



## Ultrastructural and physiological aspects of eucalyptus clones submitted to barium

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### Abstract

This work aimed evaluate ultrastructural and physiological aspects of 35-days old eucalyptus, clones 13; 59 and 131, by submitting them to the following barium doses: null, 125; 250; 500 and 1000 ppm of barium solution, with four repetitions. Due to the exposition to barium, there was appearance of phytotoxicity on the leaves. Then, it was determined the following variables: Phloem of Diameter of Leaf and Root (PDL and PDR); Xylem of Diameter of Leaf and Root (XDL and XDR) and Thickness of Palisade Parenchyma (PP); Spad Index (SPADI); Inferior Face of Stomata Functionality of the Inferior Face (IFSF) and Stomach Density of the Inferior Face (SD). Barium negatively influenced the leaf ultrastructure of eucalyptus and its physiology. Eucalyptus clones 13; 59 and 131 are susceptible to barium solution. Barium caused root and ultraestructutal leaves and root damages on eucalyptus clones. Xylem diameter of leaves of eucalyptus clones 13 and 59 were not harmed as exposed to Barium.

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## Introduction

Nowadays, it has been important the use of eucalyptus (*Eucalyptus* sp.) in different programs of reforestation, as the plant has fast growth and good adaptation to different soil and climatic conditions, which guarantees the preservation of native remain forest (Figueiredo *et al.*, 2011).

Eucalyptus has simple and opposite leaves, with entire borders, provided with oil glands (Donato and Morretes, 2006). Its planting may occur in areas that show contamination by heavy metals, such as barium (Ba). Due to the interest in reduction of soil contamination, which guarantees good quality surface water and groundwater, nowadays, reference values were set to the concentration of Ba in agricultural soils (Merlino *et al.*, 2010).

As the plant is exposed to this metal, physiological cells disorders occur that change, consequently, the inner structure of leaves and root cells. (Pookothai and Vijayavathi, 2012; Moraes *et al.*, 2014). On eucalyptus, as exposed to heavy metals, modifications occur in the leaves ultrastructure.

Changes in the anatomy of the plant visible to the naked eye are in fact derived from changes in the structures of the dermal, fundamental or vascular tissues of plants, making it necessary to have in-depth knowledge of these transformations caused by changes in the environment (Raven *et al.* 2014; Taiz and Zeiger, 2017.). The symptomatology is widely used to evaluate the damage caused by biotic or abiotic factors. In this case, structural aspects help in understanding the mechanisms that cause the injuries (Figueiredo *et al.*, 2013).

There is a lack of studies about ultrastructural and physiological aspects of eucalyptus submitted to barium, that way; it is necessary carry out researches in order to show the best use of contaminated by this metal. Therefore, this work aimed evaluates ultrastructural and physiological aspects of eucalyptus clones submitted to barium doses.

## Material and methods

### *Installing the experiment*

The experiment was carried out in September, 2017, at Integrated College Stella Maris (FISMA), Andradina, São Paulo State, Brazil. Uniform eucalyptus plants (*Eucalyptus grandis*) were selected, 35-days old clones 13; 59 and 135. Roots of the plants were transported to polyethylene vases with 5 liters capacity, which contained adapted nutritive solution, as described by Hoagland and Arnon (1950), without barium (Ba). After a 7-days term of adaptation, the treatments were done. The experimental design was completely randomized, with 05 doses of barium: null, 125; 250; 500 and 1000 ppm in solution, with four repetitions. The initial pH was  $4,0 \pm 0,5$ , daily observed.

The first and the second totally expanded eucalyptus leaves were selected from the apex of the plants four days after their exposition to barium. Fragments of the main roots, on their middle part, also were selected.

### *Ultrastructural and physiological analyzes*

The samples were transported to Laboratory of Vegetal Morphophysiology and Forages at College of Agricultural and Technological Sciences – São Paulo State University. The collected material was immersed in F.A.A. 70 (formaldehyde 37%, acetic acid and 70% ethanol in the ratio of 1.0: 1.0: 18.0 - V / V). Twenty four hours after, the fragments were washed and stored in 70% ethanol until the date of the analyzes, as described by Kraus and Arduin (1997). All fragments of plant tissues were treated with the pertinent procedures for dehydration, diaphanization, inclusion and embedding. By using a microtome Leica that contains steel razors, eight- $\mu$ m transversal sections were done in each embedded fragment.

The first transversal sections without damage caused by cut of plants tissues was chosen for preparation of the histological slides. These sections were fixed with patches (albumin), were tinted with safranin with a 1% ratio, and were set in microscope and glass slides with Entellan® patch (Kraus and Arduin, 1997).

All slides were observed with an Olympus optical microscope; model BX 43, with a attached camera in order to perform the photographs of the cuts.

Pictures were used to measure anatomic parameters through the software cellSens Standart that was calibrated with a microscopic ruler in the same gains, as described by Figueiredo *et al.* (2013).

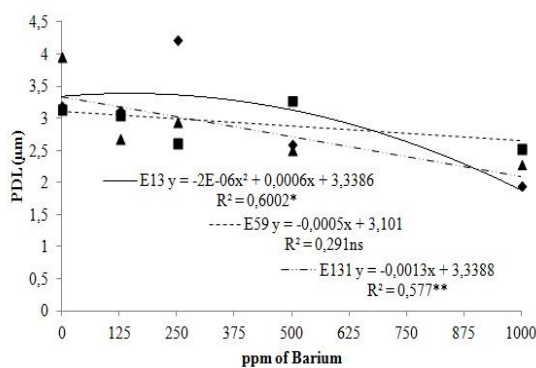
By using transversal sections, the following ultrastructural variables were measured: Phloem Diameter of Leaf and Root (PDL and PDR); Xylem Diameter of Leaf and Root (XDL and XDR) and Thickness of Palisade Parenchyma (PP). The Spad Index (SPADI) was also measured by direct reading in CCM-200 device. The lower or abaxial epidermal impression of the fragments collected using cyanoacrylate ester was used to determine: Inferior Face of Stomach Functionality (IFSF) and Stomata Density (SD) (Figueiredo *et al.*, 2013). Ten measurements were done for all characteristics in each microscope slide. Plots were represented by average value obtained on each characteristic.

*Statistical analyzes*

All variables were submitted to the F test ( $p < 0.05$ ) and the regression analysis was applied to the barium doses, in which their models were tested: linear, quadratic and cubic (Banzatto and Kronka, 2013), by using Assistat 7.7 static software (Silva and Azevedo, 2016).

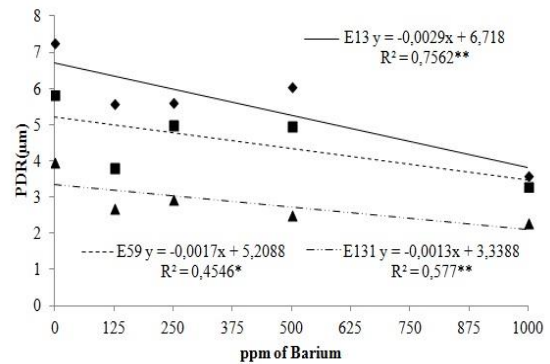
**Results and discussions**

Clone 13 presented a quadratic negative response as submitted to the different barium concentrations, presenting a maximum point of 0.5 ppm. Clone 131, has linear and in clone 59 no harmful effect was found, as exposed to barium exposure, as Fig. 1 shows.



**Fig. 1.** Phloem of Diameter of Leaf (PDL) of eucalyptus clones submitted to barium doses.

In general, the eucalyptus responded negatively to the presence of barium, even at low concentrations, which shows the harmful effect caused by this heavy metal. When the plant is submitted to barium, other damages occurred, as in the variable diameter of the eucalyptus root phloem, which demonstrated a linear negative response to the presence of barium, as Fig. 2 shows.



**Fig. 2.** Phloem of Diameter of Root (PDR) of eucalyptus clones when submitted to barium doses.

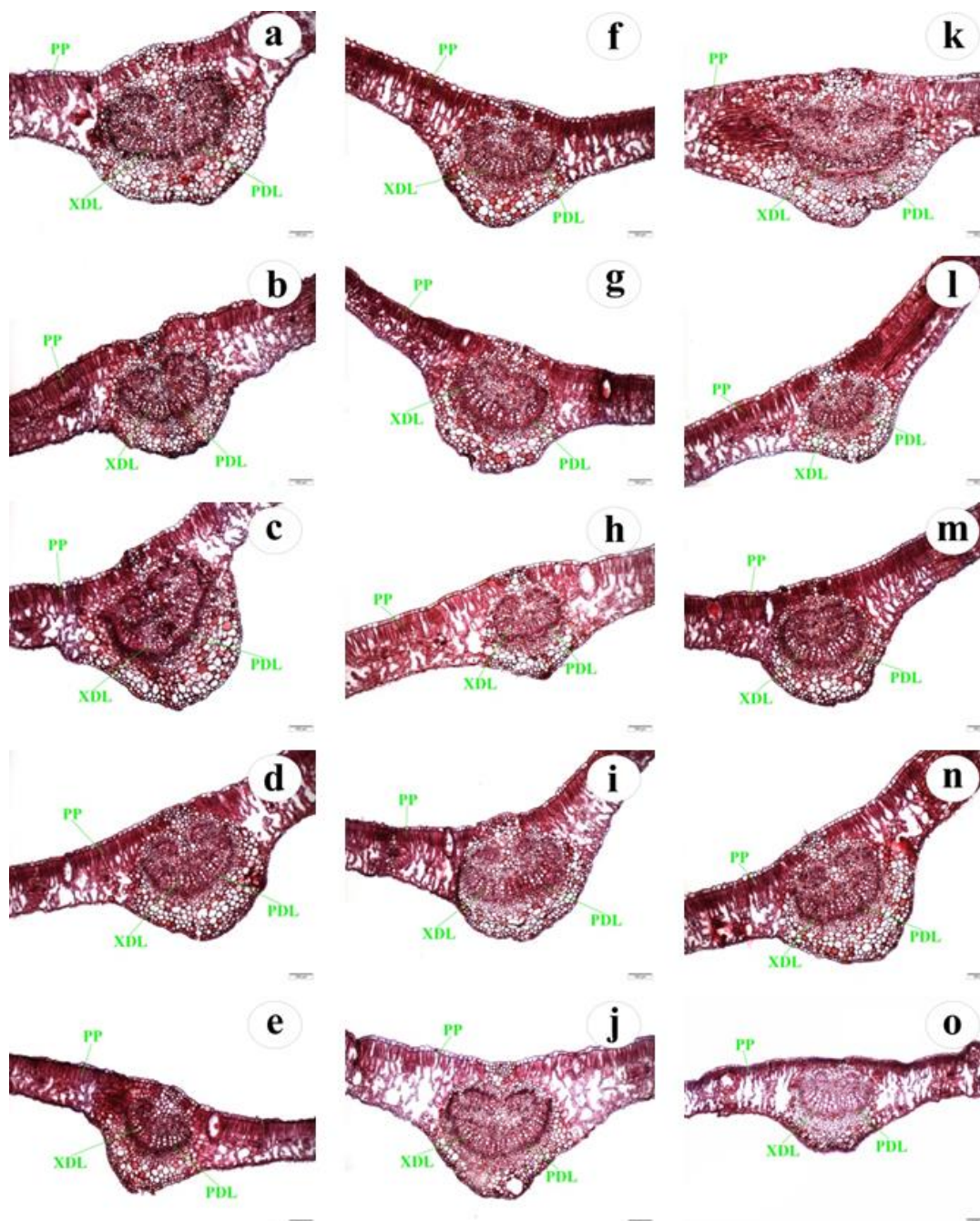
The presence of heavy metals in solution caused damage to the phloem. According to Reis *et al.* (2017), it occurs due to its property in inhibiting the biosynthesis of essential molecules for the development of the vegetable. The authors also cite that nickel has a negative influence in soybean. Barium, even at low concentrations, causes a reduction in the dry mass of the plants, as reported by Merlino *et al.* (2010), who studied the damage caused by these metals in the corn crop.

The phloem is the tissue responsible for the distribution of the photosynthesized molecules to the other organs of the plant. With metals presents in solution, the plant responds negatively, since the plant starts to divert its chemical energy to the synthesis of enzymes that have the capacity to ameliorate the damage caused (Macedo *et al.*, 2014). This phenomenon may have caused the reduction of the phloem in the leaves and root of the eucalyptus, as shown in Fig. 3.

The understanding of the ultrastructural conditions of the roots and its interaction with the soil is fundamental in decisions related to the choice of the best progeny to be implanted in a certain area.

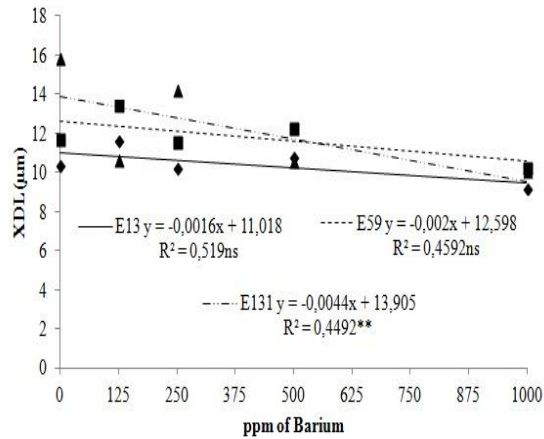
According to Thomas *et al.* (2016), the variability of the roots interferes in the ionic absorption of the metals of

the soil that start to be accumulated in its leaves.



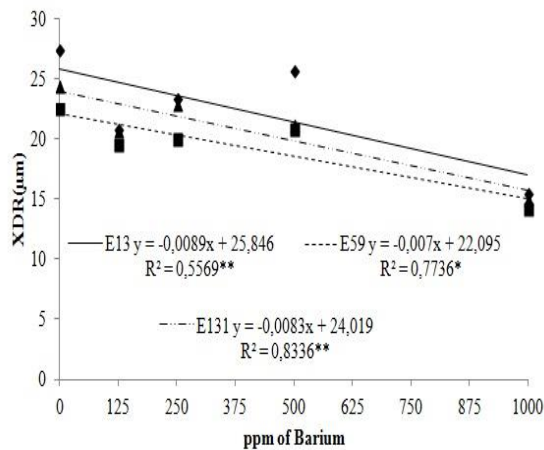
**Fig. 3.** Cross section on the leaves of eucalyptus clones when submitted to barium doses. A - Clone 13 with no barium in solution; B - Clone 13 exposed to 125 ppm of barium in solution; C - Clone 13 exposed to 250 ppm of barium in solution; D - Clone 13 exposed to 500 ppm barium in solution; E - Clone 13 exposed to 1000 ppm barium in solution; F - Clone 59 with no barium in solution; G - Clone 59 exposed to 125 ppm barium in solution; H - Clone 59 exposed to 250 ppm barium in solution; I - Clone 59 exposed to 500 ppm of barium in solution; J - Clone 59 exposed to 1000 ppm barium in solution; K - Clone 131 with no barium in solution; L - Clone 131 exposed to 125 ppm of barium in solution; M - Clone 131 exposed to 250 ppm barium in solution; N - Clone 131 exposed to 500 ppm barium in solution and o- exposed to 1000 ppm barium in solution. PP- Palisade parenchyma; PDL – Phloem of Diameter of Leaf (PDL) and XDL- Xylem of Diameter of Leaf.

Barium accumulation occurred due to the transport, by xylem, of inorganic molecules, which are essential for the biosynthesis of organic molecules in the aerial part of the plant. In barium presence in solution, the leaf xylem was linearly reduced only in clone 131, as Fig. 4 shows.



**Fig. 4.** Xylem of Diameter of Leaf (XDL) of eucalyptus clones submitted to barium doses.

This harmful effect was also observed on root xylem (Fig. 5). All eucalyptus clones displayed, in an accentuated way, a reduction of the xylem tissue.



**Fig. 5.** Xylem of Diameter of Root (XDR) of eucalyptus clones when submitted to barium doses.

The xylem has the ability to transport the inorganic soil solution to the leaves. With the increase in barium concentration in solution, the diameter is reduced due to the toxicity of this element to the plant.

When this element is within the elements of xylem vessels, the plant uses much of its chemical energy for the synthesis of callose from the nucleotide sugar UDP-glucose, which becomes a donor of monosaccharides for its biosynthesis (Buckeridge *et al.* 2000). Callose can be stored in internal walls of xylem vases, which, consequently, decreases its diameter, as well as its translocation capacity. Therefore, due to the reduction of its diameter, it occurs a smaller transport of sap in order to avoid increasing the concentration of barium (Blamey *et al.*, 2015; Santos *et al.*, 2017).

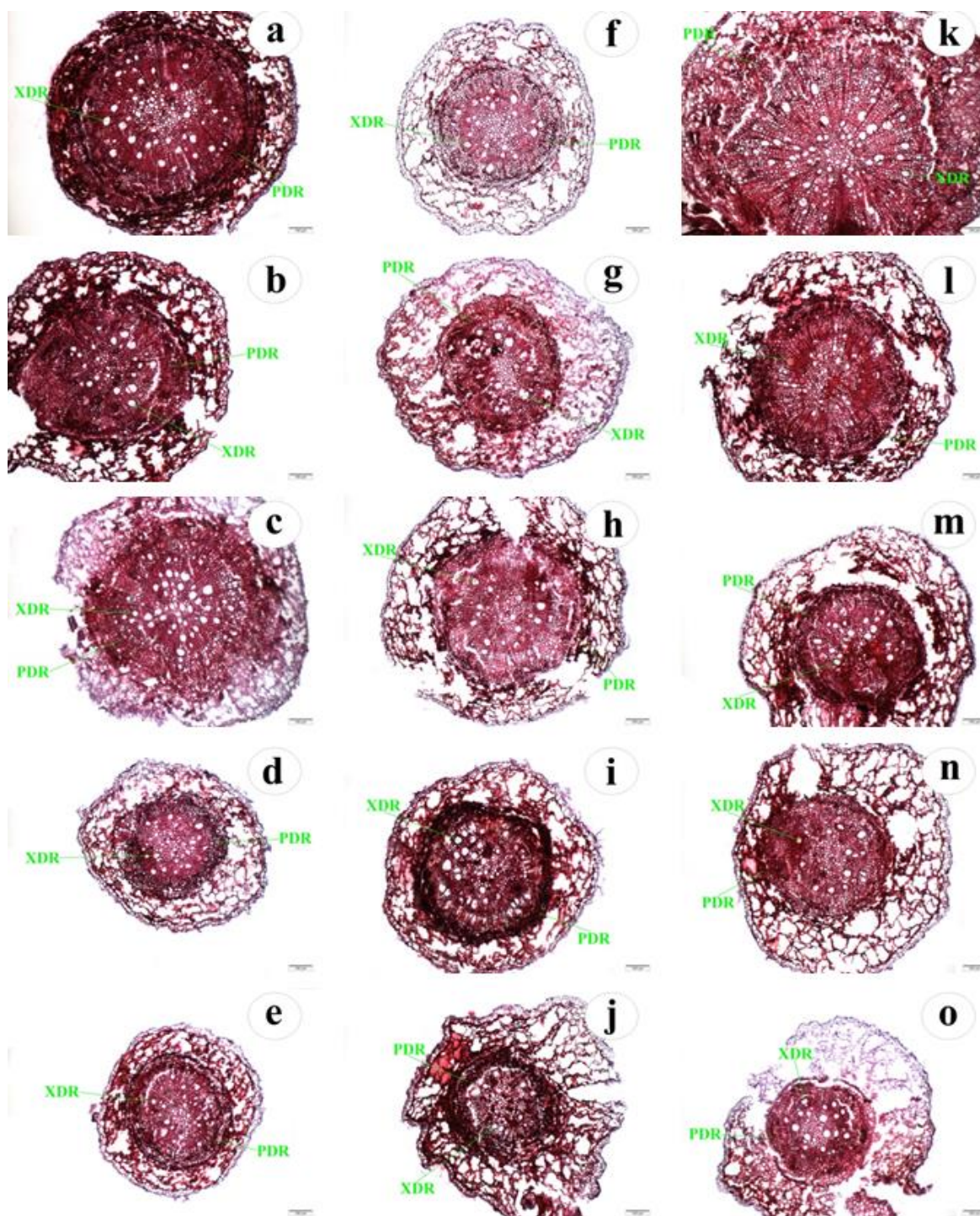
Salazar *et al.* (2012) studied the effects of Cd, Zn and Pb in different areas cultivated with soybean and reported losses of this transport due to the effect of the accumulation of the metals.

The reduction in the diameter of the xylem vessels of roots and leaves has been observed in different species of plants when they came into contact with other heavy metals (Fig. 6), which influences on the development of the plant (Ali *et al.* 2014; Santos *et al.*, 2017) due to the interference with the photosynthetic system of the plant.

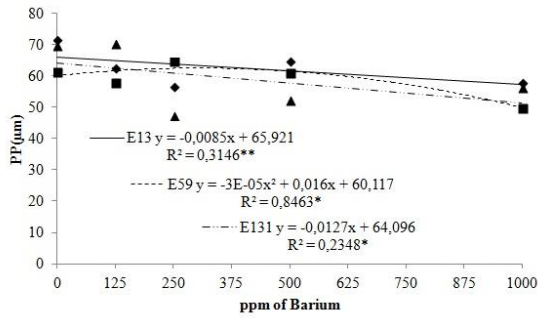
This interference occurs due the storage of metals in the upper part of the vegetables, mainly on its leaves (Macedo *et al.*, 2014; Bertolli *et al.*, 2011; Thomas *et al.*, 2016). Under conditions in which chloroplasts of the palisade parenchyma are affected, there is a decrease in the chemical reactions that compose the photosynthesis (Pookothai and Vijayavathi, 2012; Moraes *et al.*, 2014), corroborating with the results found in this work.

There was a linear reduction in the thickness of the palisade parenchyma in clones 13 and 131, while clone 51 presented a quadratic response when exposed to barium, as shown in Fig. 7.

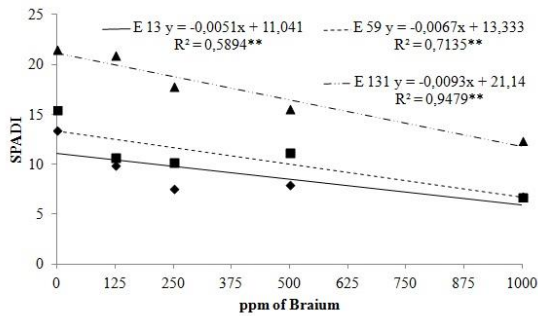
Due to the presence of the metal inside the cells, the color intensity of the leaves was reduced, which could be evaluated by the readings, through the Spad index, in the eucalyptus clones. Under these conditions, the responses were linear in a negative way, as Fig. 8 shows.



**Fig. 6.** Cross section of eucalyptus clones roots submitted to barium doses. A - Clone 13 with no barium in solution; B - Clone 13 exposed to 125 ppm of barium in solution; C - Clone 13 exposed to 250 ppm of barium in solution; D - Clone 13 exposed to 500 ppm barium in solution; E - Clone 13 exposed to 1000 ppm barium in solution; F - Clone 59 with no barium in solution; G - Clone 59 exposed to 125 ppm barium in solution; H - Clone 59 exposed to 250 ppm barium in solution; I - Clone 59 exposed to 500 ppm of barium in solution; J - Clone 59 exposed to 1000 ppm barium in solution; K - Clone 131 with no barium in solution; L - Clone 131 exposed to 125 ppm of barium in solution; M - Clone 131 exposed to 250 ppm barium in solution; N - Clone 131 exposed to 500 ppm barium in solution and O - exposed to 1000 ppm barium in solution. PDR – Phloem of Diameter of Root and XDR – Xylem of Diameter of Root.

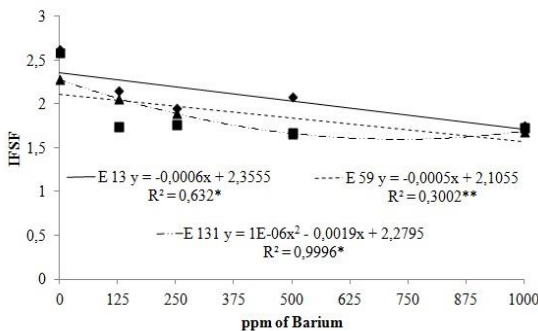


**Fig. 7.** Thickness of palisade parenchyma (PP) of eucalyptus clones when submitted to barium doses.



**Fig. 8.** Spad index (SPADI) of eucalyptus clones submitted to barium doses.

Spad Index has a high correlation with the chlorophyll index on plants as changes occur in the concentration of nutrients on its cells, which can promote interference in the photosystems presents in photosynthesis areas, mainly in leaves (Sharwood *et al.*, 2016; Brahim, 2017). In presence of barium in the inner part of the leaves, stomata suffered a negative influence. To the stomata functionality, as shown on Fig. 9, clones 13 and 59 had a negative linear response as exposed to barium, while clone 131 showed a quadratic response.

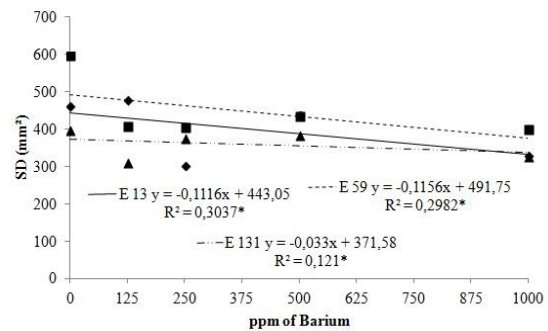


**Fig. 9.** Inferior Face of Stomach Functionality (IFSF) of eucalyptus clones submitted to barium.

Stomata functionality is obtained through the relation between its longitudinal and latitudinal lengths. When one of these lengths has a reduction, the stomata's slit is corrupted; consequently, the gases exchange and transpiration also are negatively influenced. Besides, with the reduction of the stomata functionality, the production of photoassimilates is harmed due to the decrease of the stomata conductivity and, even, the stomata density.

That way, these factors may interfered on liquid photosynthesis in the inner part of eucalyptus leaves (Reis *et al.*, 2017) resulting in a lower development of the leaf tissue. Likewise, this exposition may interfered on structure of the guard cells of the stomata.

The exposition to barium also caused reduction in the total of stomatas presents in the inferior face of the leaves. All the clones showed a linear negative response to barium, as Fig. 10 shows.



**Fig. 10.** Stomata Density (SD) of eucalyptus clones submitted to barium.

Stomata density reduction can harm all the photosynthetic system of leaves, mainly on cells of the palisade parenchyma, as Fig. 7 shows. This occurs due to the lower availability of carbon dioxide in the leaves, which starts to damage the carbon dioxide fixation on biochemical stage of the photosynthesis. As a consequence, there is a lower production of dry mass, besides the reduction of glucose availability, to gain of chemical energy.

**Conclusions**

Barium negatively influenced the eucalyptus foliar ultrastructures and their physiology. The eucalyptus clones 13; 59 and 131 are susceptible to barium in solution.

Barium caused ultrastructural foliar and root damage in eucalyptus clones. Diameter of Leaf Xylem of the eucalypt clones 13 and 59 were not harmed as exposed to barium.

#### Acknowledgement

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