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Iron Toxicity on Germination and Early Growth of *Cecropia hololeuca* Miq.

Toxicidade por Ferro na Germinação e Crescimento Inicial de *Cecropia hololeuca* Miq.

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Abstract

Iron (Fe) is as an essential nutrient for plants and is irreplaceable in many metabolic processes. However, the increase in its concentration leads to the accumulation of reactive oxygen species and oxidative stress that will result in damage to plant. The objective of this work was to verify the effect of high Fe concentrations on germination and initial development of *Cecropia hololeuca* Miq. They were submitted to concentrations of 0.045, 4 and 8mM applied as ferrous sulfate and Fe-EDTA. Germination percentage, germination speed index, shoot length and root, fresh and dry mass were analyzed. For the initial developmental the following variables were analyzed: Leaf area, stem length, root length, fresh and dry mass, chloroplast pigments, chlorophyll fluorescence analysis, analysis of element contents via EDS. The activity of enzymes (SOD, CAT, POX) was verified. Both sources of Fe resulted in damage to germination and development. Fe-EDTA treatment showed the most significant negative effects on germination, root and air growth and seed biomass accumulation. The same treatment was more detrimental in the establishment of young seedlings with decreases in chlorophyll a, root growth, aerial growth, leaf area and biomass accumulation. Seedlings exposed to Fe showed a decrease in photosynthetic performance and a decrease in leaf calcium (Ca) content. No increase in CAT and SOD enzymes activity was observed. POX increased its activity when submitted to 8mM Fe-EDTA. These results show that the species *C. hololeuca* is sensitive when exposed to toxic levels of Fe, causing damage to metabolism and initial growth.

Keywords: Embauba. Stress. Heavy Metal. Toxicity.

Resumo

O ferro (Fe) é um nutriente essencial para as plantas, sendo insubstituível ao metabolismo. Todavia, o aumento em sua concentração leva ao acúmulo de espécies reativas de oxigênio e estresse oxidativo que resultará em prejuízos aos vegetais. O objetivo deste trabalho foi verificar o efeito de elevadas concentrações de Fe na germinação e desenvolvimento de Cecropia hololeuca Miq. A espécie foi submetida às concentrações de 0,045, 4 e 8mM aplicados na forma de sulfato ferroso e Fe-EDTA. Foram analisadas a porcentagem de germinação, índice de velocidade de germinação, comprimento da parte aérea, radicular, massa fresca e seca. Para a análise de desenvolvimento foi analisado a área folhar o comprimento do caule, comprimento radicular, massa fresca e seca, os pigmentos cloroplastídeos, fluorescência da clorofila, e análise dos teores de elementos via EDS. Foi verificado a atividade enzimática (SOD, CAT, POX). Ambas as fontes de Fe resultaram em danos à germinação e desenvolvimento. O tratamento com Fe-EDTA mostrou efeitos mais significativos na germinação, crescimento aéreo e radicular e biomassa das sementes. O mesmo tratamento se mostrou mais prejudicial nas plântulas jovens com quedas na clorofila a, crescimento radicular, aéreo, área folhar e biomassa. As plântulas expostas ao Fe apresentaram queda no desempenho fotossintético e no teor de cálcio (Ca) folhar. Não foi observado aumento na atividade da CAT e SOD. A POX apresentou elevação quando submetida ao Fe-EDTA 8mM. Tais resultados evidenciam que a espécie C. hololeuca se mostra sensível a toxidez por ferro, acarretando danos ao metabolismo e crescimento inicial.

Palavras-chave: Embaúba. Estresse. Metal Pesado. Toxidez.

1 Introduction

Iron (Fe) is an abundant metal in the soil and classified as an essential nutrient for plants, participating in processes such as respiration, photosynthesis, DNA, and protein synthesis (BECANA et al., 1998; JUCOSKY, 2011). Although abundant, most of the Fe found in the environment is in its oxidized form (Fe^{+3}), which is not well absorbed by plants. However, the increased concentration of Fe, emitted mainly due to the extraction and processing that generates Fe ore dust, combined with conditions of change in pH or flooding, increases the chances of absorption of metals, which will cause various damages to the plant (FAGERIA et al., 1990; JUCOSKY, 2011).

Excess Fe is closely linked to increased production of reactive oxygen species, culminating in the establishment of oxidative stress (NEVES *et al*., 2009). Also, contact with high levels of Fe causes damage to the photosynthetic apparatus, with possible loss of pigment content, fluctuations in enzymatic metabolism, chlorotic leaf spots, and drop in growth rate (AHLERT, 2010). Another worrying factor is that Fe does not have biodegradable characteristics and remains in the environment for long periods and causing several disturbances to rivers and riparian forests (FELIPPE *et al*., 2016).

Riparian forests play an important role in soil protection and compaction, as well as in the protection of terrestrial and aquatic fauna (CAMPOS *et al*., 2008). However, this

ecosystem is susceptible to various environmental impacts, whether, of natural origin, such as erosion and sedimentation or of anthropic origin, such as agriculture and mining (BOTREL *et al*., 2002), the latter was responsible in 2015 for supplying more than 40 million cubic meters of iron-rich tailings that invaded and contaminated the riparian forests along the Rio Doce, Brazil (VIANA;COSTA, 2016).

Floristic and phytosociological studies of the riparian forests of the Rio Doce in the states of Minas Gerais and Espírito Santo indicated a great richness and diversity in the distribution of species (ALVES FERREIRA *et al*., 2014; CURTINHAS *et al*., 2015). Among the species studied, *Cecropia hololeuca* Miq. showed abundance and wide distribution in the area studied (ROLIM *et al.,* 2006; CAMPOS *et al.,* 2008; GONÇALVES *et al.,* 2011). *Cecropia hololeuca* Miq. tree is commonly called embaúba and belongs to the Cecropiaceae family. It is a heliophytic species with a height between 6 and 15 meters. It is a fast-growing species, and its fruits are sought by local fauna and is always present in reforestation programs (LORENZI, 2002; CARVALHO, 2003; IPEF, 2017), thus being a species of considerable ecological importance. It is used in the manufacture of wood products (toys, pencils, phosphorus sticks), in civil construction and as a medicinal plant.

At the expense of advances in understanding the metabolic and ecophysiological responses of plants (ALTANGEREL *et al.,* 2017; PANDEY *et al.,* 2017), there is still little data related to specific damage to riparian vegetation caused by high levels of Fe. This work evaluated the effect of high Fe concentrations on germination and initial development of *C. hololeuca.*

2 Material and Methods

Seeds of *C. hololeuca* were harvested in 2017, in the city of Rio Doce (MG) (20°15'16.7"S 42°53'15.6"W), and stored in a cold store chamber (5ºC, 15% relative humidity) until the beginning of the tests, which were carried out in Laboratory of Seeds and Forest Ecophysiology of the Federal University of Espírito Santo in Vitória, ES.

The treatments were two Fe sources - ferrous sulfate and Fe-EDTA using the concentrations - 4mM and 8mM - plus a control treatment (0,045mM) to evaluate the seed germination and initial seedlings development

We used 100 seeds with four repetitions per treatment, and the data obtained were submitted to analysis of variance (ANOVA) and *a posteriori* Tukey test ($p \leq 0.05$). We performed all statistical analyses using Sisvar program (FERREIRA, 2011).

2.1 Germination Test

The seeds were previously disinfected with a 2% sodium hypochlorite solution for 2 minutes and sown in Petri dishes lined with two sheets of filter paper moistened with the solutions corresponding to each treatment (FERREIRA; BORGUETTI, 2005). We placed the plates in a germination chamber at a constant temperature of 25 ºC and a photoperiod of 12h.

The experiment monitoring was daily, considering germinated those seeds showing a radicle protrusion equal to or greater than 2mm (BRASIL, 2009). The trials ended after 14 days. The following parameters were analyzed: germination percentage (%G) and germination velocity index according to (MAGUIRE, 1962), aerial and radicle length (cm) (FORMAGIO *et al.,* 2010) and fresh and dry mass (ECHER *et al.,* 2009).

2.2 Initial Development Analysis

We sowed *C. hololeuca* seeds in 0.5 L polyethylene pots containing 1:1 unfertilized substrate and washed sand, totalizing twenty seedlings per treatment. After 20 days from germination, we submitted seedlings to the Fe treatments using Fe solutions applied via soil. The experiment was carried out in a growth room at 25º C and a 12 h photoperiod. We kept all the seedlings in the Hoagland solution at half ionic strength and pH 5.0 (HOAGLAND; ARNON, 1950).

2.3 Growth Analysis

The seedlings were evaluated at 10 and 20 days after the start of the treatments, using the following growth measures: leaf area, stem length, root length, fresh and dry mass.

2.4 Extraction and quantification of chloroplastid pigments

We determined the chlorophyll a, b, and carotenoid levels on the 20th day after the beginning of the experiment. We used three repetitions for the analyses, each one with 20 mg of fresh foliar mass homogenized in 5 mL of cold acetone (80%). The material obtained was filtered through a funnel and filter paper, and the filtrate was stored in a 10ml volumetric flask, wrapped in aluminum (Al) foil and PVC. The quantification of the pigments was performed by reading the extract in a spectrophotometer at 470, 645, and 662 nm. We determined concentrations according to Lichtenthaler (1987).

2.5 Chlorophyll fluorescence analysis

We quantified the fluorescence of chlorophyll after 20 days of the experiment. We used fully expanded young leaves from 10 plants per treatment and previously adapted to 40 minutes of darkness by leaf clamps for complete oxidation of the photosynthetic system. The results obtained were tabulated in spreadsheets using the PEA Plus v1.11 software. From this analysis, the biophysical parameters that quantify the energy flow through the electron transport chain were calculated using the JIP test (STRASSER; STRASSER, 1995).

2.6 Microscopic Scanning and EDS Analysis

At the end of the experiments, seedlings were taken to

the drying oven and dehydrated at 70 ºC for three days. Later they were sectioned into leaf, stem, and root and covered with gold for analysis by scanning electron microscopy and then analyzed by EDS to detect the elements absorbed by their parts (DEDAVID *et al.,* 2007).

2.7 Antioxidant enzyme activities

We verified the activity of the enzymes superoxide dismutase, peroxidase, and catalase in young seedlings at the end of the experiment. We extracted the antioxidant enzymes using 300 mg of vegetable material homogenized with 0.1 M potassium phosphate buffer (pH 6.8), $EDTANa^2$ 0.1 mM, and polyvinylpolypyrrolidone (PVPP) 1% (w/v). Extractions were performed with liquid nitrogen and the homogenized centrifuged at 12000 xg for 15 min at 4°C. We used the supernatant for the superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) activities. We based the superoxide dismutase (SOD) activity on Del Longo *et al*. (1993), conducting the reaction at 25 ºC in a 15W light bulb chamber. After 6 minutes of exposure, we did the reading at 560 nm. The peroxidase activity (POX) was measured based on Kar (1976), with reaction at room temperature for 2 minutes followed by reading at 420nm; and the catalase activity (CAT) followed Anderson *et al.* (1995) protocol, performed at room temperature with reading at 240nm for 2 minutes. We used three repetitions with duplicates in an entirely random design (EDS) (PEIXOTO *et al.,* 1999).

3 Results and Discussion

In this study, we verified the effects of high Fe levels on *C. hololeuca* germination and initial growth. The results showed that exposure to this stress caused disturbances in seed metabolism. Although the metal did not affect germination velocity, it significantly reduced seedling shoot size in the most toxic treatment (8mM EDTA) and germination percentage, root growth, fresh and dry mass in all other treatments. The decrease in dry mass accumulation was 50% in the roots and 83% in the shoots when treated with 8mM Fe-EDTA, compared with the control. In the same 8mM treatment, it was verified an 80% fall in root size and a germination percentage of only 6%, compared to 84% of control seeds (Table 1).

Table 1 - Effects of Fe stress on germination percentage (% G), germination speed index (IVG), root growth (CR), shoot growth (CPA), fresh root mass (MFR), fresh mass of shoot (MFPA), dry root mass (MSR) and dry shoot mass (MSPA) of *C. hololeuca* seeds.

Treatment	%G	IVG	CR(cm)	CPA (cm)	MFR(g)	MF PA(g)	MSR(g)	MS PA(g)
Control	84a	1,8a	0,88a	0,33a	0,0029a	0,0039a	0,0008a	0,0018a
$Sulfate^{+2}$ 4mM	16b	1,5a	0,72b	0,30a	0,0022b	0,0032ab	0,0006b	0,0010ab
$Sulfate^{+2}$ 8mM	12b	1,17a	0,74b	0,35a	0,0017bc	0,0018abc	0,0004b	0,0005b
EDTA 4mM	14b	1,16a	0,2c	0,25ab	0,0011cd	0,0013bc	0,0004b	0,0005b
EDTA 8mM	6c	1,09a	0,15c	0,17b	0,0009d	0,0007c	0,0004b	0,0003b

CV (9,52%)

Different letters in the same column indicate significant difference between treatments ($p \leq 0.05$, tukey test).

Source: Research data.

Reductions in germination potential and seedling establishment are closely related to the Fe content applied to the seeds. Although metal is essential to the germination process, even contributing to overcoming plant dormancy according to Murgia and Morandini (2017), Fe above the optimum average concentration for vegetables (0.9mM) is an element that can negatively affect the seed germination reported El Rasafi *et al.* (2016). Working with heavy metal stress, especially Fe, on wheat and bean germination, these authors found that at the concentration of 750 mg L^{-1} (~ 5mM) of Fe, wheat germination percentage was severely affected. We observed a significant decrease in root and aerial growth when compared to control treatment. Verma and Pandey (2017) studied the germinative behavior of seeds of the genus *Vigna* (Fabaceae). Their results showed that an increase in Fe concentration caused a decrease in germination percentage, as well as in aerial part and root growth, measured through the mass fresh and dry. We verified similar results in this study. Fe can affect the uptake and transport of water necessary for seed imbibition, which causes permanent damage to the embryo, according to Li *et al.* (2005) and Bautista *et al*. (2013). In its reduced and/or chelated form, plants absorb Fe readily. However, if in excess, it can damage membrane structures, DNA and proteins, due to the accumulation of reactive oxygen species (ROS) as reported by Nagajyoti *et al.* (2010).

In this study, the roots were more sensitive to Fe exposure than the aerial part. Similar results were found by Lingua *et al.* (2008) when working with species of the genus *Phaseolus* (Fabaceae) and *Triticum* (Poaceae). The fact that the root is most affected may be related to plant metabolism itself, as it is the first organ to emerge and thus come into contact with the contaminant. Also, through the deterioration of plant structural components, Fe stress has negative consequences on cell division and cell wall expansion, processes that are most active in primary root growth and seedling emergence (YUSUF *et al*., 2011). After causing morphophysiological damage, Fe toxicity then leads the plant to, according to Nenova (2006) and Mehraban *et al*. (2008), lose biomass, mainly by depositing in mitochondrial ridges, causing a decrease in respiratory rate and a delay in the development.

C. hololeuca seedlings exposed to toxic Fe levels after

germination showed an increase in POX enzyme activity only in the 8mM Fe-EDTA treatment when compared to the control. There was no significant difference in CAT and SOD activity (Table 2).

Table 2. Effects of Fe stress on the activity of the enzymes superoxide dismutase (SOD), Total Peroxidase (POX) and catalase (CAT) in *C. hololeuca* seedlings

Treatment	SOD (unid. SOD g-1 MS)	CAT (µmol H2O2 min-1 g -1 MS)	POX (µmol POX min-1 g -1 MS)
Control	2,39a	0,91a	1,50a
Sulfate^{+2} 4mM	2,36a	1,23a	1,11a
Sulfate^{+2} 8mM	2,73a	2,74a	1,81a
EDTA 4mM	2,26a	3,14a	1,10a
EDTA 8mM	2,23a	3,47a	3,57b

CV (12,18%). Different letters in the same column indicate significant difference between treatments ($p \leq 0.05$, tukey test).

Source: Research data.

Fe is an element known for its pro-oxidative effects through the generation of several ROS and activator of the plant antioxidant system as stated by Stein *et al.* (2009). With the increase of ROS in cells generated by Fe accumulation, the first line of defense of the plant is the increase of SOD activity, an enzyme capable of disputing two superoxide radicals (O^{2-}) in molecular oxygen and hydrogen peroxide (H_2O_2) (SINHA;SAXENA, 2006). The generated H_2O_2 is also toxic to the cell and must be eliminated. This process is performed by CAT and/or POXs, which act preferentially on peroxisomes and chloroplasts, respectively. Besides, POXs are related to plant growth with direct action on the cell elongation process, by transforming H_2O_2 into OH^-, an important oxidizing agent in lignin formation as reported by Maia *et al.* (2012).

In this study, the CAT and SOD expressions were unchanged, and the POX enzyme had a significant increase only in the 8mM Fe-EDTA treatment. These results differ from several studies showing the positive correlation between Fe stress, increased oxidative stress in plants, and increased antioxidant enzyme activity as it was found in Pereira (2006), Jucosky (2011) and Adamski (2011). The increase in POX expression in response to Fe has been observed by several authors like Carli (2008) (*Ipomea pes-caprae*) (L.) R.Br., Kuki *et al*. (2008) (*Schinus terebinthifolius*) Raddi, and Xing *et al*. 2010 (*Spirodela polyrrhiza*) (L.) Schleid. It´s elevation has been linked to a certain degree of metabolic disturbance in chloroplasts, the site of the highest activity of POX enzymes and Fe allocation in the leaves, being the organelle that will most quickly suffer adverse effects under stress (SPEROTTO *et al.,* 2010).

Del Longo (1993) reported that its activity has been concurrent with several other enzymes, especially SOD and CAT. However, we did not verify this in present work, which possibly indicates a non-increase in ROS levels and oxidative stress in *C. hololeuca* seedlings, when subjected to high Fe content. Possibly, the species has its strategies to deal with oxidative damage, which can be neutralized through non-enzymatic antioxidants. This kind of process was already observed in the studies of Pekker *et al*. (2002) and Fourcroy *et al*. (2004), working with *Phaseolus vulgaris* L. and *Arabidopsis* (Brassicaceae) genus, in which treatment with glutathione, an efficient non-enzymatic antioxidant, resulted in reduced gene expression of antioxidant enzymes in seedlings exposed to high levels of Fe.

After analyzing the pigment content, a decrease in the chlorophyll content was observed in all treatments, except Fe^{+2} 4mM, compared to the control. The levels of chlorophyll b and carotenoid did not change (Table 3).

Table 3. Effects of Fe stress on chlorophyll a, b and carotenoid concentrations of *C. hololeuca* seedlings

Treatment	Chlorophyll*a* (mg g^{-1} MS)	Chlorophyll*b* (mg g^{-1} MS)	Carotenoids (mg g^{-1} MS)
Control	4,40 a	2,38a	1,32 a
Sulfate^{+2} 4mM	5,01a	2,96a	1,35 a
Sulfate^{+2} 8mM	2,01b	2,33a	1,01 a
EDTA 4mM	2,15b	2,09 a	0,98 a
EDTA 8mM	2,19b	2,89a	1,21a

CV (8,3%). Different letters in the same column indicate significant difference between treatments ($p \leq 0.05$, tukey test).

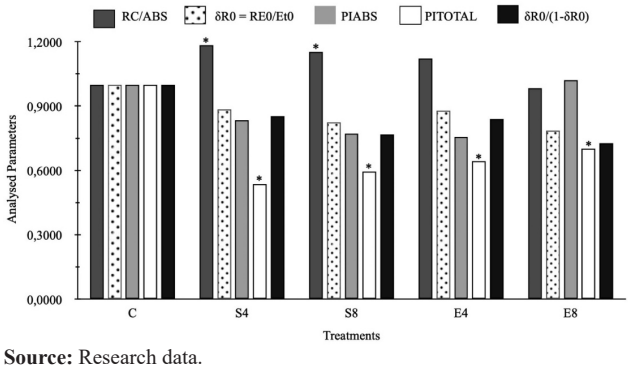
Source: Research data.

We did not observe visible chlorosis on the leaves, but it is known that Fe stress causes a reduction in the concentration of chloroplastic pigments through their binding to several enzymes, which causes their inactivation. Furthermore, Fe can affect the reduction of various compounds in the biosynthesis route of the pigments and has an exceptional power to inhibit protochlorophyllide reductase enzyme, which performs the conversion of protochlorophyll into chlorophyll (CHANDRA; KANG, 2016). In species of Fabaceae and some Poaceae such as rice, the accumulation of Fe caused a decrease in the concentration of chlorophyll and carotenoids which was highlighted by Mehraban *et al.* (2008) and Arunachalam *et al.* (2009). However, the chlorophyll b and carotenoid values of *C. hololeuca* seedlings were not affected by this metal, which shows that, in the species in question, Fe stress may be directed to the main route of light uptake and not to the accessory pigments. Streit *et al.* (2005) found, in the same pathway, that Fe through its binding power to the sulfhydryl group of proteins, can generate damage by inactivating the enzymatic mechanism responsible for the conversion of chlorophyll b (which remained unchanged) into chlorophyll a, namely, the enzyme chlorophyll a oxygenase.

We can also observe that magnesium, one of the essential elements in the formation of the chlorophyll molecule and strongly inhibited in the presence of Fe, according to Zhang *et al.* (2018), has not changed its concentration among the evaluated treatments (Supplementary file), which corroborates the role of Fe as an enzyme inhibitor in chloroplastid pigment biosynthesis. The analysis of the effects of high Fe levels

on photosynthetic variables by the JIP test showed that the density of photosynthetic FSII reaction centers (RC/ABS) showed a significant increase only in the treatment with ferrous sulfate when compared to the control (Figure 1). There was no difference between the efficiency with which electrons move from the inter-system receptors to the final FSI receptors (δR0) or a decrease in the performance of the FSI oxy reduction reactions (δR0 / (1-δR0) or a deficiency in the FSII. However, the analysis of the total performance index (PITOTAL) shows a decrease in the overall photosynthetic performance of the plant (Figure 1).

Figure 1 - Effects of ferrous stress on RC / ABS, δR0 = RE0 / ET0, PIABS, PITOTAL and δR0 / (1-δR0) of *C. hololeuca* seedlings. (C): Control (S4): Fe^{+2} 4mM (S8): Fe^{+2} 8mM (E4): FeEDTA 4mM (E8): FeEDTA 8mM. Asterisk indicates significant difference between mean and control. ($p \leq 0.05$, Tukey test).



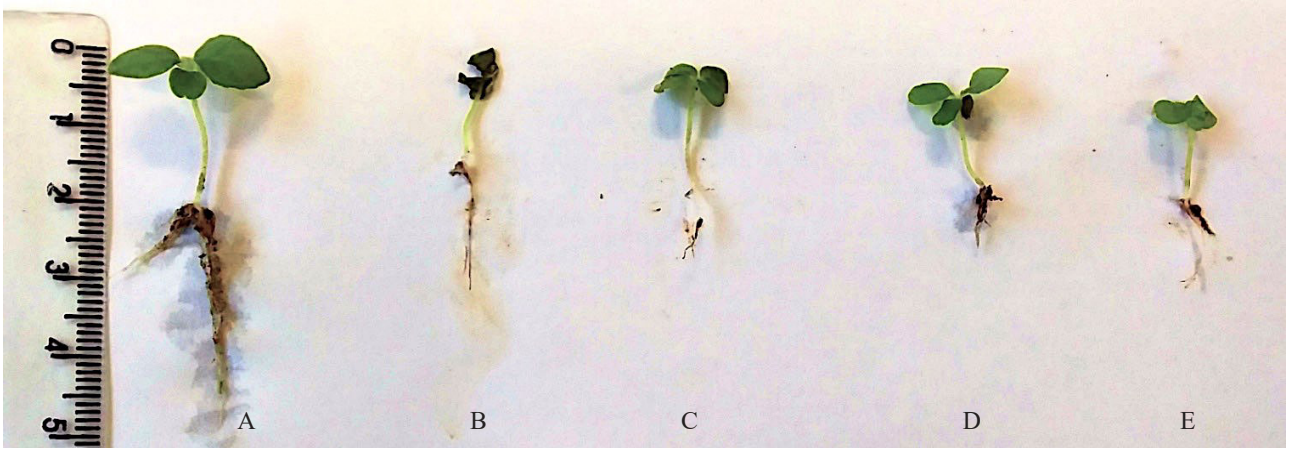
Source: Research data.

Fe is the most requested essential micronutrient in plant metabolism, acting as a co-factor in photosystems I and II, in cytochrome b6f and as a structural agent of subunits of the LHC complex. However, Pinto *et al.* (2016) highlights that in excess, it is known to negatively affect photosynthesis. According to Kalaji *et al*. (2017), among the steps in this process, the most easily affected by stress are those related to FSII due to the ease of D1 protein degeneration. RC/ABS shows the density of active FSII reaction centers, and PIABS represents all FSII activity and is commonly used as an indicator of plant vitality. δR0 and δR0/(1-δR0) represent the performance and efficiency of electrons in the oxi-reduction reactions that will culminate in the contribution of electrons to the FSII. The PITOTAL represents the total photosynthesis performance index (PSII + PSI) covering all stages of the electron transport chain and is described as the most sensitive and essential parameter of the analysis (CHEN *et al.,* 2017).

The damage caused by high amounts of Fe has little influence on the efficiency of oxi-reduction reactions of electrons migrating to the FSI conforming to Santos Junior (2018). However, the increase in density of the FSII reaction centers (RC/ABS) in plants treated with ferrous sulfate may indicate an attempt by the plant to increase the absorption and capture of electrons in order to optimize the energy process. Galazzi (2011) and Santos Junior (2018) found similar increases in *Jatropha curcas* L. plants stressed by abiotic factors, especially Fe accumulation. The drop in chlorophyll a and PITOTAL content in both Fe sources, combined with results showing that FSII activity was not affected, probably reflects damage in the FSI. Araújo (2012) found similar results for the species of the genus *Passiflora* (Passifloraceae) exposed to Al and so did Freitas (2018) when working with *Mangifera indica* L., cv. Rosa, exposed to Fe. FSI is more protected from heavy metal damage than other membranous structures, indicating a lower probability of photoinhibition/oxidation than FSII. However, according to Strasser *et al.* (2010) and Tsimilli-Michael and Strasser (2013) the impairment of the FSI is a sign that the photochemical reactions of photosynthesis have been severely affected, which will lead to low final electron assimilation and a drop in the formation of reducing compounds, $NADPH_2$ and ATP, also bringing damage to the carboxylation phase of photosynthesis with a consequent drop in the sugar rate and growth.

When checking the growth of seedlings submitted to high Fe levels, a visible reduction in both root size and the aerial part was observed already at ten days (Figure 2).

Figure 2 - Appearance of *C. hololeuca* seedlings 10 days after starting Fe treatment. (A): Control plant (B): Fe^{+2} 4mM (C): Fe^{+2} 8mM (D): FeEDTA 4mM (E): FeEDTA 8mM



Source: Research data.

We observed a reduction in root and stem growth, as well as in leaf area in all treatments, and Fe in its chelated form promoted the most significant damage with significant reductions in practically all variables (Table 4). We observed that treatment with Fe was dose-dependent, causing decreases of 54% in root growth, 46% in stem height, and 80% in leaf area at day 20 in the Fe-EDTA 8mM treatment when compared to control. The production of dry matter from the roots was compromised in the two temporal evaluations (10 and 20 days) for chelated Fe. In the aerial part, the damage was verified only at the end of the experiment for both sources of the contaminant, with Fe in the Fe-EDTA 8mM treatment causing a 75% difference in dry mass after 20 days (Table 4).

Table 4 - Effects of Fe stress on root growth (Root), stem growth (Stem), leaf area (AF), fresh root mass (MFR), fresh shoot mass (MFPA), dry root mass (MSR) and shoot dry mass (MSPA) of *C. hololeuca* seedlings.

Treat	Root (cm)		Stem (cm)		AF (cm^2)		MFR (g)	
	10d.	20d.	10d.	20d.	10d.	20d.	10d.	20d.
Control	2,64a	2,89a	1,48a	2,34a	0,65a	1,73a	0,036a	0,047a
$Sulfate^{+2}$ 4 mM	0,99b	2,11b	1,02b	1,69b	0,42b	0,9b	0,012b	0,016bc
$Sulfate^{+2}$ 8 mM	1,08b	2,08b	1,15ab	1,70b	0,24bc	0,79b	0,0048b	0,02b
EDTA 4 mM	0,95b	1,65c	1,06b	1,36c	0,26bc	0,43c	0,0088b	0,0090cd
EDTA 8 mM	0,94b	1,33c	1,24ab	1,28c	0,21c	0,35c	0,0063b	0,0063d
CV(10,23%)								
	MSR (g)		MFPA (g)		MSPA (g)			
	10d.	20d.	10d.	20d.	10d.	20d.		
Control	0,0049a	0,013a	0,031a	0,068a	0,0023a	0,0075a		
$Sulfate^{+2}$ 4 mM	0,0047ab	0,005b	0,010b	0,051b	0,0026a	0,0039b		
$Sulfate^{+2}$ 8 mM	0,0026abc	0,0028c	0,010b	0,040b	0,0030a	0,0038b		
EDTA 4 mM	0,0024bc	0,0025c	0,007b	0,020c	0,0022a	0,0026c		
EDTA 8 mM	0,0021c	0,0022c	0,0062b	0,016c	0,0018a	0,0019c		
CV(10,23%)								

Different letters in the same column indicate significant difference between treatments ($p \leq 0.05$, tukey test).
Source: Research data.

Several studies have highlighted the harmful effect of Fe stress on plant growth. Jucoski (2011) working with the accumulation of different Fe concentrations under the growth of *Eugenia uniflora* L., noticed a great decrease in leaf number, root size, and biomass allocation. Adamski (2011), when submitting *Ipomoea batatas* (L.) Lam plants to concentrations of 4 and 9 mmol of Fe, observed a decrease in the length of branches, leaf area, dry mass, and the number of roots of the specimens. Other results include a decrease in plant height, accompanied by a drop in productivity and ultimately plant death as described by Schmidt *et al.* (2013). We can correlate Fe with the decrease in growth rate, at first, by the damage caused to the biochemical metabolism due to the accumulation of oxidizing substances. However, other pronounced effects include disturbances in nitrogen and carbohydrate metabolism, changes in plasma membrane fluidity with consequent lipid peroxidation, protein inactivation in the membrane itself and damage to the structure of several cytoplasmic organelles, especially the mitochondria, which will culminate in cell overflow (RODRIGUES *et al.,* 2016).

We found, as for the assimilation of elements, that the roots had different concentrations of assimilated Fe. The treatment with Fe^{+2} 4mM was the one that achieved the highest percentage of accumulation (11.91 %), whereas Fe-EDTA 4mM had an accumulation of 5.56%. Also, we observed that, with the increase in Fe concentration of both sources used, there was a decrease in root accumulation, with Fe^{+2} 8mM composing 7.96% of the weight in the root and Fe-EDTA 8mM only 1.18%, indicating a tendency of Fe allocation in the aerial part when it reaches high concentrations (Supplementary File). In plants, the distribution of macro and micronutrients depends basically on transport via xylem and phloem. As studied by Stein *et al*. (2009) and Morrissey and Guerinot (2009) due to Fe low mobility, it encounters limitations for translocation to the aerial part (about 14%), and roots retain it extensively, with almost 75% of its content linked to the apoplast. Thus, with Fe saturation in the roots, its transport to the leaves would be deficient due to the rapid binding to chelating compounds, mainly ferritin, reducing its activity (DOS SANTOS *et al.,* 2011). However, in this study, there was an increase in Fe transport from the root at concentrations of 8 mM. This result is in agreement with Tripathi *et al*. (2018), who in their study, noticed that in very high concentrations of Fe (8mM), the plant presents the strategy of translocating the metal to the aerial part, especially the young leaves, which would balance the toxicity, not concentrating only on the root, primary target of stress.

Plants treated with Fe in its chelated form showed a 40% reduction in foliar calcium content (Supplementary File). Fe in high doses is known as a potent unbalancing agent of the metabolism of various elements, especially magnesium, phosphorus, potassium, and Ca conforming to Tanaka *et al.* (1966) and Zhang *et al.* (2018). Along the evolutionary course, plants developed several strategies to balance the

entrance of Fe in their tissues, avoiding competition with other ions. Following Morrissey and Guerinot (2009), the main mechanisms are related to the modification of the environment around the root epidermis, via acidification of the habitat by releasing protons, which will allow differences in the solubility of the element. Another strategy adopted is morpho-anatomic changes, mainly in leaves and roots by ensuring an adaptation to prevent damage caused by the absorption of high levels of Fe. The element will still be present in the soil, but almost exclusively in its oxidized and not assimilable form. The oxidation of Fe will initiate the formation of Fe plates that can, even if indirectly, harm plant growth. According to Liu *et al.* (2007), because the plate presents physical-chemical characteristics similar to the Fe oxides found in the environment, which can affect the absorption of other elements through adsorption or co-precipitation so that it may explain the decrease in the foliar Ca rate and consequently a decrease in growth. Muller *et al*. (2015) found similar results in other plants when submitted to different forms of Fe.

4 Conclusion

Fe in concentrations of 4mM and 8mM affects the germination process of *C. hololeuca* seeds, generating a drop in the percentage of germination, growth, and accumulation of biomass. The activity of the enzymes SOD and CAT was not sensitive to Fe toxicity, whereas only POX had its expression increased in *C. hololeuca* seedlings when exposed to 8mM concentration, which does not show significant oxidative stress.

Concentrations of 4mM and 8mM affect the initial development at 10^{th} and 20^{th} days evaluations, with a drop in root and aerial growth, leaf area, and biomass accumulation. Fe can negatively affect the chlorophyll a content, and the photosynthesis of *C. hololeuca* presented significant damage to the PSI, as well as a drop in the general performance of the plant. Fe in high concentrations presents allocation capacity in different parts of the seedling, and in its chelated form, showed interference in Ca metabolism.

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