



## **Effect of Application of Zinc on Seedlings of *Eucalyptus urophylla***

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/JSRR/2022/v28i1030558

### **Open Peer Review History:**

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/90113>

**Original Research Article**

**Received 02 June 2022**  
**Accepted 10 August 2022**  
**Published 25 August 2022**

### **ABSTRACT**

*Eucalyptus* represents a species with consolidated cultivation in the Cerrado and that has been cultivated in several states that cover this Biome. Thus, the objective was to evaluate the growth and development of *Eucalyptus urophylla* seedlings in response to soil and foliar zinc application in a Cerrado Red Latosol. Two experiments were set up in a completely randomized design, with five treatments and seven replications. The first experiment consisted of five doses of Zn, in the form of zinc sulfate for both experiments, 0; 2.5; 5; 10 and 20 mg/dm<sup>3</sup> applied via soil and the second experiment consisted of five doses of Zn, 0; 2.5; 5; 10 and 20 mg/L applied via foliage plus an extra dose of 20 mg/dm<sup>3</sup> of Zn applied via soil, this dose being necessary due to the low zinc content in the soil and which could compromise the initial development of seedlings that would receive applications via leaf. The experiments were carried out in a greenhouse and after 80 days of planting, the following were evaluated: stem diameter (neck), height, root length, number of leaves, shoot and root dry matter, total dry matter, of macro and micronutrients in leaves and gas exchange: internal carbon concentration, stomatal conductance, transpiration and net photosynthesis rate. Even though *Eucalyptus urophylla* seedlings did not show statistically significant differences, part of the evaluated variables showed a positive response to the application of zinc, analyzing the linear and quadratic regression graphs, mainly at doses of 10 and 20 mg/L and 10 and 20 g/dm<sup>3</sup>.

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**Keywords:** Fertilization; Cerrado tocantinense; eucalyptus; mineral nutrition.

## 1. INTRODUCTION

Brazil has ideal climatic conditions for the growth and development of species of the genus *Eucalyptus*, with a productivity 10 times higher than countries like Finland, Portugal and the United States. Currently, forestry companies in the country reach an average productivity of 40 to 50 m<sup>3</sup>/ha/year with the use of improved species and forestry technologies with areas producing around 70 m<sup>3</sup>/ha/year [1].

According to Agência Brasil [2], planted forests in Brazil in 2020 totaled an area of 9.3 million hectares, with areas covered with eucalyptus representing 80.2% of planted forests. Much of Brazil's forestry production is found in the South and Southeast regions, with Minas Gerais remaining the largest forest producer.

Among the main species of the genus cultivated in Brazil are *Eucalyptus grandis*, *Eucalyptus camaldulensis*, *Eucalyptus saligna* and *Eucalyptus urophylla*, in addition to hybrids resulting from crosses between the species, such as the cross between *E. grandis* × *E. urophylla* [3].

The Brazilian Cerrado occupies an area of approximately 150 million hectares, representing 25% of the national territory, having a part with significant potential for vegetable production. *Eucalyptus* represents an already consolidated species for cultivation in the Cerrado and has already been planted in this region in the states of Minas Gerais and São Paulo and currently has a great expansion in the Midwest region, which a large part is occupied by the Cerrado Biome [4,5].

The Cerrado of Tocantins covers 91% of the State's territorial area. *Eucalyptus* cultivation according to the 2017 Agricultural Census carried out by the IBGE corresponded to an area of 121,193 ha, with greater importance in the cities of Brejinho de Nazaré with 22,000 ha and São Bento do Tocantins with 20,300 ha, which concentrate the largest massifs of eucalyptus trees in the region intended to serve the production of pulp and paper, in addition to other purposes [5].

Among the most recommended species for the Cerrado region are *E. urophylla*, *E. camaldulensis* and *E. cloeziana*, the first species

providing wood with medium density and a light color, it is used for the production of cellulose, fiberboard, sawmill, posts, sleepers and coal. *Eucalyptus urophylla* started to be cultivated in Brazil due to the high resistance to eucalyptus canker, in addition to the properties of its wood, thus being able to be a substitute for *E. grandis* in localities where it becomes susceptible [3].

Faced with the increase in areas planted with species of the genus *Eucalyptus*, the use of good quality seedlings is one of the most important aspects for the establishment of forest plantations and to ensure success in their development, and plant nutrition plays a very important role, having a direct effect on the physical and biochemical aspect of the plant. Good quality seedlings have a higher survival rate at planting, better development, thus reducing the need for frequent maintenance of cultural treatments, in addition to guaranteeing a final product of good quality and at a lower cost [6,7].

According to Silveira [8], the practice of fertilization is one of the most important factors for increasing eucalyptus productivity, considering that most plantations are carried out in areas with low fertility soils. Zinc, due to its low availability in cerrado soils, is one of the limiting factors for agriculture.

Zinc is responsible for the biosynthesis of tryptophan, which is a precursor of the phytohormone indoleacetic acid, which acts directly on cell division and plant growth, increasing the efficiency of water and nutrients. Zinc-deficient eucalyptus plants often show stunting and the leaves are small and clumped. In *E. urophylla*, leaves that are still growing initially show interveinal chlorosis, and areas with a purplish color may appear and with the worsening of the deficiency, the leaves can reduce in size and the internodes shorten, leaf tips and spots on the interveinal tissue can become if necrotic [9].

Thus, the present work aimed to evaluate the growth and development of seedlings of the species *Eucalyptus urophylla* subjected to different doses of zinc (Zn) and application modes, via soil and leaves, in a substrate made from Cerrado soil. and its influence on photosynthesis, on the concentration of nutrients in the leaves of the seedlings.

## 2. MATERIALS AND METHODS

The experiments were conducted on a small rural property located in the municipality of Paraíso do Tocantins - TO, and evaluated in the Insect-Microorganism Symbiosis Laboratory, Federal University of Tocantins, Campus de Gurupi, from May 2021 to July 2021. Soil for the production of the seedling substrate was collected in the deeper layers (20 to 40 cm), classified as Oxisols, following the Brazilian Soil Classification System (SiBCS), with coordinates latitude 10°6'30.0222" and longitude 48°55'33.82896".

From the collected soil, a sample of 300 grams was taken for chemical analysis and following the methodology of the Embrapa Clima Temperado - RS Soil Fertility Laboratory, it was placed in a ventilated place in the shade and later, packed in a transparent plastic bag, identified and sent to the Safrar Análises Agrícolas laboratory, located in the municipality of Patrocínio - MG and the following results were obtained, shown in Table 1.

After the chemical analysis of the soil and for the composition of the substrate, 50% of the substrate volume of cerrado soil, 30% of well-tanned bovine manure and 20% of sand were used, following the recommendation of Embrapa Cerrado for the production of eucalyptus seedlings. Then, two experiments were carried out, one with applications of different doses of Zn via soil and the other with applications of different doses of Zn via foliar for treatments with applications of Zn via soil and the other for treatments with applications of Zn foliar pathway. The experimental design used was a completely randomized block (DIC) with 5 treatments and 7 replications.

The first experiment consisted of 5 doses of Zn (0, 2.5, 5, 10 and 20 mg/dm<sup>3</sup>) applied via soil and

the second experiment consisted of 5 doses of Zn (0, 2.5, 5, 10 and 20 mg/L) applied via foliar plus a Zn dose of 20 mg/dm<sup>3</sup> via soil, and this last dose was intended to avoid the appearance of possible symptoms of deficiency of the element in the initial development of the seedlings and possible interferences in the results, since the soil used for the preparation of the substrate in both experiments was found to have a low zinc content and the applications of Zn via foliar were made only at 45 days after sowing. For the choice of doses, the methodology applied by Rodrigues et al. (2012) [10].

To adapt the soil according to the nutritional requirements of eucalyptus seedlings, the recommendations of correctives and fertilizers for Goiás 5th approach [11] were followed. For the production of seedlings, polyethylene forest trays were used and the experiments were placed in a cage with a mesh to protect against insects, in order to prevent the attack of the gall wasp *Leptocybe invasa* (*Hymenoptera: Eulophiidae*) and to avoid possible interference with the results obtained in the experiments.

The zinc source used was zinc sulfate (ZnSO<sub>4</sub>) and all adjustments were made in relation to the other nutrients required by the eucalyptus crop, following the chemical analysis of the soil. The sources of the other necessary nutrients were: simple superphosphate, potassium chloride, urea, boric acid and dolomitic limestone to supply calcium and magnesium to the seedlings. For a homogeneous distribution of nutrients in the substrate, all sources of nutrients were diluted in water and subsequently applied to the substrate.

The seeds of the *E. urophylla* species were acquired from the company NC Aromas, located in the city of São João do Paraíso - MG, and were sown in polyethylene forest trays using the

**Table 1. Chemical characteristics of the soil collected in the area**

pH (H <sub>2</sub> O) <sup>(1)</sup>	Ca <sup>2+</sup> <sup>(2)</sup>	Mg <sup>2+</sup> <sup>(2)</sup>	Al <sup>3+</sup> <sup>(2)</sup>	H+Al <sup>(3)</sup>	T <sup>(7)</sup>	T <sup>(7)</sup>	V <sup>(7)</sup>	m <sup>(7)</sup>
cmolc dm <sup>3</sup>				%				
5,0	0,60	0,30	0,00	4,70	5,7	1,00	17,50	0,00
P meh. <sup>(4)</sup>	K <sup>+</sup> <sup>(4)</sup>	Fe <sup>(5)</sup>	Cu <sup>(5)</sup>	Mn <sup>(5)</sup>	Zn <sup>(5)</sup>	B <sup>(5)</sup>	M.O <sup>(6)</sup>	
mg dm <sup>3</sup>				dag kg				
1,7	38,00	18	0,6	2,9	0,7		0,32	1,70

pH in water<sup>(1)</sup>, <sup>(2)</sup> Ca<sup>2+</sup>, Mg<sup>2+</sup> e Al<sup>3+</sup>: extractor KCl 1 mol L<sup>-1</sup>, <sup>(3)</sup> H+Al: Buffer Solution SMP a pH 7,5, P e K<sup>+</sup><sup>(4)</sup>: extractor Mehlich-1, B: extractor BaCl<sub>2</sub> . 2H<sub>2</sub>O 0,125% the hot, <sup>(5)</sup> Cu, Fe, Mn, Zn: extractor: DTPA em pH 7,3, <sup>(6)</sup> M.O.: colorimetric method, T: CTC pH 7,0, t: CTC efectiva, V%: base saturation, m%: aluminum saturation.

substrate produced from the dystrophic Red Latosol. The seedlings were thinned 15 days after emergence, leaving only the most vigorous plant located in the center of each cell of the tray.

In the experiment consisting of foliar Zn applications, the doses were divided into 3 weekly applications after 45 days of seedling emergence, and the applications and the doses were divided according to the development of the seedlings and the leaf area. The first dose constituted 20% of the total dose to be applied, the second dose 30% and the third dose 50%, thus constituting 100% of all doses evaluated in the experiment. For application, the dilution of zinc sulfate was carried out in water and to improve the efficiency of application of the solution, vegetable oil was used, in view of the high waxiness contained in the surface of the leaves of the eucalyptus seedlings.

The seedlings remained in the nursery until 80 days and were later submitted to evaluation. The parameters evaluated were shoot height, stem diameter (stem base), root length, number of leaves, shoot dry mass, root dry mass, total dry mass and zinc content of shoot dry mass. For the collection of all the parameters to be evaluated, an analog caliper (stem diameter), a millimeter ruler (height of the area part and root length), a drying oven and a precision scale were used to obtain the dry mass of the shoot, root dry mass and total dry mass.

In addition to the parameters mentioned above, the gas exchange of the seedlings submitted to different doses of zinc, from 8 am to 12 am, was also evaluated. To obtain the data, an IRGA infrared gas analyzer, model LCI5D from ADC System, UK, was used and the variables

analyzed were the internal carbon concentration (Ci), stomatal conductance (gs), transpiration (E) and the net photosynthetic rate (A).

For data analysis, such as shoot height, stem diameter (stem base), root length, number of leaves, shoot dry mass, root dry mass, total dry mass, zinc content of the dry mass of the shoot, internal carbon concentration (Ci), stomatal conductance (gs), transpiration (E) and net photosynthetic rate (A), using the Sisvar software, version 5.8, applying analysis of variance and regression at 5 % probability.

### 3. RESULTS AND DISCUSSION

#### 3.1 Seedling Growth and Development

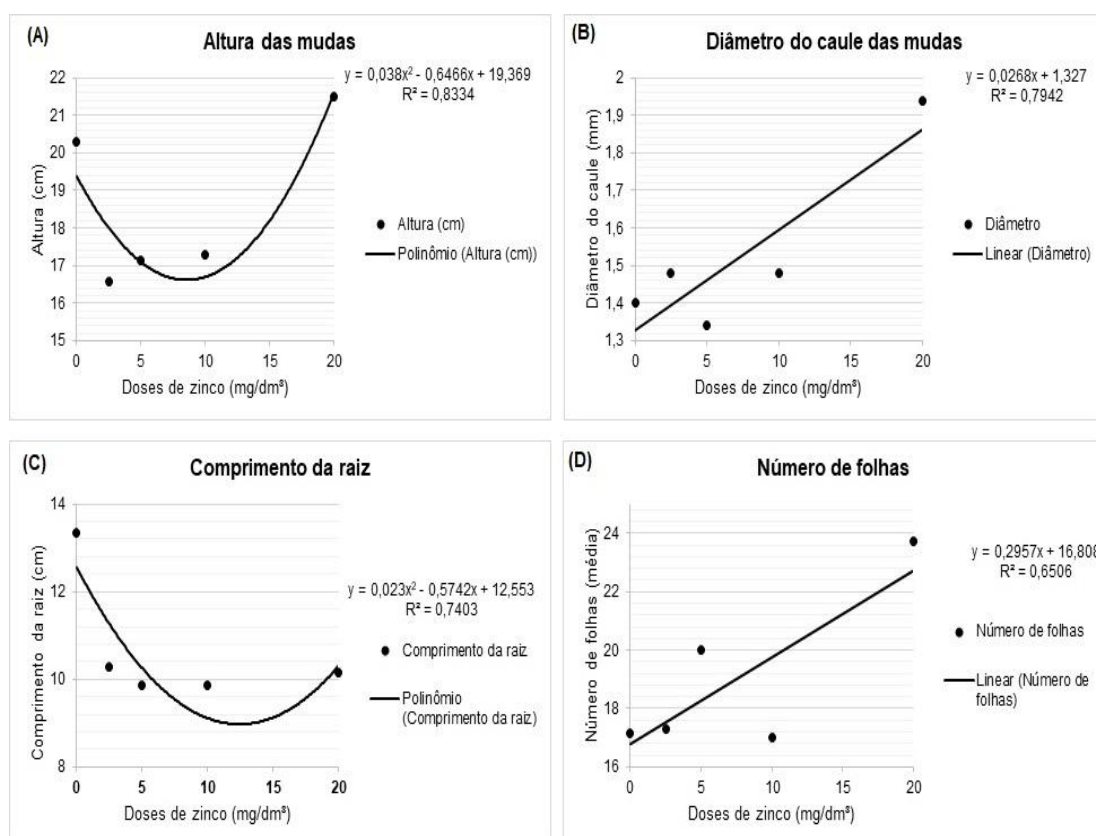
The growth and development of seedlings in height, stem diameter (neck) and number of leaves did not present significant differences in the results obtained in the evaluations carried out according to the increase of zinc doses applied via soil, however the root length showed a negative response when the seedlings were submitted to the highest doses of zinc, and the greatest reduction was observed at the dose of 2.5 mg/dm<sup>3</sup>. Seedlings that received doses of 5 to 10 mg/dm<sup>3</sup> showed no variation in the values obtained, with a slight increase in the dose of 20 mg/dm<sup>3</sup>, Table 2 and Fig. 1.

Fig. 1 (A) shows a reduction in the height of the seedlings when they were submitted to the dose of 2.5 mg/dm<sup>3</sup> of zinc via soil, with a slight increase again from the dose of 5 mg/dm<sup>3</sup> and reaching the maximum height in the soil. dose of 20 mg/dm<sup>3</sup> even though there was no significant difference in relation to seedlings that did not receive zinc via soil.

**Table 2. Mean height, stem diameter (lo), root length and number of different leaves of *E. urophylla* seedlings at 80 days, increased doses of zinc via soil**

Treatment (doses/mg/dm <sup>3</sup> )	Height (cm)	Diameter (mm)	Root length (cm)	Number of leaves/plant
0	20,28 a	1,40 a	13,35 a	17,14 a
2,5	16,57 a	1,48 a	10,28 ba	17,28 a
5	17,14 a	1,34 a	9,85 b	20,00 a
10	17,28 a	1,48 a	9,85 b	17,00 a
20	21,50 a	1,94 a	10,14 ba	23,71 a
Média	18,55	1,53	12,83	19,03
CV%	20,99	21,24	30,51	39,74

Note: Different lowercase letters in the column differ statistically from each other



**Fig. 1. Height (A), stem diameter (neck) (B), root length (C) and number of leaves (D) as a function of the different doses used via soil in *E. urophylla* seedlings**

Regarding the stem diameter and number of leaves, Fig. 1 (B and D) shows a positive and linear response from the dose of 10 mg/dm<sup>3</sup> of zinc applied via soil, and the seedlings submitted to a dose of 20 mg /dm<sup>3</sup> showed the best results in these two variables evaluated even though they did not differ significantly.

Natale et al. [12], evaluating the effects of the application of five doses 0, 2, 4, 6 and 8 mg/dm<sup>3</sup> of zinc on the development, nutritional status and dry matter production of passion fruit seedlings cultivated in substrate of a dystrophic Red Latosol verified a positive and significant effect of zinc doses on stem diameter and height in passion fruit seedlings as the doses increased.

Silva et al. [13], evaluating the accumulation and translocation of zinc in seedlings of species of the genus *Eucalyptus* and *Corymbia* applied via soil, found that *Corymbia citriodora*, *Eucalyptus saligna* and *Eucalyptus dunnii* showed a reduction of 32, 30 and 93% of the root volume according to the increase in doses. of zinc provided, but the reduction was caused by the

toxic effect of high doses that varied from 140 to 980 mg/kg of soil 280.

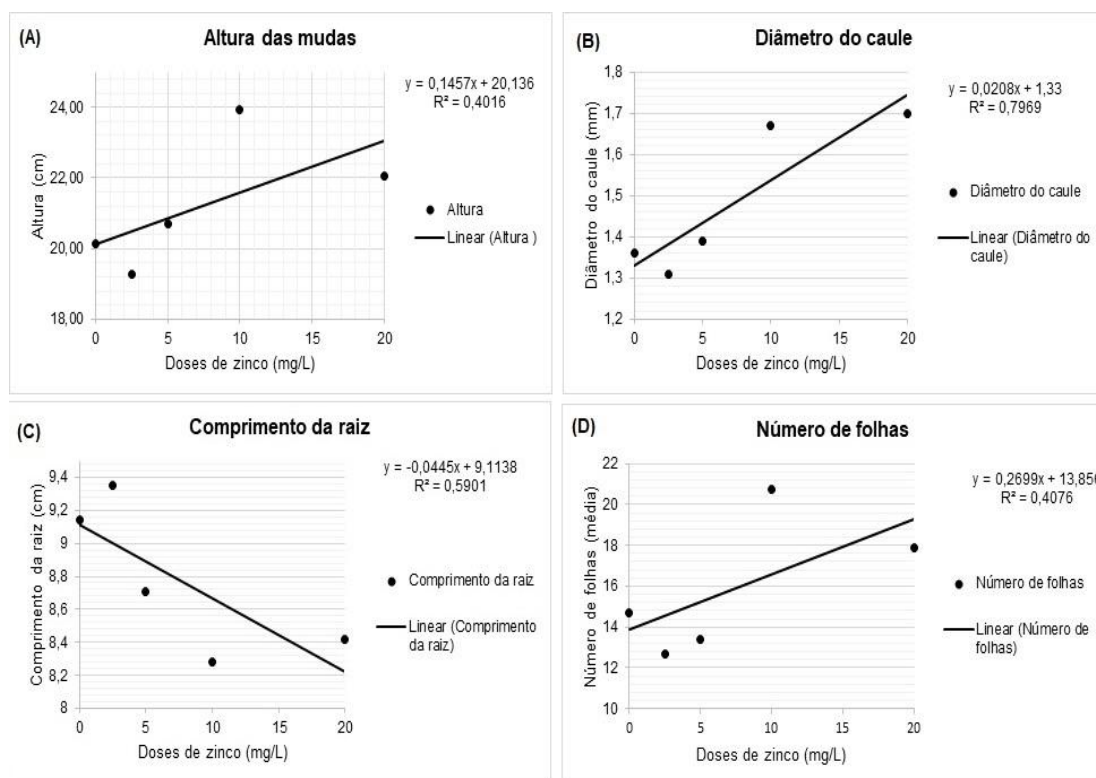
In seedlings that received applications via foliar, the growth and development in height, root length and number of leaves did not show significant differences in the results according to the increase of applied doses of zinc, however they presented significant differences in the stem diameter, and plants that received the highest doses, 10 and 20 mg/L, had a larger stem diameter.

By analyzing the regressions Fig. 2 (A and D) it can be observed that although the height and number of leaves did not present significant differences in the results, there was a positive and linear increase in the doses of 5, 10 and 20 mg/L, seedlings that received a dose of 10 mg/L showed the best results. At the highest dose of zinc, 20 mg/L, the seedlings showed a decrease in height and number of leaves in relation to the dose of 10 mg/L, but did not show symptoms of toxicity as mentioned by Malavolta et al. [14], which is characterized by general chlorosis with reddish-brown pigments.

**Table 3. Mean values of height, diameter, root length and number of leaves of *Urophylla eucalyptus* seedlings at 80 days, submitted to different doses of zinc via foliar**

Treatment (doses/mg/dm <sup>3</sup> )	Height (cm)	Diameter (mm)	Root length (cm)	Number of leaves/plant
0	20,14 a	1,36 ba	9,14 a	14,71 a
2,5	19,28 a	1,31 b	9,35 a	12,71 a
5	20,71 a	1,39 ba	8,71 a	13,42 a
10	23,92 a	1,67 ba	8,28 a	20,71 a
20	22,07 a	1,70 a	8,42 a	17,85 a
Média	21,22	1,49	8,85	15,88
CV%	16,58	16,43	16,20	36,18

Note: Different lowercase letters in the column differ statistically from each other.

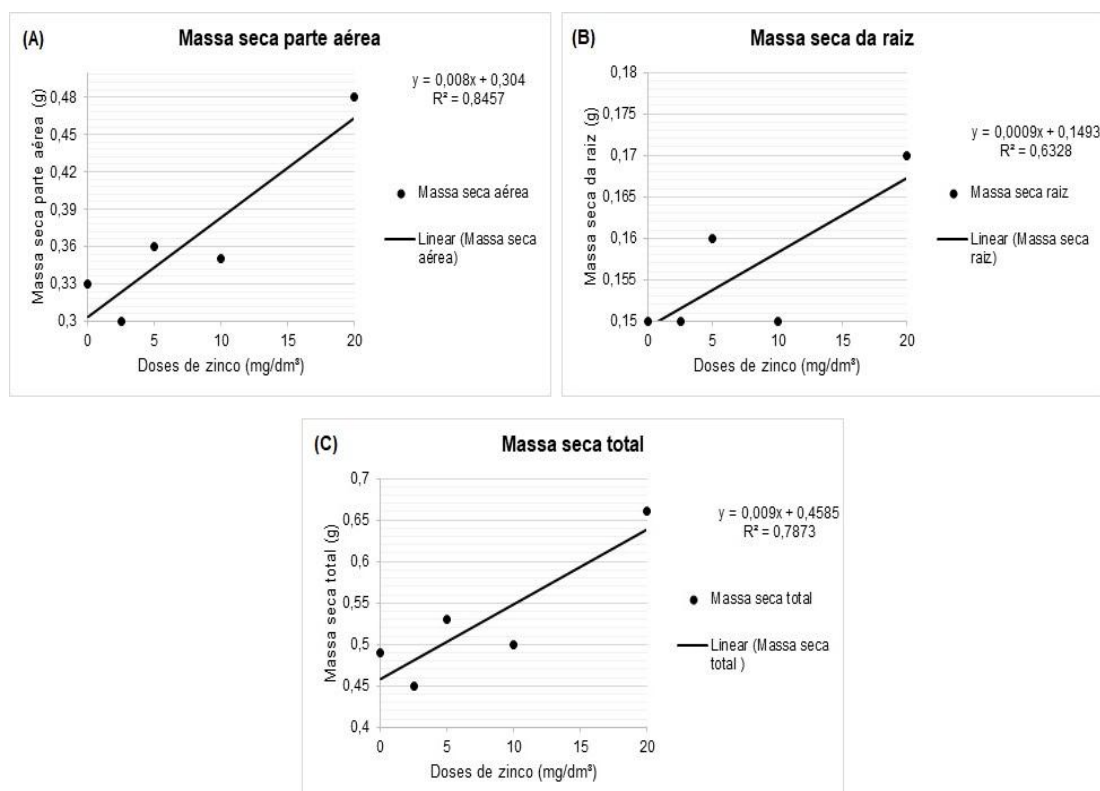


**Fig. 2. Height (A), stem diameter (neck) (B), root length (C) and number of leaves (D) as a function of the different doses used via soil in *E. urophylla* seedlings**

**Table 4. Mean shoot dry mass, root dry mass and total dry mass of *Urophylla eucalyptus* seedlings at 80 days, submitted to different doses of zinc via soil**

Treatments (doses/mg/dm <sup>3</sup> )	Aerial part dry mass (g)	Root dry mass (g)	Total dry mass (g)
0	0,33 a	0,15 a	0,49 a
2,5	0,30 a	0,15 a	0,45 a
5	0,36 a	0,16 a	0,53 a
10	0,35 a	0,15 a	0,50 a
20	0,48 a	0,17 a	0,66 a
Média	0,36	0,16	0,53
CV%	42,75	41,51	39,49

Note: Different lowercase letters in the column differ statistically from each other



**Fig. 3. Shoot dry mass (A), root dry mass (B) and total dry mass (C) as a function of the different doses used via soil in *E. urophylla* seedlings**

Stem diameter had a positive and linear increase at doses of 5, 10 and 20 mg/L, and seedlings that received a dose of 20 mg/L had greater gain, Fig. 2 (B). Root length had a negative and linear response from the 5 mg/L dose, Fig. 2 (C). The reduction in root length by increasing doses of zinc may be related to the disturbance of the physiological processes of the seedlings, and according to Hooda [15], this disturbance affects mitosis and favors cell death in the roots, causing a reduction in root development.

The accumulation in shoot dry mass, root dry mass and total dry mass did not show statistical differences in the soil treatment with different doses of zinc used, Table 4.

Rodrigues et al. [10] in a study similar to this one, found that there was no positive influence on shoot dry matter of eucalyptus seedlings under different doses of zinc via soil. A similar result was found by Couto et al. [16], growing eucalyptus seedlings in a greenhouse using 12 types of Cerrado soils with low levels of zinc, with no positive response in the dry matter of the aerial part of seedlings that received increasing doses ranging from 0 to 64 mg/dm<sup>3</sup> of zinc.

Silva [13], evaluating the accumulation and translocation of zinc in seedlings of species of the genera *Eucalyptus* and *Corymbia*, verified a reduction in the dry mass of the aerial part with the increase of doses of zinc supplied via soil, which varied from 0 to 980 mg. /dm<sup>3</sup>. Similar results were found by Soares et al. [17] on seedlings of *E. maculata* and *E. urophylla* in soil contaminated with zinc and by Ramos et al. [18] in cultivation of *E. urophylla* in nutrient solution with increasing doses of the metal, however such results were found in seedlings submitted to high doses of zinc.

However, Natale et al. [12] observed that shoot and root dry matter in passion fruit seedlings was positively affected due to the increments promoted in developmental characteristics (stem diameter, height and leaf area). Grunes et al. [19] also observed positive effects of zinc application on the growth of the aerial part and root system in several plant species, and this positive effect may be related to the role of zinc in auxin synthesis, which stimulates development and elongation. of the young parts of the plants [20].

The positive results found by Natale et al. [12] and Grunes et al. [19] show the results found when analyzing the regression graphs generated from the results obtained in the shoot dry mass, root dry mass and total dry mass in Fig. 3, although there are no significant differences in the data. a positive increase in the dose of 20 mg/dm<sup>3</sup> is observed in relation to the lowest doses used.

In the foliar treatment, there was a significant difference in the results obtained in the shoot dry mass and in the total dry mass according to the different doses of zinc used. In the dry mass of

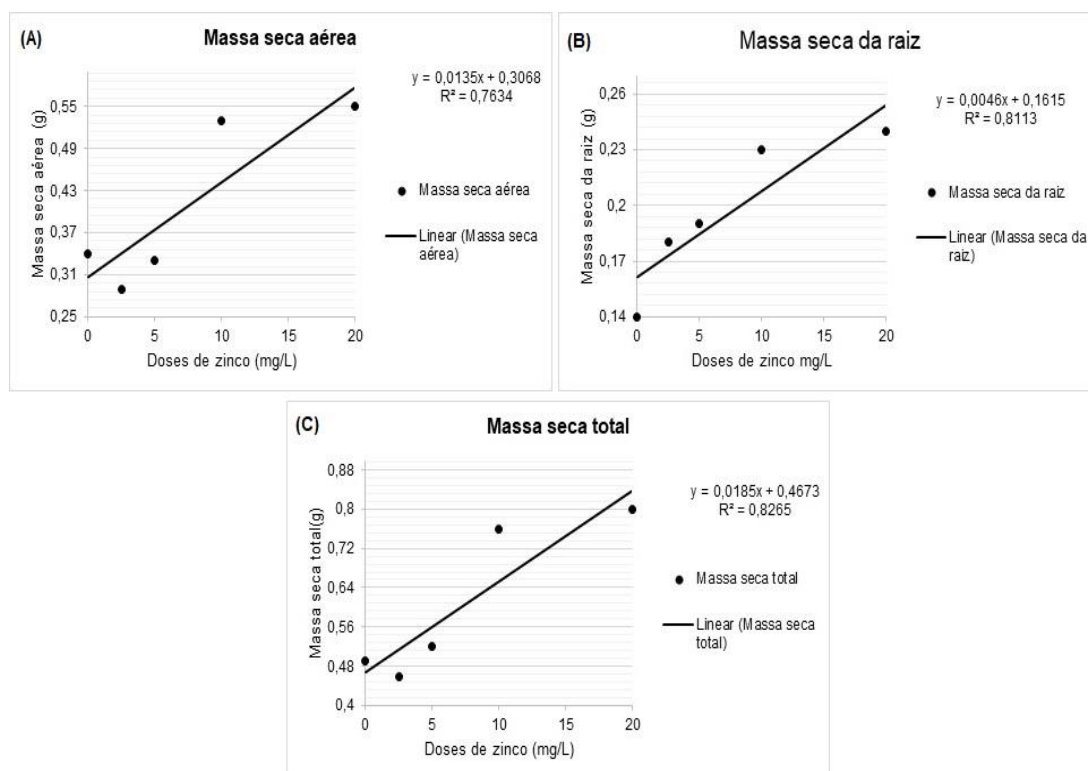
the aerial part, the doses of 10 and 20 mg/L of zinc presented the best results of mass gain. The doses of 5, 10 and 20 mg/L of zinc presented the best results in relation to the total dry mass, and the dose of 20 mg/L was the one that provided the greatest mass gain, Table 5 and Fig. 4.

According to Rodrigues et al. [10], in order to obtain gains in shoot dry matter, one of the necessary conditions is a significant increase in the amount of a given nutrient absorbed and which will be part of the constitution of the additionally produced dry matter.

**Table 5. Mean shoot dry mass, root dry mass and total dry mass of *E. urophylla* seedlings at 80 days, submitted to different doses of zinc via foliar**

Treatments (doses/mg/dm <sup>3</sup> )	Aerial part dry mass (g)	Root dry mass (g)	Total dry mass (g)
0	0,34 cba	0,14 a	0,49 b
2,5	0,29 c	0,18 a	0,46 b
5	0,33 cb	0,19 a	0,52 ba
10	0,53 ba	0,23a	0,76 ba
20	0,55 a	0,24 a	0,80 a
Média	0,41	0,20	0,61
CV%	33,73	38,19	32,35

Note: Different lowercase letters in the column differ statistically from each other



**Fig. 4. Aerial dry mass (A), root dry mass (B) and total dry mass (C) as a function of the different doses used via foliar in *E. urophylla* seedlings**



In relation to the dry mass of the root, the graph in Fig. 4 (B) shows that although the results do not differ significantly, a linear and positive increase can be observed with the increase in the doses of zinc supplied to the seedlings. Comparing with the root length where there was a reduction of this with the increase of the doses of zinc applied via soil and via foliar, Fig. 1 (C) and Fig. 2 (C), despite this reduction, there was an increase in the volume of the system root of the seedlings.

Positive effects of zinc application were also found by Lopes [20] in passion fruit seedlings cultivated in Dark Red Latosol and Red-Yellow Latosol, where the authors indicate the need for fertilization with zinc for seedling formation.

### 3.2 Nutrient Content in Leaves

Nutrient absorption was altered when eucalyptus seedlings were submitted to different doses of zinc and application method, both via foliar and soil, Figs. 5 and 6. When submitted to a dose of 20 mg/L via foliar, there was a significant increase in the macro and micronutrient content in the leaves compared to the leaves of seedlings that did not receive zinc via the foliar, except for manganese and zinc, which showed higher levels in seedlings that did not receive zinc via the foliar, Fig. 5.

The increase in the content of macro and some micronutrients in the leaves of seedlings submitted to a dose of 20 mg/L via foliar may be related to the increase in the leaf area index of these seedlings, and when the dry mass gain of the aerial part of seedlings submitted to the same dose, they presented a greater mass gain of the aerial part.

Seedlings that received a dose of zinc of 20 mg/dm<sup>3</sup> via soil showed a significant increase only in the content of nitrogen, potassium, iron, manganese and zinc in the leaves. In contrast to seedlings that did not receive zinc, the foliar content of phosphorus, calcium, magnesium, boron and copper significantly reduced, Fig. 6.

The reduction in phosphorus content in the leaves of seedlings that received a higher dose of zinc via the soil may be related to the increase in the availability of this element in the soil, thus generating a competition with phosphorus for the same active absorption site, where according to

Malavolta [21] increasing zinc in the soil can reduce the phosphorus content and vice versa. Also according to the same author, the increase in the concentration of zinc in the nutrient solution of the soil can reduce the content of calcium and magnesium in the same, confirming the result found in seedlings that received the highest dose of zinc (20 mg/dm<sup>3</sup>) in the soil, where they showed a reduction in the content of calcium and magnesium in the leaves.

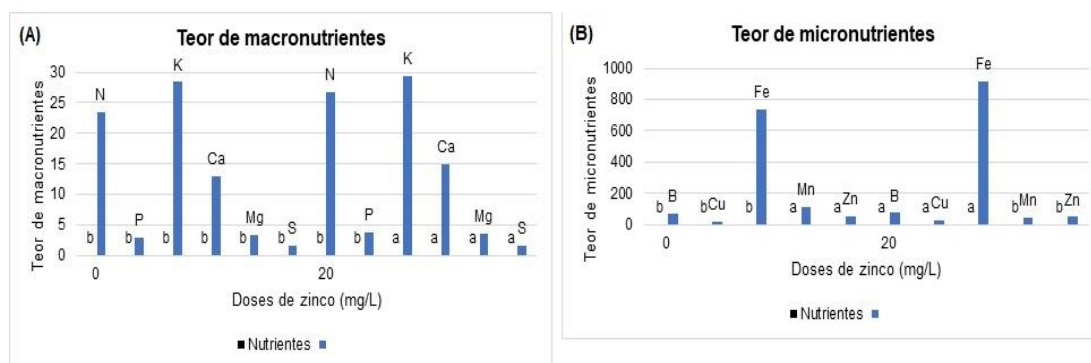
Duarte et al. [22], evaluating the nutritional status of eucalyptus as a function of different doses of zinc in Cerrado soil applied in the planting furrow, found that increasing doses of zinc had an influence on foliar concentrations of sulfur, zinc and iron. Leaf concentrations of N, P, K, Ca and Mg were within the levels considered ideal for the initial phase of eucalyptus development, except for sulfur, which was slightly below. The B concentrations were above the content considered adequate and the Cu, Mn and Iron were within the ideal indication range.

Natale et al. [12] observed that the application of zinc via soil did not change the levels of macronutrients and Mn in the aerial part of passion fruit seedlings cultivated in a dystrophic Red Latosol. B, Cu and Fe contents decreased and Zn increased with increasing zinc doses.

In the case of soil and foliar zinc applications on eucalyptus seedlings, the highest doses, 20 mg/dm<sup>3</sup> and 20 mg/L, provided an increase in the Fe content in the leaves. In the case of B and Cu, in the seedlings that received only zinc applications via soil, the foliar contents had a reduction in the dose of 20 mg/dm<sup>3</sup>. The seedlings that received foliar applications at a dose of 20 mg/L had an increase in the foliar contents of B and Cu. In the case of Zn, seedlings that received only zinc applications via soil had an increase in the foliar content of the element at the highest dose and seedlings that received applications via foliar had a reduction.

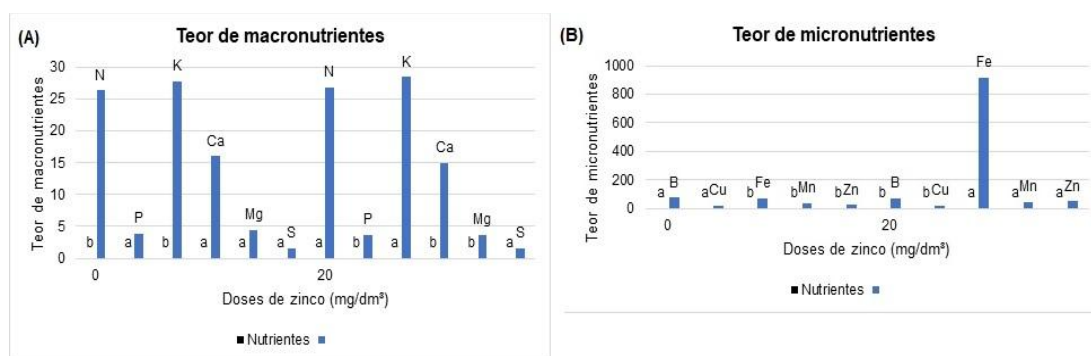
### 3.3 Gas Exchange

Regarding the gas exchange evaluations carried out in seedlings submitted to different doses of zinc via soil, the results obtained did not have significant changes in the internal carbon concentration in the seedlings, in stomatal conductance, in transpiration and in the net photosynthesis rate, Table 6.



**Fig. 5.** Contents of macronutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and micronutrients boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) in leaves as a function of different doses of zinc used via foliar in *E. urophylla* seedlings

Note: Different lowercase letters in the column differ statistically from each other



**Fig. 6.** Contents of macronutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and micronutrients boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) in leaves as a function of different doses of zinc used via soil in *E. urophylla* seedlings

Note: Different lowercase letters in the column differ statistically from each other

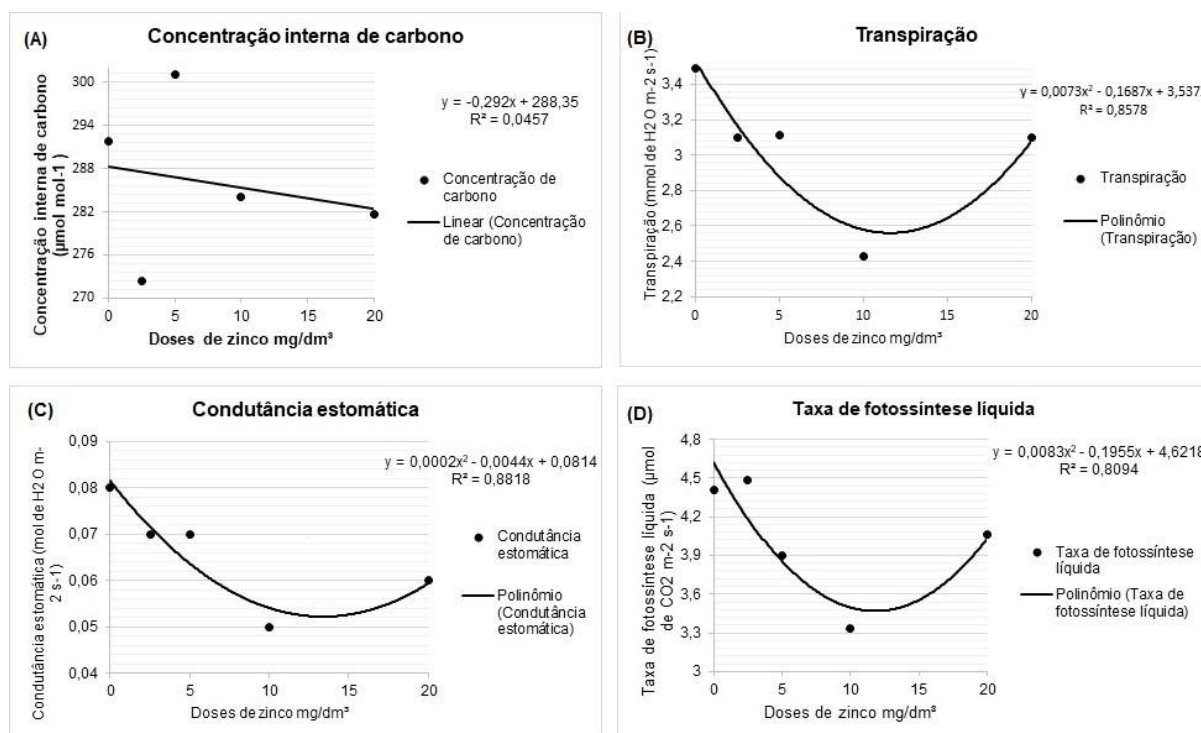
**Table 6.** Internal carbon concentration (Ci), stomatal conductance (gs), transpiration (E), net photosynthesis rate (A) in *Urophylla eucalyptus* seedlings at 80 days, submitted to different doses of zinc via soil

Tratamentos (doses mg/dm <sup>3</sup> )	Ci (μmol mol <sup>-1</sup> )	E (mmol de H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	gs (mol de H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	A (μmol de CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )
0	291,80 a	3,49 a	0,08 a	4,41 a
2,5	272,40 a	3,10 a	0,07 a	4,48 a
5	301,00 a	3,11 a	0,07 a	3,90 a
10	284,00 a	2,43 a	0,05 a	3,33 a
20	281,60 a	3,10 a	0,06 a	4,06 a
Média	286,16	3,05	0,06	4,04
CV%	12,16	39,57	45,30	45,73

Note: Different lowercase letters in the column differ statistically from each other.

Analyzing the graphs obtained through the analysis of variance and by the regression test by the linear and quadratic model, a negative

response to the increase of zinc doses supplied via soil is observed, Fig. 7.



**Fig. 7. Internal carbon concentration (Ci), transpiration (E), stomatal conductance (gs) and net photosynthesis rate as a function of different doses used via soil in *E. urophylla* seedlings**

**Table 7. Internal carbon concentration (Ci), stomatal conductance (gs), transpiration (E), net photosynthesis rate (A) in *Urophylla eucalyptus* seedlings at 80 days, submitted to different doses of zinc via foliar**

Tratamentos (doses mg/L)	Ci ( $\mu\text{mol mol}^{-1}$ )	E (mmol de H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	Gs (mol de H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	A ( $\mu\text{mol de CO}_2$ m <sup>-2</sup> s <sup>-1</sup> )
0	295,60 ba	3,28 a	0,05 a	2,75 a
2,5	285,20 ba	2,50 a	0,05 a	3,31 a
5	274,20 b	2,33 a	0,06 a	4,74 a
10	281,20 ba	1,77 a	0,03 a	1,85 a
20	325,60 a	3,99 a	0,08 a	3,41 a
Média	292,36	2,77	0,05	3,21
CV%	8,62	48,11	52,01	49,97

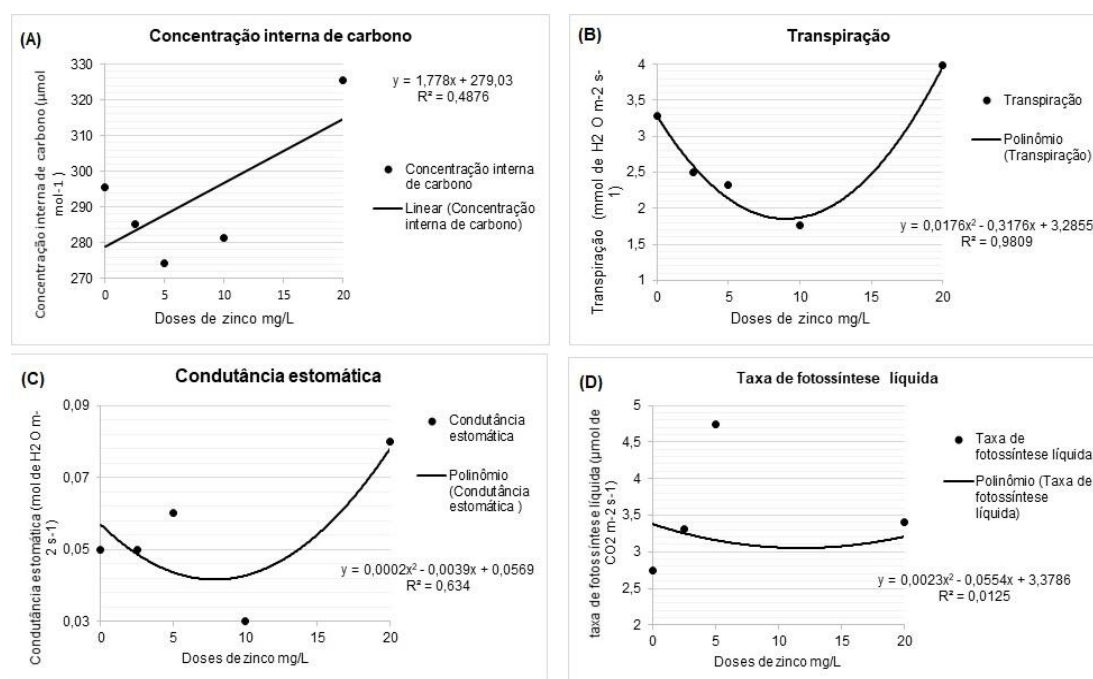
Note: Different lowercase letters in the column differ statistically from each other

In the evaluations of gas exchange performed in seedlings submitted to different doses of zinc via foliar, stomatal conductance, transpiration and net photosynthesis rate did not change significantly when submitted to different treatments, Table 7.

Teixeira et al. [23] in a study evaluating the dry mass and photosynthetic rate of eucalyptus under increasing doses of zinc, observed a reduction in this rate when plants were submitted

to increasing doses of zinc compared to plants with control treatment, but this rate decrease is related to the high doses used, which in this case were 300, 450 and 900 mg/kg<sup>1</sup>. The dose of 900 mg/kg<sup>1</sup> was considered lethal to plants.

The internal carbon concentration showed a significant difference in the results when submitted to different treatments, and seedlings that received the dose of 20 mg/L had higher internal carbon concentrations, Table 7.



**Fig. 8. Internal carbon concentration (Ci), transpiration (E), stomatal conductance (gs) and net photosynthesis rate as a function of the different doses used via foliar in *E. urophylla* seedlings**

According to Taiz and Zeiger [24], the internal concentration of carbon (CO<sub>2</sub>) is important because of its relationship with plant productivity, which can be analyzed as the product of intercepted solar energy and CO<sub>2</sub> captured during a certain period. Higher concentrations of CO<sub>2</sub> show high photosynthetic rates when in the presence of an ideal amount of light and in the absence of stress, such as water deficit, while at very low internal CO<sub>2</sub> concentrations, photosynthesis is limited.

Analyzing the graphs obtained by the analysis of variance and linear and quadratic regression in Fig. 8, it can be observed that, despite not having significant differences in the evaluated data, the internal concentration of CO<sub>2</sub>, transpiration and stomatal conductance had a positive increase in the dose of 20 mg/L in relation to the lowest doses of zinc used. The net photosynthesis rate also had a positive increase at the 5 mg/L dose, even though there were no significant differences in the evaluated data.

#### 4. CONCLUSION

Even though *Eucalyptus urophylla* seedlings did not show statistically significant differences in the data obtained, part of the evaluated variables showed a positive response to the application of

zinc, analyzing the linear and quadratic regression graphs generated, mainly at doses of 10 and 20 mg/L and 10 and 20 g/dm<sup>3</sup>.

*Eucalyptus urophylla* seedlings grown in a substrate with low zinc content may show a positive response to zinc application.

Faced with some variables evaluated which showed a great difference in the averages of the data obtained, but did not have statistically significant differences, the need arises to carry out a new experiment to carry out new evaluations.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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