Floresta e Ambiente 2020; 27(3): e20180003
https://doi.org/10.1590/2179-8087.000318
ISSN 2179-8087 (online)

ORIGINAL ARTICLE – Conservation of Nature

Growth and Tolerance of Eucalyptus Seedlings in Soil Contaminated by Copper

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Abstract

Copper damages plants when present in high concentrations in the soil. This study assessed the growth and tolerance of *Eucalyptus grandis*, *E. saligna*, *E. dunnii*, and *Corymbia citriodora* seedlings in soil contaminated by copper. The experimental design was completely randomized in a factorial scheme (4 × 6), with four eucalyptus species and six copper doses (0, 80, 160, 240, 320, 400 mg kg^{-1}) with eight replications. The experiment was performed in a greenhouse for 120 days. The height, stem diameter, root dry mass and shoot dry mass, root specific surface area, Dickson quality index, and tolerance index were assessed. The results showed that the morphological parameters of the studied eucalyptus species were reduced by the copper doses added in the soil. However, the *Corymbia citriodora* and the *Eucalyptus saligna* species had a higher tolerance index to the metal.

Keywords: tree species, exotic, heavy metal.

1. INTRODUCTION AND OBJECTIVES

Heavy metals are found in soils, sediments, water, biological samples, and in the air, becoming potentially toxic (Dghaim et al., 2015). When in high concentrations, Copper (Cu) is one of the main heavy metals with polluting potential in soil and water (Andreazza et al., 2010). When present in the soil in high concentrations, copper may affect plant growth and development (Santos et al., 2010), as it causes disturbances in the structure of proteins and prevents cell elongation, since it increases plasma membrane permeability and cell wall lignification (Yruela, 2009).

The main cause of high concentrations of Cu in the soil is anthropic, because of the intensified industrial, agricultural, and urbanization activities, causing soil pollution and developing environmental impacts (Andreazza et al., 2010). Mining, together with copper fungicide applications in vineyards, significantly contribute to Cu soil contamination (Chaignon & Hinsinger, 2003), as well as the continuous and intense use of heavy metals by anthropic activity (Andreazza et al., 2013).

Because of the increasing contaminated areas by Cu, the use of technologies that reduce the toxic effects of this element is necessary. Among them, the use of plants has been an interesting alternative and seeks to use species with the potential to develop, absorb, and retain the metal in the plant tissues, consequently reducing its toxic effect in the soil.

Regarding plant species, the *Eucalyptus* genus encompasses more than 600 species, presents good adaptation to the Brazilian climatic conditions, with relatively fast growth and development, and large biomass production (Magalhães et al., 2011). However, studies by De Marco et al. (2017) have showed that the *Eucalyptus grandis* seedlings have high capacity to accumulate Cu in their root system, which would enable its use for phytostabilization, but there are still few studies including the use of different eucalyptus species.

Several nutritional, physiological, and biochemical processes and reactions are related to the tolerance and adaptation mechanisms used by certain plants, which enable production in areas with deficiency or excess of elements, as well as the

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possibility of recovering areas with heavy metals excess (Souza et al., 2011). Thus, it is hypothesized that *Eucalyptus grandis*, *Eucalyptus saligna*, *Eucalyptus dunnii*, and *Corymbia citriodora* species differ in growth and tolerance to high copper concentrations in the soil.

Therefore, this study aimed to assess the growth and tolerance of eucalyptus species in soil contaminated by copper.

2. MATERIALS AND METHODS

The experiment was performed in a greenhouse belonging to the Departamento de Ciências Agronômicas e Ambientais da Universidade Federal de Santa Maria, Frederico Westphalen Campus, between October 2015 and January 2016.

The experimental design was completely randomized in factorial arrangement (4 × 6), with four forest species (*Eucalyptus grandis* W. Hill ex Maiden, *Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson, *Eucalyptus saligna* Smith, and *Eucalyptus dunnii* Maiden) and six copper doses added to the soil (0 – natural content of the soil –, 80, 160, 240, 320, and 400 mg kg^{-1}), with eight repetitions.

The Cu doses were mixed into the soil 30 days before the seedling transplantation in the form of copper sulphate solution ($CuSO_4.5H_2O$), being diluted in 50 mL of distilled water to allow better homogenization. Soil samples were collected for determining the pseudo-total copper contents at the time of the seedlings transplantation, according to the methodology 3050b described in USEPA (1996).

The soil used in the experiment was defined as a Red Latosol, collected in an agricultural area in the 0-20 cm layer, and is shown in Table 1 according to the methodology described by Mann & Ritchie (1993) for exchangeable copper (KCl extractor 0.005 mol L^{-1}) and by Tedesco et al. (1995) for the other attributes.

The seeds of the eucalyptus species used in the experiment were provided by the Centro de Pesquisa em Florestas da Fundação Estadual de Pesquisa Agropecuaria (Fepagro), Santa Maria, RS unit. Sowing was carried out by hand and when the seedlings presented a pair of definitive leaves they were transplanted to polyethylene plastic bags with volumetric capacity of 1,000 cm^3. Each plastic bag with its soil content and one seedling was considered one experimental unit (EU).

The experiment was performed for 120 days after transplanting the seedlings and daily irrigations were performed during this period, maintaining moisture between 70 and 80% of the field capacity. Fertilization was performed in the seedling transplant, applying 150 g of N per m^3 of soil in the form of urea; 700 g of P_2O_5 in the form of triple superphosphate; and 100 g of K_2O in the form of KCl. Then, 100 g of N in the form of urea was applied as coverage and 30 g of K_2O diluted in 10 L of water was applied in the form of 50 mL per EU. Fertilization after transplanting the seedlings was performed in three seasons: at 30 days, with application of N and K; at 60 days, only N; and at 90 days with N and K, following the instructions of Gonçalves & Benedetti (2005). The EUs rotated weekly in order to meet the designed requirements.

At the end of the experiment, shoot height (H) was assessed with a graduated ruler from the lap of the seedling to its stem apex; the stem diameter (SD) with digital caliper; the root system dry mass (RDM) and shoot dry mass (SDM), with both separated in the seedling lap region and dried in an oven at 60 ± 1°C to constant mass, weighed on an analytical balance; and the total dry mass (TDM) by the sum of RDM and SDM. Based on these variables, the specific surface area (SSA) of the roots was estimated according to the methodology of Tennant (1975) and the Dickson quality index (DQI) (Dickson et al., 1960), according to Equation 1.

$$DQI = \frac{TDM}{\frac{H}{SD} + \frac{SDM}{RDM}} \quad (1)$$

The tolerance index (Toi) was estimated based on the TDM at zero dose of Cu (d_0) and at doses of 80 to 400 mg kg^{-1} (d_n), according to the equation: Toi = (TDM_{dn}/TDM_{d0})*100, which measures the ability of seedlings to grow in environments with high contaminant concentration (Wilkins, 1978).

The results were submitted to analysis of variance, to polynomial regression analysis, and Tukey test at 5% probability of error when significant, using the procedures available in the SISVAR program (Ferreira, 2011).

Table 1. Chemical model of soil used for seedlings production of forest species.

pH_{water}	Ca+Mg	Al	H+AL	$P_{(exchangeable)}$	$K_{(exchangeable)}$	$Cu_{(exchangeable)}$	OM	Clay
1:1	cmol$_c$ kg^{-1}			mg kg^{-1}			%	
5.6	2.23	0.0	3.3	6.5	126.5	12.7	1.1	62.0

OM: organic matter.

3. RESULTS AND DISCUSSION

Cu levels applied to the soil increased the pseudo-total concentrations of the element linearly, reaching values above the limits established for research in agricultural areas, which is 200 mg kg^{-1} (Conama, 2009), also surpassing the VRQs (Quality Reference Values) defined by *Fepam* (2014), which is 203 mg kg^{-1} for soils originating from volcanic rocks of the Plateau (*Planalto*), as shown in Figure 1. Moreover, in the treatments that received the highest Cu doses (240, 320, and 400 mg kg^{-1}) the soil contents were higher than double the limit for research in agricultural areas, also shown in Figure 1.

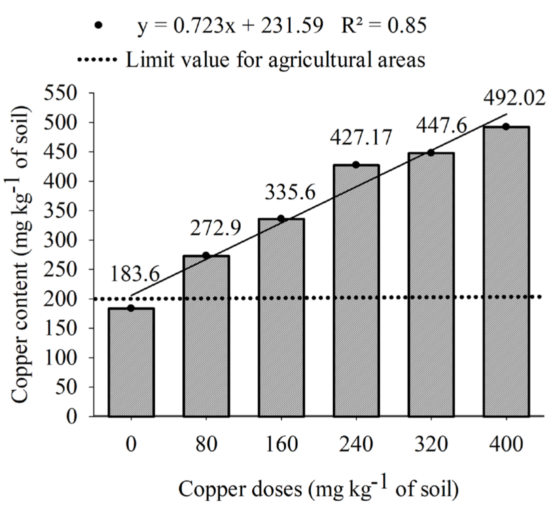

Figure 1. "Pseudo-total" copper content as a function of the Cu doses added to the soil.

The analysis of variance showed significant interaction ($p \leq 0.05$) between the studied eucalyptus species and the Cu applied to the soil for the variables plant height, SD, RDM and SDM, root SSA, and DQI, according to Figure 2.

The Cu doses applied to the soil showed different effects among the species for the seedling height, since squared regression models for this factor were adjusted for *C. citriodora*, *E. grandis* and *E. dunnii*, and linear regression for *E. saligna*, as shown in Figure 2a. There was an increase in plant height only for *E. grandis*, with a maximum value estimated at 151.7 mg kg^{-1}, showing a possible nutritional effect up to this dose with subsequent toxicity at the highest doses. According to Guo et al. (2010), Cu toxicity may be different among plant species, which can be evidenced in the eucalyptus species under study, and different responses may be observed as a result of increased Copper doses.

The stem diameter of the seedlings decreased with increasing doses of Cu in all species, but with lower intensity in *E. saligna* when comparing the higher dose with the zero dose (natural copper of the soil) showed in Figure 2b. The high Cu levels in the soil reduce the photosynthetic rate of plants, since they affect the electron transport chain, reducing photoassimilate production by plants, thereby reducing their growth (Kabata-Pendias, 2011). The effect showed by the difference between species can be attributed to plants developing separated tolerance mechanisms to the metal toxicity (Lequeux et al., 2010). However, the reduced SD observed in the studied eucalyptus species can be an indication that high metal contents reduce this morphological variable.

The SDM results showed a quadratic negative effect for *C. citriodora* and *E. dunnii* species, and positive quadratic behavior in *E. grandis* and *E. saligna*, with maximum efficiency (greater dry mass accumulation) at the estimated doses of 62.5 and 166.6 mg kg^{-1}, respectively, as shown in Figure 2c. The positive response by *E. grandis* and *E. saligna* may be due to the nutritional effect of the micronutrient, which participates in several physiological processes of plants, and the tolerance limit is dependent on each plant species (Taiz et al., 2017).

In a study with copper doses in the Brazilian orchid tree *pata-de-vaca* (*Bauhinia forficata*) and *carne-de-vaca* (*Pterogyne nitens*) seedlings, Silva et al. (2016) found that the shoot dry mass reduction with increasing pollutant levels was significant in both native forest species, with a reduction of 72 and 74% at the maximum dose of 300 mg kg^{-1}, respectively.

The RDM reduced in all studied species with the increase of the Cu applied to the soil, as shown in Figure 2d. The lower yields of RDM with the increase of copper doses obtained in this study corroborate the results of Silva et al. (2011), in which the root system was the first to be affected by copper in *açoita-cavalo* (*Luehea divaricata*) seedlings and Brazilian peppertree *aroeira-vermelha* (*Schinus terebinthifolius*) seedlings. However, the effect of RDM on the low doses of Cu in *E. saligna* may be related to the species tolerance to the metal through mechanisms that provide biochemical adaptations, thus allowing the plant to tolerate certain concentrations of this contaminant (Taiz et al., 2017).

E. dunnii and *E. grandis* species showed a linear reduction in the root SSA with increasing Cu doses. *C. citriodora* showed a quadratic reduction with a minimum estimated dose point of 344.0 mg kg^{-1}, while *E. saligna* had a maximum dose point of 142.5 mg kg^{-1} according to Figure 2e. The reduction in root growth is one of the most evident symptoms of copper's toxic effect (Kukkola et al., 2000), because the excess micronutrient reduces the longitudinal root growth because of the contaminant chemical barriers, providing greater secondary root emission (Taiz et al., 2017). It is possible that there has been a higher production of secondary roots in *E. grandis* seedlings, which is interesting because it increases the water and soil nutrients absorption area.

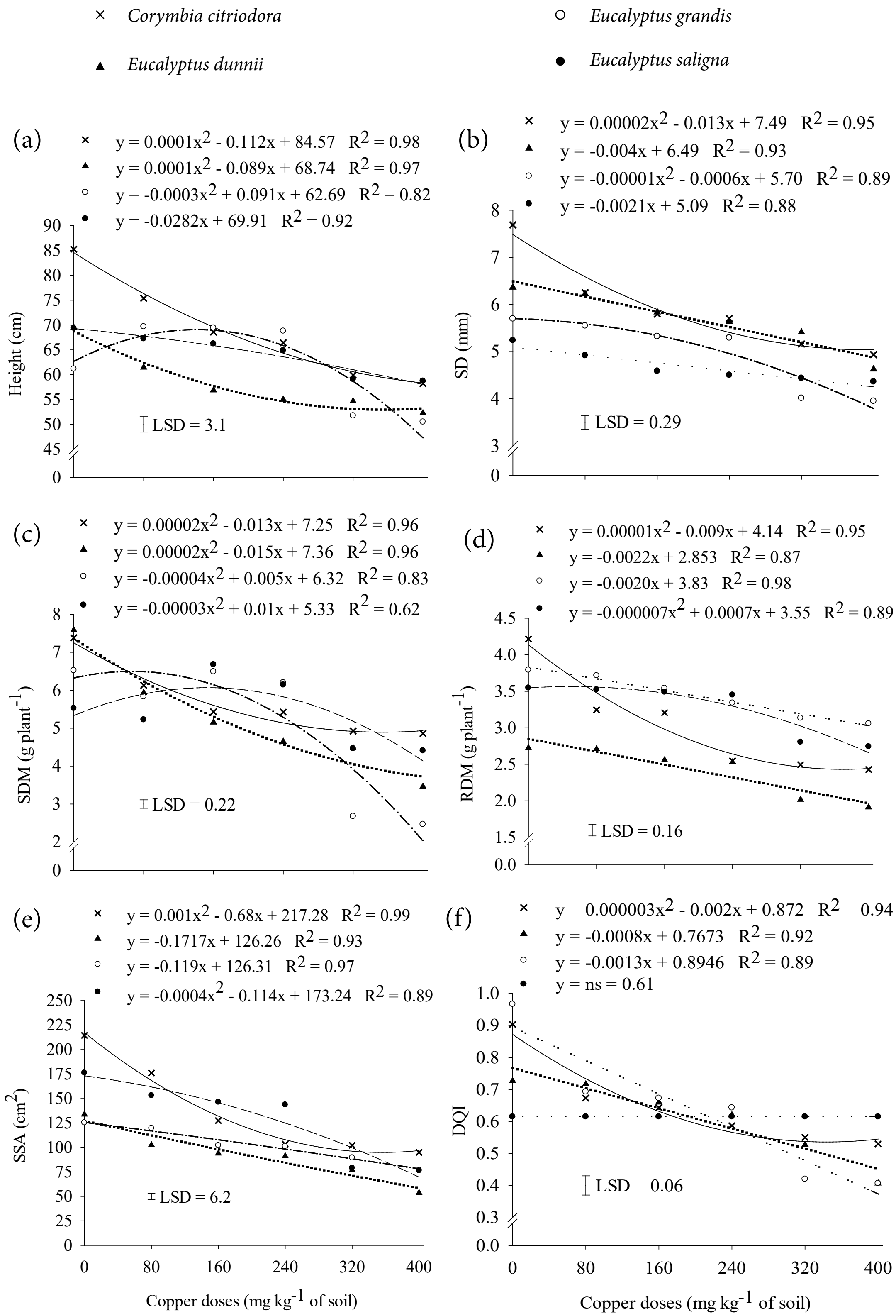

Figure 2. Height (a), stem diameter (b), shoot dry mass (c), root dry mass (d), specific surface area (e), and Dickson quality index (f) of *C. citriodora*, *E. dunnii*, *E. grandis*, and *E. saligna* seedlings in soil contaminated by copper.

SD: stem diameter; SDM: shoot dry mass; RDM: root dry mass; SSA: specific surface area; DQI: Dickson quality index; LSD: least significant difference; ns: not significant.

The quadratic regression model for the *C. citriodora* seedlings was adjusted to the DQI, with an evident reduction of this index in the first Cu doses applied to the soil, as shown in Figure 2f. There was a linear reduction of 41.7% and 58.1% in the DQI at the maximum dose of the metal in the *E. dunnii* and *E. grandis* seedlings. In order to estimate DQI, morphological attributes that show seed vigor and quality are considered (Rossi et al., 2008). Thus, it was observed that the *E. saligna* seedling quality was not influenced by the Cu applied to the soil.

The Toi was linearly reduced by Cu applied to the soil, presenting values of 28 and 41% of *C. citriodora* and *E. dunnii* at the maximum dose of the metal applied to the soil, respectively, and induced a quadratic response with a maximum point at the estimated dose of 86.3 and 231 mg kg^{-1} for *E. grandis* and *E. saligna*, respectively, as shown in Figure 3. The tolerance ability of plants to heavy metals is because of the presence of mechanisms which enable them to survive in soils in which other plants would present too much toxicity to be tolerated (Macnair et al., 2000).

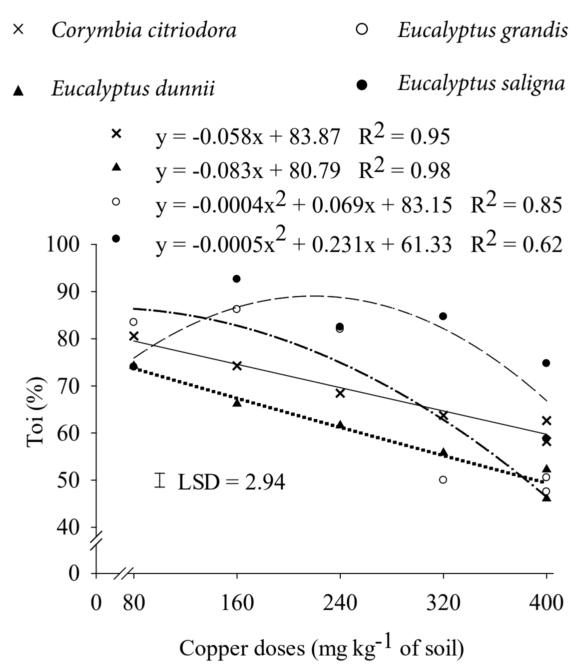

Figure 3. Regression equations for the tolerance index (Toi) of *C. citriodora*, *E. dunnii*, *E. grandis*, and *E. saligna* seedlings grown in soil contaminated by copper.

LSD: least significant difference.

Some plant species have developed tolerance or resistance to heavy metals because they present a complex mechanism of homeostasis that enables these plants to develop by controlling the absorption, accumulation, and translocation of metals in the plant (Santos et al., 2006). The ability of the plant to maintain higher levels of metals in the root system regarding the shoot indicates the presence of a separating mechanism of the metal, and consequent reduction of its translocation to the shoot (Accioly et al., 2004). In the classification scale according to the Toi, a species is considered tolerant when its Toi is higher than 60%, moderate between 60 and 35%, and sensitive when lower than 35% (Lux et al., 2004). According to the obtained data, the *C. citriodora* and *E. saligna* species were considered tolerant until the maximum studied dose, and the *E. dunnii* and *E. grandis* species up to the dose of 240 mg kg^{-1}.

In general, the morphological variables of the different studied eucalyptus species were reduced. However, different responses by the species to the Cu levels added to the soil were observed, including (according to Toi) *C. citriodora* and *E. saligna* species being considered tolerant, even when 400 mg Cu kg^{-1} of soil was used. This study shows that there are differences among plant species when submitted to high Cu doses, so that studies assessing the classification and selection of species which are capable of developing, tolerating, and decontaminating areas should be considered.

4. CONCLUSION

The implemented copper doses reduced the morphological variables of *Eucalyptus grandis*, *E. saligna*, *E. dunnii*, and *Corymbia citriodora* seedlings.

Eucalyptus saligna species had a higher tolerance index to the copper doses applied to the soil and could be considered for cultivation in areas contaminated by copper.

ACKNOWLEDGEMENTS

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes).

SUBMISSION STATUS

Received: 4 Jan. 2018
Accepted: 30 June 2018
Associate editor: José Henrique Tertulino Rocha
0000-0002-7471-4191

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FINANCIAL SUPPORT

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes).

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