Physiological and biochemical changes in tree seedlings in an urban forest soil contaminated with copper

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Abstract

Context Soil pollution by heavy metals is a worldwide environmental concern. Owing to their proximity to anthropogenic emission sources, urban forest fragments are highly affected by the excessive input of heavy metals into the soil.

Objectives This study aimed to assess the physiological and biochemical responses of two native Brazilian Atlantic Forest *Schinus terebinthifolia* Raddi (pioneer species) and *Eugenia uniflora* L. (non-pioneer species), when cultivated in soils contaminated with Cu.

Methods Plants were cultivated in soils of an urban forest fragment contaminated with 0 (control), 60, 120, 180 or 240 mg Cu kg^{-1} soil. Growth variables, Cu content in plant tissues, translocation index, bioaccumulation factor, pigment contents, leaf gas exchange and chlorophyll fluorescence were all measured to assess physiological alterations resulting from copper stress, while the enzymatic (superoxide dismutase) and nonenzymatic (ascorbic acid and glutathione) antioxidants were quantified to assess the biochemical responses of the species.

Results Both species presented high uptake and accumulation of Cu in roots with low translocation rates to shoots; however, *S. terebinthifolia* showed higher Cu restriction in roots than *E. uniflora*. *S. terebinthifolia* and *E. uniflora* showed distinct responses in growth and leaf gas exchange. The species showed neither difference in enzymatic contents nor oxidative reduction.

Conclusion The restriction of copper in roots appears to be the principal protective mechanism against copper phytotoxicity, preventing negative effects on the physiological and biochemical status of the species. *S. terebinthifolia* shows potential as a Cu phytostabilizer, while the *E. uniflora* has potential as a Cu phytoextractor.

Introduction

The atmospheric deposition of heavy metals in urban forests affects human quality of life since these forests conserve biodiversity and provide ecosystem services, acting, for example, as a buffer against air pollution (Alarsa et al. 2018). In urban areas, the emission of atmospheric particulate matter via industry and automobiles may impose risks to urban forest fragments by increasing heavy metal concentrations in soils and other ecosystem compartments (Ferreira et al. 2019, Nakazato et al. 2021). The excessive input of heavy metals into the soil leads to bioaccumulation since these elements are not degraded by physical, chemical or biological processes (Mishra et al. 2019).

Copper (Cu) is a heavy metal that is both a contaminant and a micronutrient required by plants, playing a fundamental role in several physiological and biochemical processes necessary for plant growth and normal development (Shabbir et al. 2020). The main anthropogenic sources of Cu are mining, refining, waste incineration, sewage effluent, continuous use of fertilizers/pesticides, industrial waste and fossil fuel combustion (Kumar et al. 2020). High concentrations of Cu in soils can cause toxic effects on plants, including reduced growth rate (Singh et al. 2016), negative impact on physiological functions (Zaouali et al. 2020) and increased reactive oxygen species (ROS) (Zeng et al. 2019).

Species tolerant to the toxicity of contaminated soil are indispensable for vegetation restoration in contaminated areas since they can grow and develop in spite of these limiting factors (Silva et al. 2011). Some species have acquired the ability to tolerate heavy metals and can flourish in contaminated soils, finding a use in

phytoremediation for removing/retaining contaminants (Vendruscolo et al. 2018). Tree species native to the Atlantic Forest display favorable characteristics for phytoremediation of soils contaminated with heavy metals as a result of their dynamic growth, high biomass production and the presence of adaptive mechanisms to new environments (Caires et al. 2011). Current studies on physiology and biochemical responses, as well as the possibility of using plants in phytoremediation for copper-contaminated soils, are frequently carried out with conventional agricultural/crop species (Saleem et al. 2020; Zaouali et al. 2020).

Pioneer and non-pioneer trees native to the Atlantic Forest have distinct strategies to tolerate environmental stressors, such as high levels of heavy metals in soils (Brandão 2017). Non-pioneer tree species seem to have a greater ability than pioneer species to tolerate and, hence, bioaccumulate the current panorama of contamination and bioavailability levels of heavy metals in Atlantic Forest remnants (Nakazato et al. 2021). On the other hand, pioneer species frequently have higher photosynthetic rates, higher biomass accumulation and lower pigment content than non-pioneer species (Calzavara et al. 2019; Macieira et al. 2020). However, specific responses to excess copper in the soil are still poorly understood in tree species native to tropical ecosystems, especially the Atlantic Forest. Based on the available knowledge, we hypothesized that high Cu concentrations in the soil of urban Atlantic Forest remnants cause more physiological and biochemical changes in non-pioneer tree species, resulting in reduced growth and development, compared to pioneer tree species. Therefore, the present study aimed to experimentally compare physiological and biochemical responses of pioneer and a non-pioneer tree species cultivated in soil from an urban forest fragment contaminated with increasing copper concentrations.

Material And Methods

Initial characterization of plants and soil

Seedlings of a pioneer tree species (*Schinus terebinthifolia* Raddi) 46.2 cm in height, 4.8 mm in stem diameter and with 0.33 g of leaf DW, 1.67 g of stem DW and 0.83 g of root DW and a non-pioneer tree (*Eugenia uniflora* L.) 46.7 cm in height, 4.7 mm in stem diameter, and with 0.66 g in leaf DW 2.62 in stem DW and 2.18 g of root DW were used in this experimental study. The natural soil used in the experiment (Table 1) was classified as acidic (pH < 7.0), eutrophic (V > 50%), and loamy, presenting high organic matter levels and consisting of highly weathered mineral material (Nakazato et al. 2021). The soil was collected at a depth of approximately 40 cm in the Atlantic Forest fragment inside the Parque Estadual Fonte dos Ipiranga (PEFI) located in São Paulo City, São Paulo State, Brazil.

Experimental conditions and design

The experiment was carried out in a greenhouse located within PEFI (23º30' S and 46º40' W; 770 m altitude) under natural lighting, air temperature and relative humidity. At the beginning of the experiment, the plants were transplanted into 2.6 L pots containing the natural soil and acclimatized in this new condition for 1 month. The experiment consisted of the application of 75 mL of Hoagland and Arnon nutrient solution n. 1 (Hoagland and Arnon 1950) modified with 0, 60, 120, 180 or 240 mg Cu ($CuSO_4 \cdot 5H_2O$) kg^{-1} soil DW. The copper concentrations were based on the prevention value for Cu (60 mg Cu kg^{-1} soil DW), as defined by the Companhia Ambiental do Estado de São Paulo (CETESB), responsible for environmental monitoring of São Paulo State, Brazil (CETESB 2016). The ionic balance of the solutions was accomplished to keep the macro- and micronutrient concentrations constant and pH adjusted to 5.8 (Table 2). The plants were irrigated weekly with ¼ of the total concentration,

repeating the procedure 4 times to obtain the total values of copper in the soil for each treatment. Irrigation with 75 mL of distilled water was performed at application intervals.

The experiment lasted 120 days after copper contamination, between the spring and summer seasons in Brazil (September to December 2019). During this period, the plants were grown inside the greenhouse under a temperature of 26 °C and 56% of relative humidity, on average.

Growth parameters

Plant height (cm) and stem diameter (mm) were measured. Old and new leaves, stem and roots were detached. The samples were oven-dried at 60 °C until constant weight for dry weight (DW) determination.

Cu concentration in soil and plant tissues

Copper concentration in soil (mg Cu dm^{-3} soil) was determined by flame atomic absorption spectrometry (AAS) after extraction in DTPA solution pH 7.3 (Raij et al. 2001). Old leaves, new leaves, stem and roots were digested in nitro-perchloric solution, and Cu concentration (mg Cu kg^{-1} DW) was determined by flame atomic absorption spectrophotometry (AAS) (Raij et al. 2001).

Translocation index (Ti) and Bioaccumulation factor (Bf)

The translocation index (Ti) was determined by dividing copper concentration in shoots ($Cu_{leaves} + Cu_{stem}$) by copper concentration in roots (Vendruscolo et al. 2018): Cu_{shoots} / Cu_{roots}. The Bioaccumulation factor (Bf) was calculated by dividing copper concentration in root tissue by copper concentration in the soil (Zang et al. 2020): Cu_{roots} / Cu_{soil}.

Relative chlorophyll (SPAD index) and pigment contents

The relative chlorophyll content was determined using a SPAD-502 (Soil Plant Analysis Development, Konica Minolta) manual chlorophyll meter. Measurements were performed on the 3rd fully expanded leaf (counting from the apex to the base) of all plants in triplicate. The values were expressed as the SPAD index (Amarante et al. 2009). In the same leaves used for relative chlorophyll content, three samples (Ø = 0.5 cm, n = 10) from the leaf blade were removed, and fresh weight (≈ 0.02 g) was determined. The pigment contents (Chl a, Chl b and carotenoids in µg g^{-1} FW-fresh weight) and Chl a/b ratio were determined in samples immersed in 2.5 mL DMF (Dimethylformamide) and kept in dark for 24 h. The solutions were analyzed on a spectrophotometer at λ = 664 nm (Chl a), 647 nm (Chl b), 480 nm (carotenoids) and 750 nm (residual) wavelengths (Minocha et al. 2009).

Leaf gas exchange and chlorophyll fluorescence

The gas exchange parameters [net CO_2 assimilation (A, µmol CO_2 m^{-2} s^{-1}), leaf transpiration (E, mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs, mol H_2O m^{-2} s^{-1}) and intercellular carbon concentration (Ci, µmol m^{-2} s^{-1})] were determined using a portable infrared gas analyzer (IRGA, LCpro+, ACD BioScientific Ltd.). The measurements were performed in the morning (8 to 11 am), using ambient relative humidity, CO_2 and air temperature. The ideal PAR value for obtaining maximum net CO_2 assimilation in *S. terebinthifolia* (1,655 mmol m^{-2} s^{-1}) and *E. uniflora* (1,056 mmol m^{-2} s^{-1}) was previously determined by a net CO_2 assimilation curve in response to a decreasing flux of photosynthetically active radiation (from 2,000 to 0 mmol m^{-2} s^{-1}) performed in healthy individuals of each

species. Minimum (F_0), maximum (F_m) and variable (F_v) fluorescence was determined in dark-adapted leaves over 30 minutes time in the same plants used for gas exchange measurements, using a portable modulated pulse fluorometer OS5p (Opti-Sciences, Hudson, NH, USA). Variable fluorescence (F_v) was calculated as $F_v = F_m - F_0$, and the maximum quantum yield of photosystem II was determined by the F_v / F_m ratio (Campostrini 2001).

Antioxidants

Samples of fully expanded leaves (n = 5) inserted in the central region of the stem were removed and immediately weighed to obtain fresh weight, then dipped in liquid nitrogen and stored in a -80 ºC freezer. The concentration of ascorbic acid in reduced (AA), oxidized (DHA) and total (AA+DHA) forms (mg g^{-1} leaf FW) was determined in leaf extracts crushed within metaphosphoric acid (HPO_3) in a mechanical mixer, followed by centrifugation at 10,000 rpm for 15 minutes at 2 ºC. The supernatant was collected and filtered (20 μm). AA concentrations were determined by high-performance liquid chromatography (HPLC) with UV detector (λ = 245 nm), C18 column, and 1.00 mL min^{-1} flow rate, using aqueous solution mobile phase acidified with orthophosphoric acid (H_3PO_4) (López et al. 2005). The concentrations of glutathione in reduced (GSH), oxidized (GSSG) and total (GSH+GSSG) forms (mg g^{-1} leaf FW) were determined by spectrophotometer reading (λ = 412 nm) after extraction with sulfosalicylic acid solution (Israr et al. 2006). The oxidized form of the nonenzymatic antioxidants (ascorbic acid and glutathione) was calculated by subtracting total content and the reduced form. The ratios between the reduced and total forms (*AA / AA+DHA* and *GSH / GSH+GSSG*) were calculated to determine the oxi-reduction capacity of these antioxidants (Burkey et al. 2006).

SOD activity (unit SOD g^{-1} leaf FW) was determined in leaf extracts homogenized with PVPP 0.5% and extraction solution (50 mM phosphate buffer, pH 7.5, containing 1 mM EDTA-Na2, 50 mM NaCl and 1 mM ascorbic acid) in a mechanical mixer. The extract was then centrifuged at 10,000 rpm at 2 ºC for 25 minutes, and the supernatant was collected. The absorbances of the extract exposed to fluorescent light and the extract kept in the dark were determined in a spectrophotometer (λ = 560 nm). SOD activity was determined by the difference between absorbances, which is based on inhibiting the reduction of NBT by enzymatic dismutation of the superoxide. One unit of SOD enzymatic activity was defined as the amount of SOD enzyme causing 50% inhibition of NBT reduction (Zeng et al. 2019).

Statistical analyses

The experimental design was based on completely randomized blocks, consisting of 5 blocks with 5 treatments and 20 plants per plot, totaling 100 plants of each species. The data were submitted to two-way analysis of variance (ANOVA-2) and the means compared by the Tukey test ($p \leq 0.05$) to evaluate the differences between treatments and species, using the statistical package Sisvar 5.7. Gas exchange parameters were also submitted to analysis of variance (ANOVA), followed by regression analyses ($p \leq 0.05$).

Results

Copper concentration in soil and plant tissues

Cu concentrations in the soils (mg Cu dm^{-3} soil) were 4.1, 11.3 21.6 25.6 and 31.2 (*S. terebinthifolia*) and 2.2, 9.0, 17.8 23.8 and 28.3 (*E. uniflora*) in treatments of 0, 60, 120, 180 or 240 mg Cu kg^{-1}, respectively (Figure 1). The soils of control and 60 mg Cu kg^{-1} treatments showed the lowest values of Cu concentration, while in the 180 and 240

mg Cu kg^{-1} treatments, the highest values of Cu concentrations were observed. No statistical difference was observed for copper concentrations in soil between *S. terebinthifolia* and *E. uniflora* in any treatment.

In all treatments, copper concentrations in *S. terebinthifolia* tissues showed significantly higher values in roots when compared to new and old leaves and stems (Figure 2). *S. terebinthifolia* showed higher copper concentration in roots in 180 and 240 mg Cu kg^{-1} treatments compared to control. *E. uniflora* showed significantly higher copper concentration in roots for 120, 180 and 240 mg Cu kg^{-1} treatments compared to control. The 120 mg Cu kg^{-1} treatment in *E. uniflora* roots resulted in a higher value of Cu concentration than that in stem, whereas 180 and 240 mg Cu kg^{-1} treatments resulted in statistically higher values of copper concentrations in roots compared to the values of Cu concentration in all above-ground tissues, i.e., new/old leaves and stem. No statistical difference was noted for Cu concentrations among the treatments in new leaves, old leaves, or stem of *S. terebinthifolia* and *E. uniflora*. Also, there were no difference in root Cu concentration between the species for control treatment; however, the roots of *S. terebinthifolia* exposed to 60, 120, 180 and 240 mg Cu kg^{-1} showed a higher value of copper concentration than that of roots of *E. uniflora* exposed to the same treatments. Copper concentrations in old leaves, new leaves and stem were no different between *S. terebinthifolia* and *E. uniflora* for all treatments.

Translocation index (Ti) and Bioaccumulation factor (Bf)

S. terebinthifolia and *E. uniflora* cultivated in 120, 180 and 240 mg Cu kg^{-1} showed the lowest translocation values (0.15, 0.13 and 0.14 for *S. terebinthifolia* and 1.25, 1.06 and 0.95 for *E. uniflora*, respectively) when compared to control treatment (Table 3). *S. terebinthifolia* showed the highest Bf value in the 60 mg Cu kg^{-1} treatment (4.79), when compared with the 240 mg Cu kg^{-1} treatment (2.87). *E. uniflora* showed no statistical difference for Bf among treatments. *S. terebinthifolia* showed higher translocation index and bioaccumulation factor than *E. uniflora* in all treatments.

Relative chlorophyll (SPAD index) and pigment contents

No statistical difference was observed for chlorophyll *a*, *b*, total (*a* + *b*), carotenoids, chlorophyll *a/b* ratio and SPAD index among treatments for both species (Online Resource 1).

Leaf gas exchange and chlorophyll fluorescence

S. terebinthifolia and *E. uniflora* showed an increase in net carbon assimilation (*A*) up to 60 mg Cu kg^{-1} and 120 mg Cu kg^{-1} compared to control, respectively, followed by a decline at higher concentrations (Figure 3). *S. terebinthifolia* showed a linear decline in stomatal conductance (*gs*) and leaf transpiration (*E*) as copper concentration increased, whereas *E. uniflora* presented a linear increase for these parameters. The intercellular carbon concentration (*Ci*) of *S. terebinthifolia* showed a linear increase with increasing Cu concentration, while *E. uniflora Ci* was negatively affected up to a concentration of 120 mg Cu kg^{-1}, followed by an increase at higher concentrations. No statistical difference was noted for chlorophyll fluorescence in any treatment for both species (Online Resource 2).

Growth parameters

S. terebinthifolia showed a reduction in leaf dry weight in the 240 mg Cu kg^{-1} treatment (Figure 4) compared to plants cultivated in the 60 mg Cu kg^{-1} treatment. *S. terebinthifolia* stem, roots, total dry weight, as well as height

and diameter (Figure 5), showed no statistical differences among treatments. *E. uniflora* showed a reduction in height in the 60 and 180 mg Cu kg^{-1} treatments when compared to control, but no statistical differences among treatments for stem diameter and all dry weight analyzed.

Antioxidants

S. terebinthifolia and *E. uniflora* showed no statistical difference for reduced, oxidated and total ascorbic acid and glutathione contents, as well as oxidation-reduction capacity among treatments (Figure 6). Neither species showed statistical difference for superoxide dismutase enzymatic (SOD) activity among treatments (Online Resource 3).

S. terebinthifolia showed higher reduced and total ascorbic acid contents than *E. uniflora* in all treatments, as well as higher oxidation-reduction capacity in the 60, 120 and 180 mg Cu kg^{-1} treatments. *S. terebinthifolia* showed higher reduced glutathione than *E. uniflora* in the control and 60 mg Cu kg^{-1} treatments and higher total glutathione in the control and 60 and 120 mg Cu kg^{-1} treatments. No statistical difference was seen for superoxide dismutase enzymatic (SOD) activity between *S. terebinthifolia* and *E. uniflora* in all treatments.

Discussion

The increase of copper concentration in nutrient solution was followed by a corresponding increase of copper concentration in the soil. However, the values of Cu concentration in the soil at the end of the experiment were lower than the values of Cu concentration in the nutrient solutions and the CETESB prevention value of 60 mg Cu kg^{-1} for soils contaminated with Cu (CETESB 2016). These results indicate that some Cu applied was retained in the soil particles, but most was absorbed by the two species. Cu bioavailability in soils is facilitated by several physical characteristics of soil, such as acidic pH (< 7.0), high levels of organic matter in the surface horizons and clay content (Campillo-Cora et al. 2019, 2020; Nakazato et al. 2021). The PEFI soil used in the experiment, therefore, showed favorable characteristics (Table 1) to copper bioavailability and uptake by *S. terebinthifolia* and *E. uniflora*.

S. terebinthifolia and *E. uniflora* cultivated in soils with copper concentrations above 120 mg Cu kg^{-1} accumulated Cu mainly through the root system. After Cu uptake, it was mostly retained in root cells owing to high affinity to root cell wall ligands; otherwise, Cu had low mobility and translocation in plants (Ambrosini et al. 2017; Zaouali et al. 2020). Under toxicity situations, copper accumulation in the root system is the most common behavior pattern observed in several species of different functional groups and botanical families (Marques et al. 2018; Torasa et al. 2019; Tiecher et al. 2017; Li et al. 2019). This behavior is one of the tolerance mechanisms in higher plants, acting as a strategy to preserve normal metabolic activities and mitigate the potential harmful effects to the photosynthetic mechanism in leaf tissues (Comin et al. 2018). Some strategies, such as chelation, sequestration and storage of copper ions in the vacuole, are used by higher plants to retain Cu in roots (Klaumann et al. 2011; Kumar et al. 2020). Moreover, the transport and accumulation of Cu phytotoxicity in shoots can be decreased since Cu stress induces a reduction of water and solutes uptake from soil, consequently decreasing the rates of transpiration that contribute to ion translocation through xylem (Farias et al. 2018).

S. terebinthifolia and *E. uniflora* showed a reduction in the Translocation Index as Cu concentration in the soil increased; however, the pioneer species *S. terebinthifolia* had less translocation of Cu than that of *E. uniflora*. Furthermore, except for the control treatment, *S. terebinthifolia* had more accumulation of Cu in the roots than that of *E. uniflora*. Species belonging to different successional groups from the Brazilian Atlantic Forest have distinct

Cu translocation capacities when cultivated in Cu-contaminated soils (Asensio et al. 2019). The pioneer *S. terebinthifolia* accumulates Cu in roots, but shows low translocation to shoots, while the non-pioneer species *E. uniflora* shows greater translocation, and Cu accumulation occurs in both roots and shoots (Zabotto et al. 2020). The differences in Cu translocation rates and root accumulation capacity between *S. terebinthifolia* and *E. uniflora* may be explained by different biochemical mechanisms governing the levels of root retention existing in each species (Silva et al. 2015). The ability to limit the translocation of heavy metals to the shoots and retain them in the roots is a tolerance indicator (Vendruscolo et al. 2018). Therefore, it is possible to assume that *S. terebinthifolia* is more tolerant to excess copper in the soil than *E. uniflora*. Despite that, neither species showed a reduction in biomass production or negative impacts on development, indicating that both *S. terebinthifolia* and *E. uniflora* could tolerate the Cu concentrations used in the experiment.

S. terebinthifolia and *E. uniflora* showed a bioaccumulation factor greater than 1 for all treatments, indicating that both species have characteristics of other phytoextracting species able to bioaccumulate soil pollutants, as indicated by Oguh and Obiwulu (2020). However, *S. terebinthifolia* showed a higher Bf than *E. uniflora* in all treatments, indicating that the pioneer species has a greater capacity for uptake, accumulation and extraction of copper than that of the non-pioneer species. As Cu concentrations applied to the soil increase, the bioaccumulation factor for both *S. terebinthifolia* and *E. uniflora* reduced significantly, implying that roots had reached a saturation point and thus restricted further Cu uptake to prevent phytotoxicity (Chua et al. 2019).

Excessive Cu concentrations can damage the photosynthetic apparatus, and plants under Cu toxicity generally present reductions in biosynthesis and concentration of photosynthetic pigments (Goswami and Das 2016; Zaouali et al. 2020). In our study, *S. terebinthifolia* and *E. uniflora* did not show alterations in relative levels of chlorophyll and carotenes in any treatment, nor did they show any significant changes in the efficient use of light by chlorophylls (e.g., *Fv/Fm* ratio). The deleterious effects on the synthesis and concentration of photosynthetic pigments under Cu toxicity occur only when the concentrations of Cu are high in the leaf tissues (Saleem et al. 2020). No increase in Cu concentration occurred in the leaves of *S. terebinthifolia* and *E. uniflora* among treatments, significantly increasing only in the root system. Consequently, the retention and accumulation of Cu in the roots seems to be an effective mechanism to preserve photosynthetic pigments in both species, avoiding the phytotoxic effects of Cu stress on the biosynthesis of pigments and the efficient use of light by chlorophyll.

S. terebinthifolia and *E. uniflora* showed a decline in carbon assimilation when cultivated in soils with the highest concentration of Cu. This decrease in carbon net assimilation (*A*) in response to Cu stress seems to be related to non-stomatal limitations [independent of the variables transpiration (*E*) and stomatal conductance (*gs*)] since the intercellular carbon concentration (*Ci*) increased in the highest concentrations of Cu, indicating that CO_2 acquisition was not the factor causing the decrease in carbon assimilation. Decrease in *A* concurrent with increase, or no change, of *Ci* is a pattern found in several species grown on substrates contaminated with excess Cu (Vinit-Dunand et al. 2002; Kevat and Sharma 2016; Hippler et al. 2018, Li et al. 2019) and probably caused by a decrease in rubisco activity and in the effectiveness of the Calvin-Benson cycle (Shahbaz et al. 2015; Suganami et al. 2020). These effects may result from changes in the plant's nutritional homeostasis caused by excess Cu in root cells, such as cell plasmolysis, retraction of the tonoplast and rupture / dysfunction of plasma membrane (Souza et al. 2014), all of which can trigger changes in uptake, distribution, mobilization and regulation of other essential nutrients (Janicka-Russak 2012; Marques et al. 2018; Zaouali et al. 2020).

Studies with several Atlantic Forest tree species cultivated in contaminated soil observed that the impacts on biometric parameters occur differently, depending on the species, concentration and time of exposure to excess

copper (Silva et al. 2010; Silva et al. 2011; Caires et al. 2011; Silva et al. 2015; Marco et al. 2016; Asensio et al. 2019; Zabotto et al. 2020). Excess Cu had negative effects on leaf dry biomass of the pioneer species *S. terebinthifolia*, but did not affect height or stem diameter, while the non-pioneer species *E. uniflora* showed no reduction in biomass but did show a decrease in height. The initial increase in dry leaf mass of *S. terebinthifolia* and in the height of *E. uniflora* are likely caused by the necessity of the element for plant growth and the beneficial effects to development when in low concentrations (Yruela 2009). However, excess of Cu causes ultrastructural damage to the root cells, impairing the growth and functionality of roots, and, as a consequence, negatively impacting the uptake of water and other nutrients (Li et al. 2019), which is a determining factor for reduction in biomass and biometric parameters in tree species (Yuan et al. 2018).

The non-enzymatic (AA) and enzymatic (GSH and SOD) antioxidant levels in the leaves of *S. terebinthifolia* and *E. uniflora* showed no significant changes among treatments, as already observed in other studies (Goswami and Das 2016; Tiecher et al. 2017; Zaouali et al. 2020; Saleem et al. 2020; Giampaoli et al. 2012; Hippler et al. 2018), in order to avoid oxidative stress and harmful effects on plant tissues. However, the increase in the synthesis of antioxidants and ROS production in leaves triggered by Cu toxicity occurs only when concentrations of this element are high in the leaf tissues (Li et al. 2019). Thus, it is possible to assume that the retention of Cu in the roots and inhibition of translocation to the shoots effectivly prevented potential oxidative stress; as a result, the levels of leaf antioxidants were essentially unchanged among treatments. Another important indicator of protection against oxidative damage is the antioxidant's redox capacity. Except for the control treatment, *S. terebinthifolia* showed higher capacity for oxidation-reduction of ascorbic acid compared to that of *E. uniflora*. The response in the synthesis of antioxidant molecules of plants under Cu stress shows species-specific behavior; they can be stimulated, inhibited or unchanged, depending on the species and concentration of the metal (Weckx and Clijters 1996; Nagae et al. 2008; Buapet et al. 2019; Zeng et al. 2019). In situations of stress induced by excess Cu, this indicates that *S. terebinthifolia* has a more active antioxidant system than *E. uniflora* and, therefore, a greater capacity for tolerance against oxidative stress.

Implications and final remarks

The pioneer species *S. terebinthifolia* and non-pioneer species *E. uniflora* present high extraction capacity with accumulation and root Cu restriction behavior when cultivated in soil contaminated with copper. However, the intensity of copper accumulation in the roots and its translocation to the shoots occurs in a species-specific way. *S. terebinthifolia* presents higher copper restriction capacity in the roots, showing potential as a Cu phytostabilizer, while *E. uniflora* has potential as a phytoextractor by accumulating and translocating part of the copper to the leaves. For both species, restricting the translocation of copper to shoots is indicative of a key protective mechanism against excess copper, effectively preventing negative effects on the photosynthetic pigments and oxidative damage to the leaves. *S. terebinthifolia* and *E. uniflora* have no visible symptoms of toxicity or developmental abnormalities, demonstrating that they are tolerant and have the ability to grow in soils contaminated with copper.

Declarations

Conflicts of interest/Competing interests

The authors declare that there is no conflict of interest.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Data is available by request to the authors

Code availability

Not applicable

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Matheus Casarini Siqueira, Shoey Kanashiro and Armando Reis Tavares. The first draft of the manuscript was written by Matheus Casarini Siqueira and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 Initial soil chemical composition

pH	O.M.	P_{resin}	H+Al	K	Ca	Mg	BS	CEC	V%	B	Cu	Fe	Mn	Zn
$CaCl_2$	g dm^{-3}	mg dm^{-3}	------------—----- mmolc dm^{-3} -----------							----------mg dm^{-3}------				
5.1	42.0	5.5	33.6	1.6	50.7	9.6	61.9	95.5	64.8	0.6	1.3	25.7	21.3	9.0

Table 2 Ion balance of Hoagland & Arnon nutritional solution modified with 0, 60, 120, 180 or 240 mg Cu kg^{-1}

Ion source	Treatments (mg Cu kg^{-1} soil DW)				
	0	60	120	180	240
	Released Ions (mmol L^{-1})				
NH_4^{1+}- $(NH_4)_2SO_4$	18	13.65669	9.313379	4.970069	0.626759
NH_4^{2+} - NH_4NO_3	1	3.171655	5.34331	7.514966	9.686621
NO_3^{1-} - NH_4NO_3	1	3.171655	5.34331	7.514966	9.686621
$[NH_4^{2+}] + [NO_3^{2-}]$	20	20	20	20	20
SO_4^{2-} - $(NH_4)_2SO_4$	9	6.828345	4.65669	2.485034	0.313379
SO_4^{2-} - $CuSO_4.5H_2O$	0	2.171655	4.34331	6.514966	8.686621
$[SO_4^{2-}]$	9	9	9	9	9
Cu^{2+}- $CuSO_4.5H_2O$	0	2.171655	4.34331	6.514966	8.686621
$[Cu^{2+}]$	0	2.171655	4.34331	6.514966	8.686621

Table 3 Translocation index and bioaccumulation factor of *S. terebinthifolia* and *E. uniflora*. Lowercase letters compare between treatments, and * indicates differences between species within each treatment. Means followed by same letters do not differ by Tukey's test at 5% probability

Species	Treatments	Translocation index (Ti)		Bioaccumulation factor (Bf)	
	(mg Cu kg^{-1} soil DW)				
S. terebinthifolia	0	0.26	a	–	
	60	0.17	ab	4.79	a *
	120	0.15	b	3.55	ab*
	180	0.13	b	3.48	ab*
	240	0.14	b	2.87	b*
E. uniflora	0	2.44	a *	–	
	60	1.71	ab*	2.02	a
	120	1.25	b*	1.57	a
	180	1.06	b*	1.40	a
	240	0.95	b*	1.22	a

Figures

Soil copper concentration
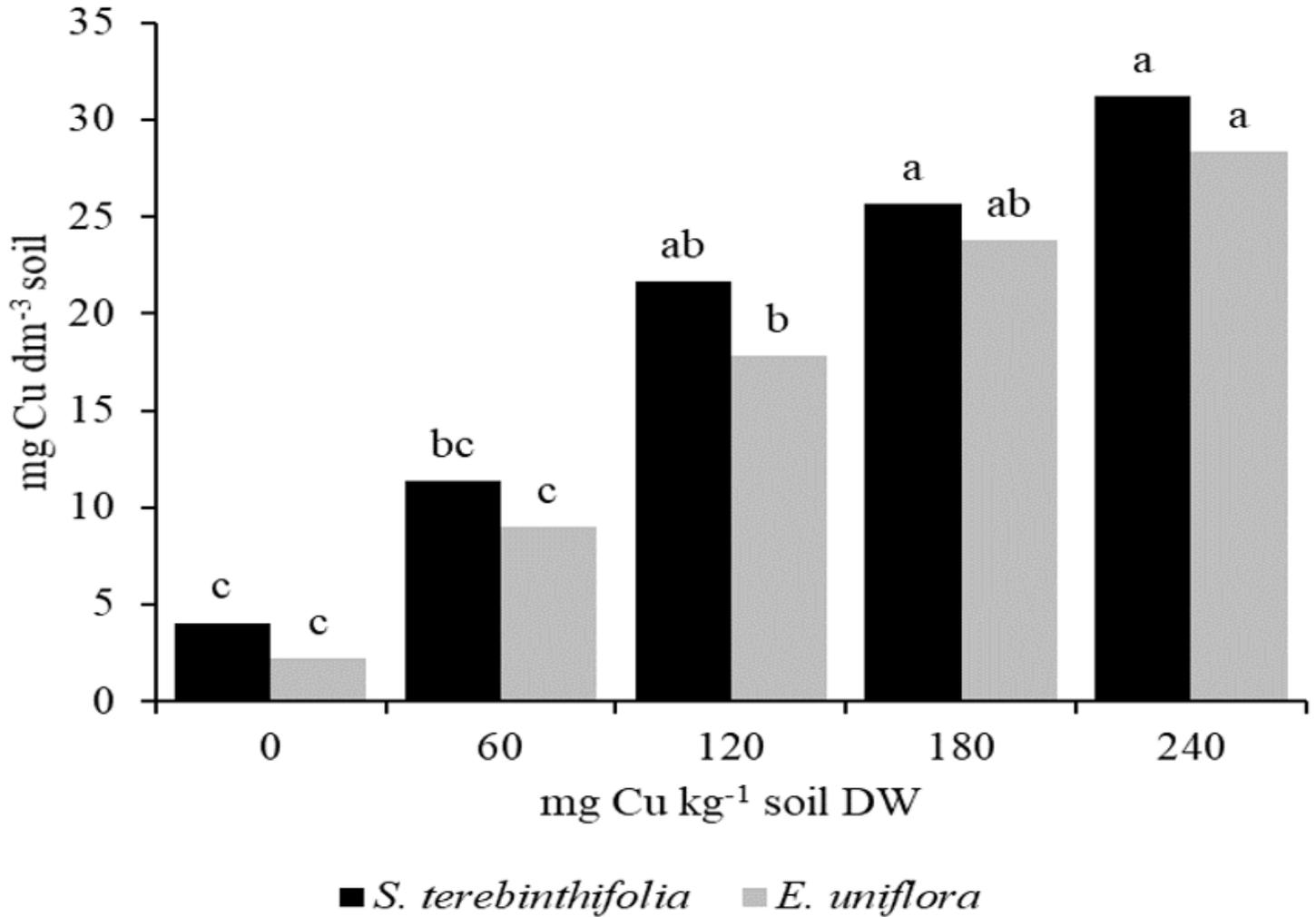
Figure 1

Copper concentration in soil for S. terebinthifolia and E. uniflora. Lowercase letters compare treatments within each organ of each species. Means followed by equal letters do not differ by Tukey's test at 5% probability
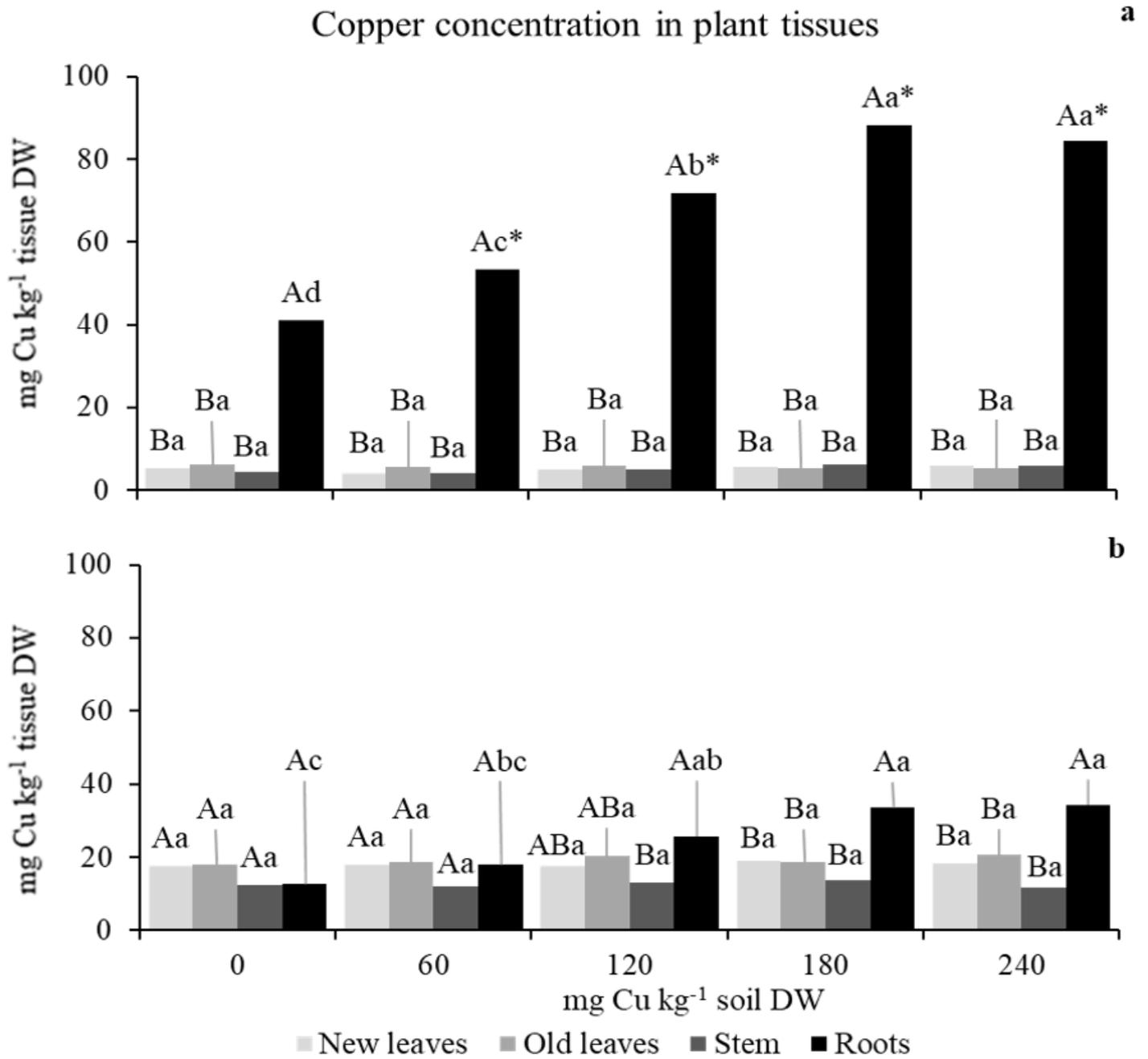
Figure 2

Copper concentration in new leaves, old leaves, stem and roots of S. terebinthifolia (a) and E. uniflora (b). Uppercase letters compare organs within each treatment and species, and lowercase letters compare treatments within each organ of each species. * Indicates differences between species within each organ and treatment. Means followed by equal letters do not differ by Tukey's test at 5% probability
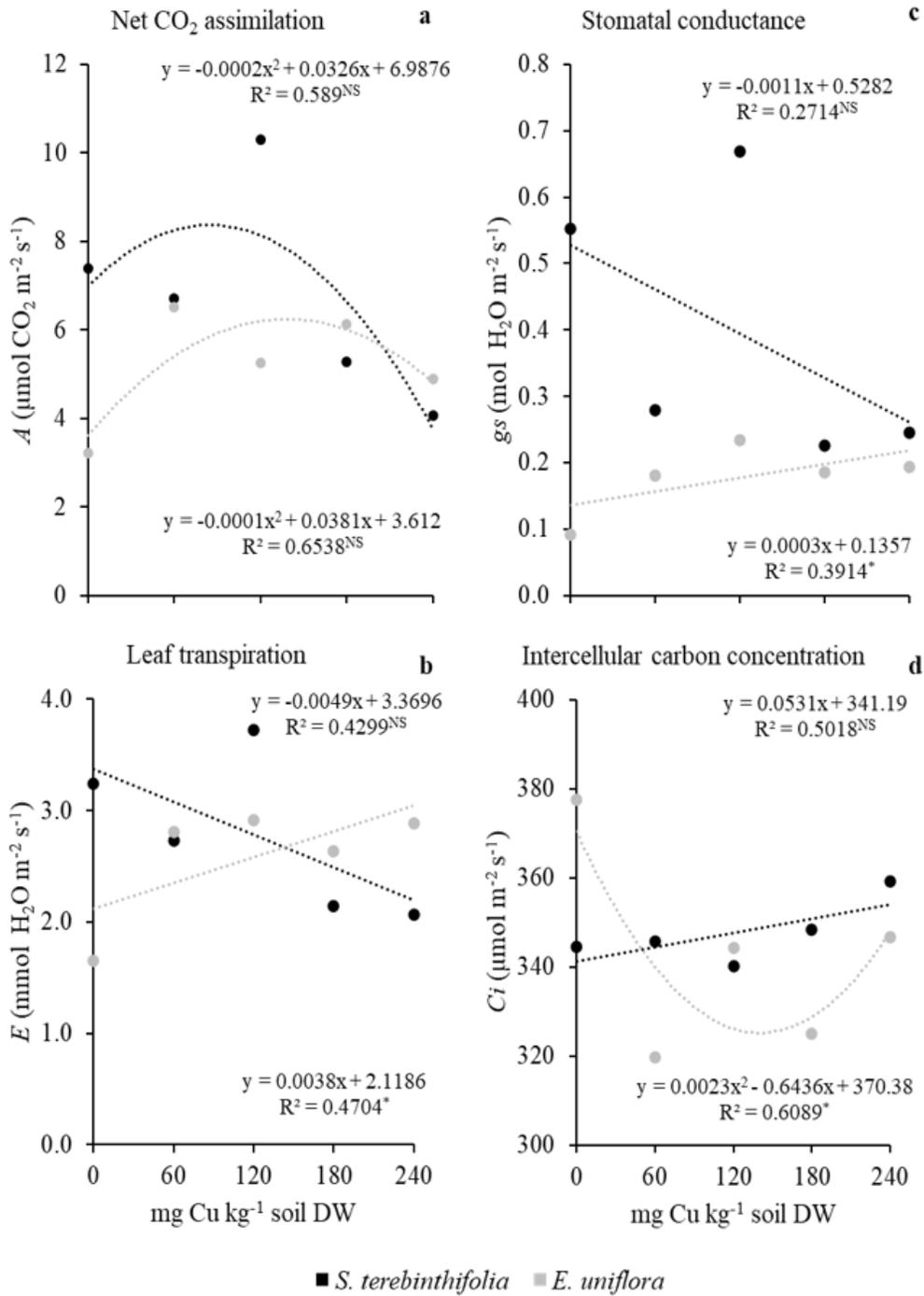
Figure 3

Net CO2 assimilation (a), stomatal conductance (b), leaf transpiration (c), and intercellular carbon concentration (d) of S. terebinthifolia and E. uniflora
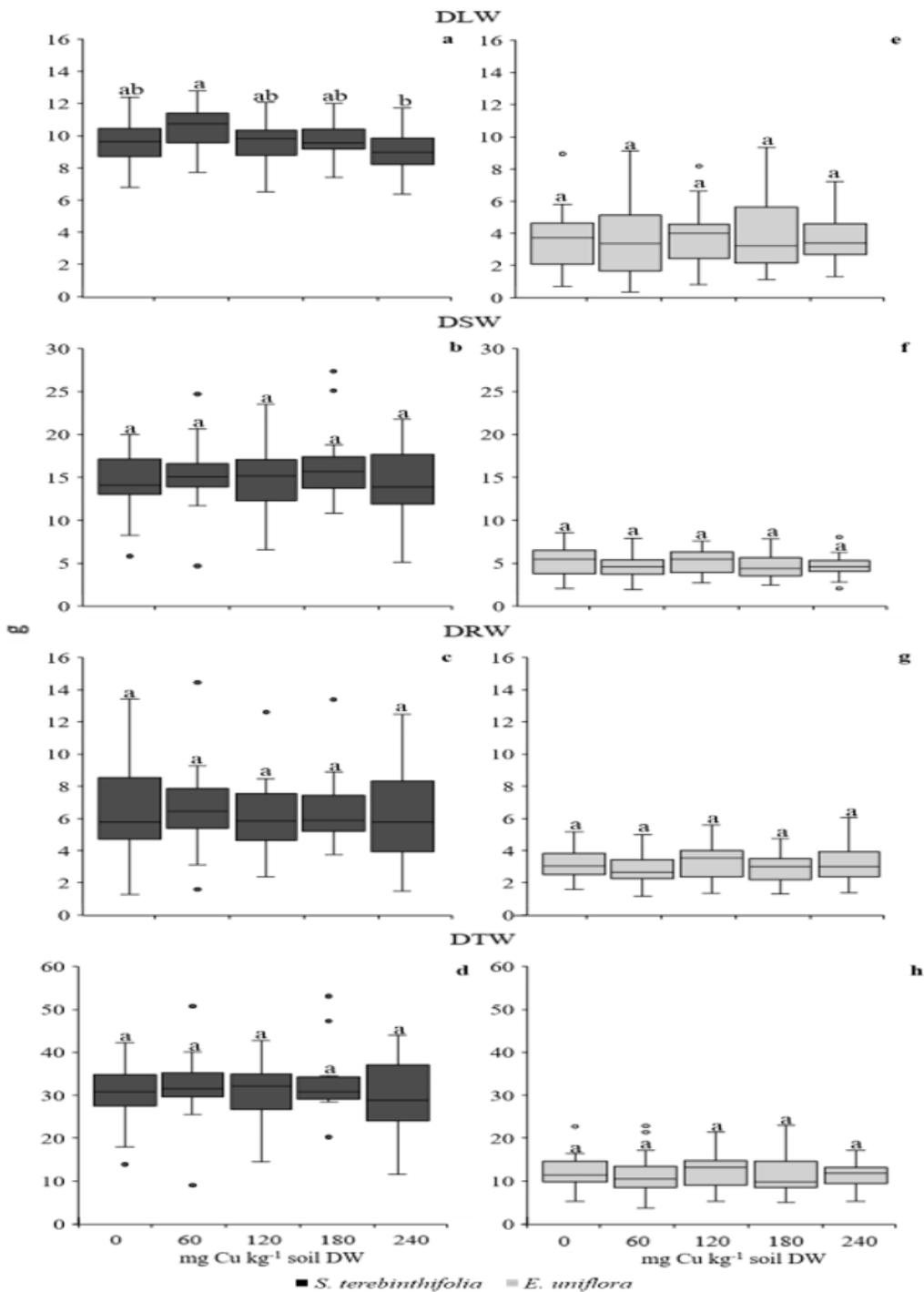
Figure 4

Dry leaf weight (DLW), stem (DSW), roots (DRW) and total (DTW) of S. terebinthifolia (a-d) and E. uniflora (e-h). Lowercase letters compare treatments within each species. Means followed by equal letters do not differ by Tukey's test at 5% probability
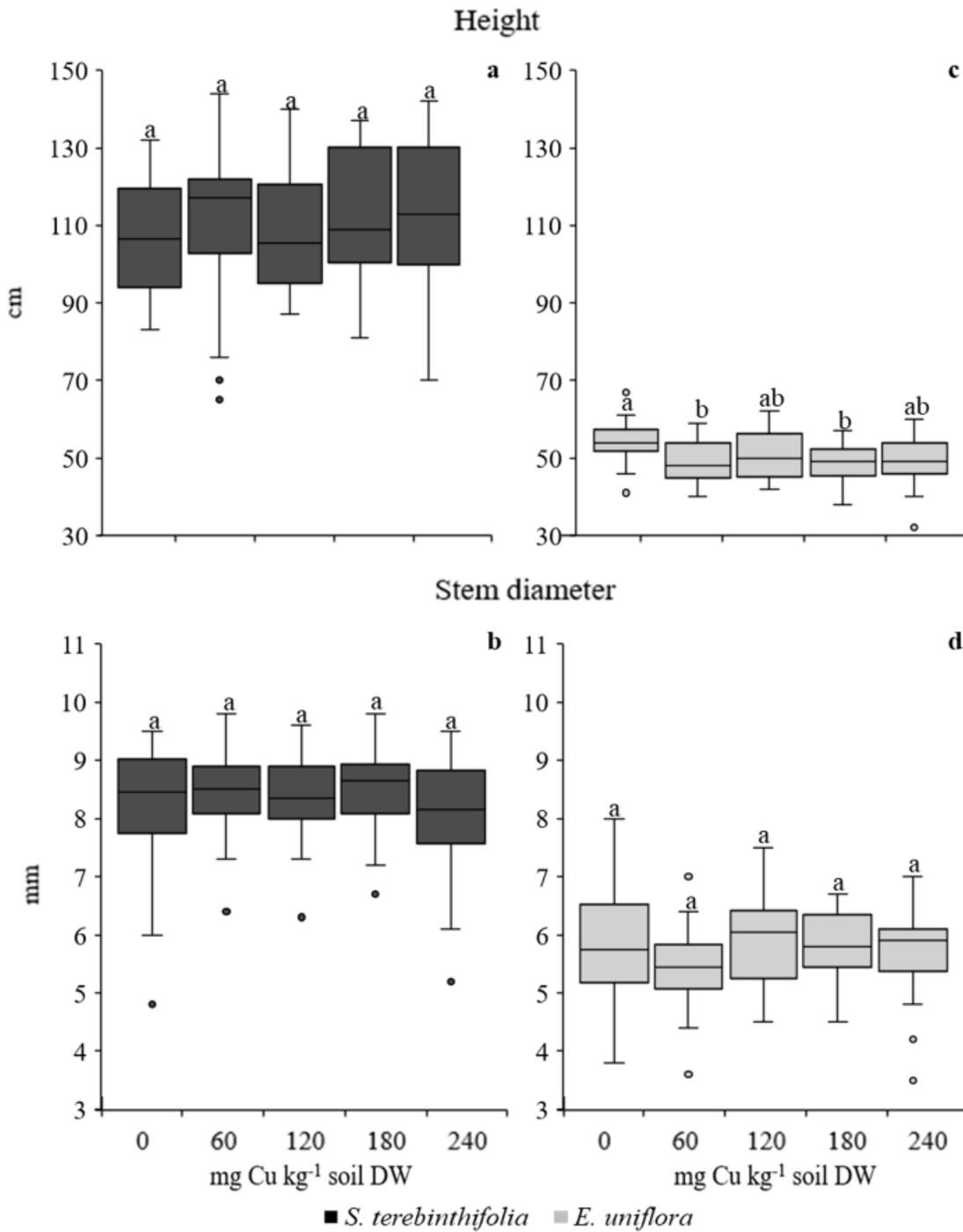
Figure 5

Height and diameter of S. terebinthifolia (a-b) and E. uniflora (c-d). Lowercase letters compare treatments within each species. Means followed by equal letters do not differ by Tukey's test at 5% probability
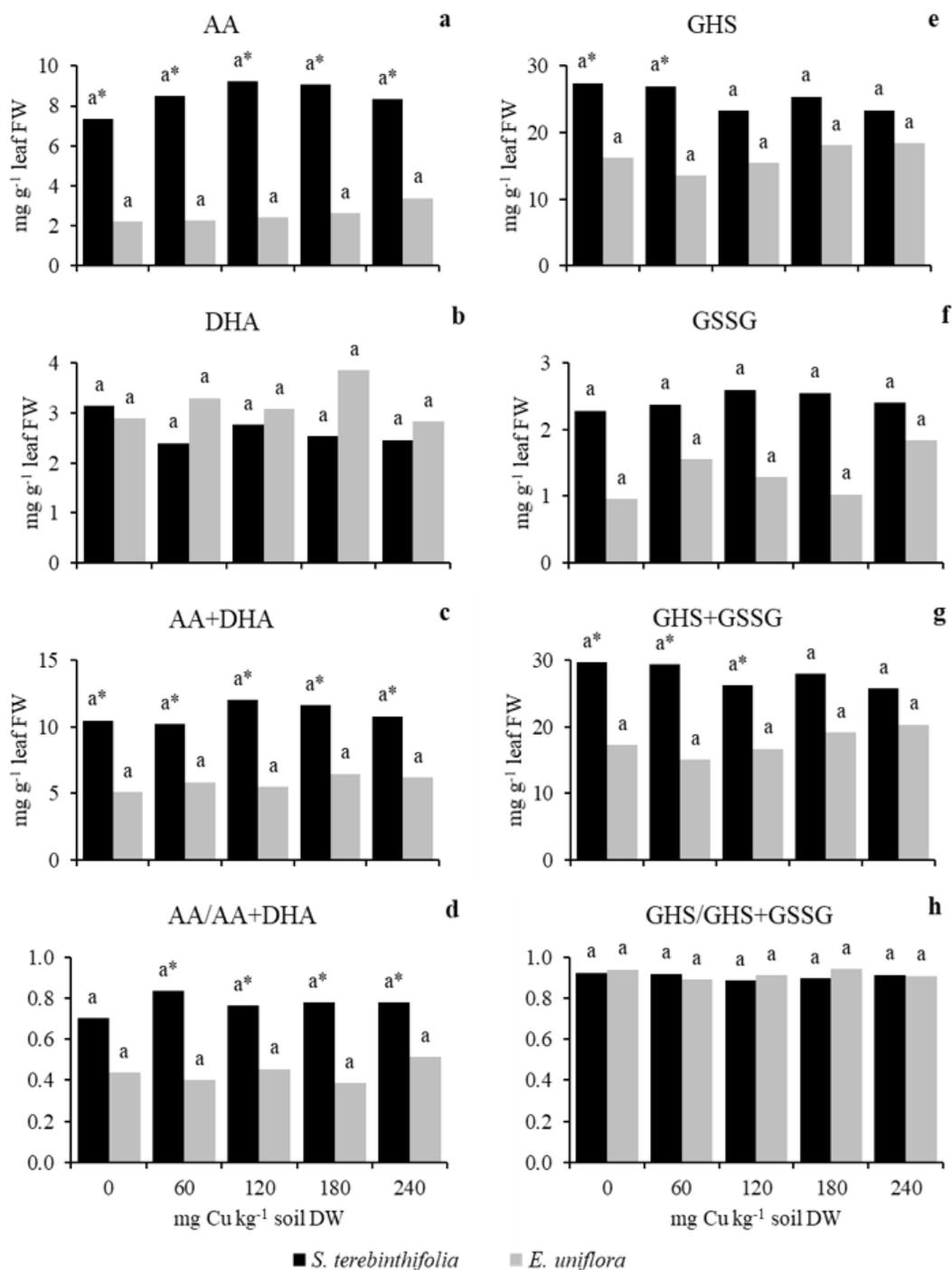
Figure 6

Concentration of ascorbic acid in the reduced (a), oxidized (b) and total (c) forms; glutathione in the reduced (e), oxidized (f) and total (g) forms; and the oxidation-reduction potential of ascorbic acid (d) and glutathione (h) in S. terebinthifolia and E. uniflora. Lowercase letters compare treatments within each species. * Indicates differences between species within each treatment. Means followed by equal letters do not differ by Tukey's test at 5% probability

Supplementary Files

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