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Toxicity of Iron Mining Tailings and Potential for Revegetation Using *Schinus terebinthifolia* Raddi Based on the Emergence, Growth, and Anatomy of the Species

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Abstract: This study aimed to evaluate the emergence, early growth, and anatomy of *Schinus terebinthifolia* Raddi cultivated in iron mining tailings. The seeds were obtained from trees used in urban afforestation and cultivated on two substrates: sand and iron mining tailings. The chemical composition of the mining tailing was characterized. The experiment was conducted in a growth room for 60 days. The emergence rate, seedling survival, height, number of leaves, chlorophyll content, and leaf and root anatomy were evaluated. The analysis of the composition of the mining tailings indicated that macro- and micronutrients were present, as well as potentially toxic elements such as Al, Cd, Cr, and Pb. The mining tailings reduced the emergence rate, and 25% of the seedlings died in this substrate. In addition, the mining tailings promoted a significant reduction in all parameters investigated, including seedling height, number of leaves, chlorophyll content, total leaf thickness, abaxial and adaxial epidermis thickness, palisade parenchyma thickness, and the length and width of the seeds. Additionally, the chloroplasts, the metaxylem vessel diameter, and the phloem proportion were evaluated. Interestingly, the tailings promoted an increase in the secretory channel. In the roots, no significant changes were observed in the parameters analyzed. Thus, the seeds of *S. terebinthifolia* germinated in the iron mining tailings, and 75% of the seedlings survived, showing their potential for reforestation. Nonetheless, iron mining tailings exhibited toxicity to *S. terebinthifolia* seedlings, reducing their photosynthetic tissues and, consequently, their growth; this toxicity is likely related to potentially toxic elements present in tailings.

Keywords: Brazilian Peppertree; potentially toxic elements; reforestation; seed germination; seedling early growth

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1. Introduction

Mining is among the main economic activities in Brazil [1], and Brazil is the second largest exporter of iron worldwide [2]. However, mining is also a main factor leading to the accumulation of pollutants in the environment [3]. Mining activity generates tailing with no commercial value that may contain potentially toxic elements [PTEs]. The volume of tailings produced can be almost the same as the raw product processed since the amount of concentrated product can represent only 1–3% of the raw material [4]. The method most commonly used for disposing of this tailing is containment dams [1], which must be frequently monitored to prevent serious tragedies. The use of chemicals to reduce tailings' toxicity is challenging and costly, emphasizing the importance of avoiding accidents with dams [4] or even the necessity of alternative ways to improve environmental quality, such as phytoremediation and reforestation of impacted areas.

Events involving tailings dams have occurred with some frequency over the years; one of the more recent events was the rupture of the Fundão dam, an iron mine which is located in the municipality of Mariana, Minas Gerais, Brazil [2]. In November 2015, the Fundão dam ruptured and released approximately 50 million m^3 of tailings into the environment, which traveled along the Rio Doce Basin and caused catastrophic environmental damage and 19 human deaths [2,5]. According to Silva et al. [6], 1289 ha was destroyed by dam failure. A potential strategy to minimize the damage caused in this area is revegetation, which is a very efficient and economically viable strategy. The success of revegetation depends on the chemical, physical, and biological characteristics of the soil, as well as the tolerance of each species to stress conditions [7]. The mining tailings contain potentially toxic elements (PTEs), such as Cd, Cr, Pb, and Al [8,9], and may limit the establishment and growth of plants [10–13]. Thus, there is a need to identify native species that are tolerant to iron mining tailings for revegetation projects.

Schinus species are known for their ability to withstand adverse environments and are often used in the restoration of degraded areas, being tolerant to Pb and Cd [13–15].

Schinus terebinthifolia Raddi, the species investigated in this study, is native to South America and is widely found in the Brazilian territory [16]. According to Scarpa et al. [17], the species is tolerant to iron mining tailings based on relevant parameters; however, some effects were not addressed. There is a lack of studies about the effect of iron mining tailings on leaf and root anatomical traits because most studies have focused on survival and plant growth parameters; in particular, no information has been found about chloroplast-specific changes in trees growing in iron mining tailings. It is important also to investigate visual symptoms in newly germinated seeds because iron mining tailings may promote some degree of physical inhibition to seedling development. Therefore, the objective of this study was to evaluate the emergence, initial growth, and leaf and root anatomy of *S. terebinthifolia* cultivated in iron mining tailings from the rupture of the Fundão dam, MG.

2. Materials and Methods

2.1. Plant Material

The cultivation of the species and the experiments were performed at the Laboratory of Environmental Biotechnology and Genotoxicity at the Federal University of Alfenas (Unifal-MG). The fruits of *S. terebinthifolia* were collected from matrices cultivated in urban forestry areas in Alfenas-MG, separated, and dried in an oven at 40 °C for 7 days. After the fruits were dried, the seeds were separated and stored in a refrigerator at 4 °C until the experiment. The temperatures for seed drying and storage were determined by previous tests and works with *Schinus* species [13,17].

2.2. Iron Mining Tailings

Samples of mine tailings were collected 4.0 km from the Fundão dam in Mariana, MG (20°22′40″ S, 43°106 24′57″ W), at 1 m depth; further, they were transported to the Federal University of Alfenas where they were stored in plastic bags and a room protected from rain and sunlight. Recent studies, such as those by [8,9], have shown that macronutrients (P, Ca, K, and Mg) and micronutrients (Mn, Fe, Zn, Cu, and N), as well as potentially toxic metals (Al, Cd, Cr, and Pb), are present in the tailings at variable concentrations. The chemical compositions of the tailings samples were analyzed to determine the macro- and micronutrient contents, as well as the presence of potentially toxic elements, and the results are shown in Table 1. The analysis methodology was performed using the protocol from AOAC [18]. Mining tailings were oven-dried at 70 °C until reaching a constant mass, homogenized in a 1.5 mm mesh sieve, and submitted to different extraction methods for elemental quantification. For this, 500 mg of the tailing was extracted using 50 mL of KCl for Ca^{2+}, Mg^{2+}, and Al^{3+} solubilization, while the K^+ and PO_4^- contents were extracted with 50 mL of 0.025 mol L^{-1} H_2SO_4 + 0.05 mol L^{-1} HCl. Micronutrients were measured using a chelating solution and toxic elements were measured with nitroperchloric digestion. The levels of macro- and micronutrients and potentially toxic elements were quantified using an atomic absorption spectrophotometer, AAnalyst 800 (PerkinElmer, Waltham, MA, USA) [19]. The pH under saturated water conditions was assessed with a portable soil pH analyzer, HPH-125—Homis (Homis, São Paulo, Brazil).

Table 1. Concentrations of macro- and micronutrients as well as potentially toxic elements in Fe mining tailings resulting from the failure of the Fundão dam in Mariana, MG. * Maximum value considered [20].

Macronutrients	**mg kg^{-1} Dry Mass**	**PTE Maximum Concentrations [mg kg^{-1}] ***
P	142.7	-
Mg	84.6	-
K	106.5	-
Ca	3274.4	-
Micronutrients	µg kg^{-1}	
Mn	440.9	-
Fe	31,509.1	-
Zn	5.3	300.0
Cu	6.1	63.0
Na	49.6	-
Potentially toxic elements	µg kg^{-1}	
Al	2472.1	-
Cr	11.3	75.0
Cd	2.3	1.4
Pb	5.3	72.0
Other characteristics		
pH	6.2	-

2.3. Experimental Design

First, *S. terebinthifolia* seeds were scarified with 5 mL of sulfuric acid (H_2SO_4) for one minute and neutralized with 20 mL of 0.1 M $NaHCO_3$ twice for 1 min or until the neutralization reaction stopped; then, the seeds were washed in running water [21]. After scarification, five seeds [per pot] were placed in 500 mL pots containing 400 mL of substrate and maintained at the maximum water-holding capacity. The treatments consisted of two different substrates, namely, iron mining tailings (that were previously dried and sieved) and washed sand, and 20 replicates (n = 40). The plants were kept in a growth room at 22 °C, 60% relative humidity, a radiation intensity of 40 µmol m^{-2} s^{-1}, and a photoperiod of 12 h for a period of 60 days. During this period, water was replaced daily according to the amount lost by evapotranspiration, which was measured by gravimetry. The nutrient solution used by Hoagland and Arnon [22] was added to the sand substrate and replaced weekly; this was necessary to compensate for the macro- and micronutrients present in mining tailings, and no nutrient solution was necessary to complement the nutrition of saplings in iron mining tailings with similar composition [19]. The experimental design was completely randomized.

2.4. Emergence and Biometrics of the Seedlings

Seedling emergence was evaluated daily, and after 60 days, the percentage of emergence (E%) was calculated according to the following equation: E% = (number of emerged seedlings per pot/number of seeds sown per pot) × 100. Seedling survival was evaluated weekly, and survival percentage (S%) was calculated according to the following equation: S% = [number of surviving seedlings/number of emerged seedlings] × 100. The leaves were counted, and the seedling height was measured with a ruler at 7-day intervals. The experiment ended after 60 days, and the chlorophyll content was estimated at three points on the leaf to obtain an average chlorophyll content using a chlorophyll meter model SPAD-502 (Minolta, Tokyo, Japan). Visual symptoms, including the presence of leaf chlorosis (yellowing), necrosis, and possible morphological deformations, were observed and photographed.

2.5. Anatomical Analysis

At the end of the experiment, leaves and roots were collected and fixed in 70% FAA (formaldehyde, glacial acetic acid, and 70% ethanol in a ratio of 0.5:0.5:9) for 72 h [22] and stored at 70% until analysis. Freehand cross-sections of the leaves and roots were obtained using steel slides and stained with safranin solution (0.1% safranin and 1% astra blue in a ratio of 7:3). Semipermanent slides were mounted according to the method described by Kraus and Arduim [23], observed under a trinocular CX31 light microscope (Olympus, Tokyo, Japan) with a coupled capture system, and digitized for later analysis with the image analysis software ImageJ. Three slides were used per replicate. In the interveinal region of the leaf, the epidermal thickness of the adaxial and abaxial surfaces, the thickness of the palisade [PP] and spongy [SP] parenchyma, the ratio between them [PP/SP], the total thickness, and the length and width of the leaves were evaluated. Chloroplasts of the palisade and spongy parenchyma were also analyzed, and the areas of the vascular bundle, xylem, phloem, and secretory channels in the midrib region were evaluated. The proportions of the xylem, phloem, and secretory canal were calculated according to the following equation: proportion = (area of tissue/area of the vascular bundle) $\times$ 100. The diameter of the metaxylem vessel elements was also evaluated, as well as the diameter of the vascular cylinder, the root diameter, the thickness of the cortex, and the proportion of the vascular cylinder in the pilifer root zone.

2.6. Statistical Analysis

The data were subjected to the Shapiro–Wilk normality test and, subsequently, to analysis of variance (ANOVA), and the means were compared by the Scott–Knott test with a 5% probability of error. For ANOVA analyses, we considered the substrate as the source of variation (sand or tailings) and 20 replicates. This gave n = 40, 39 total degrees of freedom, and 38 degrees of freedom of the error. For the normality test (Shapiro–Wilk), a 5% error probability was considered. SISVAR 5.6 software [24] was used for these analyses. This software was selected because it shows tools for the Shapiro–Wilk test and for the ANOVA that we required in this experiment; in addition, it has a friendly and efficient interface.

3. Results

3.1. Growth Parameters

Compared with sand, mining tailings reduced the emergence of *S. terebinthifolia* seedlings (Figure 1A). Variables such as seedling height (Figure 1B), number of leaves (Figure 1C), and the estimated chlorophyll content (Figure 1D) of the species also decreased in the plants cultivated in mining tailings. All plants grown in the sand survived, but only 75% of those grown in the mining tailings survived (p = 0.0162).

3.2. Qualitative Effects

The plants that grew in the mining tailings exhibited visible qualitative effects. Notably, it was difficult for the seedlings to emerge, as the cotyledons were attached to the substrate, hindering the vertical growth of the aerial part (Figure 2A); this result was not observed for the seedlings grown in sand. The tailings inhibited the development of the root system of the seedlings (Figure 2B). At the time of collection, the seedlings grown in sand had three leaflets per leaf (Figure 2C); however, the mining tailings hindered the development of these leaves, which exhibited a lower number of leaflets and deformation at the base and edges of these structures (Figure 2D). In addition, chlorosis was observed in the leaves of plants grown in the tailings (Figure 2D).

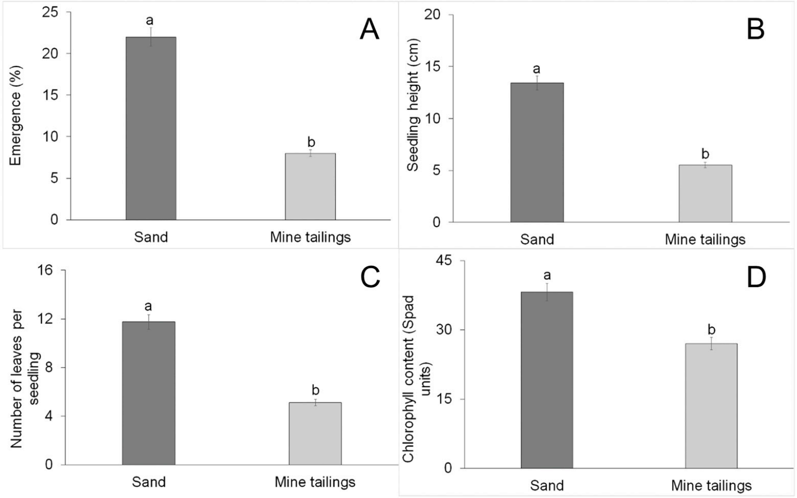

Figure 1. Growth parameters of *Schinus terebinthifolia* seedlings grown on the following substrates: iron mining tailings and sand. The bars indicate the standard error. For graphs (**A**–**D**), the means followed by different letters indicate significant differences according to the Scott–Knott test at $p < 0.05$.

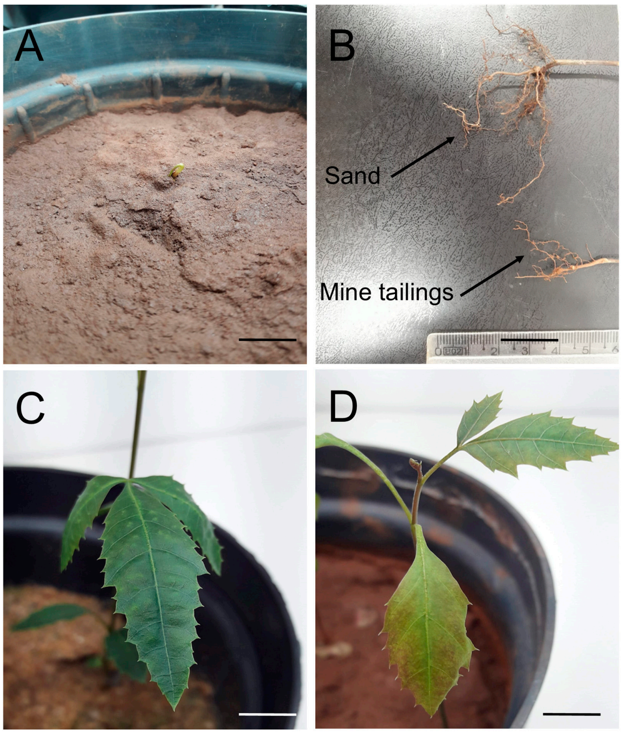

Figure 2. Qualitative effects of iron mining tailings from the Fundão dam failure in Mariana, MG, on *Schinus terebinthifolia* seedlings. (**A**) = Seedling emergence; the white arrow indicates the effects caused by the tailings trapping the cotyledons and hindering the vertical growth of the aerial part. The white arrow indicates cotyledons trapped in the substrate. (**B**) = Inhibition of the growth of the root system of the seedlings that were cultivated in the tailings. (**C**) = Compound leaves of the seedlings grown for 60 days in sand. (**D**) = Leaves composed of seedlings grown in tailings for 60 days. The black arrow indicates negative effects on the development of leaves with a lower number of leaflets and deformation of the leaf blade with a change in the edge of the leaflet base [black arrow]. Bars = 2 cm.

3.3. Leaf Anatomy

The mining tailings reduced the leaf thickness (Figures 3A and 4B); however, no effect on the epidermis thickness of the adaxial surface (Figures 3B and 4B) was detected. In addition, the tailings reduced the thickness of the palisade parenchyma (Figures 3C and 4B); however, compared to the seedlings grown in sand, no changes in the thickness of the spongy parenchyma were observed (Figures 3D and 4A,B). The tailings also reduced the thickness of the epidermis of the abaxial surface (Figures 3E and 4B) and the ratio of palisade parenchyma/spongy parenchyma (Figures 3F and 4B).

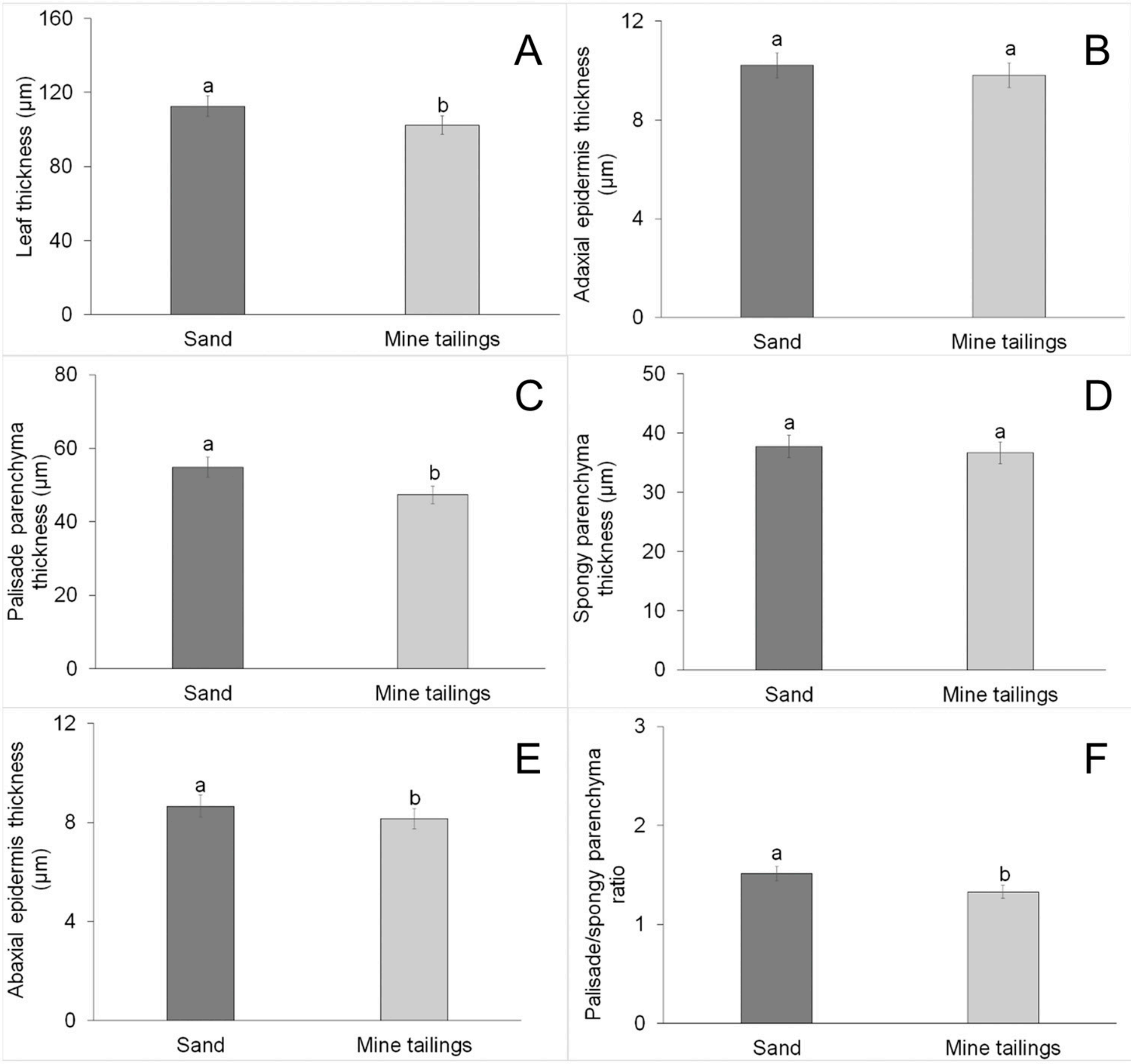

Figure 3. Anatomical characteristics of the interveinal region of *Schinus terebinthifolia* leaves grown on the following substrates: iron mining tailings and sand. The bars indicate the standard error. For graphs (**A**,**C**,**E**), the means followed by different letters indicate significant differences according to the Scott–Knott test for $p < 0.05$. For graphs (**B**,**D**,**F**), the means followed by the same letters did not differ significantly according to the Scott–Knott test at $p < 0.05$.

In the plants grown in tailings, the length of the chloroplasts in the palisade parenchyma did not change (Figure 5A). However, the tailings treatment reduced the length of the chloroplasts in the spongy parenchyma (Figure 5B), the width of the chloroplasts in the palisade parenchyma (Figure 5C), and the width of the chloroplasts in the spongy parenchyma (Figure 5D).

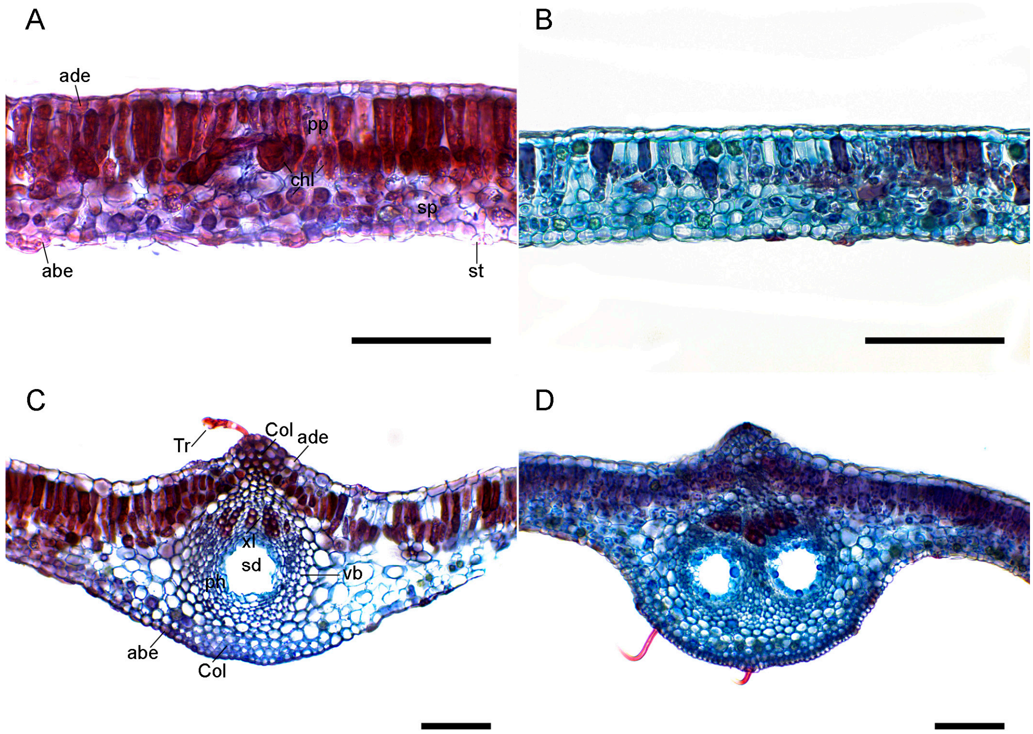

Figure 4. Leaf anatomy of *Schinus terebinthifolia* grown on the following substrates: sand (**A**,**C**) and iron mining tailings (**B**,**D**). Images A and B show the interneural region, and images (**C**,**D**) show the midrib. ade = adaxial epidermis; pp = palisade parenchyma; sp = spongy parenchyma; abe = abaxial epidermis; chl = chloroplast; st = stomata; xl = xylem; ph = phloem; sd = secretory channel; vb = vascular bundle, Col = collenchyma; Tr = trichome. Bars = 100 µm.

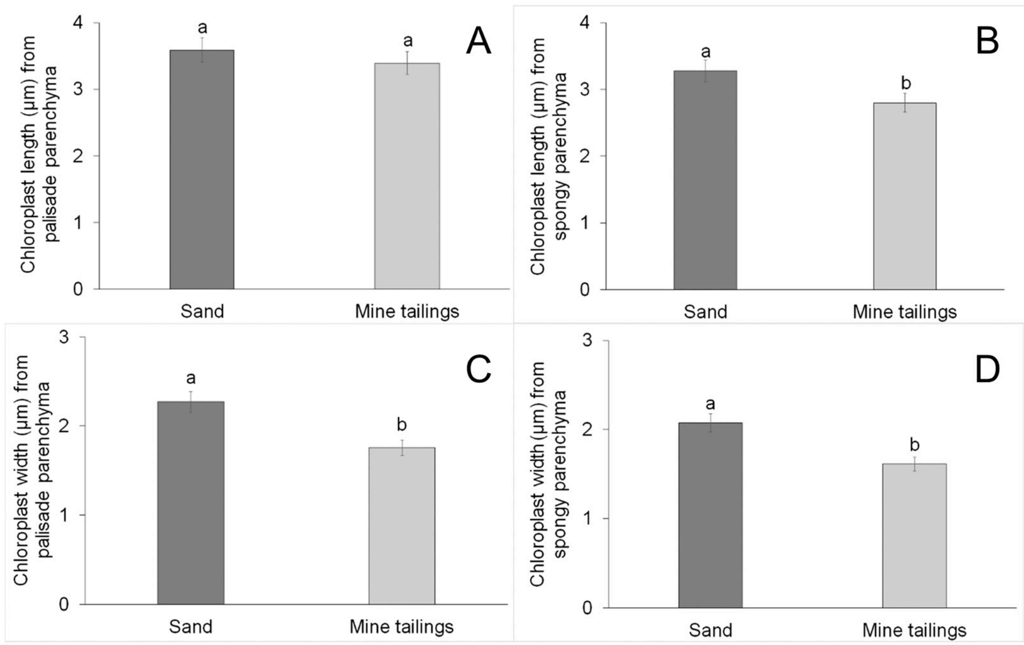

Figure 5. Chloroplast characteristics of *Schinus terebinthifolia* leaves grown on the following substrates: iron mining tailings and sand. The bars indicate the standard error. For graphs (**B**–**D**), the means followed by different letters indicate significant changes according to the Scott–Knott test for $p < 0.05$. For graph (**A**), the means followed by the same letters did not significantly differ according to the Scott–Knott test for $p < 0.05$.

The tailings did not change the area of the vascular bundle in the midrib region (Figures 4C and 6A); however, the proportion of the secretory channel increased (Figures 4C and 6B). Mining tailings reduced the proportion of phloem (Figures 4C and 6C) and xylem (Figures 4C and 6D) in the midrib. The tailings also reduced the diameter of the metaxylem vessel elements (Figures 4C and 6E).

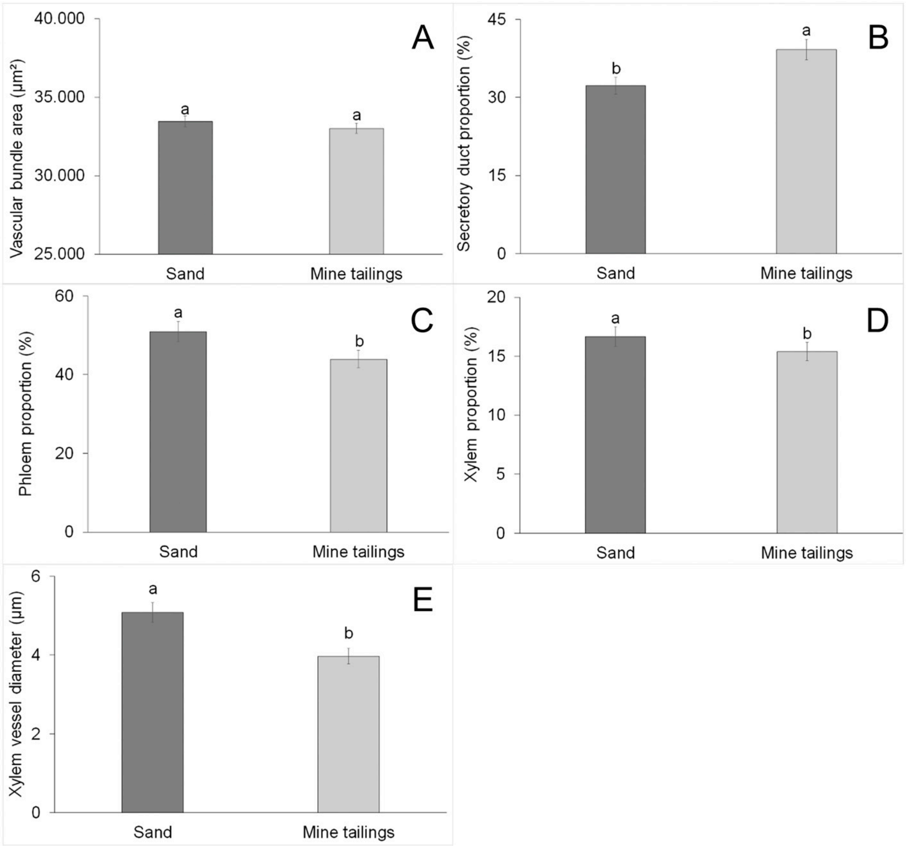

Figure 6. Anatomical characteristics of the midrib of *Schinus terebinthifolia* cultivated on the following substrates: iron mining tailings and sand. The bars indicate the standard error. For graphs (**B**,**C**,**E**), the means followed by different letters indicate significant changes according to the Scott–Knott test for $p < 0.05$. For graphs (**A**,**D**), the means followed by the same letters did not differ significantly according to the Scott–Knott test for $p < 0.05$.

3.4. Root Anatomy

Mining tailings did not generate significant changes in the root anatomy of *S. terebinthifolia* or the diameter of the vascular cylinder ($p = 0.93$), the root diameter ($p = 0.73$), the thickness of the cortex ($p = 0.85$), the diameter of the xylem vessel ($p = 0.44$), and the proportion of the vascular cylinder ($p = 0.75$). The cross-section of the primary roots of *S. terebinthifolia* in the pilifer zone revealed the initial development of the secondary structure, as the roots still contained the epidermis as a protective tissue but secondary phloem and xylem began to form in the vascular cylinder (Figure 7a). The epidermis is a single layer in which most cells are elongated in the anticlinal direction, and the cuticle is very thin and imperceptible (Figure 7b). Most of the cortex is formed by cortical parenchyma cells that are distributed in five to six layers; these cells exhibit different morphologies and intercellular space (Figure 7b). The innermost layer forms a limiting parenchyma around the entire vascular cylinder, and this layer has thick anticlinal walls (Figure 7b). The secondary phloem forms a continuous band on the outside of the vascular cylinder, and most of the cells are composed of sieve elements, companion cells and parenchyma that develop large secretory ducts (Figure 7c); however, some fiber cells are also present. The secondary xylem develops internally, forming several layers of vessel elements, fibers, and parenchyma that can deposit crystals (Figure 7c). The pith contains only ground parenchymal cells that are round in shape and form narrow intercellular spaces (Figure 7d). There was no detectable effect of mining tailings in this root anatomy which was similar in plants grown both in sand and or the residue.

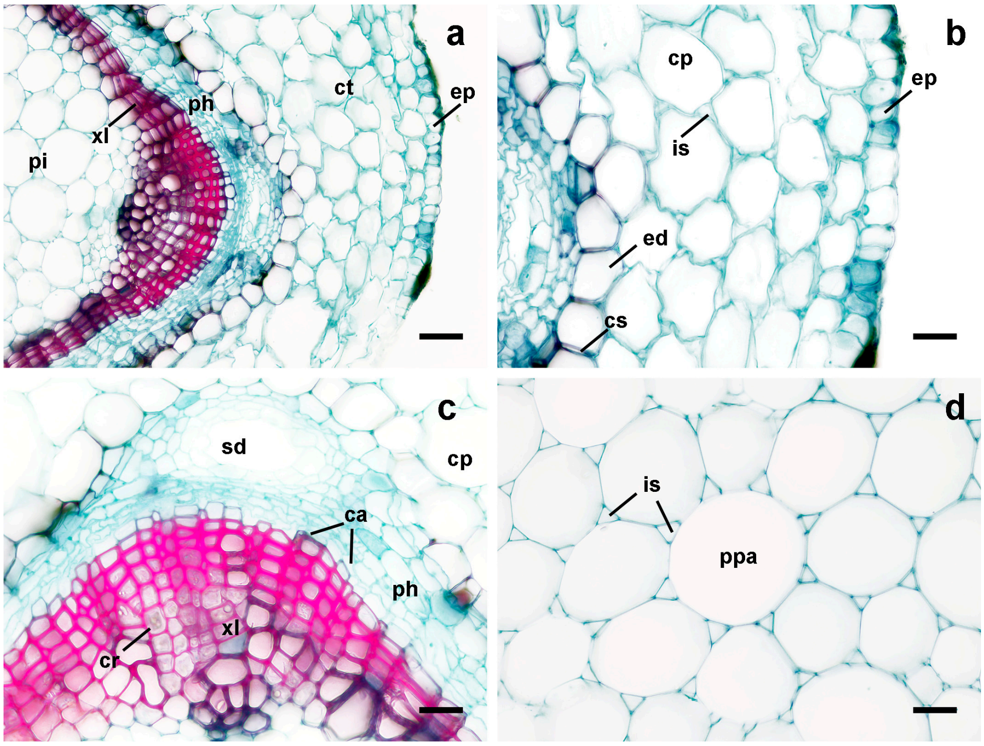

Figure 7. Cross-sections of the primary roots of *Schinus terebinthifolia*. (**a**) = overview, (**b**) = cortex and epidermis, (**c**) = vascular system, (**d**) = pith. ep = epidermis, ed = endodermis, cs = Caspary band, ca = cambium, ct = cortex, ph = phloem, xl = xylem, pi = pith, cp = cortical parenchyma, is = intercellular space, sd = secretory channel, cr = crystal, ppa = ground parenchyma. Bars = 50 µm (**a**); 25 µm (**b**–**d**).

4. Discussion

4.1. Toxicity of Iron Mining Tailings to Schinus terebinthifolia

The toxicity of the tailings to *S. terebinthifolia* may be caused by potentially toxic elements (PTEs) in the iron mining tailings (Table 1). Based on the EPT concentration limits for soil quality [25], the permitted concentration of Cd is 1.4 mg kg^{-1}; this element concentration was 64% above this level in the samples analyzed. Other authors have also reported the presence of PTEs in iron mining tailings [8,9,17,26], but the concentrations found in these cited studies and the present study are different because this type of residue is variable. Notably, the toxicity of PTEs in plants depends on their concentration, availability, and the tolerance of each species [8,9,17,19]. Cd, Al, Pb, and Cr are nonessential elements [10–13] that can enter cells through channels that normally transport essential elements; this process may trigger the production of excess reactive oxygen species [ROS] that promote lipid peroxidation, disrupting the structure of membranes and inhibiting enzymatic reactions [27,28]. Consequently, these processes induced by PTEs in mining tailings can affect photosynthetic cells, reducing photosynthesis and growth in *S. terebinthifolia*.

The reduced emergence of *S. terebinthifolia* plants grown in the mining tailings may result from the toxicity of this substrate. According to Scarpa et al. [17], the emergence of native tree species of the genus *Handroanthus* Mattos is reduced when the seeds germinate in iron mining tailings. However, the seedling emergence index of *S. terbinthifolia* was not affected by the mining tailings, and this parameter increased in the tolerant species *Schinus molle* L. under the same conditions [17]. Importantly, the concentration of

PTEs varies greatly between samples of mining tailings [8,9], and the seed germination varies between different seed lots [29] and under the experimental conditions described by Scarpa et al. [17] compared to those of the present study. Gibberellin concentrations and the gibberellin/abscisic acid ratio may vary in different seed lots, and higher values of these parameters may facilitate germination under different conditions [29]. Therefore, since the reduced emergence of *S. terbinthifolia* seedlings observed in the present study after germination in mining tailings was not observed in the study by Scarpa et al. [17], there may be variation in the tolerance of the species; this variance may depend on the seed lot sampled and endogenous factors of the seed, which should be further investigated.

The results of the present study suggest that this element is among the main PTEs that inhibit the emergence of *S. terebinthifolia*. The mining tailings used by Scarpa et al. [17] did not contain the same PTEs as those used in the present study, and the presence of Cd was not demonstrated. Cd is among the most toxic elements for plants [10]. This reduction can be attributed to the oxidative stress induced by PTEs, which is a type of stress closely related to Cd [30,31]. Oxidative stress occurs when ROS formation is directly induced or ROS removal is decreased [28]. Excess ROS can cause oxidative damage to proteins, lipids, carbohydrates, and DNA, affecting the entire metabolism of the plant [32] and triggering an autocatalytic process of membrane oxidation; these processes result in the degradation of organelles and the plasma membrane, as well as cell death [28,31]. Therefore, based on these results, oxidative stress may explain why the emergence and lethality of 25% of *S. terebinthifolia* seedlings grown in mining tailings were reduced. The work from Scarpa et al. [17] did not report lethality in *S. molle* or *S. terebinthifolia* species cultivated in mining tailings; however, compared to *S. molle*, *S. terebinthifolia* was more sensitive. In addition, the absence of lethality may be related to the variation in the chemical composition of the mining tailings, which lacks Cd.

Restrictions on the growth of *S. terebinthifolia* seedlings cultivated in mining tailings also characterize the toxicity of this substrate and are possibly related to the presence of PTEs. A reduction in growth is frequently observed in plants exposed to PTEs [33–36]. The mining tailings that reduce the growth of *S. terebinthifolia* seedlings may exert toxic effects by restricting photosynthesis since several leaf traits are reduced. The reduction in the number of leaves of *S. terebinthifolia* may be related to Cd toxicity, and leaf chlorosis was also frequently reported as a typical feature of Cd toxicity [14,37,38]. Chlorosis is closely related to the lower chlorophyll content under Cd toxicity, as this element can interfere with the synthesis of chlorophylls [14] and affects the leaf ground meristem which originates the chlorophyll parenchyma [15]. However, other PTEs present in the mining tailings may also have promoted this toxicity, but, as described by Scarpa et al. [17], there were no great signs indicating the tailings' toxicity in *S. terebinthifolia*; the only EPT absent in this study was Cd, which may have increased the toxicity of the tailings in the present study.

A reduction in root development may restrict the absorption of water and nutrients, which may affect growth and cause deformations in plants. The root development of *S. terebinthifolia* plants grown in the mining tailings was severely impaired, which may have contributed to the decreased growth of *S. terebinthifolia* on this substrate. However, the internal structure of the root did not show deformations, as observed in studies with *S. molle* exposed to lead and cadmium [13,39]. The maintenance of root tissues is essential for plants to efficiently function, as these tissues ensure that water and nutrients absorbed from the soil are transported to the photosynthetic area. These results suggest that the species can maintain its root anatomical structure when exposed to iron mining tailings.

The restriction of root development may also be related to the compaction of the mining tailings due to its clayey texture [8,9], which hinders the growth of *S. terebinthifolia* roots. The compaction of the substrate may also cause other negative effects, such as hindering the growth of the aerial part of the plant and trapping the cotyledons in the substrate (Figure 2A). The compaction of mining tailings and its possible consequences should be further investigated because these factors may be important for establishing seedlings of species used in reforestation.

Toxicity in *S. terebinthifolia* can be better understood through its leaf anatomy because this factor is directly related to photosynthesis and growth. The reduction in palisade parenchyma thickness and the proportion of phloem in *S. terebinthifolia* seedlings grown in mining tailings is probably related to the presence of PTEs and may indicate that the tailings are toxic. The thickness of the chlorophyll parenchyma is directly related to the photosynthetic capacity of *S. molle* under Cd contamination [14]. Scarpa et al. [17] reported a reduction in the palisade parenchyma in two species of *Handroanthus* and the spongy parenchyma in *S. molle* and *S. terebinthifolia* in mining tailings; the researchers associated this reduction with a possible reduction in the photosynthetic rate, which consequently reduced seedling growth, especially in *Handroanthus* spp. A reduction in total leaf thickness was also reported by Baroni et al. [39] in ferns cultivated under Cr contamination. Another result related to this reduction was the smaller size of the chloroplasts and the lower chlorophyll content of *S. terebinthifolia* cultivated under mining tailings, which can be directly associated with lower photosynthetic rates. The reduction in chlorophyll parenchyma and total leaf thickness may be related to the lower photosynthetic rate of *S. terebinthifolia* plants grown in mining tailings; these reductions may have reduced the photosynthetic capacity, resulting in a lower chlorophyll content and consequently decreasing plant growth. Other anatomical changes may be related to the toxicity of the PTEs present in the mining tailings. The reduction in the diameter of the xylem vessel may be associated with the presence of PTEs. This reduction in xylem vessel elements may reduce the flow of water and nutrients to the aerial part and may decrease the flow of PTEs to photosynthetic tissues. Thus, this reduction in vessel diameter is a defense response of the plant but has negative consequences for growth because it reduces the flow of water and nutrients to photosynthetic tissues.

Interestingly, larger secretory channels were observed in the leaves of *S. terebinthifolia* plants cultivated in the mining tailings compared with plants grown in sand. Sridhar et al. [40] reported that when *S. molle* was subjected to moderate doses of Pb, the size of the secretory channels, the volume of essential oils, and the complexity of these compounds increased, enhancing their allelopathic capacity. These increases may be a mechanism of tolerance, as essential oils are associated with allelopathic effects [41]. Thus, *S. terebinthifolia* and *S. molle* seem to develop larger secretory channels in the presence of pollutants, which may help these plants become established in reforestation systems because competition with other species is reduced due to the allelopathic effects of essential oils. This is possibly a response to the toxicity of the substrate because the substrate stimulates plant defenses, which decrease competition and increase the chances of plant survival.

4.2. Potential of S. terebinthifolia for Reforestation in Areas Impacted by Fe Mining Tailings

Despite the reduction in the growth of *S. terbinthifolia* seedlings, a high percentage of these plants (75%) survived in the mining tailings and demonstrated the ability to grow and develop. The thickness of the leaf tissues decreased, but there were no deformations in the tissues that prevented photosynthesis and growth. Species of the genus *Schinus* may be important for the successful recovery of degraded areas because their seeds can be stored for a year or more [20,41]; thus, planting can be staggered over time and the period of seed viability in the field is long. In addition, these native species are extremely important for the recovery of degraded areas because the biodiversity of the region is conserved and the costs of seedling production and transportation are reduced [42]. Notably, *S. molle* showed tolerance to Pb during germination and early growth [13], as well as to Cd [14,15]. While Cd inhibited the germination and growth of *S. terebinthifolia* [43], this inhibition depends on several factors, such as the concentration and availability of the pollutant. Scarpa et al. [17] reported that both *S. molle* and *S. terebinthifolia* demonstrate the potential for revegetating areas impacted by iron mining tailings, with *S. molle* being more tolerant, and the results of the present study corroborate these observations. In addition, despite the possibility of using *S. terebinthifolia* for revegetating areas impacted by iron mining tailings, its germination and growth depend on the chemical composition of the tailings. Recent studies have also demonstrated the tolerance of tree species to Fe mining tailings generated from the failed Fundão dam [8,9,17]. José et al. [44,45] reported that *S. terebinthifolia* also showed great potential for recovering areas degraded by bauxite mining. Therefore, *S. terebinthifolia* shows potential for revegetating areas impacted by Fe mining tailings, but its growth and development are limited by the composition of the tailings.

5. Conclusions

Iron mining tailings cause partial toxicity to *S. terebinthifolia*, promoting reductions in emergence and seedling growth; nonetheless, the species survived in the tailings as the damage to seedlings was not harsh since the plants preserved their overall anatomical structure in leaves and roots. A reduction in the growth of *S. terebinthifolia* is associated with a reduction in the thickness of leaf photosynthetic tissues, the size of chloroplasts, and the chlorophyll content, thus reducing the photosynthetic capacity of the plant. Nevertheless, the species survived in the tailings, and the internal structure of the roots was maintained, demonstrating the potential of the species for cultivation in tailings and achieving reforestation. Tailings showed the presence of potentially toxic elements (Al, Cd, Cr, and Pb), which may be related to its toxicity; however, macro- and micronutrients necessary to plants were also found. This work focused on seed germination and early seedling growth and further works should focus on experiments testing the species adaptation in field conditions and also the effect of a longer exposition time on iron mining tailings.

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References

1. IBRAM—Instituto Brasileiro de Mineração. *Gestão E Manejo de Rejeitos de Mineração*; IBRAM: Brasilia, Brazil, 2016; ISBN 978-85-61993-10-8.
2. Lacaz, F.A.C.; Porto, M.F.S.; Pinheiro, T.M.M. Tragédias brasileiras contemporâneas: O caso do rompimento da barragem de rejeitos de Fundão/Samarco. *Rev. Bras. Saúde Ocup.* **2017**, *42*, e9. [CrossRef]
3. Andrade, G.F.; Paniz, F.P.; Martins, A.C.J.R.; Rocha, B.A.; Lobato, A.K.S.; Rodrigues, J.L.; Gustavson, P.C.; Masuda, H.P.; Batista, L.B. Agricultural use of Samarco's spilled mud assessed by rice cultivation: A promising residue use? *Chemosphere* **2018**, *193*, 892–902. [CrossRef] [PubMed]
4. Adiansyah, J.S.; Rosano, M.; Vink, S.; Keir, G. A framework for a sustainable approach to mine tailings management: Disposal strategies. *Clean. Prod.* **2015**, *108*, 1050–1062. [CrossRef]
5. Carmo, F.F.; Kamino, L.H.Y.; Tobias Junior, R.; Campos, I.C.; Carmo, F.F.; Silvino, G.; Castro, K.J.S.X.; Mauro, M.L.; Rodrigues, N.U.A.; Miranda, M.P.S.; et al. Fundão tailings dam failures: The environment tragedy of the technological disaster of Brazilian mining in global. *Perspect. Ecol. Conserv.* **2017**, *15*, 145–151. [CrossRef]
6. Silva, C.A.; Oliveira-Júnior, J.F.; Teodoro, P.E.; Lima, M.; Shakir, M.; Gois, G.; Johann, J.A. Analysis of the impact on vegetation caused by abrupt deforestation via orbital sensor in the environmental disaster of Mariana, Brazil. *Land Use Policy* **2018**, *76*, 10–20.
7. Guimarães, L.A.O.P.; Assis, I.R.; Dias, L.E.; Cordeiro, A.L.; Freire, A.S. Espécies arbóreas potenciais para a revegetação de rejeito salino contaminado com arsênio. *Ciênc. Florest.* **2017**, *27*, 871–881. [CrossRef]

8. Pádua, M.P.; Caetano, A.L.; Polo, M.; Pasqual, M.; Pereira, F.J. Ecophysiological responses of *Copaifera langsdorfi* grown in mining tailing under lower water availability. *Water Air Soil Pollut.* **2021**, *232*, 57. [CrossRef]
9. Caetano, A.L.; Pádua, M.P.; Polo, M.; Pasqual, M.; Pereira, F.J. Growth, anatomy, and gas Exchange of *Cenostigma pluviosum* cultivated under reduced water levels in iron mining tailing. *J. Soils Sediments* **2021**, *22*, 381–391. [CrossRef]
10. Benavides, M.P.; Gallego, S.M.; Tomaro, M.L. Cadmium toxicity in plants. *Braz. J. Plant Phisiol.* **2005**, *17*, 21–34. [CrossRef]
11. Quintal, E.B.; Magana, C.E.; Machado, L.E.; Estevez, M.M. Aluminum, a friend or foe of higher plants in acid soils. *Front. Plant Sci.* **2017**, *8*, 1767.
12. Sharma, P.; Dubey, R.S. Lead toxicity plants. *Braz. J. Plant Physiol.* **2005**, *17*, 35–52. [CrossRef]
13. Pereira, M.P.; Pereira, F.J.; Corrêa, F.F.; Oliveira, C.; Castro, E.M.; Barbosa, S. Lead tolerance during germination and early growth of the Brazilian Peppertree and the morpho-physiological modifications. *Rev. Ciênc. Agrár.* **2013**, *56*, 72–79. [CrossRef]
14. Pereira, M.P.; Rodrigues, L.C.A.; Correa, F.P.; Castro, E.M.; Ribeiro, V.E.; Pereira, F.J. Cadmium tolerance in *Schinus molle* trees is modulated by enhanced leaf anatomy and photosynthesis. *Trees* **2016**, *22*, 163–170. [CrossRef]
15. Pereira, M.P.; Corrêa, F.F.; Castro, E.M.; Oliveira, J.P.V.; Pereira, F.J. Leaf ontogeny of *Schinus molle* L. plants under cadmium contamination: The meristematic origin of leaf structural changes. *Protoplasma* **2017**, *254*, 2117–2126. [CrossRef] [PubMed]
16. Lorenzi, H. *Árvores Brasileiras: Manual de Identificação e Cultivo de Plantas Arbóreas Nativas do Brasil*; Editora Plantarum: Nova Odessa, Brazil, 1992.
17. Scarpa, A.L.M.; Rodrigues, F.A.; Cruz, Y.C.; Duarte, V.P.; Castro, E.M.; Pasqual, M.; Pereira, F.J. Seed germination, initial growth and leaf anatomy of seedlings of four tree species grown in mine tailings in Brazil. *Seed Sci. Res.* **2022**, *32*, 104–114. [CrossRef]
18. AOAC. *Official Methods of Analysis of the Association Analytical Chemists*; AOAC: Rockville, MD, USA, 2005.
19. Scarpa, A.L.M.; Cruz, Y.C.; Duarte, V.P.; Castro, E.M.; Pasqual, M.; Oliveira, J.P.V.; Pereira, F.J. Growth response, gas exchange, and leaf anatomy of *Handroanthus* spp. seedling in mine tailings enriched with nutrient solution. *J. Soil Sci. Plant Nutr.* **2022**, *22*, 3774–3787. [CrossRef]
20. Pereira, M.P.; Correa, F.F.; Polo, M.; Castro, E.M.; Cardoso, A.A.; Pereira, F.J. Seed germination of *Schinus molle* L. [Anacardiaceae] as related to its anatomy and dormancy alleviation. *Seed Sci. Res.* **2016**, *26*, 351–361. [CrossRef]
21. Hoagland, D.R.; Arnon, D.I. *The Water-Culture Method for Growing Plants without Soil*; California Agricultural Experiment Station: Salinas, CA, USA, 1950; Volume 347.
22. Johansen, D.A. *Plant Microtechnique*; McGraw-Hill: New York, NY, USA, 1940.
23. Kraus, J.E.; Arduin, M. *Manual Básico de Métodos em Morfologia Vegetal*; EDUR: Seropédica, Brazil, 1997.
24. Ferreira, D.F. Sisvar: A computer statistical analysis system. *Ciênc. Agrotecnol.* **2011**, *35*, 1039–1042. [CrossRef]
25. CONAMA. Conselho Nacional do Meio Ambiente. Resolução n° 420, de 28 de dezembro de 2009. Dispõe sobre critérios e valores orientadores de qualidade do solo quanto à presença de substâncias químicas e estabelece diretrizes para o gerenciamento ambiental de áreas contaminadas por essas substâncias em decorrência de atividades antrópicas. *Diário União* **2009**, *249*, 81–88.
26. Guerra, M.B.B.; Teaney, B.T.; Mout, B.J.; Asunskis, D.J.; Jordan, B.T.; Barker, R.J.; Santos, E.E.; Schaefer, C.E.G.R. Post-catastrophe analyses of the Fundão tailing dan failure in the doce river system, southeast Brazil: Potentially toxic elements in affectes soils. *Water Air Soil Pollut.* **2017**, *228*, 252. [CrossRef]
27. Apel, K.; Hirt, H. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* **2004**, *55*, 373–379. [CrossRef] [PubMed]
28. Moller, I.M.; Jensen, P.E.; Hansson, A. Oxidative Modifications to cellular components in Plants. *Annu. Rev. Plant Biol.* **2007**, *58*, 459–481. [CrossRef] [PubMed]
29. Zhang, Z.; Fran, J.; Jinghua, W.; Zhang, L.; Wang, J.; Zhand, B.; Pruski, G.W. Alleviating effectof silicon on melon seed germinationunder autotoxicity stress. *Ecotoxicol. Environ. Saf.* **2020**, *188*, 109901. [CrossRef] [PubMed]
30. Gonçalves, J.F.; Becker, A.G.; Cargnelutti, D.; Tabaldi, L.A.; Pereira, L.B.; Battisti, V.; Spanevello, R.M.; Morsch, V.M.; Nicoloso, F.T.; Schetinger, M.R.C. Cadmium toxicity causes oxidative stress and induces response of the antioxidant system in cucumber seedlings. *Braz. J. Plant Physiol.* **2007**, *19*, 223–232. [CrossRef]
31. Vestena, S.; Cambraia, J.; Ribeiro, C.; Oliveira, J.A.; Oliva, M.A. Cadmium-induced oxidative stress andantioxidative enzyme response in water Hyacinth and Salvinia. *Braz. J. Plant Physiol.* **2011**, *23*, 131–139. [CrossRef]
32. Gill, S.S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **2010**, *48*, 909–930. [CrossRef]
33. Chaves, L.H.G.; Souza, R.S. Crescimento, distribuição e acumulação de cádmio em plantas de *Jatropha curcas*. *Rev. Ciênc. Agrár.* **2014**, *37*, 286–291.
34. Romeiro, S.; Lagoa, A.M.M.A.; Furlani, P.R.; Abreu, C.A.; Pereira, P.F.F. Absorção de chumbo e potencial de fitorremediação de *Canavalia ensiformes* L. *Solos Nutr. Plantas* **2007**, *66*, 327–334. [CrossRef]
35. Oliveira, N.P.; Bastos, A.R.R.; Costa, A.L.; Cipriani, H.N. *Eucalyptus urophylla* cuttings under different concentrations of Cr: Toxicity symptoms and effects on Growth. *Braz. J. Dev.* **2022**, *8*, 33990–34001. [CrossRef]
36. Steiner, F.; Zoz, T.; Pinto, A.S.; Castagnara, D.D.; Dranski, J.A.L. Effects of aluminum on plant growth and nutrient uptake in young physic nut plants. *Semin. Ciênc. Agrár.* **2012**, *33*, 1779–1788. [CrossRef]
37. Ferreira, M.M.A.A.S.; Santos, J.A.G.; Moura, S.C.; Abreu, C.B.; Bomfim, M.R.; Azevedo Neto, A.D.A. Cadmium effects on sunflower growth and mineral nutrition. *Afr. J. Agric. Res.* **2016**, *11*, 3488–3496.

38. Cunha, K.P.V.; Nascimento, C.W.A.; Pimentel, R.M.M.; Accioly, A.M.A.; Silva, A.J. Disponibilidade, acúmulo e toxidez de cádmio e zinco em milho cultivado em solo contaminado. *Rev. Bras. Ciênc. Solo* **2008**, *32*, 1319–1328. [CrossRef]
39. Baroni, G.R.; Pereira, M.P.; Corrêa, F.F.; Castro, E.M.; Pereira, F.J. Cadmium tolerance during seed germination and seedling growth of *Schinus molle* (Anacardiaceae). *Floresta Ambiente* **2020**, *27*, e20170502. [CrossRef]
40. Sridhar, B.B.M.; Han, F.X.; Diehl, S.V.; Monts, D.L.; Su, Y. Effect of phytoaccumulation of arsenic and chromium on structural and ultrastructural changes of brake fern (*Pteris vittata*). *Braz. Soc. Plant Physiol.* **2011**, *23*, 288–293. [CrossRef]
41. Ribeiro, L.P.; Leite, A.T.; Silva, L.B.J.; Silva, V.F.; Borges, E.E.L. Physiological and biochemical changes in Brazilian Pepper (*Schinus terebinthifolia* Raddi) seeds during storage. *Rev. Árvore* **2018**, *42*, E420105. [CrossRef]
42. Almeida, V.G.; Moura, E.N.; Vieira, G.T. Espécies vegetais utilizadas em áreas degradadas pela mineração. *Res. Soc. Dev.* **2019**, *8*, e3583710. [CrossRef]
43. Silva, E.S.; Guilherme, M.F.S.; Oliveira, H.M.; Araújo, L.N.C.; Viana, Z.C.V.; Santos, C.S. Ecotoxicological effects of cadmium on the germination and initial development of *Schinus terebinthifolius*. *Rev. Ciênc. Agrár.* **2017**, *40*, 311–318. [CrossRef]
44. José, A.C.; Davide, A.C.; Oliveira, S.L. Produção de mudas de aroeira (*Schinus terebinthifolia* Raddi) para áreas degradadas pela mineração de bauxita. *Cerne* **2005**, *11*, 187–196.
45. Ribeiro, V.E.; Pereira, M.P.; Castro, E.M.; Correa, F.F.; Cardoso, M.G.; Pereira, F.J. Enhanced essential oil and leaf anatomy of *Schinus molle* plants under lead contamination. *Ind. Crops Prod.* **2019**, *132*, 92–98. [CrossRef]