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POLIANA NOEMIA DA SILVA

GERMINATION AND GROWTH Of *Schinus Terebinthifolia* Raddi GROWN ON COMPACTED IRON MINING TAILINGS

LAVRAS-MG
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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Botânica Aplicada, área de concentração em Botânica Aplicada, para a obtenção do título de Mestre.

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GERMINATION AND GROWTH Of *Schinus Terebinthifolia* Raddi GROWN ON COMPACTED IRON MINING TAILINGS

GERMINAÇÃO E CRESCIMENTO DE *Schinus terebinthifolia* Raddi CULTIVADA EM RESÍDUO DE MINERAÇÃO DE FERRO

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Botânica Aplicada, área de concentração em Botânica Aplicada, para a obtenção do título de Mestre.

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ABSTRACT

Soil compaction is a well-known harmful process for germination and seedling growth and development. Nonetheless, studies about this process in different pollutants such as mining tailings have been neglected despite being essential for the revegetation of impacted areas. This work aimed to evaluate the effects of compaction of iron mining tailings on the germination, early growth, and photosynthesis of *Schinus terebinthifolia* Raddi, which is a tolerant species with potential for use in revegetation systems. Experiments were implemented in a greenhouse where seeds of *S. terebinthifolia* were sown in iron mining tailings with four compaction treatments: 1) uncompacted dry tailing, 2) uncompacted moistened tailing, 3) compacted dry tailing, and 4) compacted moistened tailing. Penetration resistance, seed germination, germination speed index, seedling biometry, and photosynthesis were evaluated. Compacted moistened tailings showed the highest penetration resistance and the uncompacted dry tailings the lowest, other treatments showed intermediate means. The compacted moistened tailings produced the lowest germination rate and germination speed index and these parameters showed the highest means at uncompacted dry tailings with other treatments showing intermediate means. Compaction did not affect seedling biometric parameters with similar growth among all treatments. *Schinus terebinthifolia* seedlings showed improved growth parameters during the experimental period evidencing the potential of the species for reforestation systems in impacted areas by iron mining tailings.

Keywords: solid wastes; brazilian pepper tree; recovering of impacted areas; trees.

RESUMO

A compactação do solo é um processo nocivo para a germinação, crescimento e desenvolvimento de mudas. No entanto, estudos sobre esse processo em diferentes poluentes, como resíduo de mineração de ferro, têm sido negligenciados, apesar de serem essenciais para a revegetação de áreas impactadas. Este trabalho teve como objetivo avaliar os efeitos da compactação de resíduos de mineração de ferro na germinação, crescimento inicial e fotossíntese de *Schinus terebinthifolia* Raddi, que é uma espécie tolerante com potencial para uso em sistemas de revegetação. Os experimentos foram implementados em casa de vegetação onde sementes de *S. terebinthifolia* foram semeadas em resíduos de mineração de ferro com quatro tratamentos de compactação: 1) resíduo seco não compactado, 2) resíduo umedecido não compactado, 3) resíduo seco compactado e 4) resíduo umedecido compactado. A resistência à penetração, a germinação das sementes, o índice de velocidade de germinação, a biometria das mudas e a fotossíntese foram avaliados. Os resíduos umedecidos compactados apresentaram a maior resistência à penetração e os resíduos secos não compactados a menor, outros tratamentos apresentaram médias intermediárias. Os resíduos umedecidos compactados produziram a menor taxa de germinação e índice de velocidade de germinação e esses parâmetros apresentaram as maiores médias em resíduos secos não compactados com outros tratamentos apresentando médias intermediárias. A compactação não afetou os parâmetros biométricos das mudas com crescimento semelhante entre todos os tratamentos. As mudas de *Schinus terebinthifolia* apresentaram parâmetros de crescimento melhorados durante o período experimental, evidenciando o potencial da espécie para sistemas de reflorestamento em áreas impactadas por resíduos de mineração de ferro.

Palavras- chave: resíduos sólidos; aroeira-brasileira; recuperação de áreas impactadas; árvores.

INDICADORES DE IMPACTO

Em primeiro lugar, é importante ressaltar o impacto científico do presente trabalho. É a primeira vez em que se avaliou o impacto da compactação do resíduo de mineração de ferro sobre a germinação e o crescimento inicial de uma espécie nativa. Isto pode guiar novas pesquisas que avaliem o efeito de diferentes níveis de compactação de resíduo de mineração de ferro sobre outras espécies vegetais da flora nativa, buscando avaliar espécies que possam ser tolerantes e utilizadas para restauração e recuperação de áreas degradadas. Para além do avanço científico, este trabalho também apresenta impactos potencialmente econômicos e sociais. Em relação aos impactos econômicos, ao levar em consideração os resultados do trabalho, *Schinus terebinthifolia* é uma espécie tolerante a compactação de resíduos de mineração de ferro. Isso pode, potencialmente, levar a utilização dessa espécie em programas de restauração e recuperação florestal em áreas afetadas por solos compactados, tanto no caso de resíduo de mineração de ferro quanto potencialmente outros tipos de solo. Sistemas de reflorestamento tem o potencial de gerar mão-de-obra local, além da oportunidade de parcerias entre a sociedade civil, o poder público e a iniciativa privada. Entre os impactos sociais, pode-se citar o potencial que uma espécie tolerante tem para recuperar áreas impactadas e, assim, envolver as comunidades locais afetadas no processo de recuperação das áreas afetadas. Munidas de trabalhos científicos que demonstram como determinadas espécies reagem as condições ambientais, as pessoas podem exigir dos órgãos competentes que o processo de restauração das áreas afetadas seja feito da melhor maneira possível. Assim, os cidadãos se tornam agentes ativos e conhecedores do processo de recuperação das áreas nas quais vivem.

IMPACT INDICATORS

Firstly, it is important to emphasize the scientific relevance of the present study. This is the first known assessment of the effects of iron mining waste compaction on the germination and early development of a native plant species. The findings may serve as a foundation for future research investigating the impact of varying levels of compaction on other native plant species, with the aim of identifying those that demonstrate tolerance and potential applicability in the restoration and rehabilitation of degraded areas. In addition to contributing to scientific advancement, this study also presents potential economic and social implications. From an economic standpoint, the results indicate that *Schinus terebinthifolia* is tolerant to iron mining waste compaction, suggesting its suitability for use in forest restoration and rehabilitation programs in areas affected by compacted soils—whether resulting from mining activities or other sources. Reforestation initiatives have the potential to generate local employment opportunities, while also fostering collaboration among civil society, public institutions, and the private sector. From a social perspective, the use of tolerant species in ecological restoration can promote the recovery of degraded areas and actively engage affected local communities in the restoration process. Supported by scientific evidence on species-specific responses to environmental stressors, local populations are empowered to demand that the restoration of impacted areas be conducted in an effective and evidence-based manner. In this way, citizens become informed and proactive participants in the ecological recovery of the environments in which they live.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

Em novembro de 2015, ocorreu o rompimento da barragem de Fundão, localizada no município de Mariana, Minas Gerais, Brasil. Esse rompimento despejou mais de 34 milhões metros cúbicos de resíduo de mineração de ferro no ambiente, causou 18 mortes e gerou impactos irreversíveis ao ecossistema (Carmo *et al.* 2017; Lacaz *et al.* 2016). Estima-se que cerca de 1200 ha da vegetação local foram totalmente destruídas (Silva Junior *et al.* 2018). Nesse contexto, o reflorestamento é uma alternativa na recuperação destas áreas, e para isso busca-se identificar espécies nativas que sejam tolerantes ao resíduo de mineração de ferro.

Elementos potencialmente tóxicos, como o Al, Cr, Cd e Pb foram encontrados no resíduo de mineração de ferro proveniente do rompimento da barragem de Fundão (Pádua *et al.* 2021; Scarpa *et al.* 2022b; Caetano *et al.* 2021; Silva *et al.* 2024). Geralmente a redução no crescimento das plantas é atribuída a altos níveis de elementos potencialmente tóxicos no solo (Chaves e Souza, 2014; Romeiro *et al.* 2007; Oliveira *et al.* 2022; Steiner, 2012), mas isso não impossibilita que a germinação, crescimento e estabelecimento das plantas seja influenciado por outros fatores.

O resíduo de mineração de ferro não afetou a germinação de espécies nativas da Mata Atlântica, mas promoveu efeitos negativos no crescimento; os autores atribuíram esses resultados a baixa disponibilidade de nutrientes, baixo teor de matéria orgânica e baixa capacidade de troca catiônica (Cruz *et al.* 2020). No entanto, Scarpa *et al.* (2022a) constatou que os nutrientes presentes no resíduo de mineração de ferro são suficientes para o crescimento de *Handroanthus serratifolius* e *Handroanthus impetiginosus*. A heterogeneidade do resíduo de mineração de ferro, bem como a demanda nutricional de cada espécie poderia explicar esses resultados contrastantes.

De acordo com Pádua *et al.* 2021 e Matos *et al.* 2021), as partículas de resíduo de mineração de ferro são muito pequenas (com diâmetro igual ou inferior a 2 μm), o que pode aumentar o risco de compactação desse material, uma vez que podem se alojar entre partículas maiores presentes no meio. A compactação do solo é um fator secundário que pode influenciar na disponibilidade de recursos para as plantas, afetando o seu crescimento e estabelecimento (Durr e Aubertol 2000; Foloni *et al.* 2003), é definida como “o processo pelo qual as partículas do solo são reorganizadas para diminuir o espaço vazio e colocá-las em contato mais próximo umas com as outras, aumentando assim a densidade aparente” (Soil Science Society of América, 1996). Os principais fatores que influenciam na compactação do solo são teor de água, textura, matéria orgânica e pressão sobre o solo, sendo essa pressão ocasionada por pisoteio de animais ou pelo uso de maquinários sobre o solo (Nawaz *et al.* 2013). Considerada uma forma grave de degradação do solo, a compactação é muitas vezes responsável pela redução do rendimento das culturas agronômicas e pela redução da taxa de crescimento das florestas (Nawaz *et al.* 2013).

A espécie escolhida para este estudo é *Schinus terbinthifolia* Raddi, conhecida popularmente como aroeira mansa, aroeira pimenteira e aroeirinha (Carvalho, 2003). *S. terebinthifolia* é uma espécie com múltiplas aplicações, como madeireira, medicinal e ornamental, podendo também ser utilizadas para arborização urbana e recuperação de áreas degradadas (Scarpa *et al.* 2022b). Além disso, o fruto da aroeira é muito utilizado como tempero culinário e na produção de óleos essenciais, e a semente, seu principal meio de propagação, é de fácil armazenamento (Ribeiro *et al.* 2018). *S. terebinthifolia* ocorre de forma natural em diversas formações vegetais no Brasil, onde as condições ambientais são muito distintas (Souza *et al.* 2022). Isso sugere que a espécie tem capacidade de desenvolver características particulares em resposta aos fatores ambientais (Souza *et al.* 2022). Estudos recentes mostraram que *S. terebinthifolia* é tolerante ao resíduo de mineração de ferro e tem potencial para recuperar áreas degradadas por este material (Scarpa *et al.* 2022b; Silva *et al.* 2024).

Este trabalho levanta a hipótese de que resíduos de mineração de ferro compactados podem prejudicar a germinação de sementes e o crescimento de plântulas, mas *S. terebinthifolia* apresenta algum grau de tolerância a esse fator secundário. Assim, o objetivo deste estudo foi avaliar o efeito de diferentes níveis de compactação do resíduo de mineração de ferro na germinação e crescimento inicial de plântulas de *S. Terebinthifolia*.

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SEGUNDA PARTE - ARTIGO

ARTIGO - Compaction of iron mining tailings impairs seed germination of *Schinus terebinthifolia* but its seedling show tolerance early growth

Artigo elaborado de acordo com as normas do periódico Forests.

Compaction of iron mining tailings impairsseed germination of *Schinusterebinthifolia* but its seedling show tolerance early growth

Abstract: Soil compaction is a well-known harmful process for germination and seedling growth. Studies about compaction in mining tailings have been neglected despite being essential for the reforestation of impacted areas. This work evaluated the effects of compaction of iron mining tailings on the germination, early growth, and photosynthesis of *Schinus terebinthifolia* Raddi, a tolerant species with potential for reforestation. Experiments were implemented in a greenhouse where seeds of *S. terebinthifolia* were sown in iron mining tailings with four compaction treatments: 1) uncompacted dry tailing, 2) uncompacted moistened tailing, 3) compacted dry tailing, and 4) compacted moistened tailing. Penetration resistance, seed germination, germination speed index, seedling biometry, and photosynthesis were evaluated. Compacted moistened tailings showed the highest penetration resistance and the uncompacted dry tailings the lowest, other treatments showed intermediate means. The compacted moistened tailings produced the lowest germination rate and germination speed index and these parameters showed the highest means at uncompacted dry tailings with other treatments showing intermediate means. Compaction did not affected seedling's biometric parameters with similar growth among all treatments. *Schinus terebinthifolia* seedlings showed improved growth parameters during the experimental period evidencing the potential of the species for reforestation of impacted areas by iron mining tailings.

Keywords: **Keywords:** Solid wastes; Brazilian pepper tree; Reforestation; Tree's ecophysiology

1. Introduction

Brazil is the second largest iron exporter in the world and its Minas Gerais state is where the largest iron mines are found. Tragic events involving mining activities have been registered for many years [1]. In November 2015 in Mariana City located in Minas Gerais state, Brazil, a massive disaster occurred being considered the most harmful in the country to this date and one of the largest ever worldwide [1, 2]. More than 34 million cubic squares of iron mining tailing were released in this accident contaminating more than 1200 ha [3].

Iron mining tailings from Fundão dam showed several potentially toxic elements such as Al, Cd, Cr, and Pb most of these below the toxicity levels (3, 4, 5, 6, 7). Nonetheless, secondary factors can increase the toxicity of the iron mining tailings such as lowering the pH to 5 or below [8]. In this context, one secondary factor never investigated for iron mining tailings is the compaction effect in this pollutant. According to Pádua et al. [4] and Matos et al. [9], the particles of iron mining tailings from Fundão dam failure are small (2 µm or less in diameter), these particles may increase the risk of compaction of this pollutant being inserted among bigger particles present. Soil compaction is a known process of degradation that impairs seed germination and plant growth [10]; nonetheless, the compaction effects in mining tailings are unclear.

Reforestation of areas impacted by mining tailings is an effective method to acceler-ate recovering of these sites. *Schinus terebinthifolia* Raddi is tolerant to iron mining tail-ings, capable of germinating and growing in the pollutant despite some degree of toxic-ity is evident [3, 7]. This species shows potential for reforestation of areas impacted by iron mining tailings [7]. *S. terebinthifolia* belongs to the Anacardiaceae family and is native to South America but is cultivated worldwide for urban forestation, reforestation of impacted areas, and as an ornamental tree [11].

This work hypothesizes that compacted iron mining tailings can impair seed germination and seedling growth but *S. terebinthifolia* shows some degree of tolerance to this secondary factor. Thus, the objective of this work was to evaluate the effect of compaction of iron mining tailings on seed germination, seedling growth, and photosynthesis of *S. terebinthifolia*.

2. Material and methods

2.1 Iron mining tailings traits

Iron mining tailings samples were collected 4 km away from Fundão dam in Mariana, state of Minas Gerais, Brazil (20° 22′ 40″ S 43° 24′ 57″ W) and transported to Universidade Federal de Alfenas, Alfenas, state of Minas Gerais, Brazil (21° 25′ 44″ S 45° 56′ 49″ W). Iron mining tailings were stored in plastic bags in a room protected against sources of water, heat, and radiation until the start of experiments. Iron mining tailings were then transported to the Universidade Federal de Lavras, Lavras, state of Minas Gerais, Brazil (21° 14′ 43″ S 44° 59′ 59″ W) for the implementation of experiments.

Iron mining tailings samples were analyzed in the Soil Analysis Laboratory from the Universidade Federal de Lavras, Lavras, state of Minas Gerais, Brazil. The micro and macronutrient contents as well as potentially toxic elements were analyzed. In addition, the organic matter, pH, and granulometry were also evaluated.

Mining tailings were oven-dried at 60 °C until reaching a constant mass and submitted to different extraction methods for elemental quantification. For this, 500 mg of the tailing was extracted using 50 ml of extractors, being: mehlich-1 extractor for P, Na, K, Fe, Zn, Mn, Cr, Pb, and Cu [12], Ca, Mg, and Al were extracted using KCl 1.0 M [13], and H+Al was determined using according to Raij et al. [14]. The levels of macro and micronutrients and potentially toxic elements were quantified using an atomic absorption spectrophotometer, AAnalyst 800 (PerkinElmer, Waltham, MA, USA). The pH was measured using the electrode immersed in a solution containing the tailings suspension in KCl and $CaCl_2$ (1:2.5). The granulometry of iron mining tailings was determined according to Bouyoucos [15]. Values for macronutrients, micronutrients, potentially toxic elements, organic matter, pH, and granulometry are shown in Table 1.

Table 1. Granulometry and 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 permited [7].

Macronutrients	mg kg^{-1} dry mass	**PTE maximum concentrations [mg k^{-1}]***
P	10.7	-
Mg	18.2	-
K	41.6	-
Ca	226.4	-
Micronutrients	**mg Kg^{-1}**	
Mn	200.4	-
Fe	189.3	-
Zn	0.7	300.0
Cu	1.1	63.0
Na	37.0	-
Potentially toxic elements	**mg Kg^{-1}**	
Al	80.9	-
Cr	0.07	75.0
Pb	0.02	72.0
Other characteristics		
pH	5.7	-
OM (mg Kg^{-1})	4,3	-
Granulometry	**%**	
Clay Silt Sand	12	-
	39	-
	49	-

2.2 Plant material and experimental design

Seeds of *Schinus terebinthifolia* Raddi were collected from trees used in urban forestation in Alfenas, state of Minas Gerais, Brazil (21° 25′ 44″ S 45° 56′ 49″ W). Fruits were oven-dried at 40ºC for five days and then stored in glass flasks at 4ºC until the installation of the experiment. Seeds were removed from dried fruits and submitted to a pre-treatment being immersed in a 1.0 mM GA_3 for 24 h before sowing. This treatment showed necessary by preliminary tests done by the group to improve seed germination of *S. terebinthifolia*, which is naturally very low [6, 7].

Seeds were sown in gerbox flasks containing 250 ml of sifted and dried iron mining tailings from the Fundão dam being previously submitted to four compaction treatments: 1) uncompacted dry tailings (DT), 2) uncompacted moistened tailings (MT), 3) compacted dry tailings (CDT), and 4) compacted moistened tailings (CMT).

Compaction was performed by promoting a pressure of 0.017 kgf cm^{-2} (1.67 kPa) by 24 h before sowing. For this, a load weighing 2 kg was placed on the surface of tailings in each gerbox from compacted treatments staying in this position for 24 h. The pressure was determined by dividing the weight of the load (2 kg) by the surface area of the gerbox (121cm^2). We considered 1 kgf cm^{-2} equal to 98.07 kPa and the kPa values applied were calculated by multiplying 0.017 by 98.07 giving 1.67 kPa. For both MT and CMT treatments 100 ml of water per gerbox was added, which is about 40% of the volume of tailings used (250 ml) being sufficient to saturate the tailings according to Pádua et al. [4]. For the CMT treatment tailings were moistened before the applica-tion of the load.

Seeds were sown after the compaction treatments and penetration resistance analyses were performed. Tailings were re-moistened using 100 ml of water per gerbox and further 100 seeds per gerbox were added in 0.5 cm depth pits later covered with tailings. Seeds were sown after compaction to avoid damage to their tissues. The experiment was carried out for 60 days in a greenhouse located at Universidade Federal de Lavras, Lavras, state of Minas Gerais, Brazil (21° 14′ 43″ S 44° 59′ 59″ W) with a mean temperature of 26°C, 12 h photoperiod, and 60% relative humidity. Water was replaced daily weighing each gerbox and adding a volume of water equal to the mass lost compared to the previous day, considering that 1 ml of water is equal to 1 g.

For the germination percentage, germination speed index, and penetration resis-tance the experiment was completely randomized with four treatments (DT, MT, CDT, and CMT) and eight replicates (n = 32). The experimental design was completely ran-domized in a factorial 2x4 scheme with four compaction levels (DT, MT, CDT, and CMT) and ages for the seedlings (15 and 30 days) and eight replicates (n = 64) for the biometric and photosynthetic analyses from seedlings. Each replicate was considered as a gerbox (penetration resistance, germination percentage, and germination speed index) or a seedling (for seedling biometric and photosynthetic analyses).

2.3 Penetration resistance analysis

CMT and MT treatments were oven-dried at 40°C for 5 days before the penetration resistance measurement so that tailings from all tailings were dry at the moment of the test. The penetration resistance was measured using a bench-top penetrometer model MA933 (Marconi, Piracicaba, São Paulo). The cylindric register comprises a conic tip with an angle of 30° and

3.84 mm in diameter. Penetration was performed perpendicular to the sample (90º) at 10 cm min^{-1} and results were given in Kgf.

2.4 Germination analysis

The number of seeds germinated per gerbox was counted daily and at the end of the experiment, the germination percentage (G%) was calculated as follows: G% = (number of emerged seedlings per gerbox/number of seeds sown per gerbox) *100. We considered the emergence percentage equivalent to germination because of the difficulty of visualizing the radicle protrusion. The germination speed index (GSI) was calculated as follows: GSI = Σ(Nn/Dn), where Nn = number of emerged plants counted in a given day and Dn = the number of days passed since sowing.

2.5 Seedling biometry analysis

Seedlings were collected passed 15 and 30 days after emergence and were immedi-ately photographed. Images were used for counting the number of leaves and roots and also for the measurements of the leaf area, stem diameter, stem length, and the length of the main and lateral roots. Images were analyzed in the Image J software version 1.45s [16].

Seedlings were separated into roots, stems, and leaves and the fresh mass of each organ was measured in an analytical scale model AY220 (Shimadzu, Tokyo, Japan) for the dry mass, each plant part was oven-dried at 60 ºC for 72 h, and weighed again. The water content (WC) was calculated as follows: WC = [(FM-DM)/FM]*100, where FM is the fresh mass and DM is the dry mass results are given in percentage. The biomass allocation to each organ (R%) was calculated as follows: OR% = (DMO/TDM)*100 where DMO is the dry mass of the organ and TDM is the total dry mass. Total fresh and dry masses were obtained by the sum of the respective mass of each organ.

2.6 Photochemical analysis of the photosynthesis

Photochemical analyses were performed at the first two fully expanded leaves (cotyledons in the case of *S. terebinthifolia*) per seedling, at the middle region of the leaf on the adaxial side during the morning (between 8-11 a.m.). The leaves selected were in good phytosanitary conditions without evident damage, chlorosis, or infections. Photochemical parameters were evaluated using a portable fluorometer MINI-PAM (Walz, Wetzlar, Germany). The effective photochemical yield (ϕPSII) and the electron transport rate were evaluated. We could not evaluate the Fv/Fm because the leaves were too fragile and did not support the clip for dark measurements. The chlorophyll content was estimated using a portable chlorophyll meter SPAD-502 (Konica Minolta, Tokyo, Japan).

2.7 Statistical analyses

Data was submitted to the Shapiro-Wilk test for normality and then to one-way ANOVA (for penetration resistance, germination percentage, and germination speed in-dex) or two-way ANOVA (for seedling traits). Further data was submitted to the Tukey test for 5% of error probability. Statistical analyses were performed using the SISVAR 5.6 software [17].

3. Results

Results from one-way ANOVA and two-way ANOVA are shown in Table 2.

Table 2. Summarized ANOVA results for all variables analyzed, including mean square values, *F* test results, and *P* values. CV% = coefficient of variation.

Variable	**CV%**	**n square value**	***F* test value**	***P* value**
Penetration resistance (C)	50.43	5.894046	53.517	<0.0001
Germination percentage (C)	44.37	73.083333	5.852	0.0038
Germination speed index (C)	43.33	0.186249	4.575	0.0118
Number of leaves (C)	15.74	0.454327	1.101	0.3539
Number of leaves (A)	15.74	24.740540	59.949	<0.0001
Number of leaves (C x A)	15.74	0.712271	1.726	0.1685
Leaf area (C)	31.01	0.116012	0.422	0.7374
Leaf area (A)	31.01	25.731590	93.707	<0.0001
Leaf area (C x A)	31.01	0.151009	0.550	0.6498
Stem length (C)	20.53	0.465704	1.658	0.1830
Stem length (A)	20.53	2.603987	9.269	0.0032
Stem length (A x C)	20.53	0.299690	1.067	0.3681
Stem diameter (C)	23.48	0.000381	1.029	0.3845
Stem diameter (A)	23.48	0.017878	48.292	<0.0001
Stem diameter (C x A)	23.48	0.000250	0.674	0.5705
Number of roots (C)	49.67	19.715615	0.753	0.5239
Number of roots (A)	49.67	662.209710	25.290	<0.0001
Number of roots (C x A)	49.67	7.467980	0.285	0.8361
Main root length (C)	46.81	1.888468	1.921	0.1341
Main root length (A)	46.81	0.280585	0.285	0.5949
Main root length (C x A)	46.81	0.454257	0.462	0.7096
Lateral root length (C)	47.12	2.029913	2.747	0.0494
Lateral root length (A)	47.12	22.311022	30.188	<0.0001
Lateral root length (C x A)	47.12	0.766689	1.037	0.3816
Total fresh mass (C)	33.95	135.832458	0.528	0.6646
Total fresh mass (A)	33.95	16287.778125	63.278	<0.0001
Total fresh mass (C x A)	33.95	190.291125	0.739	0.5320
Total dry mass (C)	30.95	6.427458	0.332	0.8024
Total dry mass (A)	30.95	831.405125	42.901	<0.0001
Total dry mass (C x A)	30.95	11.767458	0.607	0.6124
Water content (C)	11.94	465.573548	7.232	0.0002
Water content (A)	11.94	1514.530888	23.526	<0.0001
Water content (C x A)	11.94	293.643410	4.561	0.0055*
Leaf dry mass (C)	28.85	2.820333	0.647	0.5872
Leaf dry mass (A)	28.85	329.672000	75.648	<0.0001
Leaf dry mass (C x A)	28.85	5.224333	1.199	0.3164
Stem dry mass (C)	34.36	0.467000	0.643	0.5897
Stem dry mass (A)	34.36	24.8645000	34.250	<0.0001
Stem dry mass (C x A)	34.36	0.697500	0.961	0.4160
Root dry mass (C)	55.94	3.973792	0.625	0.6013
Root dry mass (A)	55.94	32.385125	5.091	0.0271
Root dry mass (C x A)	55.94	3.108125	0.489	0.6912
Leaf dry mass allocation (C)	18.52	130.353371	1.448	0.2361
Leaf dry mass allocation (A)	18.52	581.850781	6.462	0.0132
Leaf dry mass allocation (C x A)	18.52	77.094288	0.856	0.4679

Stem dry mass allocation (C)	25.43	5.500327	0.269	0.8480
Stem dry mass allocation (A)	25.43	5.325120	0.260	0.6116
Stem dry mass allocation (C x A)	25.43	12.748707	0.623	0.6026
Root dry mass allocation (C)	55.94	3.973792	0.625	0.6013
Root dry mass allocation (A)	55.94	32.385125	5.091	0.0271
Root dry mass allocation (C x A)	55.94	3.108125	0.489	0.6912
Chlorophyll content (C)	21.68	114.811673	1.396	0.2457
Chlorophyll content (A)	21.68	302.279391	3.676	0.0569
Chlorophyll content (C x A)	21.68	151.295337	1.840	0.1418
Effective photochemical yield (C)	12.21	0.005205	0.923	0.4316
Effective photochemical yield (A)	12.21	0.745344	132.175	<0.0001
Effective photochemical yield (C x A)	12.21	0.003778	0.670	0.5718
Electron transfer rate (C)	42.83	56.578512	1.240	0.2969
Electron transfer rate (A)	42.83	4179.755792	91.616	<0.0001
Electron transfer rate (C x A)	42.83	63.801778	1.398	0.2452

The *P* value limit of the software was 0.0001, so results lower than this limit are given as $p < 0.0001$. (C) = Compaction (Compacted iron mining tailing when moistened to field capacity (CMT), Compacted dried iron mining tailing (CDT), Not compacted iron mining tailing when moistened to field capacity (MT), Not compacted dried iron mining tailing (DT)); Age (15 days or 30 days). All variables showed no significant interaction at $p < 0.05$, except for those indicated by *

The compaction of iron mining tailings promoted significant effects on the penetraion resistance (Table 2). Moistened treatments showed higher means for penetration resistance, CMT showed the highest values followed by MT and the dry treatments (MT and CDT) showed the lowest means with almost no resistance (Fig. 1).

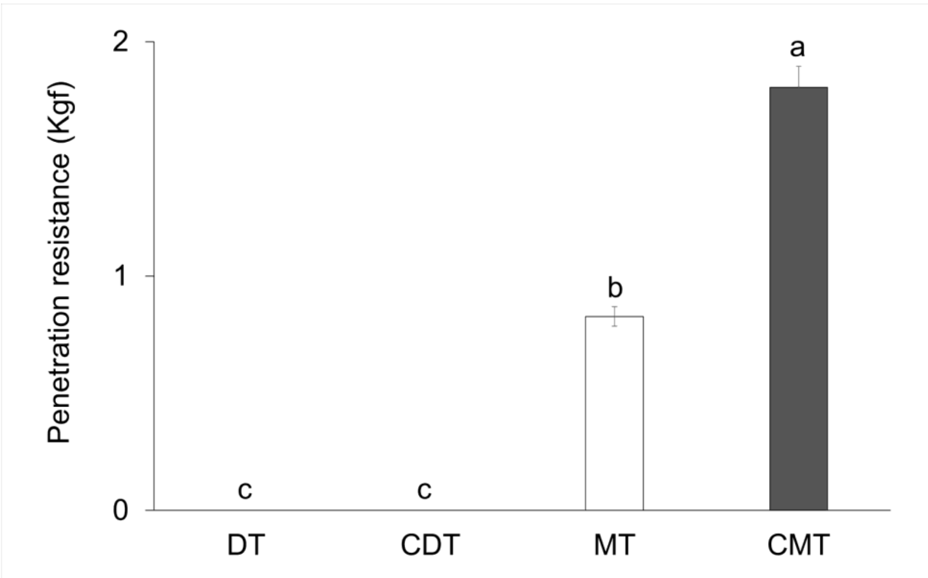

Figure 1: Penetration resistance from four different compaction treatments in iron mining tailings. DT = uncompacted dry tailings, MT= uncompacted moistened tailings, CDT= compacted dry tailings, and CMT= compacted moistened tailings. Means followed by the same letter do not differ significantly according to Tukey's test to $p<0.05$ ($n = 32$). Bars = standard error.

Compaction caused significant effects on seed germination and germination speed index (Table 2). Compaction reduced the germination percentage being the CMT and MT the treatments with the lowest means and the DT showed the highest germination, and CDT showed intermediate means (Fig. 2A). Similar results were found for the germination speed index, being the lowest mean promoted by CMT and the highest by DT being CDT and MT treatments with intermediate means (Fig. 2B).

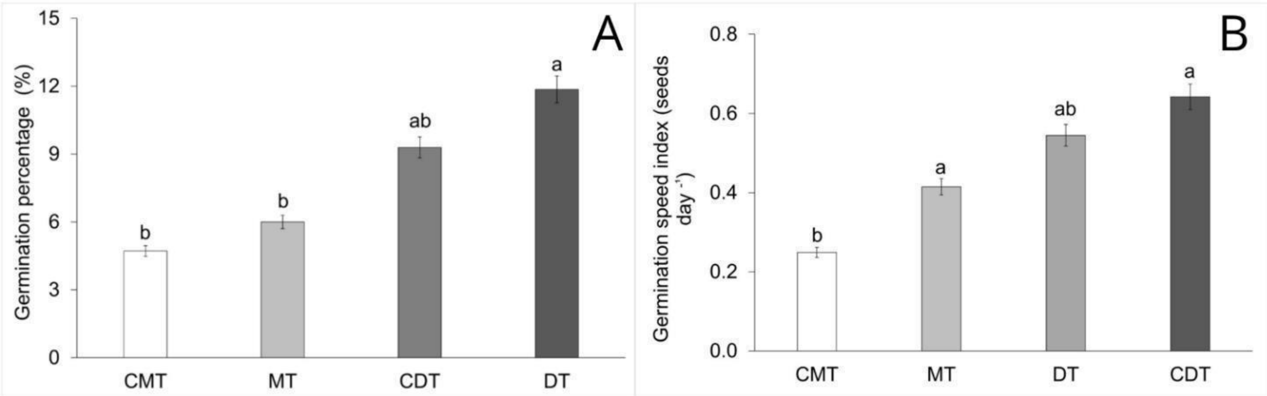

Figure 2: Germination (A) and germination speed index (B) from *Schinus terebinthifolia* seeds sown in iron mining tailings with different compaction levels. DT = uncompacted dry tailings, MT= uncompacted moistened tailings, CDT= compacted dry tailings, and CMT= compacted moistened tailings. Means followed by the same letter do not differ significantly according to Tukey's test to $p<0.05$ (n = 32). Bars = standard error.

No significant interaction was found for the seedling's age and compaction treatments for the leaf area and number of leaves per seedling (Table 2). Compaction promoted no significant effect on the leaf area (Fig. 3A) or the number of leaves (Fig. 3C) from *S. terebinthifolia* seedlings growing in iron mining tailings. *S. terebinthifolia* seedlings showed increased leaf area (Fig. 3B) and the number of leaves (Fig. 3D) from 15 to 30 days independently of compaction treatments.

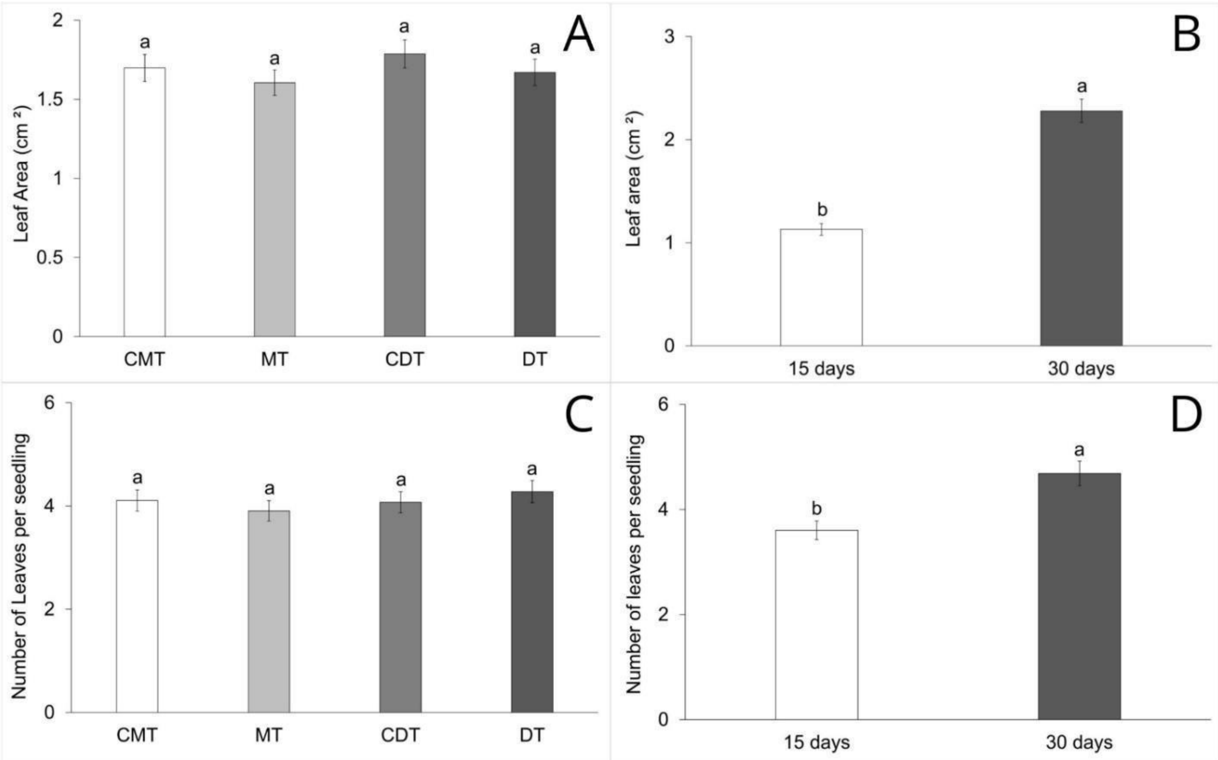

Figure 3: Leaf area (A and B) and number of leaves per seedling (C and D) from *Schinus terebinthifolia* seedlings growing in iron mining tailings with different compaction levels and seedling age. DT = uncompacted dry tailings, MT= uncompacted moistened tailings, CDT= compacted dry tailings, and CMT= compacted moistened tailings. Means followed by the same letter do not differ significantly according to Tukey's test to $p<0.05$ (n = 64). Bars = standard error.

There was no significant interaction between tailings compaction and seedling age for stem length, stem diameter, and seedling quality index (Table 2). Compaction promoted no significant modification in the stem length (Fig. 4A) and diameter (Fig. 4C). Seedlings of *S. terebinthifolia* increased their stem length (Fig. 4B) and diameter (Fig. 4D) from 15 to 30 days.

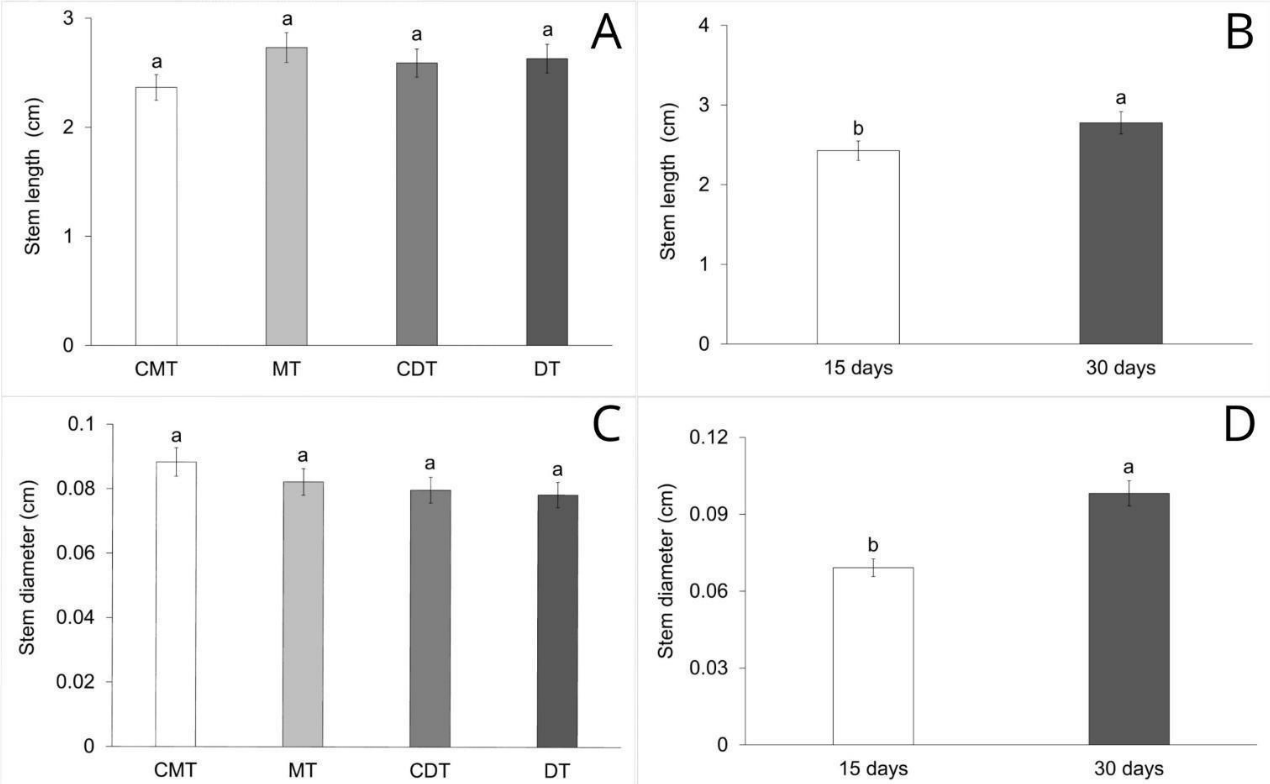

Figure 4: Stem length (A and B) and stem diameter (C and D) from *Schinus terebinthifolia* seedlings growing in iron mining tailings with different compaction levels and seedling age. DT = uncompacted dry tailings, MT= uncompacted moistened tailings, CDT= compacted dry tailings, and CMT= compacted moistened tailings. Means followed by the same letter do not differ significantly according to Tukey's test to $p<0.05$ ($n = 64$). Bars = standard error.

There was no significant interaction between compaction and seedling age for main root length, lateral root length, and the number of roots per seedling (Table 2). Compaction promoted no significant effect in the main root length of *S. terebinthifolia* seedlings (Fig. 5A) and no significant differences were found between 15 and 30 days of seedling's age (Fig. 5B). Compaction increased the lateral root length being the highest means found in CMT treatment and the lowest in DT seedlings, MT and CDT showed intermediate means (Fig. 5C). Lateral root length increased from 15 to 30 days in *S. terebinthifolia* leaves, independently of compaction (Fig. 5D). Compaction promoted no significant modification in the number of roots per seedling (Fig. 5E) but this parameter increased from 15 to 30 days in *S. terebinthifolia* seedlings (Fig. 5F).

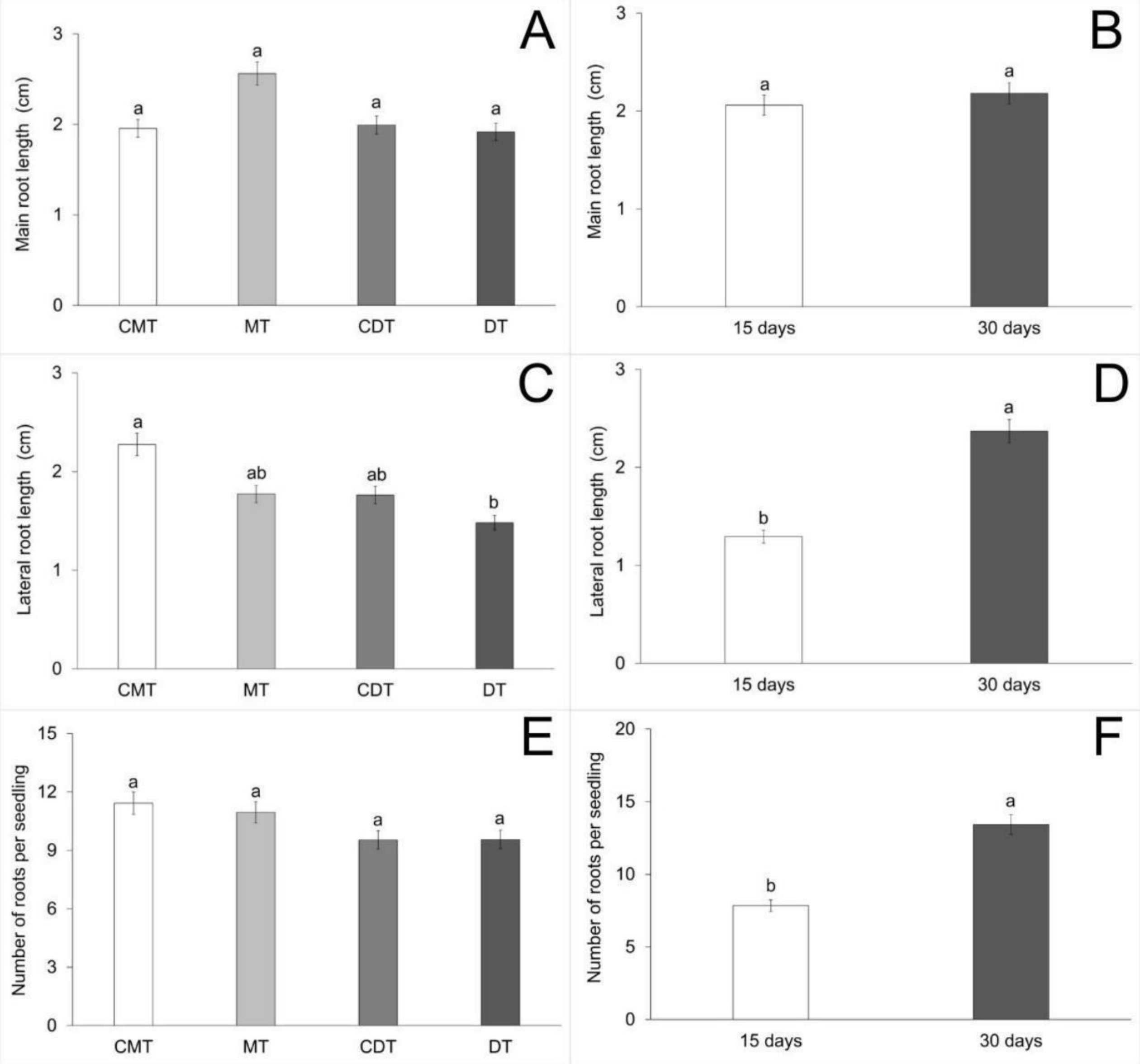

Figure 5: Main root length (A and B), lateral root length (C and D), and number of roots per seedling (E and F) from *Schinus terebinthifolia* seedlings growing in iron mining tailings with different compaction levels and seedling age. DT = uncompacted dry tailings, MT=

uncompacted moistened tailings, CDT= compacted dry tailings, and CMT= compacted moistened tailings. Means followed by the same letter do not differ significantly according to Tukey's test to $p<0.05$ ($n = 64$). Bars = standard error.

No significant interaction between compaction and seedling age was found for the total fresh mass and total dry mass (Table 2); however, significant interaction was found for the water content (Table 2). Compaction promoted no significant effect on total fresh mass (Fig. 6A) and total dry mass (Fig. 6C) of *S. terebinthifolia* seedlings. Both total fresh (Fig. 6B) and dry masses (Fig. 6D) increased from 15 to 30 days in *S. terebinthifolia* grown in iron mining tailings, independently of the compaction. Compaction and pre-moisture of iron mining tailings promoted higher water content in 15 days seedlings of *S. terebinthifolia* since CMT, MT, and CDT showed higher means compared to DT at this age; however, no significant differences in this parameter were found for 30 days seedlings (Fig. 6E). In addition, compacted treatments (CMT and CDT) showed no significant differences for the seedling water content comparing 15 to 30 days of seedling's growth; nonetheless, uncompacted treatments (MT and DT) showed increase in this parameter from 15 to 30 days (Fig. 6E).

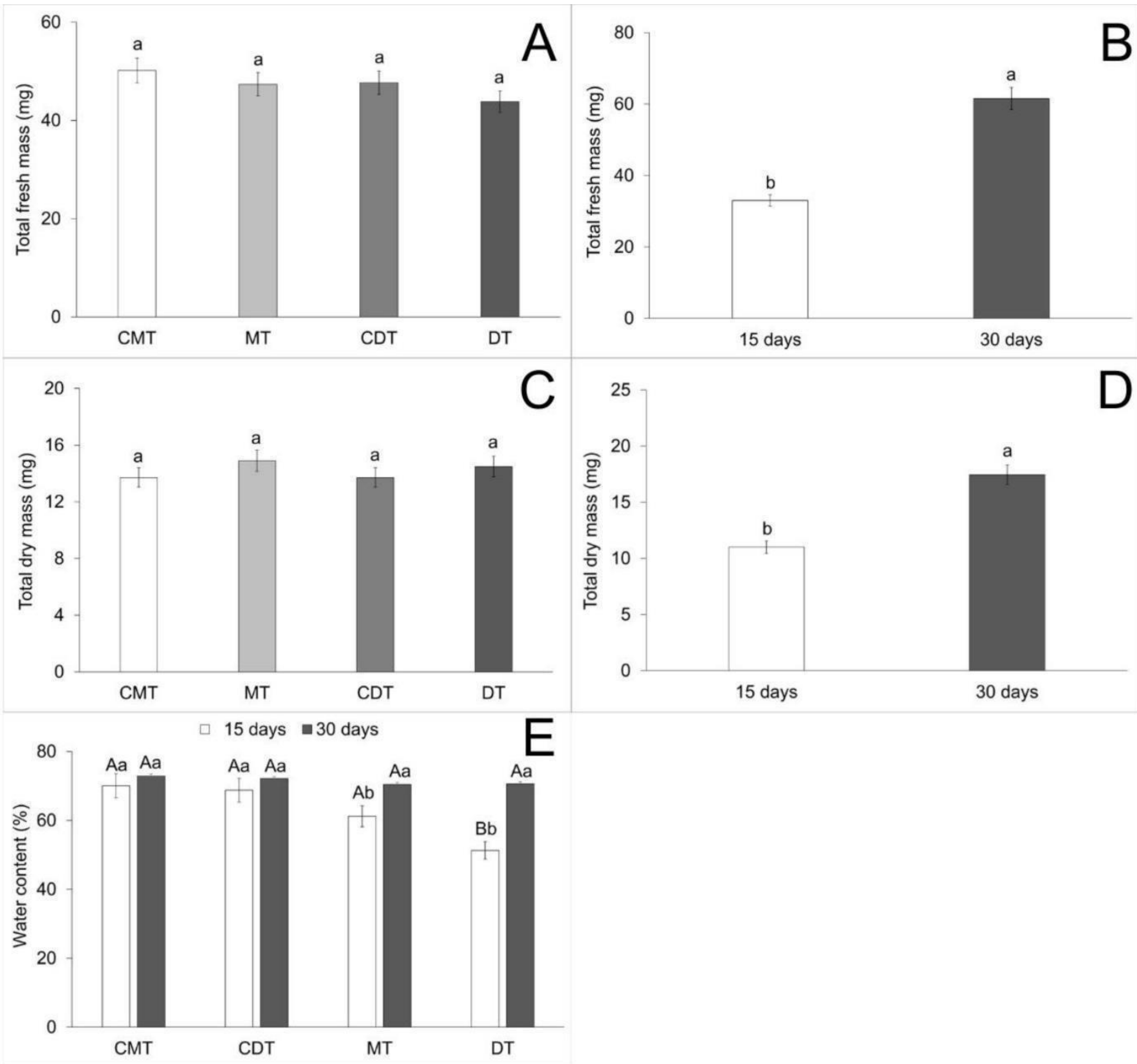

Figure 6: Total fresh mass (A and B), total dry mass (C and D), and water content (E and F) from *Schinus terebinthifolia* seedlings growing in iron mining tailings with different compaction levels and seedling age. DT = uncompacted dry tailings, MT= uncompacted moistened tailings, CDT= compacted dry tailings, and CMT= compacted moistened tailings. Means followed by the same letter do not differ significantly according to Tukey's test to $p<0.05$ (n = 64). Bars = standard error.

There was no significant interaction between compaction and seedling age for the leaf, stem, and root dry masses (Table 2). Compaction promoted no significant effect for the leaf (Fig. 7A), stem (Fig. 7C), and root (Fig. 7E) dry masses from *S. terebinthifolia* growing in iron mining tailings. Seedlings from *S. terebinthifolia* increased their leaf (Fig. 7B), stem (Fig. 7D), and root (Fig. 7F) dry masses from 15 to 30 days independently from tailings compaction.

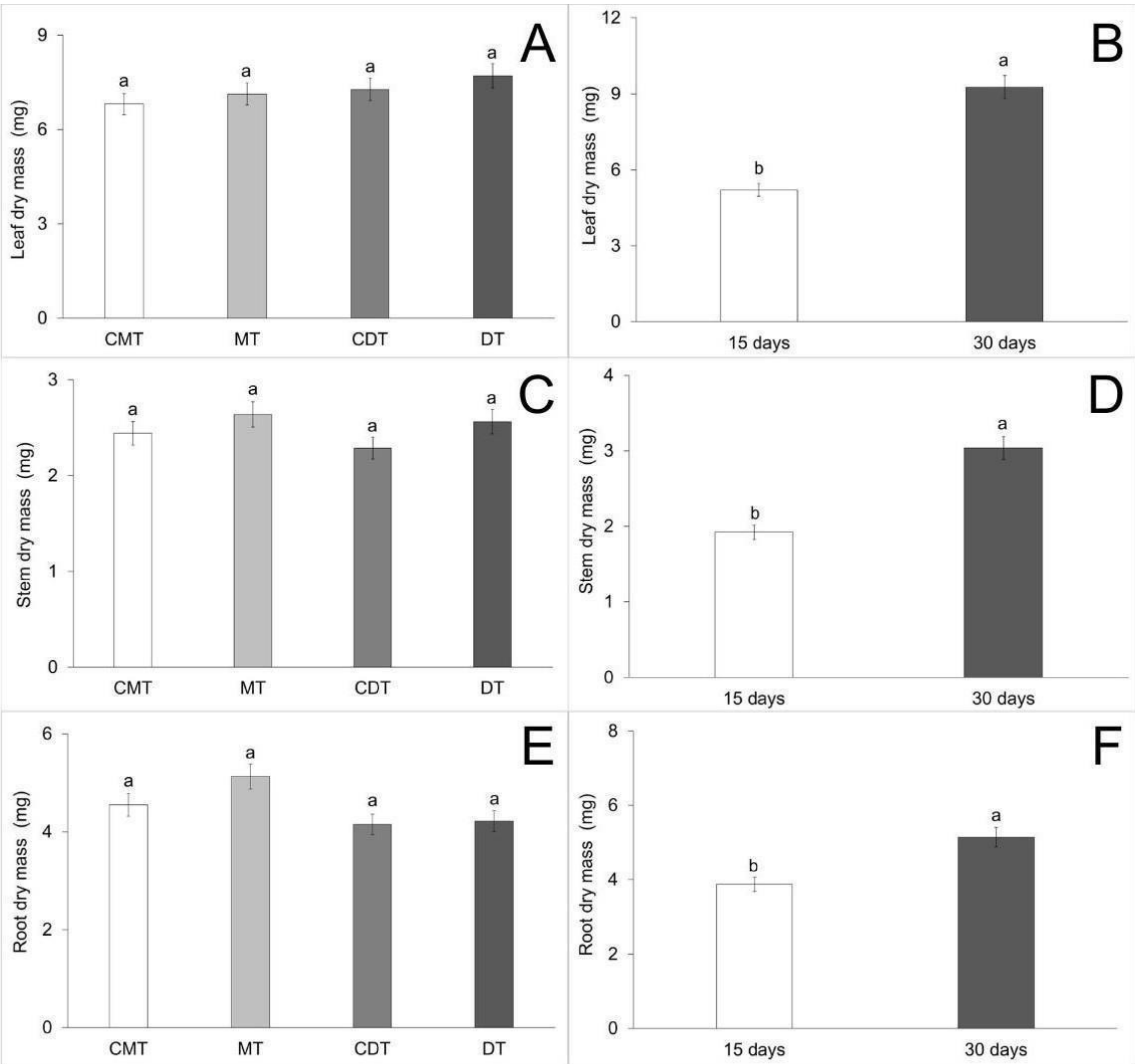

Figure 7. Leaf (A and B), stem (C and D), and root (E and F) dry masses from *Schinus terebinthifolia* seedlings growing in iron mining tailings with different compaction levels and seedling age. DT = uncompacted dry tailings, MT= uncompacted moistened tailings, CDT= compacted dry tailings, and CMT= compacted moistened tailings. Means followed by the same letter do not differ significantly according to Tukey's test to $p<0.05$ (n = 64). Bars = standard error.

There was no significant interaction between compaction and seedling age for the leaf, stem, and root biomass allocation (Table 2). Compaction promoted no significant effect for the leaf (Fig. 8A), stem (Fig. 8C), and root (Fig. 8E) biomass allocation from *S. terebinthifolia* growing in iron mining tailings. Seedlings from *S. terebinthifolia* increased their leaf biomass allocation (Fig. 8B) from 15 to 30 days independently from tailings compaction while allocation to roots decreased (Fig. 8F) and no significant modification was observed in stem allocation (Fig. 8D) in the same period.

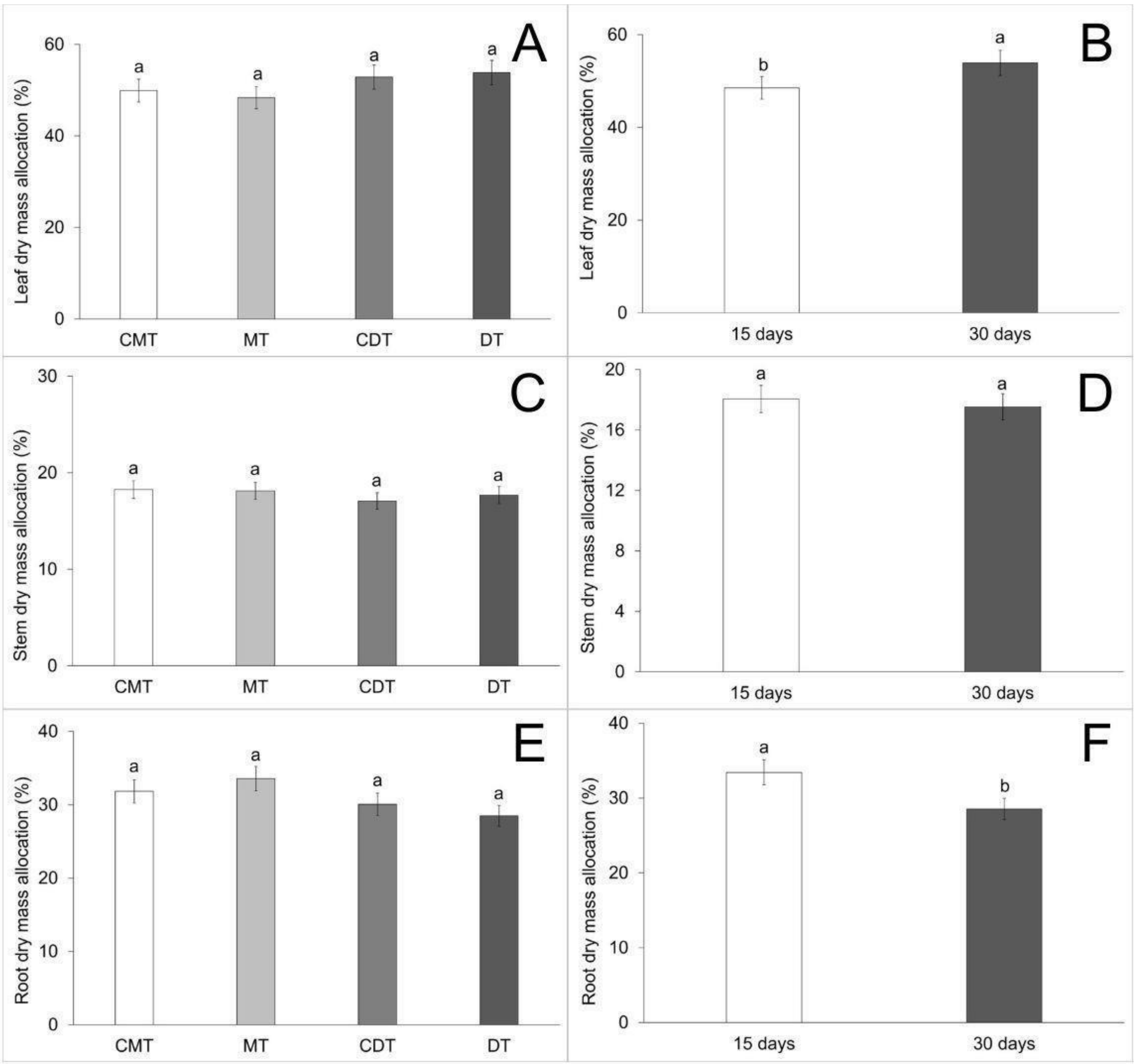

Figure 8. Biomass allocation to leaves (A and B), stem (C and D), and roots (E and F) from *Schinus terebinthifolia* seedlings growing in iron mining tailings with different compaction levels and seedling age. DT = uncompacted dry tailings, MT= uncompacted moistened tailings, CDT= compacted dry tailings, and CMT= compacted moistened tailings. Means followed by the same letter do not differ significantly according to Tukey's test to $p<0.05$ ($n = 64$). Bars = standard error.

No significant interaction between compaction and seedling age was found for the chlorophyll content, electron transfer rate, and effective photochemical yield (Table 2). Compaction promoted no significant differences in the chlorophyll content (Fig. 9A), electron transfer rate (Fig. 9C), and effective photochemical yield (Fig. 9E) of *S. Terebinthifolia* seedlings growing in iron mining tailings. The chlorophyll content was not significantly modified between 15 to 30 days of *S. terebinthifolia* seedling's growth in iron mining tailings (Fig. 9B). The electron transfer rate of *S. terebinthifolia* photosynthetic cotyledons increased from 15 to 30 days of seedling's growth in iron mining tailings (Fig. 9D) but the effective photochemical yield decreased in this period (Fig. 9F).

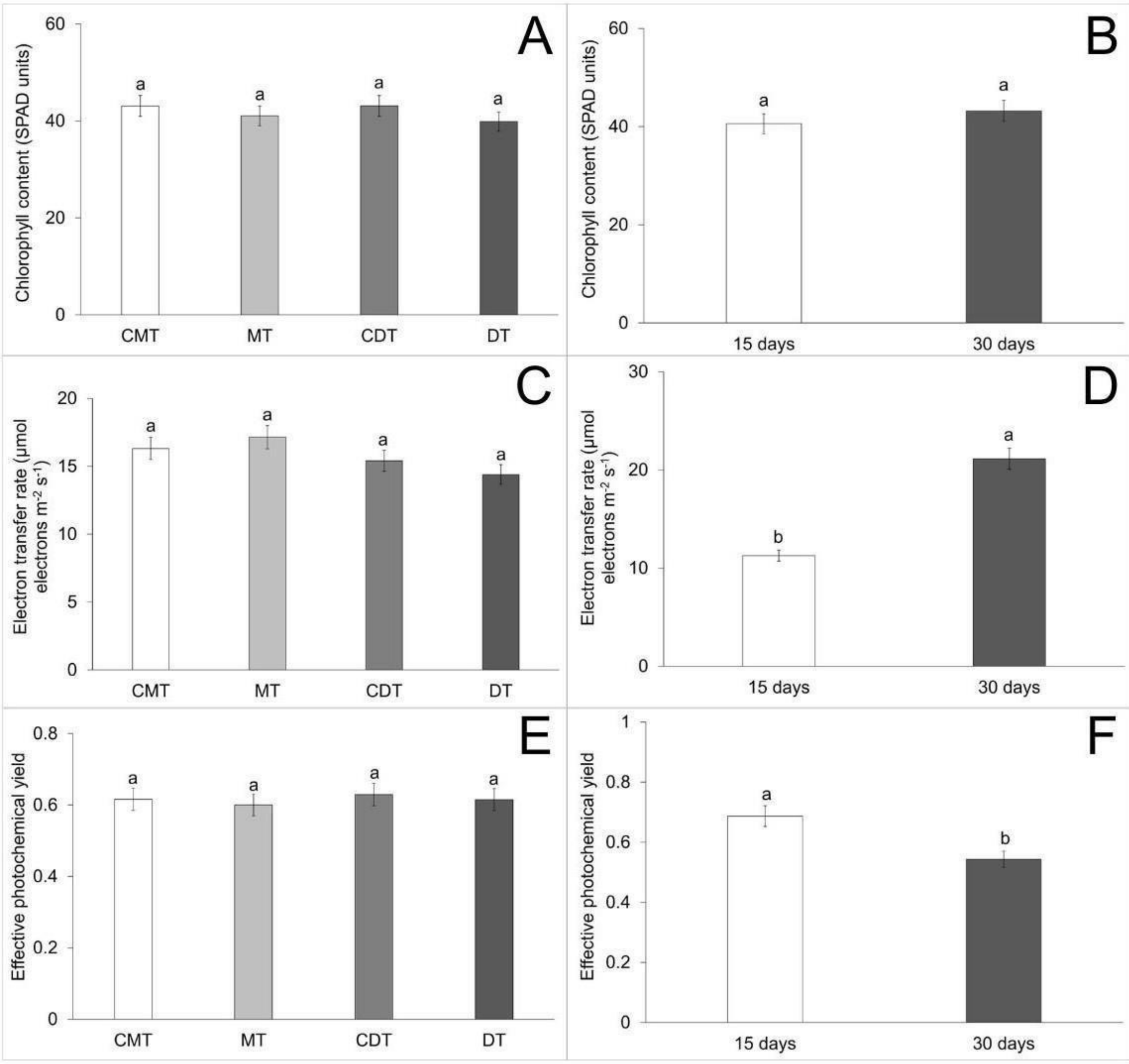

Figure 9. Chlorophyll content (A and B), electron transfer rate (C and D), and effective photochemical yield (E and F) from *Schinus terebinthifolia* photosynthetic cotyledons growing in iron mining tailings with different compaction levels and seedling age. DT = uncompacted dry tailings, MT= uncompacted moistened tailings, CDT= compacted dry tailings, and CMT= compacted moistened tailings. Means followed by the same letter do not differ significantly according to Tukey's test to $p<0.05$ (n = 64). Bars = standard error.

4. Discussion

4.1 Compaction of iron mining tailings and its effect on *S. terebinthifolia* germination

The compaction methods applied in this experiment were effective, increasing the penetration resistance of pre-moistened (MT) and compacted moistened tailings (CMT). The penetration resistance varies with soil texture and hydration which can change by natural or anthropic influence [10, 18]. Results showed that pre-moistened tailings which were dried previously to the penetration resistance analysis were a determinant factor for the compaction of the pollutant because both compacted (CMT) and uncompacted (MT) moistened tailings increased their penetration resistance. The increase in the penetration resistance is related to higher density and lower porosity in soils [10]. Drying the soil removes water located on the surface of its particles or stored in pores causing tailings compaction by approaching its particles and reducing porosity. Previous studies show that the penetration resistance increased with soil water loss [18, 19]. In addition, compaction decreases soil porosity increasing its density [20]. The moisture and humidity increased iron mining tailings compaction but the surface pressure promoted by the load further increased the penetration resistance as shown in CMT treatment which showed the highest means for penetration resistance. Plant survival and establishment are more difficult in compacted soils [20] and this can add a secondary factor impeding reforestation programs in areas impacted by iron mining tailings. It is important to note that only the compaction of dry tailings caused no significant effect on penetration resistance (CDT treatment), indicating that the tailing's water content is important for the compaction of this pollutant. Thus, both surface pressure and cycles of moisture and evaporation may cause compaction of iron mining tailings adding a secondary factor for plants to overcome.

The iron mining tailings showed a heterogeneous texture being 51% of its parti-cles comprised of silt and clay which are smaller than sand. This is corroborated by other works with iron mining tailings from Fundão dam which showed small particles comprised mainly of clay with a very small size (1.2 μm) [4, 21]; however, these works used a different method, with direct measuring of the diameter of the particles in the microscope. Clay soils show higher water retention capacity compared with sandy soils [10, 23]. According to Pádua et al. [4] irrigation to field capacity of iron mining tailings caused water-logging because of the lower porosity reducing growth and development of *Copaifera langsdorffii* Desf. suggesting that particles in iron mining tailings are small promoting lower porosity in this pollutant. Thus, compaction promoted in moistened iron mining tailings (CMT treatment) or by water evaporation in moistened tailing (MT) may have reduced its porosity approaching its particles which can harm plant establishment.

Similar to previous works, the iron mining tailing comprises mostly silt and sand [23, 24]. According to Zanchi et al. [25] this composition can reduce the porosity of the substrate. It is also important to note that small particles can insert among bigger ones, reducing the soil porosity [26] which can harm the growth and development of plants by limiting water and aeration [25]. In addition, the iron mining tailing shows limited organic matter content (Table 1), and this can reduce the formation and stabilization of particle aggregates which are important components of the soil's structure [27]. Aggregates help reduce the compaction process improving the porosity, aeration, and water content in the soil [28, 29].

The significant reduction of germination traits from *S. terebinthifolia* seeds in compacted and moistened tailings (CMT and MT treatments) supports the hypothesis that compaction is harmful to seed germination and is a relevant secondary factor affecting iron mining tailings. This can be associated with a lower porosity found in compacted soils that decreases water and oxygen availability [18]. Germination is one of the most sensitive stages of plant development and it is highly dependent on water and oxygen availability [30, 31]. Thus, the compaction of iron mining tailings may have caused limitations to water and oxygen availabilities to *S. terebinthifolia* seeds. Soil compaction reduces seed germination of other plant species [32, 33, 34]. Methods like soil scarification can be used to improve the permeability of compacted soils [35, 36], which can enhance water and air availability in the substrate being potential to the management of areas impacted by compacted iron mining tailings.

4.2 Tolerance of *S. terebinthifolia* seedlings to compaction of iron mining tailings

Although compaction impaired seed germination, *S. terebinthifolia* seedlings showed tolerance to compacted tailings because no significant modification was found for the majority of growth parameters evaluated (leaf area, number of leaves, stem length, and diameter, main root length, number of roots, fresh and dry masses and their allocation). The absence of significant growth modification is an important trait for compaction-tolerant species such as *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S. Moore [37] and *Moringa oleifera* Lam.

[35] which showed no significant growth changes in compacted soils. There are no comparable works with iron mining tailings compaction effects in seedling growth, be-ing this the first report of such effects but the absence of growth restriction from *S. terebinthifolia* in compacted treatments supports its tolerance and potential for the revegeta-tion of impacted areas.

The increase of the lateral root length in compacted iron mining tailings can be an interesting tolerance trait of *S. terebinthifolia* (Fig. 5C). Because roots are responsible for water and nutrient uptake the longer roots produced in compacted iron mining tailings by *S. terebinthifolia* seedlings may be more efficient compensating compaction negative effects. Similar results were found for *Hordeum vulgare* L. grown in growth chambers with an externalpressure system [38]. Increased lateral root growth was also reported for conifer trees growing in compacted soils, improving their root system [39]. In addition, the development of the root system is an important vigor trait in compacted soils because it promotes better shoot growth [40]. The increased proportion of lateral roots may be stimulated by the shoot, compensating for its development under stress conditions [41]. The improved growth of *S. terebinthifolia* roots in compacted iron mining tailings supports its tolerance to this pollutant and potential for revegetation of impacted areas.

The absence of negative effects in photochemical traits of *S. terebinthifolia* seedlings like the chlorophyll content (Fig. 9A), electron transfer rate (Fig. 9C), and effective photochemical yield (Fig. 9E) indicate that this species is tolerant to compacted tailings, maintaining its photosynthesis. This is an important result since non-tolerant species show decreased photosynthesis under compacted soils [42, 43]. Reduced photosynthesis may harm plant growth [44] being important in its stability for efficient plant growth. Soil compaction reduces photochemical traits in beans [45] and wheat [46] causing problems for their growth and development. Thus, stable photosynthesis is an important tolerant trait in *S. terebinthifolia* supporting the capacity of this species for reforestation of areas impacted by compacted iron mining tailings.

4.3 Early growth and development of *S. terebinthifolia* seedlings in compacted iron mining tailings

Most growth parameters and photochemical traits from *S. terebinthifolia* seedlings showed an increase from 15 to 30 days after germination independently of com-paction treatment supporting its tolerance to this pollutant and potential for use in refor-estation systems. This species was already reported as tolerant to iron mining tailings showing capacity to germinate early growth [6]. This species can suf-fer some degree of toxicity to its germination and early growth despite being capable to survive and grow in iron mining tailings [7]. Variation in tolerance capacity may be caused by the seed set used [7] or the great variability in the iron mining tailings composition [4, 5, 6, 7, 8, 21]. It is also important to note that factors such as the pH of iron mining tailings affect its toxicity, being more toxic at pH values of 5 or lower due to increased Al and Fe availabilities [8]. Although the species showed variations to its iron mining tailing tolerance, in this work results from growth, development, and photochemical traits support its tolerance to the pollutant most of which increased during early growth. Because this investigation lasted 60 days and seedlings were just 30 days old, works with longer experimental times may be important to understand the responses of the species in reforestation systems and also to evaluate gas exchange parameters since leaves of the species are fragile at the early stage not allowing the use of most gas exchange devices.

Conclusion

Surface pressure and moisture followed by drying promote compaction of iron mining tailings. Compacted mining tailings reduce seed germination traits in *S. terebinthifolia*; however, seedlings of this species show no effects of compaction to its early growth improving most growth parameters from 15 to 30 days of experiment. Compaction of mining tailings promotes no significant effects in the photochemical stage of photosynthesis of *S. terebinthifolia* seedlings. The capacity to grow in compacted mining tailings and the absence of significant negative effects support the tolerance of *S. terebinthifolia* for compacted iron mining tailings. This section is not mandatory but can be added to the manuscript if the discussion is unusually long or complex.

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TERCEIRA PARTE

CONSIDERAÇÕES FINAIS

A compactação do resíduo de mineração afetou negativamente a germinação de sementes de *S. terebinthifolia*, mas seu crescimento inicial não foi prejudicado, contudo, ainda não se conhece o efeito da compactação do resíduo de mineração de ferro em outras espécies nativas e sua capacidade de estabelecimento nestas áreas. Desse modo, experimentos com maior tempo de duração e estudos com outras espécies nativas são necessários para melhor compreensão dos efeitos da compactação no resíduo de mineração de ferro em espécies vegetais, bem como seu potencial para reflorestamento de áreas impactadas.