.

Keywords: Phytoremediation, Heavy Metals, Gas Exchange, Morphophysiological Variables

Introduction

Toxic metals associated with industrial and agricultural expansion stand out among the main abiotic agents capable of causing stress in living organisms. The bioaccumulation of these metals can be found at different environmental levels (Huihui et al. 2020). In particular, metals such as cadmium (Cd) and aluminum (Al) are potentially toxic to different organisms even at

low concentrations (Cunha Neto et al. 2020).

Cadmium is widespread in different environments and is extremely toxic to plants and animals (Hassan et al. 2020). It is mainly released into the environment through human activities such as mining, metallurgy, cement factories, waste combustion and use of pesticides and fertilizers comprising Cd (Huang et al. 2017). Although Cd

is not essential for plant nutrition, it can be easily absorbed by roots and accumulated in all plant tissues, from roots to shoot. Average Cd rate in Brazilian soils seen as uncontaminated is 0.18 mg Kg⁻¹, whereas contaminated soils worldwide present Cd content ranging from 5.9 to 531 mg Kg⁻¹, depending on the cause of pollution (Kubier et al. 2019).

The main Cd-induced toxicity symptoms observed in plants comprise stunted growth, chlorosis, leaf epinasty, abnormal chloroplast ultrastructure, photosynthesis inhibition, enzyme inactivation in CO₂ fixation processes, lipid peroxidation, disturbance in nitrogen and sulfur homeostasis, biological membrane oxidation and reduced root and stem growth, and concomitant reduction in biomass production (Anjum et al. 2017).

Aluminum is one of the most abundant metals on Earth's crust (Zhou et al. 2017). Its toxicity is one of the biggest limitations to plant yield in acidic soils in tropical and subtropical sites worldwide (Cunha Neto et al. 2020). Brazilian soils are mostly weath-

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ered and acidic due to high Al and manganese (Mn) contents, as well as to low sum and saturation of bases (Cunha et al. 2018). These soils show $\text{pH} < 5.0$; under these conditions, Al is ionized to form phytotoxic ions (Al^{3+}) that are promptly absorbed by plant roots, thus inhibiting root elongation and reducing crop yield (Guo et al. 2018). Since amelioration processes are hard to accomplish and highly expensive, soil acidity has become a major issue in subsurface soils; therefore, it is necessary to find crop plant species capable of tolerating acidic soils or high Al accumulation rates.

Root growth inhibition – which may occur after brief plant exposure to Al – is the first symptom of Al toxicity. Root meristems are considered the plant part most sensitive to Al toxicity, suggesting that Al actively interacts in cell division and elongation processes (Cárcamo et al. 2019). Plants' response to exposure to Al are associated with changes in physiological and biochemical processes, such as increased reactive oxygen species (ROS) production and damage to biological membranes. In addition, Al has negative effects on photosynthetic activity, such as decreased photosynthetic pigments and fluorescence parameters, reduced enzyme activity and carbohydrate metabolism, decreased stomatal conductance, and programmed cell death induction (Xu et al. 2018).

Given the harmful effect of these metals on plant components, it is essential to identify species capable of tolerating such contamination or accumulating these metals though time in order to revegetate and/or even decontaminate contaminated sites (Yan et al. 2020). Also, it is necessary understanding the mechanisms developed by tolerant and resistant plants to support the selection of species suitable to be used in sites contaminated with toxic metals.

Cedrela fissilis Vell. (cedar) stands out among several species with potential to recover degraded areas (Silva et al. 2020). This native woody species grows fast and is widely distributed in tropical America. *C. fissilis* has light and soft timber, which can be easily used for several purposes, such as plywood, moldings, sculptures, civil construction and as ornamental species (Goeten et al. 2016). In addition, it presents high biomass production and filtering capacity, due to its extensive root system, which are features highly desirable for phytoremediation programs (Covre et al. 2020).

The aim of the present study is to investigate the suitability of *C. fissilis* to be used in remediation of areas contaminated with Al and Cd, by evaluating morphological, physiological and biochemical variables in hydroponic system.

Material and methods

Study site

The study was conducted in a greenhouse at the Biology Department of Fed-

eral University of Santa Maria (UFSM), Santa Maria Campus, RS, Brazil, under controlled temperature of approximately 25 °C, and mean humidity of 60%. Analyses were carried out at the Plant Physiology and Nutrition Laboratory of the Biology Department.

Experimental design

Cedrela fissilis seedlings were subdivided into two experiments, which followed a completely randomized design. The first experiment evaluated the effect of four aluminum (Al) concentrations: 0 (complete nutrient solution without phosphorus), 25, 50 and 100 mg L^{-1} . Control treatment had nutritive solution without phosphorus (P) to avoid physical-chemical interactions between P and Al. The second experiment evaluated four cadmium (Cd) concentrations: 0 (complete nutrient solution), 25, 50 and 100 μM . Each sample unit consisted in a pot with four plants. The above Cd and Al concentrations were selected based on the scientific literature and on studies conducted by our research group with other plant species.

Seeds purchased in the UFSM Forest Nursery, Santa Maria Campus, were used to produce *C. fissilis* seedlings. They were sown in Carolina Soil® commercial substrate composed of *Sphagnum* sp. and vermiculite. Plastic trays (38 × 56 cm) were used as containers for seedling germination and initial growth. Substrate humidity was kept close to 60% of the field capacity and monitored on a daily basis throughout the experimental period.

Seedlings were irrigated with complete nutrient solution at $\text{pH } 4.5 \pm 0.1$ on a weekly basis, from the 15th day after sowing (DAS) onwards. Nutrient solution was formed by 6090.5 μM of nitrogen (N); 974.3 μM of magnesium (Mg); 4986.76 μM of chlorine (Cl); 2679.2 μM of potassium (K); 2436.2 μM of calcium (Ca); 359.9 μM of sulfur (S); 243.592 μM of phosphorus (P); 0.47 μM of copper (Cu); 2.00 μM of manganese (Mn); 1.99 μM of zinc (Zn); 0.17 μM of nickel (Ni); 24.97 μM of boron (B); 0.52 μM of molybdenum (Mo) and 47.99 μM of iron (as $\text{FeSO}_4/\text{Na-EDTA}$ – Hoagland & Arnon 1950).

Seventy-three days after sowing, when seedlings were approximately 10 cm tall, they were carefully removed from the substrate in the trays and transferred to hydroponic system. Each seedling was placed in a 6-L pot filled with Hoagland & Arnon (1950) complete nutrient solution. Polystyrene blade with four central holes was added to the surface of each pot to allow plants to pass through it. The polystyrene blade allowed plants to be fixed and reduced the solution evaporation from each pot.

Seedlings were left to acclimate for seven days in Hoagland's nutrient solution, at 100% of its original concentration. Nutrient solution in each pot was aerated through PVC microtubes connected to air compres-

or. Its original form comprised the following concentrations (in mg L^{-1}): $\text{NO}_3^- = 196$; $\text{NH}_4 = 14$; $\text{P} = 31$; $\text{K} = 234$; $\text{Ca} = 160$; $\text{Mg} = 48.6$; $\text{S} = 70$; $\text{Fe-EDTA} = 5$; $\text{Cu} = 0.02$; $\text{Zn} = 0.15$; $\text{Mn} = 0.5$; $\text{B} = 0.5$; $\text{Mo} = 0.01$.

After the acclimation period, seedlings were subjected to treatments based on different Al and Cd availability conditions for 15 days; they remained in hydroponic system for 22 days, in total. Plants were collected as soon as metal toxicity symptoms were observed, mainly at the highest Al and Cd concentrations. Nutrient solution in each pot was replaced twice a week and its pH was adjusted on a daily basis to 4.5 ± 0.1 using 1.0 mol L^{-1} HCl or 1.0 mol L^{-1} NaOH.

Growth variables

One plant from each experimental unit was collected for growth assessment purposes. Shoot height and root length of each seedling were measured by a millimeter ruler. Measurements were taken before and after treatment application, and plant growth derived from the increase recorded within that period.

Seedlings were harvested and separated into shoots and roots, washed in running water and dried in forced air circulation oven at approximately 65 °C, until they reached constant weight. Shoot (SDW, g plant⁻¹) and root dry weight (RDW, g plant⁻¹) were determined.

Roots were subjected to morphological characterization based on digitized images using the software WinRhizo™ Pro 2013 (Regent Instruments Inc., Sainte-Foy, Quebec, Canada) coupled to a scanner Expression 11000® (EPSON Corp., Nagano, Japan) equipped with additional light (TPU) at 600 DPI resolution. Root length (cm plant⁻¹), mean root diameter (mm) and number of branches were measured.

Photosynthetic variables

The third fully expanded leaf of each seedling was used to evaluate photosynthetic variables in an infrared gas analyzer (model Li-COR® 6400 XT, Lincoln, NE, USA) at 500 μmol photosynthetic radiation² s⁻¹ and CO_2 concentration of 400 $\mu\text{mol mol}^{-1}$. Measurements were carried out in the morning between 8:00 and 10:00 am, before plants were collected for growth analysis. The following variables were determined: net CO_2 assimilation rate (A), transpiratory rate (E), stomatal conductance (G_s), intercellular CO_2 concentration (C_i), Rubisco's instant carboxylation efficiency (A/C_i , based on the ratio between photosynthetic rate and intercellular CO_2 concentration), and water use efficiency (WUE, based on the ratio between photosynthetic and transpiration rates).

Biochemical variables

Twelve plants from each treatment were collected and subjected to biochemical variable analysis, totaling 48 plants per experiment. Seedlings were separated into shoot and roots, washed in distilled water,

placed in aluminum foil envelopes, and frozen with liquid nitrogen to avoid sample degradation. They were kept in ultra-freezer at -80°C until they were pre-prepared for analysis. Sample preparation was carried out through manual maceration with liquid nitrogen until a fine powder was obtained for each sample. Subsequently, the specific amount of powder necessary for each analysis was weighted using a precision digital scale: 0.05 g of fresh sample was used for determining leaf pigments, 0.5 g for antioxidant enzymes, 0.3 g for hydrogen peroxide, and 0.5 g for lipid peroxidation.

Total chlorophylls and carotenoids content

The Hiscox & Israelstam (1979) method was used for total chlorophyll and carotenoid extraction, whereas the Lichtenthaler (1987) equation was used for their estimates. Previously weighed samples were added with 5 ml of dimethylsulfoxide (DMSO). Tubes were incubated at 65°C for approximately 90 min, until full pigment release in a dark green solution was achieved. Subsequently, this solution was separated into two repetitions of 2 ml each. Solution absorbance was measured in UV-visible spectrophotometer (model 1105, Bel Photonics, Piracicaba, Brazil), at wavelength of 663, 645 and 470 nm for chlorophyll *a*, chlorophyll *b* and carotenoids, respectively. Total chlorophyll values corresponded to the sum of chlorophylls *a* and *b*.

Antioxidant enzyme activity

Antioxidant enzymes were determined by adding 0.5 g of sample to 3 mL of 0.05 M homogenization extraction buffer (pH 7.8) comprising 1 mM EDTA and 2% (w/v) polyvinylpyrrolidone (PVP). Homogenate was centrifuged in a high-speed refrigerated centrifuge (model CR22 N, Eppendorf Himac Technologies Co., Hitachinaka, Japan) at $13,000\times g$ at 4°C for 20 minutes. Supernatant was used to determine enzyme activity and protein concentration (Zhu et al. 2004).

Guaiaicol peroxidase (POD) enzyme activity was determined according to Zeraik et al. (2008); guaiaicol was used as substrate. Reaction mixture comprised 1.0 ml of potassium phosphate buffer (100 mM, pH 6.5), 1.0 ml of guaiaicol (15 mM) and 1.0 ml of H_2O_2 (3 mM) in quartz cuvette. When the homogenization was complete, 50 μL of plant extract was added to the solution. Enzyme activity was measured through guaiaicol oxidation into tetraguaiaicol by increasing absorbance at 470 nm at 15-second reading intervals. Results were expressed in enzyme units per mg of protein (U mg^{-1} protein). The molar extinction coefficient of $26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ was used for calculation purposes.

Superoxide dismutase (SOD) activity was determined based on the spectrophotometric method described by Giannopolitis

& Ries (1977). Reaction mixture (MIX), which was kept in the dark, comprised 50 mM of potassium phosphate buffer (pH 7.8), 13 mM of methionine, 0.1 mM of EDTA, 75 μM of nitrobluetetrazolium (NBT) and 2 μM of riboflavin. Photochemical production of blue formazan from NBT was monitored by increasing absorbance at 560 nm. Reaction process was carried out at 25°C in test tubes (13 \times 100 mm) filled with 2.8 ml of reaction mixture (MIX) and 200 μl of enzymatic extract from the respective samples. After pipetting, tubes were placed in the reaction chamber under 15 W fluorescent lamp. Reaction started when the light was turned on and stopped 2 minutes later, when the light was turned off; samples were read in UV-visible spectrophotometer. SOD unit was defined as the number of enzymes inhibiting NBT photoreduction by 50% (Beauchamp & Fridovich 1971). During the test, photochemically excited riboflavin was reduced by methionine in semiquinone, which donated an electron to oxygen and formed the superoxide radical, which in turn converted NBT into blue formazan. Superoxide dismutase has catalyzed this reaction.

Hydrogen peroxide content

Hydrogen peroxide content was determined according to Loreto & Velikova (2001). Root and leaf samples were homogenized in 3.0 mL of 0.1% trichloroacetic acid (TCA). Homogenized samples were centrifuged, 0.5 ml of the supernatant was added to 0.5 ml of potassium phosphate buffer (10 mM) and 1 ml of KI (1 M), and samples were absorbed in spectrophotometer at 390 nm. H_2O_2 concentration in the supernatant was evaluated by comparing its readings to a standard calibration curve. H_2O_2 concentration was expressed as $\mu\text{mol g}^{-1}$ fresh weight.

Membrane lipid peroxidation

Lipid peroxidation was determined based on malondialdehyde (MDA) concentration, based on the method by El-Moshaty et al. (1993). Leaf and root samples were homogenized in 4.0 mL of sodium citrate buffer (pH 6.5) and centrifuged. In total, 1 ml of supernatant was added to 1 ml of 20% (w/v) trichloroacetic acid (TCA) added with 0.5% (w/v) thiobarbituric acid (TBA). The mix was heated to 95°C for 40 min and cooled in ice bath for 15 min. Subsequently, it was centrifuged at $10,000\times g$ for 15 minutes. Supernatant absorbance was read at 532 and 600 nm (to correct non-specific turbidity). Lipid peroxidation was expressed as nmol of MDA mg^{-1} protein.

Statistical analysis

Results were subjected to analysis of variance, based on the factorial model (factorial ANOVA). Significant differences were checked by F-test and treatment means compared by Tukey test ($\alpha = 0.05$) using the Sisvar statistical software (Ferreira 2014).

Results

Cadmium effects on Cedrela fissilis

ANOVA results showed significant differences ($p \leq 0.05$) among Cd treatments (different cadmium concentrations) for most growth-related morphological variables in *C. fissilis* seedlings reared in a hydroponic system. Cd concentrations of 50 and 100 μM promoted a significant reduction in root length increment (ILR – Fig. 1A), while seedlings grown at Cd concentration of 25 μM did not show difference in ILR in comparison to control (0 μM Cd). On the other hand, *C. fissilis* seedlings did not show significant difference in increment in shoots (IS) regardless of the tested concentrations (data not shown).

Cadmium concentrations of 25, 50 and 100 μM had negative effects on shoot dry weight (SDW – Fig. 1C) and root dry weight (RDW – Fig. 1B), in comparison to the control treatment. Seedlings exposed to 100 μM of Cd had the shortest mean root length (Fig. 1D), as well as the smallest diameter (Fig. 1E) and number of branches (Fig. 1F). However, there was no significant difference in root volume at all tested Cd concentrations (data not shown).

Different Cd concentrations had significant effect ($p \leq 0.05$) on most physiological variables analyzed in the present study. Net CO_2 assimilation rate (*A* – Fig. 2A), stomatal conductance (*G_s* – Fig. 2B) and transpiration rate (*E* – Fig. 2C) showed significant decrease as Cd concentrations in the nutrient solution increased. The highest Cd concentration treatment (100 μM) had the most negative effects on the aforementioned parameters.

Cadmium concentration of 100 μM had the most significant effect on internal CO_2 concentration (*C_i* – Fig. 2D). Cadmium levels of 0 and 25 μM were statistically similar in terms of both internal CO_2 concentration (Fig. 2 D) and water use efficiency (*WUE* – data not shown). On the other hand, Cd treatments at 50 and 100 μM recorded the highest mean *WUE* in comparison to other treatments (data not shown). However, there was no significant difference between treatments on Rubisco carboxylation efficiency (*A/C_i* – data not shown), regardless of the tested concentrations.

Different Cd concentrations had a significant effect ($p \leq 0.05$) on most biochemical variables analyzed in this study. Total chlorophyll increased at Cd concentration of 50 μM (Fig. 2E), whereas an increased content in carotenoids was found at Cd concentrations of 25 and 50 μM (Fig. 2F).

A significant increase in the activity of superoxide dismutase (SOD) in the shoot of *C. fissilis* seedlings was detected when Cd concentration in the nutrient solution increased (Tab. 1). SOD activity reached the highest values at Cd concentrations of 50 and 100 μM , i.e., an increase by 37% in shoot at 100 μM . SOD activity increase by 10.83% was observed in the roots at Cd concentration of 100 μM , in comparison to the

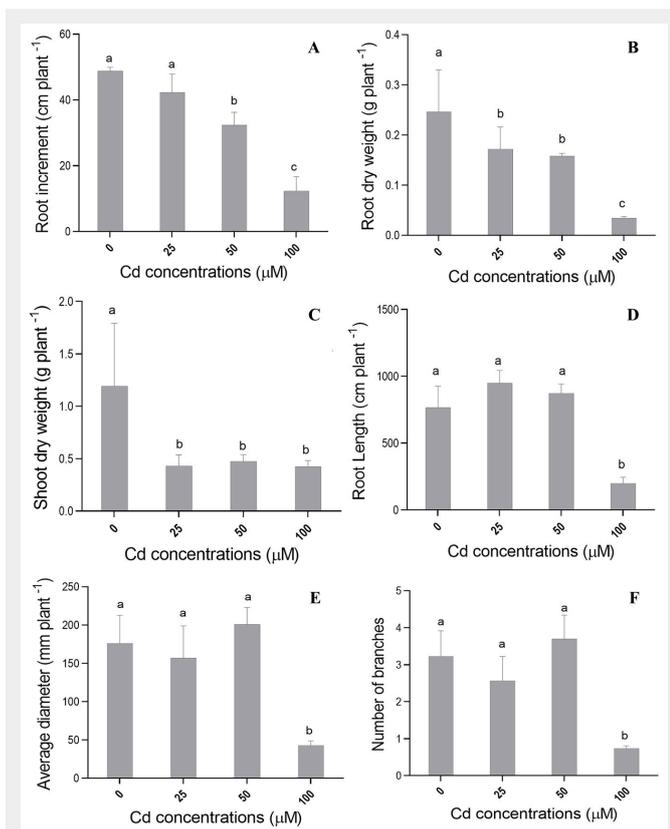


Fig. 1 - Mean values recorded for (A) largest root length increment (ILR), (B) root dry weight (RDW), (C) shoot dry weight (SDW), (D) root length, (E) root diameter and (F) number of branches in *C. fissilis* seedlings subjected to different Cd concentrations. Bars represent the mean ± standard deviation. Different lowercase letters indicate significant ($p \leq 0.05$) differences between treatment means after Tukey test.

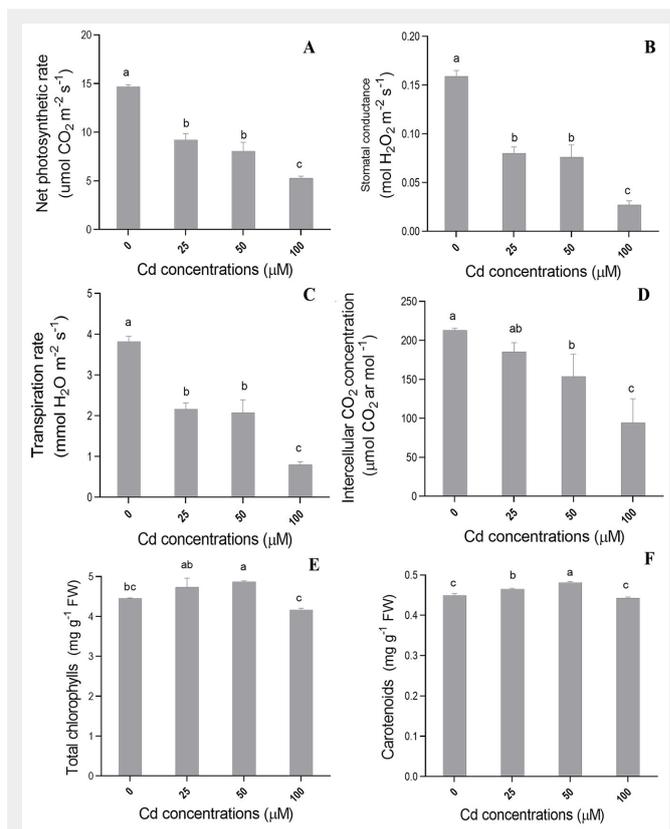


Fig. 2 - Mean values recorded for (A) net photosynthetic rate, (B) stomatal conductance (G_s), (C) transpiration, (D) intercellular CO_2 concentration (C_i), (E) total chlorophyll (total Chl) and (F) carotenoids in *C. fissilis* seedlings subjected to different Cd concentrations. Bars represent the mean ± standard deviation. Different lowercase letters indicate significant ($p \leq 0.05$) differences between treatment means after Tukey test.

control treatment (Tab. 1).

The highest values recorded for guaiacol peroxidase (POD) activity in the shoots were observed in seedlings subjected to Cd concentrations of 25, 50 and 100 μM , which were significantly different from those recorded in control plants (Tab. 1). Contrastingly, a significant reduction in POD activity in roots was observed at the same Cd concentrations (25, 50 and 100 μM – Tab. 1).

A significant increase in hydrogen perox-

ide (H_2O_2) content in the shoot was detected at Cd concentrations of 50 and 100 μM (Fig. 2A). On the other hand, H_2O_2 content in the shoot and roots were similar ($p > 0.05$) at Cd concentrations of 25 and 0 μM . However, there was negative correlation between H_2O_2 content and Cd concentration in the roots (Tab. 1), since H_2O_2 content decreased at Cd concentrations of 50 and 100 μM , in comparison to the control seedlings.

Lipid peroxidation values in the shoot at Cd concentrations of 25, 50 and 100 μM were higher than those recorded in the control plants (Tab. 1). On the other hand, lipid peroxidation in the roots at Cd concentrations of 25 and 100 μM was higher when compared with that in control seedlings (Tab. 1).

Aluminum effects on *Cedrela fissilis*

Analysis of variance revealed significant

Tab. 1 - Mean values recorded for superoxide dismutase (SOD) enzyme activity, guaiacol peroxidase enzyme (POD) activity, hydrogen peroxide content, and membrane lipid peroxidation in the shoot and roots of *C. fissilis* seedlings subjected to different Cd concentrations. Data represent the mean ± standard deviation. Different letters in rows indicate significant ($p \leq 0.05$) differences between treatment means after Tukey test.

Variable	Compartment	Treatment (Cd concentration)			
		0 μM	25 μM	50 μM	100 μM
SOD activity (U mg^{-1} protein)	Shoot	327.2 ± 4.5 ^c	351.6 ± 18 ^{bc}	418.3 ± 23.0 ^b	524.5 ± 6.4 ^a
	Root	338.3 ± 27 ^b	322.7 ± 20.1 ^b	315.7 ± 17.7 ^b	379.3 ± 23.7 ^a
POD activity (U mg^{-1} protein)	Shoot	80.3 ± 10.3 ^c	177.8 ± 5.6 ^b	253.4 ± 7.6 ^a	235.9 ± 10.5 ^a
	Root	1358.6 ± 10.8 ^a	774.7 ± 23.6 ^b	736.4 ± 32.9 ^b	510.3 ± 0.6 ^c
H_2O_2 ($\mu mol g^{-1}$ FW)	Shoot	0.86 ± 0.02 ^b	0.91 ± 0.02 ^b	1.0 ± 0.01 ^a	1.04 ± 0.05 ^a
	Root	0.17 ± 0.02 ^a	0.18 ± 0.01 ^a	0.13 ± 0.01 ^b	0.0 ± 0.01 ^c
MDA (nmol mg^{-1} protein)	Shoot	0.05 ± 0.01 ^c	0.10 ± 0.02 ^a	0.07 ± 0.0 ^b	0.07 ± 0.0 ^b
	Root	0.03 ± 0 ^c	0.08 ± 0.001 ^a	0.05 ± 0.0 ^{bc}	0.05 ± 0.0 ^b

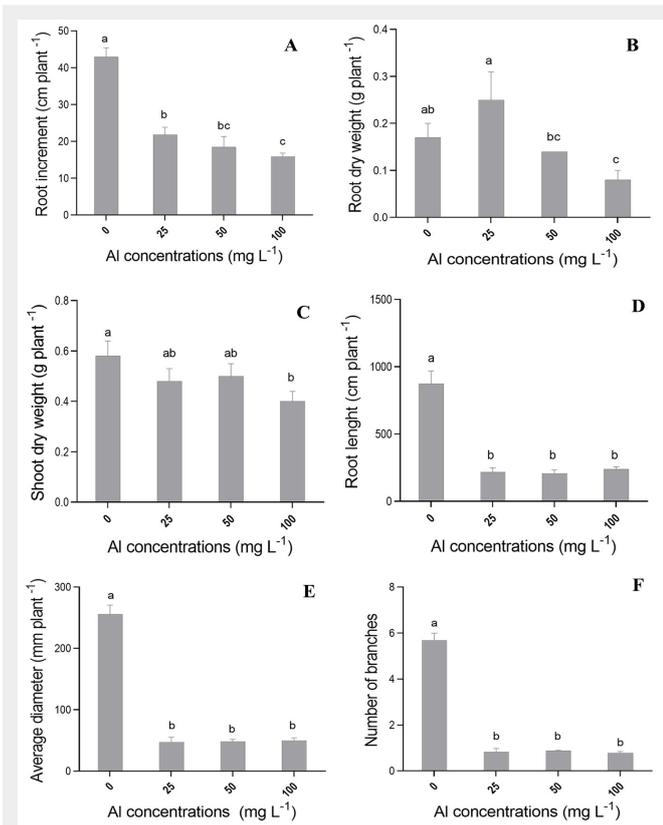


Fig. 3 - Mean values recorded for (A) largest root length increment (ILR), (B) root dry weight (RDW), (C) shoot dry weight (SDW), (D) root length, (E) root diameter, and (F) number of branches in *C. fissilis* seedlings subjected to different Al concentrations. Bars represent the mean \pm standard deviation. Different lowercase letters indicate significant ($p < 0.05$) differences between treatment means after Tukey test.

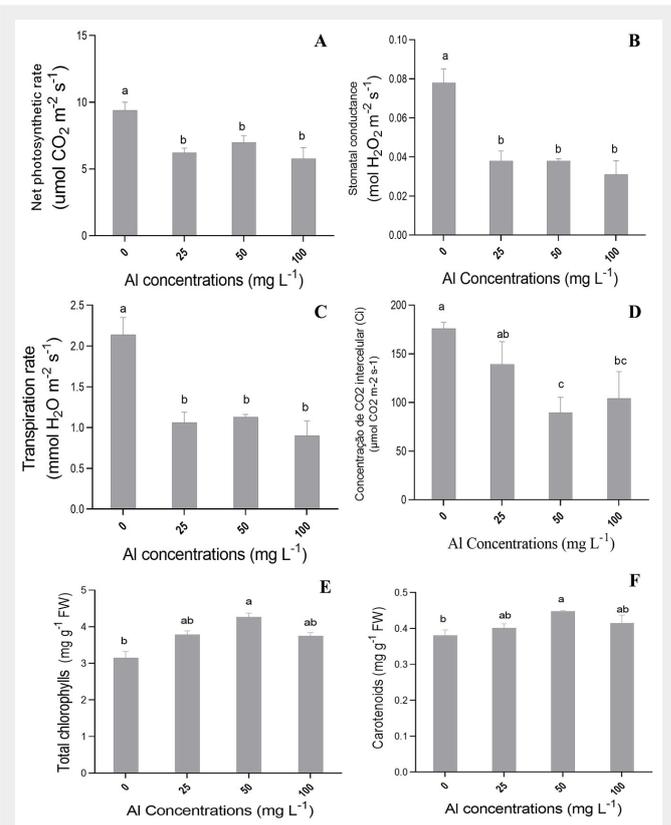


Fig. 4 - Mean values recorded for (A) net photosynthetic rate, (B) stomatal conductance (G_s), (C) transpiration, (D) intercellular CO_2 concentration (C_i), (E) total chlorophyll (total Chl), and (F) carotenoids in *C. fissilis* seedlings subjected to different Al concentrations. Bars represent the mean \pm standard deviation. Different lowercase letters indicate significant ($p < 0.05$) differences between treatment means after Tukey test.

differences ($p < 0.05$) among different Al treatments (different Al concentrations) on most growth-related morphological variables.

The ILR (increment of the largest root) variable showed the lowest mean (15.93 cm) at Al concentration of 100 mg L⁻¹, which was significantly ($p < 0.05$) different from that recorded for control seedlings (42.97 cm – Fig. 3A). Al concentration of 100 mg L⁻¹ reduced ILR by 37.07%, in com-

parison to the control treatment (Fig. 3A). In addition, seedlings grown at Al concentrations of 25 and 50 mg l⁻¹ showed a significant ILR reduction. On the other hand, we found no significant differences in height growth (SI) of *C. fissilis* seedlings, regardless of Al concentrations (data not shown).

Shoot dry weight (SDW – Fig. 3C) and root dry weight (RDW – Fig. 3B) also had the lowest means at the highest Al concentration (100 mg l⁻¹), in comparison to the

control treatment. However, SDW and RDW means at Al concentrations of 25 and 50 mg l⁻¹ did not differ from those recorded for the control treatment (Fig. 3).

The application of different Al concentrations had the most severe effects on seedling root length (Fig. 3D), root diameter (Fig. 3E) and number of branches (Fig. 3F), in comparison to the control treatment. The highest means recorded for the above variables were observed in the control

Tab. 2 - Mean values recorded for superoxide dismutase (SOD) enzyme activity, guaiacol peroxidase enzyme (POD) activity, hydrogen peroxide content, and membrane lipid peroxidation in the shoot and roots of *C. fissilis* seedlings subjected to different Al concentrations. Data represent the mean \pm standard deviation. Different letters in rows indicate significant ($p < 0.05$) differences between treatment means after Tukey test.

Variable	Compartment	Treatment (Al concentration)			
		0 mg l ⁻¹	25 mg l ⁻¹	50 mg l ⁻¹	100 mg l ⁻¹
SOD activity (U mg ⁻¹ protein)	Shoot	365.5 \pm 4.5 c	500.91 \pm 7.2 b	522.1 \pm 6.0 b	1180.5 \pm 9.6 a
	Root	267 \pm 4.8 c	342.56 \pm 2.8 b	388.5 \pm 1.6 b	549.1 \pm 3.1 a
POD activity (U mg ⁻¹ protein)	Shoot	245.7 \pm 9.7 a	192.22 \pm 18 b	148.8 \pm 7.7 c	143.8 \pm 11 c
	Root	1090 \pm 8.3 a	1202 \pm 8.7 a	1207 \pm 12.6 a	1203 \pm 20 a
H ₂ O ₂ (μmol g ⁻¹ FW)	Shoot	0.55 \pm 0.02 c	0.87 \pm 0.01 b	1.10 \pm 0.06 a	0.87 \pm 0.04 b
	Root	0.07 \pm 0.00 c	0.12 \pm 0.01 b	0.15 \pm 0.00 b	0.22 \pm 0.02 a
MDA (nmol mg ⁻¹ protein)	Shoot	0.06 \pm 0.00 bc	0.04 \pm 0.00 c	0.07 \pm 0.00 bc	0.13 \pm 0.00 a
	Root	0.03 \pm 0.00 b	0.02 \pm 0.00 b	0.03 \pm 0.00 b	0.06 \pm 0.00 a

treatment (Fig. 3). Only the highest Al concentration (100 mg l⁻¹ of Al) had negative effect on root volume.

There was significant effect ($p \leq 0.05$) of different Al concentrations on most photosynthetic variables analyzed in the present study. Aluminum concentrations of 25, 50 and 100 mg l⁻¹ negatively affected net CO₂ assimilation rate (*A* – Fig. 4A), stomatal conductance (*G_s* – Fig. 4B), and transpiratory rate (*E* – Fig. 4C) in *C. fissilis* seedlings in comparison to the control treatment. The highest means for these variables were recorded in the control treatment. The lowest mean internal CO₂ concentrations (*C_i*) were recorded at Al concentrations of 50 and 100 mg l⁻¹ (89.858 and 104.200 μmol CO₂ m⁻² s⁻¹, respectively – Fig. 4D). All tested Al concentrations promoted a significant increase in water use efficiency (*WUE* – data not shown) in comparison with the control. On the other hand, there was no significant effect between treatments on Rubisco carboxylation efficiency (*A/C_i* – data not shown).

Significant effects ($p \leq 0.05$) of different Al concentrations on biochemical variables assessed were also observed. Indeed, 50 mg l⁻¹ of Al promoted significant increase in total chlorophyll (Fig. 4E) and carotenoid contents in comparison to the control (Fig. 4F). Superoxide dismutase (SOD) activity in the shoot and roots of seedlings showed a significant increase at all tested Al concentrations (Tab. 2).

Lower guaiacol peroxidase (POD) activity was observed in the shoot of seedlings subjected to Al concentrations of 25, 50 and 100 mg l⁻¹, in comparison to the control (Tab. 2). On the other hand, there was not significant difference in POD activity in the roots, regardless of the Al concentrations (Tab. 2).

The highest hydrogen peroxide (H₂O₂) content was observed in the shoot and in the roots of plants subjected to all tested Al concentrations (Tab. 2).

Lipid peroxidation in the shoot and roots was significantly increased in plants subjected to the highest Al concentration (100 mg l⁻¹ – Tab. 2). On the other hand, lipid peroxidation in the shoot and roots of seedlings subjected to Al concentrations of 25 and 50 mg l⁻¹ did not significantly differ from that of the control treatment (Tab. 2).

Discussion

Cadmium

Growth reduction in the shoot and roots is one of the main Cd toxicity symptoms in plants, leading to a decrease of biomass production in plants belonging to different species (Deng et al. 2014). However, shoot growth (*SI* – data not shown) was lesser affected than root growth in the present study (Fig. 1A) due to Cd addition to the nutrient solution. This could be attributed to the fact that roots are often in direct contact with contaminants (Huang et al. 2017), thus affecting the physiological response

of plants to stress. Moreover, Cd is mostly retained in the roots and only smaller amounts are translocated to the shoots, whereby causing lesser damage to the latter organs (Hassan et al. 2020).

Root increment inhibition (Fig. 1A) induced by Cd was observed at concentrations of 50 and 100 μM, corroborating the results reported by Ullah et al. (2020) in *Cicer arietinum* L. cultivars subjected to high Cd stress (50 μM). This response can be attributed to cell cytoskeleton microtubule depolymerization and to chromosomal aberration formation, which lead to lower mitotic activity of meristematic cells (He et al. 2017).

Changes in root morphology may be part of integrated signal response to metal stressors. In fact, a 1g reduction in the length, diameter and number of root branches (Fig. 1D-F) were observed under the highest Cd stress level (100 μM). These changes in root morphology under Cd stress may stem from changes in root development, such as reduced parenchymal cell and cortical tissue size, which decreased plants' resistance to radial water flows.

Although different Cd concentrations did not affect shoot growth (data not shown), a reduced shoot dry weight was observed (Fig. 1C). This may be due to the reduced stem diameter or the decreased number of leaves, which resulted in lower shoot dry weight production from cedar seedlings (Fig. 1C). In addition, reduced root growth, along with changes in root morphological variables (Fig. 1D-F), have also negatively affected the biomass production of cedar seedling, since smaller roots absorb lesser water and nutrient amounts.

Plants subjected to stress conditions adjust their relative biomass allocation in their organs, in a process known as allocation plasticity. They also change basic processes such as photosynthesis and respiration (Dhir et al. 2011). Photosynthetic ability in plants is mainly regulated by photochemical reactions, which enable energy production, gas exchange, as well as CO₂ fixation and assimilation. However, Cd can damage plants' photosynthetic ability, induce oxidative stress, inhibit stomatal opening and reduce nitrate and iron absorption (Yang et al. 2015).

In this study, significant reductions in CO₂ assimilation rate (*A* – Fig. 2A), stomatal conductance (*G_s* – Fig. 2B), and transpiration rate (*E* – Fig. 2C) was observed as Cd concentrations in nutrient solution increased. The most severe effect of Cd on these variables was recorded at Cd concentration of 100 μM. Decreased net assimilation rate (Fig. 2A) may be associated with limited CO₂ diffusion to the Rubisco carboxylation site, due to decreased stomatal conductance (Fig. 2B). Heavy metal-related stress can lead to decreased stomatal conductance, thus limiting the photosynthetic ability (Huihui et al. 2020).

Cadmium decreases partial CO₂ pressure

in the stomata, which leads to stomatal closure and decreased transpiration. One of the harmful effects of Cd lies on calcium induction in the endoplasmic reticulum and vacuole. This process increases cation levels in the cytosol, which is associated with stomatal closure (Nogueira 2018). Thus, Cd affects stomatal opening and closure, mainly due to increased leaf osmotic potential and to direct Cd action on guard cells. In addition, *G_s* reduction (Fig. 2B) may occur due to root growth inhibition, which limits water absorption and leads to stomatal closure. Moreover, high chemical element concentration leads to metabolic decline, leaf turgidity loss and hydropassive stomatal closure (Sousa 2018). Therefore, the observed reduction in transpiration rates is directly linked to the reduction in assimilation rate and stomatal conductance (Fig. 2).

The highest water use efficiency (*WUE*) values (data not shown) were found at Cd concentrations of 100 and 50 μM, as the stress caused by Cd forced plants to close their stomata. This decreased the stomatal conduction (Fig. 2B) and led to reduced transpiration rate (Fig. 2C), which in turn resulted in higher *WUE*.

The photosynthetic performance of seedlings is also affected by the pigments involved, such as total chlorophyll and carotenoids (Taiz et al. 2017). The observed increase in total chlorophyll and carotenoid contents in plants exposed to Cd concentration of 50 μM (Fig. 2E-F) was not sufficient to counterbalance the reduction in photosynthetic performances due to Cd stress. This response may be associated with the fact that Cd stress impairs chloroplast structure and affects their stable binding to proteins, thus damaging the photosynthetic apparatus. In fact, Cd ions may change the activity center of enzymes and replace Mg²⁺ in the chlorophyll molecule (Zhang et al. 2019), decreasing the light harvesting ability of chlorophyll and affecting the activity of enzymes associated with chlorophyll synthesis. This process has negative effects on photosynthetic variables, leading to the reduction of seedling dry weight (Zhang et al. 2019). These inhibitory effects may also be associated with indirect Cd interaction with micronutrients, which act as cofactors for enzymes, pigments and structural components of the photosynthetic apparatus.

Carotenoid contents in seedlings subjected to 50 μM of Cd have increased to protect the photosynthetic apparatus and avoid singlet oxygen formation (reactive oxygen species – ROS). This occurs because carotenoids are non-enzymatic antioxidants acting as photoprotective pigments in the photosynthetic system by suppressing ROS and free radicals' formation (Reyes-Díaz et al. 2009). Increased carotenoid content is the result of a natural strategic defense mechanism to combat the toxic effect of oxidative stress generated under Cd stress.

Plants are equipped with a natural defense system made by enzymatic and non-enzymatic antioxidants, which act as a protection from the oxidative damage induced by different environmental stress types (Hassan et al. 2020). Indeed, ROS can be produced through different routes, such as the imbalance in electron transport chains of chloroplasts and mitochondria. Cadmium can lead to ROS generation by inducing chloroplast disturbance (Yang et al. 2015). Antioxidant enzymes such as superoxide dismutase (SOD) and guaiacol peroxidase (POD), play a key role in maintaining homeostasis in plant cells (Zhang et al. 2019) by maintaining cellular redox status and prevent damage caused by ROS accumulation. In particular, SOD plays crucial role in removing the radical $O_2^{\cdot-}$, whose decomposition is always followed by H_2O_2 production which acts as oxidizer and reducer (Weifeng et al. 2018). The results of this study showed that SOD activity in the shoot gradually increased as Cd concentrations in the nutrient solution also increased (Tab. 1), as a consequence of the activation of the existing enzyme stock by the increased free radical production (Hassan et al. 2020). The increased SOD activity in the shoot (Tab. 1) indicated that free radicals resulting from stress were neutralized via the formation of H_2O_2 . However, high H_2O_2 accumulation is extremely harmful to the cellular metabolism, and POD converts H_2O_2 into water and oxygen. POD plays an essential role in providing tolerance to plants exposed to unfavorable conditions (Li et al. 2014). In this study we observed an increased POD activity in the shoot, which may due to the increase in H_2O_2 released by SOD activity as a consequence of Cd stress (Tab. 1). On the other hand, POD activity inhibition was detected in the roots of seedlings subjected to all tested Cd concentrations (Tab. 1). This may be associated with a decreased enzyme synthesis or with changes in the assembly of its subunits (Xin et al. 2019). Excessive metal accumulation can inhibit enzymes by binding to catalytic active groups or by causing protein denaturation. Thus, POD activity often decreases when stress exceeds plants' regulatory capacity (Zhang et al. 2020). The decrease in POD activity suggests a likely delay in ROS removal, which implies an increase in lipid peroxidation which contributes to seedling growth inhibition.

Nonetheless, the increased POD activity in the shoot, mainly at Cd concentrations of 50 and 100 μM , was unsuccessful in counteracting the H_2O_2 accumulation (Tab. 1), as H_2O_2 content in the shoot significantly increased as Cd concentrations increased in the nutrient solution (Huang et al. 2017). According to Weifeng et al. (2018), the high H_2O_2 rates observed under Cd stress were likely accountable for lipid peroxidation, as indicated by the excessive malondialdehyde (MDA) accumulation. MDA is an oxidized product of membrane lipids; it accumulates in plants exposed to oxidative

stress. Therefore, MDA concentration in plants is often seen as an indicator of lipid peroxidation, as well as of the level of oxidative stress (Huang et al. 2017). In this study, the highest MDA content observed in the shoot was found at Cd concentrations of 25, 50 and 100 μM (Tab. 1), while the highest MDA content in the seedling roots was recorded at Cd concentrations of 25 and 100 μM . Thus, our results indicate that different Cd concentrations can change the original balance of plasma membrane permeability, as well as increase ROS production and induce antioxidant protein expression as adaptive response to neutralize ROS excess and minimize damage.

Aluminum

Root growth in this study was significantly inhibited by aluminum in the nutrient solution, especially at concentrations of 50 and 100 $mg\ l^{-1}$ (Fig. 3A). On the other hand, shoot growth was lesser affected by Al, regardless of the tested concentrations (data not shown). Indeed, roots are one of the organs mostly sensitive to Al, resulting in the inhibition of main axis elongation, as well as in the limited development of lateral roots, which leads to short and poorly-developed root system (Bose et al. 2015, Zhao et al. 2017).

In addition, different Al concentrations significantly reduced the length, diameter and number of branches in cedar seedling roots (Fig. 3D-F). This is likely due to the strong binding of Al to negatively charged carboxylic groups on roots' cortical and epidermal cell walls, thus changing ions binding and distribution in the apoplast and directly affecting root growth (Dornelles et al. 2016). Also, changes in morphological characteristics of roots can be caused by apoplastic lesions and interactions in root plasma membrane (Zhou et al. 2017, Zhao et al. 2017), which leads to deficient mineral nutrient and water acquisition (Guo et al. 2017).

Root growth inhibition and changes in root morphological variables negatively affected root and shoot biomass production in plants exposed to 100 $mg\ l^{-1}$ of Al (Fig. 3). The aluminum translocation to the shoot may have negatively influenced the formation and growth of these organs, which implied reduced photosynthetic rate (Fig. 4A) and lower biomass production (Guo et al. 2018). Moreover, Al stress may be responsible for determining structural changes in plant tissues (Mota et al. 2020) and impairing the functioning of photosynthetic apparatus, as suggested by the low net photosynthetic rates (A – Fig. 4A), associated with the decrease in stomatal conductance (G_s – Fig. 4B) and internal CO_2 concentration (C_i – Fig. 4D) observed in seedlings under Al stress.

Aluminum may change stomatal behavior and directly influence stomatal closure (Cárcamo et al. 2019); consequently, it inhibits plant transpiration (E – Fig. 4C) and

increases water use efficiency, especially at the highest Al concentration (100 $mg\ l^{-1}$). A possible explanation is that higher Al concentrations in the nutrient solution enhances cuticle and epicuticular wax development and leads to higher WUE (Tabaldi et al. 2011). In addition, seedlings exposed to Al showed reduced stomatal conductance, which may also result in higher WUE.

Photosynthetic pigments are extremely sensitive to stress caused by toxic metals and can be used as reliable markers of stress caused by these metals (Anjum et al. 2017). Aluminum concentration of 50 $mg\ l^{-1}$ led to significant increase in total chlorophyll content in comparison to the control (Fig. 4E). However, the increase in photosynthetic pigment contents did not hinder a decrease in photosynthesis rates and, consequently, in dry biomass production (Fig. 3B-C). Moreover, high concentrations of Al have been reported to damage thylakoid membranes and replace compounds in the chlorophyll metabolic pathway (Marques et al. 2018). Aluminum can also affect chlorophyll biosynthesis by inhibiting δ -aminolavulinic acid dehydratase activity, which accounts for chlorophyll molecule and cytochrome formation (Cunha Neto et al. 2020). Therefore, higher concentrations of this ion can change chlorophyll and carotenoid composition, which can limit plant metabolic potential (Mendes et al. 2018).

Al concentration of 50 $mg\ l^{-1}$ has also promoted significant increase in carotenoid content in *C. fissilis* seedlings compared to control (Fig. 4F). Carotenoids play an essential role in protecting the photosynthetic apparatus against the harmful effects of light and oxygen by dissipating the excess of light in the form of heat in antenna pigment complexes (Reyes-Díaz et al. 2009). However, whenever carotenoids fail to dissipate ROS, antioxidant enzymes such as SOD and POD dismutate ROS and maintain the cell homeostasis. In this study, we observed a gradual SOD activity increase in the shoot and roots as Al concentrations increased (Tab. 2). This suggests that plants' ability to detoxify ROS was positively regulated (Zhao et al. 2017). However, long-term Al stress can overcome the antioxidant capability of plant tissues. This results in ROS overproduction, which can damage biological membranes, have negative effects on photosynthetic activity, reduce enzyme activity and, finally, lead to programmed cell death (Zhang et al. 2019).

The presence of aluminum at any concentration in the nutrient solution has significantly reduced POD activity in the shoot of cedar seedlings, but not in the roots (Tab. 2). Conversely, the increased SOD activity in seedling tissues indicated that free radicals were neutralized and detoxified as H_2O_2 (Tab. 2). This may explain the observed accumulation of H_2O_2 , which is released as a product of SOD activity but not properly removed by POD activity in the

shoot.

H₂O₂ also plays an important role in signal transduction during plant abiotic stress, since it acts as biomarker of tissue damage induced by ROS overproduction (Yusuf et al. 2016). However, excessive H₂O₂ accumulation can be extremely harmful, leading to lipid peroxidation and increased plasma membrane permeability. Indeed, Al can bind to phospholipids and/or change the fatty acid composition in the plasma membrane, reducing its fluidity and increasing its permeability, and ultimately leading to lipid peroxidation (Bose et al. 2015).

Lipid peroxidation in the shoot and roots has significantly increased at the highest Al concentration (100 mg l⁻¹ – Tab. 2). On the other hand, MDA contents in the roots of seedlings subjected to Al concentrations of 25 and 50 mg l⁻¹ did not differ from that of control plants (Tab. 2). The higher MDA content observed at the highest Al concentration (100 mg l⁻¹) can be the direct effect of Al toxicity, as well as indicate oxidative stress in cedar seedlings, which can cause irreversible damage to plant tissue development and function in the long run.

Our results demonstrate that *Cedrela fissilis* can tolerate moderate Al concentrations in the growth medium. Aluminum tolerance mechanisms involve the exclusion via root organic acid exudation, changes in carbohydrate components of cell walls capable of binding Al in roots, as well as the uptake and subsequent sequestration either in the root or leaf cell vacuoles, where it is detoxified and sequestered (Kochian et al. 2015). Besides, symplasmic tolerance to internal Al is also achieved by complexing Al with several organic compounds such as citrate, malate, and oxalate, as well as by secreting or sequestering the resulting complexes in vacuoles through several membrane-localized transport proteins, or by activating enzymatic and non-enzymatic antioxidants.

Conclusion

The presence of cadmium in the nutrient solution has negatively affected morphophysiological and biochemical variables in *Cedrela fissilis* seedlings, hindering their growth. Therefore, the species is sensitive to the exposure to cadmium. On the other hand, it has tolerated high Al concentrations (up to 50 mg l⁻¹), which may indicate its tolerance to this metal.

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Authors' contributions

CCK, MVMA, GSWO, DB, MB and LAT con-

ceived and designed the experiments and analyzed the data; CCK, MVMA and GSWO carried out the physiological and photosynthetic analyses; CCK, MVMA, GSWO, DB, MB and LAT carried out the biochemical analyses; CCK, MVMA and GSWO wrote the manuscript, and LAT revised it.

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