Luehea divaricata Martius et Zuccarini Is a Sensitive Species to Aluminum, Not Presenting Phytoremediation Potential

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Abstract
The aim of this work was to evaluate the effect of different concentrations of aluminum (Al) on physiological and biochemical parameters of Luehea divaricata seedlings grown in a hydroponic system under greenhouse conditions to verify the possible tolerance to Al or phytoremediation potential of this species. Seeds of Luehea divaricata were placed to germinate in commercial substrate and after 30 days the seedlings were transferred to a hydroponic system with a complete nutrient solution, pH at 4.5±0.1, with daily adjustment. After 20 days of acclimatization, homogenous plants were selected and transferred to a new nutrient solution (without phosphorus (P) and pH at 4.5±0.1) with different concentrations of Al: 0, 25, 50, 75 and 100 mg L⁻¹, each treatment being composed of 10 replicates of one plant each. The experiment was conducted in a completely randomized design. After seven days of exposure to the treatments, plants were collected for physiological and biochemical analyzes.

Aluminum promoted a significant reduction in fresh and dry weight of roots, stems and leaves; in plant height; leaf number; leaf area; and pigment content. On the other hand, Al promoted an increase in lipid peroxidation and guaiacol peroxidase enzyme activity. Therefore, the presence of Al in the growth medium, for the studied conditions, altered significantly both physiological and biochemical parameters in Luehea divaricata seedlings, presenting a sensitive behavior to this element. Due to these characteristics, the studied species does not show phytoremediation potential.

Keywords: acidic soils, antioxidant enzymes, açoita-cavalo, lipid peroxidation

1. Introduction
Acidic soils, which occupy about 30% of ice-free areas in the world (Brunner & Sperisen, 2013), directly influence on plant development. A large proportion of these soils is covered by forests (Fao & Iiasa, 2007). These soils are present in several parts of the world, including in Brazil, the state of Rio Grande do Sul being one where they appear more significantly (Abreu et al., 2003). This acidity promotes a decrease in plant growth due to aluminum (Al) toxicity, as well as low base saturation, and deficiency of phosphorus, calcium, magnesium and molybdenum (Poschenrieder et al., 2008).

Aluminum is the third most abundant element in Earth’s crust, after oxygen and silicon. In acidic soils, where pH is less than or equal to 5.5, Al is able to solubilize itself and become possibly toxic to some plants (Singh et al., 2017). In addition, it is noteworthy that the effect of this toxicity on the root system is that it inhibits its growth, fixes phosphorus in forms which are less available in the soil and/or roots, and reduces root respiration (Gupta et al., 2013; Schmitt et al., 2016). Furthermore, it has the potential to interpose in enzymatic reactions, to promote oxidative stress, as well as to interfere in the absorption, transportation and utilization of other nutrients, inducing nutritional deficiency in the plant (Tabaldi et al., 2009; Dorneles et al., 2016; Singh et al., 2017).

Aluminum is not an essential element to plant growth, since it does not meet any essentiality criteria. However, under special conditions, from the element in the culture medium and depending on the species, Al it can induce an increase in plant growth and even produce other positive effects on growth. Moreover, physiological,
molecular and biochemical mechanisms of Al tolerance in plants have been proposed to try to explain the tolerance presented by some plants to the excess of this metal (Foy et al., 1978; Sousa et al., 2016).

Unlike annual crops (Tabaldi et al., 2009; Carlin et al., 2012; Bojórquez-Quintal et al., 2014), little attention has been given to forest species, especially the native ones, in relation to their behavior in relation to Al (Alves et al., 2001; Brunner & Sperisen, 2013). In synthesis, the effect of this element varies according to the studied species or even to the genotype within the same species. Forest species that are able to grow and complete their life cycle on acid soils rich in Al can be grown in these soils, which are often unsuitable for growing food or medicinal plants. Therefore, the selection of species of trees tolerant to Al toxicity may be an option for plant restoration in acidic soils and metal contaminated areas, besides being used as phytoremediation species.

The *Luehea divaricata* (Martius et Zuccarini) species, popularly known as *açoita-cavalo* in Brazil, is native to the state of Rio Grande do Sul (RS), belonging to the Malvaceae family (Carvalho, 2008). It is of great importance, especially in the Mixed Ombrophilous Forest of this state. Due to its easy adaptability, it is a target species for the recovery of degraded areas and reforestation. Its wood is widely used for construction, furniture and energy production, as well as medicinal uses (IBGE, 2012). Its vast use, along with its natural distribution in the soils of RS, suggests that the species could present a certain tolerance to Al and, therefore, have phytoremediation potential.

The previous selection of seedlings exposed to toxic concentrations of Al in a hydroponic system may provide adequate information about the survival capacity of the seedlings after their transplantation to the field. Thus, the objective of this study was to evaluate the effect of different concentrations of Al on physiological and biochemical parameters of *Luehea divaricata* seedlings grown in a hydroponic system to verify the possible tolerance or phytoremediation potential of this species.

2. Method

The experiments were carried out in the Plant Biotechnology Laboratory and in greenhouses belonging to the Biology Department of the Federal University of Santa Maria, RS. Seeds of *Luehea divaricata* were germinated in plastic trays with commercial substrate Plantmax and irrigated daily with a complete nutrient solution, keeping pH at 4.5±0.1, with daily adjustment. The nutrient solution had the following composition (in μM): 6090.5 of N; 974.3 of Mg; 4986.76 of Cl; 2679.2 of K; 2436.2 of Ca; 359.9 of S; 243,592 of P; 0.47 of Cu; 2.00 of Mn; 1.99 of Zn; 0.17 of Ni; 24.97 of B; 0.52 of Mo; 47.99 of Fe (FeSO₄/Na-EDTA).

After 30 days, the initial growth period of the seedlings, 75 homogenous plants with the height of about 10cm were transferred to trays in a hydroponic system, where they were fixed by means of sponges in polystyrene plates, for acclimatization. The same nutrient solution was used, with pH at 4.5±0.1, with daily adjustment and constant aeration system. The volume of the trays was replaced daily and the solution changed every 3 days.

At 20 days of acclimatization, new homogeneous plants were selected, which were transferred to the final hydroponic system, in 1 L pots, with one plant each. In a new nutrient solution (without P and pH at 4.5±0.1), five treatments were applied: 0, 25, 50, 75 and 100 mg L⁻¹ of Al, each consisting of 10 replicates. The experiment was conducted in a completely randomized design, the nutrient solution was replaced every two days, with daily replenishment and pH level adjusted at each replacement. After seven days of exposure to the treatments, the plants were collected for physiological and biochemical analyzes.

2.1 Physiological Parameters

For the physiological evaluations, four plants per treatment were collected, having their roots washed in distilled water, and then divided into leaves, stem and root system. It was determined the number of leaves, aerial part length (using a ruler graduated in millimeters), leaf area (with leaf area integrator), and fresh and dry weight of leaves, stems and roots. The parts were collected separately and immediately weighed in a digital scale to determine fresh weight. Then they were dried in an oven at 65 °C with forced ventilation until constant weight, and the appropriate dry weights were measured.

2.2 Biochemical Parameters

For the biochemical analyzes, six plants per treatment were collected and separated into leaves and roots. The material was washed with distilled water, immediately frozen in liquid nitrogen and then stored in a freezer at -80 °C in order to maintain its characteristics. Chlorophylls a and b and carotenoids were extracted according to the method of Hiscox and Israelstan (1979) and estimated using the equation of Lichtenthaler (1987).

The lipid peroxidation was determined in the plants root system, according to the method of El-Moshaty et al. (1993). For the preparation of extracts for the determination of antioxidant enzymes activity, it was initially used
the protocol of Zhu et al. (2004) to determine the enzyme activity, and later the protocol of Bradford (1976) to
determine the concentration of proteins. The guaiacol peroxidase (POD) activity was determined according to
Zeraik et al. (2008) and the superoxide dismutase (SOD) activity was determined according to the
spectrophotometric method, described by Giannopolitis and Ries (1977).

2.3 Tolerance Index
The Al tolerance index was calculated according to Trannin et al. (2001), through Equation 1:

\[ TI = \frac{TDW_{Al}}{TDW_{C}} \times 100 \]  

where, \( TI \) = tolerance index; \( TDW_{Al} \) = total dry weight in each concentration of aluminum; \( TDW_{C} \) = total dry
weight in control.

2.4 Statistical Analysis
Data normality and homogeneity of variances were tested through the Shapiro-Wilk and Bartlett tests,
respectively, both using the Action-Excel Software. The data were submitted to variance analysis and tested by
the 5% probability of error regression models, through the SISVAR Software (Ferreira, 2011). The graphics
program used was SigmaPlot 12.5.

3. Results
3.1 Analysis of Fresh and Dry Weight
Based on tests of normality and homogeneity, the data showed to be normal and the variances are homogeneous.
Tables 1 and 2 show the results of the analysis of variance for the physiological and biochemical parameters,
demonstrating a significant effect at 5% of error probability in all study analyzes.

Table 1. Results of the analysis of variance for the physiological parameters in Luehea divaricata Martius et
Zuccarini seedlings submitted to different aluminum concentrations

<table>
<thead>
<tr>
<th>Variables</th>
<th>RFW</th>
<th>LFW</th>
<th>SFW</th>
<th>RDW</th>
<th>LDW</th>
<th>SDW</th>
<th>LN</th>
<th>APL</th>
<th>LA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VF</strong></td>
<td>GL</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
</tr>
<tr>
<td>Concentration</td>
<td>4</td>
<td>0.0000*</td>
<td>0.0000*</td>
<td>0.0000*</td>
<td>0.0000*</td>
<td>0.0000*</td>
<td>0.0021*</td>
<td>0.0000*</td>
<td>0.0000*</td>
</tr>
<tr>
<td>Error</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total corrected</td>
<td>29</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DF (%)</td>
<td>-</td>
<td>30.74</td>
<td>32.55</td>
<td>25.58</td>
<td>13.56</td>
<td>26.9</td>
<td>0.18</td>
<td>20.59</td>
<td>11.36</td>
</tr>
<tr>
<td>Average overall</td>
<td>-</td>
<td>1.66</td>
<td>1.35</td>
<td>0.84</td>
<td>0.14</td>
<td>0.43</td>
<td>26.04</td>
<td>9.33</td>
<td>13.88</td>
</tr>
</tbody>
</table>

Note. VF: Variation factor; CV: Coefficient of variation; DF: Degrees of freedom; RFW: Fresh weight of roots;
LFW: Fresh weight of leaves; SFW: Fresh weight of stem; RDW: Dry weight of roots; LDW: Dry weight of leaves;
SDW: Dry weight of stem; LN: Leaves number; APL: aerial part length; LA: leaf area. * Significant at 5%
probability of error (Pr < 0.05).

Table 2. Results of the analysis of variance for the biochemical parameters in Luehea divaricata Martius et
Zuccarini seedlings submitted to different aluminum concentrations

<table>
<thead>
<tr>
<th>Variables</th>
<th>Chlorophyll a</th>
<th>Chlorophyll b</th>
<th>Totals Chlorophyll</th>
<th>Carotenoids</th>
<th>MDA</th>
<th>POD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VF</strong></td>
<td>GL</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
<td>Pr &gt; F</td>
</tr>
<tr>
<td>Concentration</td>
<td>4</td>
<td>0.0000*</td>
<td>0.0000*</td>
<td>0.0000*</td>
<td>0.0000*</td>
<td>0.0000*</td>
</tr>
<tr>
<td>Error</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total corrected</td>
<td>29</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DF (%)</td>
<td>-</td>
<td>12.32</td>
<td>10.34</td>
<td>11.45</td>
<td>13.73</td>
<td>14.87</td>
</tr>
<tr>
<td>Average overall</td>
<td>-</td>
<td>1.57</td>
<td>0.51</td>
<td>3.41</td>
<td>0.44</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Note. VF: Variation factor; CV: Coefficient of variation; DF: Degrees of freedom; MDA: malondialdehyde; POD:
guaiacol peroxidase. * Significant at 5% probability of error (Pr < 0.05).
Figure 1 (A and B) shows the regression equations among averages observed for fresh and dry weight, both for stem, leaves and root system. The best adjusted regression to the model for all biomass analyzes was the linear regression.

The presence of aluminum (Al) in the growth medium promoted a significant reduction in both fresh and dry weight of roots, stems and leaves, presenting the lowest values in the highest concentration of Al (100 mg L⁻¹), in all analyzed variables. According to Furtini Neto et al. (1999), Al is generally the limiting factor for seedlings development and growth of some forest species, such as Senna multijuga (Cássia verrugosa/November shower), Schizolobium stans (Ipê mirim), Anadenanthera falcata (Angico do cerrado) and Cedrela fissilis (Cedro), corroborating with the obtained results in this study.

![Graph](image1.png)

Figure 1. Fresh weight of roots (RFW), leaves (LFW) and stem (SFW) (A) and dry weight of roots (RDW), leaves (LDW) and stem (SDW) (B) in *Luehea divaricata* Martius et Zuccarini seedlings submitted to different aluminum concentrations

According to Silva et al. (2004), different species of the *Eucalyptus* genus showed to be sensitive to Al toxicity, presenting a decrease in root growth. This relation was portrayed in 15 other different forest species by Vale (1996). Since Al is a potentially reactive element with biological ligands (Singh et al., 2017), studies have suggested that inhibition of root growth caused by Al could be a consequence of interactions of the element with several different sites within the cell wall, plasma membrane and protoplasm (Tabaldi et al., 2009; Sun et al., 2016), once the solubilized Al in the solution first encounters the roots. Kopittke et al. (2015) reported that the primary Al lesion is apoplastic, considering that Al, when in contact with the root system, immediately binds to outer cell walls of the root surface.

Aluminum exposure induces cell cycle deregulation, reduction of mitotic and interphase activity, leading to inhibition in cellular elongation, and possibly, as a consequence, immediate inhibition of root growth (Kochian et al., 2004; Rossiello & Netto, 2006; Panda et al., 2009; Zelinova et al., 2011; Silva, 2012; Kopittke et al., 2015).

Because of the damages to the root system, the plant presents difficulties in the absorption of essential elements, consequently compromising its aerial part. Alves et al. (2001), when studying forest species, showed that the initial effect of Al toxicity was inhibition in root growth and development, and later progressive reduction in the shoot growth, resulting in lower root and shoot dry weight production.

Although the physiological effects of *Luehea divaricata* plants are also general symptoms of nutritional deficiency, it is important to emphasize that the hydroponic system allows to develop the best growth conditions, isolating the variables to be considered. Thus, it is possible to consider that the symptoms presented by the plants were not caused by a nutrient imbalance, but by the toxicity of the isolated element, Al.

### 3.2 Tolerance Index for Total Dry Weight

The tolerance index for total dry weight of *Luehea divaricata* seedlings to Al toxicity generally showed a decreasing behavior with increasing element concentration, mainly from the concentration of 50 mg L⁻¹ of Al, where the tolerance index was approximately 40% (Table 3). The lower tolerance index at the intermediate dose may be related to the tolerance mechanisms of the plant. That is, a plant when exposed to Al, suffered a stress of
the element, which led to a decrease in tolerance index. However, from the concentration of 50 mg/L, with the increase in stress caused by Al, it may also have increased the capacity of the plant to investigate defense mechanisms, a factor that resulted in the stability of the tolerance index in the highest concentrations studied.

Although the growth of the species occurs naturally in acid soils, this behavior suggests that it presents sensitivity to the element, being recommended its planting in places with low Al concentrations. Due to its low tolerance, the species has potential to be used as a signal for contaminated areas. However, Al concentrations employed in this study may have been higher than that found in acid soils. In addition, the use of the hydroponic system and daily adjustment of pH level, maintaining the constant availability of all ions to the plant, is different from what occurs in soils, factors that may have been crucial for such results.

Table 3. Tolerance index for total dry weight of *Luehea divaricata* Martius and Zuccarini seedlings submitted to different aluminum concentrations

<table>
<thead>
<tr>
<th>Aluminum concentrations (mg/L)</th>
<th>Tolerance index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.00</td>
</tr>
<tr>
<td>25</td>
<td>68.46</td>
</tr>
<tr>
<td>50</td>
<td>38.46</td>
</tr>
<tr>
<td>75</td>
<td>40.77</td>
</tr>
<tr>
<td>100</td>
<td>40.00</td>
</tr>
</tbody>
</table>

3.3 Analysis of Leaves Number, Aerial Part Length and Leaf Area

The regression equation among the observed means for leaf number, aerial part length and leaf area are presented in Figure 2, where the quadratic function was the most adjusted to the model, as for plants height the linear function was the most adjusted to the model. The Al presence in growth medium reduced all parameters evaluated in *L. divaricata* seedlings. In an analogous way, in an experiment carried out by Alves et al. (2001), the presence of Al drastically reduced the variables of the shoot of *Senna multijuga* and *Handroanthus stans* plants.

The Al, damaging the root system of the plants, indirectly affects their absorption, translocation and transportation of nutrients to the aerial part, which can result in nutritional deficiency symptoms (phosphorus, potassium, calcium, magnesium and molybdenum) and consequent reduction of leaf area and leaf number (Poschenrieder et al., 2008). The absorption of solar radiation in these plants may be impaired, causing lower photosynthetic rates and, with this, lower accumulation of biomass, as may be observed in Figure 1. The reduction of plant growth may be due to a decrease in photosynthetic activity, which in turn may be related to both stomatal and non-stomatal factors (Konrad et al., 2005).
3.4 Analysis of Pigment Content

Regarding the biochemical parameters, Al in the nutrient solution promoted a significant reduction both in chlorophyll a, b and total content, as well as in the carotenoid content of the plants. The linear regression was the best adjusted to the model for all pigment analyzes (Figure 3), thus presenting the lowest pigment content in the highest Al concentration in the solution (100 mg L⁻¹). The results of this study were similar to those reported in literature (Kuo & Kao, 2003; Guo et al., 2007; Carlin et al., 2012).

Figure 3. Chlorophyll a, b, total and carotenoids contents of Luehea divaricata Martius et Zuccarini seedlings submitted to different aluminum concentrations
Metals which are toxic to plants, such as Al, for generating oxidative stress, are capable of degrading aminolevulinic acid, an important enzyme component involving chlorophyll biosynthesis, thus affecting the plant photosynthetic route and consequently reducing chlorophyll content (Vajpayee et al., 2000). Similarly, Al induces carotenoid degradation in plants (Panda & Chudhury, 2005).

The decrease of chlorophyll content may still be associated with lower plant capacity to absorb different nitrogen forms (Oliveira et al., 2017), interfering in the synthesis of photosynthetic pigments. In addition, Al toxicity impairs the absorption and translocation of magnesium, calcium and potassium, by competing for sites on cell wall and especially on the plasma membrane (Ma et al., 2014). As a consequence, the direct relationship of these elements with chlorophyll molecules may lead to a decrease in the pigment content of plants (Milivojevic & Stojanovic, 2003).

In some species, Al toxicity is also related to decreased stomatal conductance and biochemical CO₂ fixation reactions. This is because any environmental or biotic stress may cause changes or injuries in the formation or functional status of the thylakoid membranes of the chloroplasts, affecting electron transportation and consequently interfering directly on the CO₂ assimilation rate (Peixoto et al., 2002), which can also translate into photosynthetic alteration (Konrad et al., 2005).

3.5 Analysis of Lipid Peroxidation

In relation to lipid peroxidation (TBARS) in the root system of L. divaricata plants, the linear regression was kept as the best adjusted to the model, increasing the concentration of malondialdehyde (MDA, one of the products of lipid peroxidation) with the increase in concentrations of Al (Figure 4). Aluminum is not a redox metal, but acts as a catalyst in the generation of reactive oxygen species (ROS) that cause oxidative stress in plants (Gupta et al., 2013). The increase in the production of ROS, such as superoxide radical (O₂⁻), hydroxyl radical (OH) and hydrogen peroxide (H₂O₂), causing oxidative stress in cells, is a common consequence when plants are exposed to various biotic and abiotic stresses, including the Al toxicity (Sharma & Dubey, 2007; Pandey et al., 2013).

![Figure 4. Lipid peroxidation in root system of Luehea divaricata Martius et Zuccarini seedlings submitted to different aluminum concentrations](image)

The oxidative stress corresponds to a state where the pro-oxidant cellular mechanisms overcome antioxidants, obtaining the MDA as one of lipid peroxidation products. The linear increase of the MDA concentration in relation to Al concentrations shows that Luehea divaricata seedlings went through a period of stress caused by the toxicity of the element present in the nutrient solution. A similar effect of lipid peroxidation and Al toxicity was also observed by Yamamoto et al. (2001) in peas; Kuo and Kao (2003) in rice; Meriga et al. (2004) also in rice; Jin et al. (2011) in Festuca arundinacea; and Couto et al. (2012) in sunflower. ROS production may also contribute significantly to Al-induced inhibition of root elongation (Tabaldi et al., 2009), which is why it was observed the previously described reduction in fresh and dry weight of roots.

3.6 Analysis of Enzyme Guaiacol Peroxidase in Root System

To combat ROS, plants use a non-enzymatic antioxidant system, along with enzymatic antioxidants such as Guaiacol peroxidase (POD) and superoxide dismutase (SOD) (Silva et al., 2000; Tabaldi et al., 2009). Figure 5
shows the regression equation for POD activity. The most adjusted regression to the model was the linear one, obtaining higher enzyme activity in the highest Al concentration in the nutrient solution. On the other hand, the SOD activity was not significantly influenced by different Al concentrations (data not shown).

Figure 5. Enzyme guaiacol peroxidase (POD) in root system of Luehea divaricata Martius et Zuccarini seedlings submitted to different aluminum concentrations

The increase of the antioxidant activity has the purpose of alleviating the ROS lesion and helping to prevent membrane lipid peroxidation (Jin et al., 2011). The increase of POD enzyme activity due to an increase of Al concentrations occurred similarly to the increase of membrane lipid peroxidation, corroborating the attempt of the plants to reduce the oxidative stress that was shown in the presence of this element. However, the increase in POD activity was not sufficient to avoid oxidative stress, since there was a significant increase in lipid peroxidation in the plants exposed to Al. Similar studies were evidenced by Kuo and Kao (2003) in rice; Šimonovičová et al. (2004) in barley; and Guo et al. (2007) also in barley.

4. Conclusion

Although the species occurs naturally in acidic soils, the presence of Al in the growth medium for the conditions studied significantly altered both biochemical and physiological parameters in Luehea divaricata seedlings, showing a sensitive behavior of this species. Due to these characteristics, the species studied does not present phytoremediation potential and has potential to be used as a signal for contaminated areas.

References


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