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Mining Tailings Effects on Soil Quality and Performance of Two Native Species of Atlantic Forest: Implications for Restoration

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Abstract Mining activities significantly contribute to environmental pollution by releasing chemical contaminants into ecosystems, making natural recovery challenging. In this study, we amended the mining tailings from the Fundão dam with reference soil to simulate the heterogeneity of tailings concentrations to which native species are exposed. We aimed to identify the tailings concentration that best supports the development of two native species, *Deguelia costata* and *Peltophorum dubium*. Our results showed that substrates with lower concentration of tailings had an increase in organic matter content and higher acidity. In contrast, substrates with higher tailings concentration showed increased levels of toxic metals; yet these metals seemed not to be readily available to plants, as evidenced by the enhanced biomass accumulation and improved seedling quality in these subtracts. Conversely, the reference soil, being nutritionally deficient and acidic, may have led to increased absorption of toxic metals by the plants. This resulted in reduced growth and increased activity of antioxidant enzymes and synthesis of flavonoids. Both species selectively accumulated iron in the roots and manganese in the leaves, without reaching phytotoxic levels. The findings unequivocally demonstrate that *D. costata* and *P. dubium* exhibited remarkable resilience by successfully thriving and growing in highly contaminated substrates (up to 100% mining tailings). Hence, both *D. costata* and *P. dubium* prove to be highly suitable candidates for the restoration of areas devastated by iron mining activities.

Keywords Ecological Restoration · Samarco Dam Breach · Gas Exchange · Oxidative Stress · Mineral Nutrition · Plant Development

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1 Introduction

Mining activities significantly contribute to environmental pollution by releasing chemical contaminants into the air, water, and soil. The process of extracting and processing minerals produces tailings that are laden with toxic metal ions (Adiansyah et al., 2015; Andrade et al., 2018). These activities not only contribute to environmental pollution but also hinder plant growth and development, along with negatively affecting soil microbiota health (Santolin et al., 2015). The resultant degraded soils demonstrate low resilience, struggling to support the natural colonization of native plant species. Such a backdrop highlights the extensive and often catastrophic impacts of mining, underscored by numerous dam failures worldwide, illustrating the pressing need for comprehensive understanding and action (Fernandes & Ribeiro, 2017).

Over 100 years (1915 to 2019), there have been 356 cases of mining dam's failures worldwide, with at least 38 mining-related disasters considered extremely severe in the last 50 years (Freitas & Silva, 2019; World Mine Tailings Failures, 2019). In Brazil, over 80 mining-related disasters have been reported (Cruz et al., 2022), and it is estimated that 126 mining dams are at imminent risk of failure in the coming years (Garcia et al., 2017). The dam failure at Fundão was the largest in terms of the volume of material released into the environment and affected area. Over 50 million cubic meters of tailings were dumped into the Doce River basin, covering 663.2 km until it reached the Atlantic Ocean, causing negative impacts on 41 municipalities along the way and affecting millions of human lives (Fernandes et al., 2016a; Marta-Almeida et al., 2016). The area flooded by the contaminated tailings was 1,176.6 ha, destroying 457.6 ha of native vegetation in the Atlantic Forest (Omachi et al., 2018).

Soils contaminated with mining tailings, such as those from the Fundão Dam, are characterized by their high density, compactness, and low porosity (Caetano et al., 2022; Silva Júnior et al., 2018). These physical properties negatively affect water retention and nutrient mobility, severely inhibiting plant germination and growth (Caetano et al., 2022; Silva Júnior et al., 2018). Furthermore, these tailings significantly lack organic matter and essential macronutrients (N, P, K, Mg), and exhibit a low effective cation exchange capacity (CEC), compounding the challenge by also containing high concentrations of toxic metals like Cr, Fe oxides, Mn, and Si (Araújo et al., 2022; Hatje et al., 2017; Schaefer et al., 2016; Segura et al., 2016). This nutrient depletion, combined with metal accumulation, creates an adverse environment that significantly impairs plant health by disrupting essential physiological processes, including chlorophyll synthesis, thereby undermining plant metabolism (Andrade et al., 2018; Matos et al., 2020; Lana-Costa et at., 2021). Such conditions lead to diminished growth, flowering, and fruiting (Grotz & Guerinot, 2006). The high concentrations of metals such as Fe and Mn in the soil can cause plant toxicity, resulting in severe physiological damage (Andrade et al., 2018; Araújo et al., 2022). The resultant physiological stress, further exacerbated by nutrient scarcity, induces oxidative stress marked by an overproduction of reactive oxygen species (ROS; Mittler, 2002; Rodriguez & Redman, 2005). However, plants have evolved sophisticated antioxidant defence systems, including enzymes such as ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) which confer stress tolerance and aid in the detoxification of ROS within cells (Gill & Tuteja, 2010; Mehes-Smith et al., 2013).

The natural regeneration of local vegetation can be significantly impaired by limitations imposed by physical and chemical characteristics of the mining tailings, which often interfere with plant development (Cruz et al., 2020). Therefore, it is essential to select appropriate species for the success of the restoration of degraded sites (Balestrin et al., 2019; Figueiredo et al., 2022, 2024; Ramos et al., 2023). The most suitable approach to achieving effective biodiversity, ecosystem functionality, and structure comparable to natural or near-natural reference ecosystems is the use of native species that can thrive under conditions of multiple stresses (Fernandes et al., 2016b; Toma et al., 2023). Even though ecological restoration, by definition, requires the use of native species from the local area, this approach has been inadequately explored and studied in numerous restoration initiatives within the Doce River basin (Cordeiro et al., 2022). The native vegetation has not yet recovered due to several issues related to the direct impact of the mining tailings. The changes in soil conditions are leading to the spread of various allochthonous (invasive) and pioneer species, such as *Urochloa* spp. and *Ricinus communis* in the affected and adjacent areas (Cordeiro et al., 2022; Figueiredo et al., 2022).

This reality underscores the need for a more effective and targeted approach in the selection of species for ecological restoration, prioritizing those with better performance and adaptability to the specific conditions created by mining tailings (e.g., Campanharo et al., 2021; Pires et al., 2017; Prado et al., 2019). In this context, the Fabaceae family emerges as the most prominent and influential plant family in the affected region (Figueiredo et al., 2022; Ramos et al., 2023). It plays a crucial role in soil protection and ecosystem health, thanks to its unique ability to enhance water retention capacity and soil fertility through symbiotic interactions with nitrogen-fixing bacteria (Gei et al., 2018; Temperton et al., 2007).

In this context, the study utilizes a method involving the dilution of tailings from the Fundão dam with uncontaminated soil to accurately simulate the range of contamination levels that native species encounter in natural environments. This methodology offers insights into the viability of employing native species for the ecological restoration of areas contaminated by mining tailings. For this purpose, we selected two native tree species from the Fabaceae family, *Deguelia costata* and *Peltophorum dubium*, both of which are pioneer species with low requirements for soil fertility and moisture. Furthermore, these species are characterized by their rapid growth rates and extensive distribution across the Rio Doce basin (Lorenzi, 2016). Specifically, we determine how different tailings concentrations influence: i) the physicochemical quality of the soil; ii) the seed germination rate; iii) the physiological traits; iv) the quality of the seedlings; and v) the translocation of nutrients from the soil to the roots and leaves. The hypothesis was that substrates with higher concentrations of tailings pose more significant challenges to the development of these tree species. The aim was to identify the levels of tailings dilution that allows effective ecological restoration with *D. costata* and *P. dubium* in areas affected by mining activity.

2 Material and Methods

2.1 Plant Species

Deguelia costata (Benth.) AMG Azevedo & RA Camargo (Fabaceae, Faboideae) is a native, endemic tree of the Atlantic Forest, exhibiting characteristics of a semi-deciduous, heliophytic, selective xerophyte, and pioneer species (Lorenzi, 2016). *Peltophorum dubium* (Spreng.) Taub. (Fabaceae, Caesalpinioideae) is classified as a heliophyte with initial secondary attributes, yet it displays pioneering traits. Notable for its extensive geographic range, this tree species is encountered frequently, both in Brazil and beyond its borders. It thrives in diverse phytophysiognomies and is especially prevalent in secondary ecosystems within the entirety of the semi-deciduous seasonal forest domain (Donadio & Demattê, 2000). These species are not very demanding regarding soil fertility and moisture and are associated with atmospheric nitrogen-fixing bacteria (Helene et al., 2019). The seeds of *D. costata* and *P. dubium* species were randomly collected from five to ten individuals of each species from non-contaminated soils located adjacent to the regions affected by the Fundão dam in June 2021 in Aimorés, state of Minas Gerais, Brazil.

2.2 Experimental Design

To prepare the growth substrates for the selected plant species, soil samples were collected from two distinct areas in the district of Barra Longa (Mariana, MG), which was the epicenter of the accident. The first set of soil samples was obtained from an area directly affected by tailings deposition. The second set of soil samples was collected from a nearby forest area that was unaffected by the contamination and thus served as the reference soil. To conduct the experiment, we prepared five treatments with varying percentages of contaminated material (hereafter referred to as "tailings") mixed with the reference soil: T100 (100% tailings), T75 (75% tailings), T50 (50% tailings), T25 (25% tailings), T0 (tailings-free reference soil). Each treatment consisted of 30 seven-litter pots, resulting in a total of 150 pots for each plant species used. The pots were transported to a greenhouse fitted with a 30% light reduction screen (Nortene®) located at the Federal University of Minas Gerais, Brazil. The microclimatic conditions of the greenhouse during the experiment are shown in Table S1. The pots were randomly placed in the greenhouse and were rotated monthly to avoid bias due to any microenvironmental influence on the development of the species. The pots were irrigated by a micro-sprinkler twice a day (at 6 a.m. and 6 p.m.) for five min. The approach of amending a reference soil with increasing amounts of mining tailings aimed to replicate the conditions resulting from the Mariana accident, both in the short and long term, considering that the soil microbiota metabolizes the mining tailings with time the diminishing the availability of some of its constituents to plants.

2.3 Physical–chemical Analysis of Soils

The physico-chemical attributes of the reference soils were analyzed before planting the seedlings to evaluate whether the presence of mining tailings improved the soil quality (n = 5 composite samples per treatment). The content of coarse and fine sand (kg/kg) was analyzed using the sieving method, while the particle density (g cm^{-3}) was analyzed using the volumetric flask method. Additionally, the content of silt (kg/kg) and clay (kg/kg) were analysed using the slow agitation pipette method (Teixeira et al., 2017). The physical characteristics of the soils were classified according to the United States Department of Agriculture (USDA) (Soil Survey Staff, 1993).

The chemical attributes analyzed were the concentrations of P, K, Na, Fe, Zn, Mn, Cu, Cd, Pb, Ni, and Cr using the Mehlich-1 method; Ca^{2+}, Mg^{2+}, and Al^{3+} using 1 mol/L KCl; B in hot water; S with monocalcium phosphate in acetic acid. In addition, pH in H_2O, $H^{+}Al$ with 0.5 mol/L calcium acetate at pH 7.0, CEC, and OM (organic carbon × 1.724) were also analyzed using the Walkley–Black method (Teixeira et al., 2017).

2.4 Seed Germination

The percentage of germination was quantified to assess whether presence of mining tailings improved the seed germination. To break dormancy, the seeds were immersed in 98% sulfuric acid (H_2SO_4) for 15 min followed by a five-minute wash with running water (Seneme et al., 2012). Subsequently, five seeds were placed in each of 30 pots per treatment per species (n = 30). The number of germinated seeds was recorded daily for 30 days. After this period, the uniformly grown seedlings were selected, leaving only one healthy plant in each pot.

2.5 Physiological Parameters

To evaluate whether the presence of mining tailings improves the physiological parameters of plants and consequently promotes development, measurements of nitrogen balance index (NBI, proxy for foliar nitrogen content), chlorophyll (μg cm^{-2}), flavonoids (μg cm^{-2}), and gas exchange—stomatal conductance (g_s, μmol CO_2 m^{-2} s^{-1}), photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}), and transpiration rate (E, mmol m^{-2} s^{-1}) were performed. These measurements were taken in the 10th, 19th, and 29th weeks of plant development (in December 2021, February, and April 2022, respectively). Additionally, it was evaluated the level of oxidative stress in leaves by quantifying H_2O_2 and the activity of the antioxidant enzymes APX, CAT, and SOD. These parameters were analyzed in the 29th week of species development.

The non-destructive analysis of NBI and the amounts of chlorophyll and flavonoid were carried out using the Dualex® instrument (Fernandes et al., 2022; Goulas et al., 2004). The measurements were recorded on the adaxial part of three expanded leaves free from pathogen attack in the middle portion of the stem of each individual (n = 15 individuals per treatment per species). Three leaflets were measured on each leaf. Dualex® uses the ratio of chlorophyll to flavonoid content to obtain the NBI.

Gas exchange measurements were analyzed using a portable photosynthesis system, LCpro + (LCpro®, ADC Bioscientific Ltd. UK), coupled to a wide leaf chamber. For each individual, analysis was performed on one of the three selected leaves used for previous measurements (n = 15 individuals per treatment per species). The apparatus was calibrated to maintain a temperature of 30 °C, a vapour pressure deficit of approximately 1.5 kPa, and a flow rate of 300 μmol of air min^{-1}. The ambient CO_2 concentration of ~ 410 μmol mol^{-1} and photosynthetic photon flux density of 1500 μmol m^{-2} s^{-1} were used. Measurements were taken when a steady state was reached and conducted between 7:00 and 11:00 a.m.

The amount of lipid peroxides and the activity of antioxidant enzymes were evaluated in a healthy and expanded leaf located between the third and fifth node of the stem (n = 4 individuals per treatment per species). Samples were collected from the same individuals selected for gas exchange measurements. The production of ROS was determined by quantifying H_2O_2 using the method of DeLong et al. (2002). Lipid hydroperoxide content was determined based on H_2O_2 equivalents, utilizing a standard curve for calibration. The activity of APX was measured following the protocol of Nakano and Asada (1981), while CAT activity was determined according to da-Silva et al. (2017). Superoxide dismutase (SOD) activity was evaluated using the method outlined by Giannopolitis and Ries (1977). All assays were conducted in triplicate on a Multiskan Spectrum© microplate reader (Thermo Fisher Scientific Oy, Vantaa, Finland) to ensure accuracy and reproducibility of the results (For more detailed information, please refer to the supplementary material).

2.6 Seedling Quality

The total dry mass production and seedling quality were assessed using the Dickson Quality Index to evaluate whether the presence of mining tailings improved plant development (DQI; Dickson et al., 1960). This index reduces the incidence of errors associated with interpreting only one or two developmental traits, as it provides a comprehensive and accurate assessment of the seedling development potential. The DQI was calculated using the following equation:

$$DQI = TDM/(H/D) + (SDM/RDM)$$

where DQI = Dickson Quality Index, H = height, D = stem diameter, SDM = shoot dry mass shoot, RDM = root dry mass, TDM = total dry mass.

To calculate the DQI, measurements were taken of stem diameter at soil level (digital caliper; 0.01 mm), seedling height (tape measure; cm), shoot dry mass, root dry mass, and total dry mass. All measurements were determined in the 37th week of development on the same individuals selected for physiological analyses (n = 15 individuals per treatment per species). Firstly, the diameter and height of the aboveground portion of the plants were measured. Subsequently, the roots were removed from the soil and underwent a thorough wash with running water. All plant material was left in a forced-air oven at 60 °C until it reached constant weight. Finally, the dry mass of both shoots and roots was quantified using an analytical scale with a precision of 0.001 g.

2.7 Nutritional Analysis of Plant Material

To assess the potential impact of mining tailings dilution on nutrient allocation within plant tissues, chemical attributes of leaves and roots were analyzed in a sample set of five composite specimens per treatment per species. For iron plaque removal, roots were washed with a solution of dithionite-citrate-bicarbonate, containing 0.03 M sodium citrate, 0.125 M sodium bicarbonate, and 1.0 g of sodium dithionite (Huang et al., 2017), then dried in a circulating oven at 60 °C to reach constant. Subsequently, dried samples of roots and leaves were ground (MA048® mill, Moinho Marconi, SP, Brazil). Concentrations of P, K, Ca, Mg, Zn, Fe, Mn, Cu, and B were determined by perchloric acid digestion. Concentrations of N by sulfuric digestion by Kjeldahl distillation method (Sáez-Plaza et al., 2013). The mineral content of substrates and plant material was determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Optima 3300 DV, Perkin Elmer®, Norwalk, CT, USA).

2.8 Statistical Analyses

For each plant species, linear mixed-effects models (LMMs) were fitted for each of the parameters: NBI, chlorophyll, flavonoids, g_s, A, E, H_2O_2, APX, CAT and SOD. The *lme4* package was used to fit the models (Bates et al., 2015). In maximal models, the treatment was included as a fixed effect, time (in weeks) as a covariate with interaction, and the individual as a random effect. For the parameters $H_2O_{2,}$ APX, CAT and SOD, the treatment was included as a fixed effect and the technical replicates were included as a random effect. Generalized linear model (GLM) with a binomial distribution was fitted for the parameter germination percentage. Additionally, simple linear models (LMs) were used to evaluate the parameters: coarse sand, fine sand, silt, clay, particle density, pH, N, P, K, Na, Ca, Mg, H^+Al, Al, CEC, OM, S, B, Cu, Mn, Fe, Zn, Cr, Ni, Cd, Pb, total dry mass and DQI. For all these variables, treatment was included as the predictor variable in the models. The residuals of all models were graphically analyzed with the *DHARMa* package, and when necessary for LMMs and LMs, data were lo or square root transformed to improve the homoscedasticity of residuals (Hartig, 2022). Simplifications of the complete models were performed by removing non-significant fixed effects to obtain minimal adequate models (Crawley, 2013). The p-values of fixed effects were generated by likelihood ratio tests of the complete model with and without the explanatory variables (Crawley, 2013). The evaluation of the proportion of variance explained by the model was conducted using the *MuMIn* package. Specifically, the marginal R^2, which quantifies the variance explained by the fixed effects, and the conditional R^2, which includes both fixed and random effects, were calculated to provide a comprehensive understanding of the model's fit to the data. Post-hoc tests were conducted using the *emmeans* package (Lenth, 2022). Principal Component Analysis (PCA) was performed using the stats package. The analysis involved examining the variables associated with soil chemical attributes before the experiment, as well as the chemical attributes of the leaves and roots for each treatment. All statistical analyses were performed in the R software (R Core Team, 2022), and the graphs were constructed in GraphPad Prism, version 9.5.0.

3 Results

3.1 Physical–chemical Analysis of Substrates

The analysis of the physical properties of the substrates indicates that the proportions of coarse sand, fine sand, silt, and particle density decreased with a decrease of the mining tailings proportion ($T100 > T75 > T50 > T25 > T0$) (Table S2, S7). Coarse sand was 40% higher in T100 and T75 compared to T25 and T0 ($F_{treatment} = 60.47$, $p < 0.001$). Fine sand was 36.8% higher in T100 than in T75 and T50, which did not differ from each other, and was even higher compared to T25 and T0 ($F_{treatment} = 98.71$, $p < 0.001$). Silt did not differ among T100, T75, and T50 but was on average 48.2% higher in these treatments than in T25, and 74% higher than T0 ($F_{treatment} = 36.92$, $p < 0.001$). In contrast, the clay proportion increased with mining tailings dilution. The clay content was 73.7% higher in T0 compared to T100. Particle density was higher in T100 compared to T0, showing an increase of 14.6% ($F_{treatment} = 396.22$, $p < 0.001$).

The textural classification we found was: T100 (loam), T75 (sandy loam), and T50, T25, and T0 (clay) (Fig. S1). The PCA substrate accounted for 88.4% of the data variability, with 81.2% of this total explained by the PC1 and 7.2% by the PC2 (Fig. 1). It was observed that the main axis separated the five treatments. Substrates with higher mining tailings concentration, T100, and T75, positioned on the positive side of axis 1 (right side), exhibited higher levels of micronutrients (e.g., Fe, Mn, Ni, Zn, and Cu), macronutrients (e.g., P, K, Mg, and Ca), higher pH, and Na. Consequently, these same substrates had lower concentrations of parameters pointing towards the opposite end (negative side of axis 1). Substrates

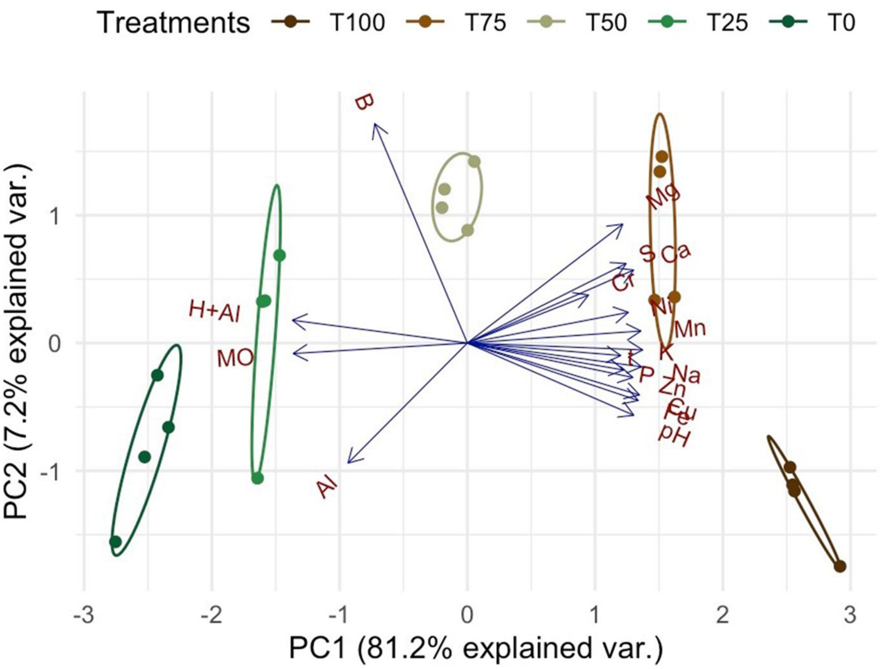


Fig. 1 Relationship between chemical parameters of the reference soils amended by increasing amounts of mining tailings such as T100 (100% mining tailings), T75 (75% mining tailings), T50 (50% mining tailings), T25 (25% mining tailings), T0 (mining tailings-free reference soils) ($n = 5$)

with lower mining tailings concentration, T25, and T0, positioned on the negative side of axis 1 (left side), had higher organic matter content but were also rich in aluminium and more acidic. These substrates had lower concentrations of parameters pointing towards the right side (Fig. 1).

3.2 Seed Germination

The germination percentage of *D. costata* was 68, 62, 71, 71 and 81% for treatments T100, T75, T50, T25 and T0, respectively. In contrast, the germination percentage of *P. dubium* was 27, 34, 36, 37, and 34% for treatments T100, T75, T50, T25 and T0, respectively. No significant differences were observed for the germination rate among the treatments for either species ($p > 0.05$).

3.3 Physiological Parameters

In the 10th week, the NBI of *D. costata* was 29.3% higher under T75 compared to T100 and T0 and did not differ from T50 and T25. In the 19th week, the NBI of *D. costata* under T25 was 25.7% higher compared to T100 or T0 and did not differ from T75 and T50 treatments. In the 29th week, the NBI of *D. costata* under T50 differed from all other treatments. During the same period, the highest NBI values were found for plants under the treatments T100 and T75, and these were 46% higher than in T25 and T0 ($\chi^2_{\text{treatment*week}} = 264.74$, $p < 0.001$; Fig. 2a; Table S7). In the 10th week, the NBI of *P. dubium* was 12.35% higher under T25 and T0 compared to T100, T75, and T50. In the 19th week, the NBI of *P. dubium* in T0 was 18.8% higher compared to other treatments that did not differ from each other. Moreover, at the 29th week, the highest NBI values were found for plants under T100, and these were 12% higher than those of plants under other treatments, which did not differ from each other ($\chi^2_{\text{treatment*week}} = 57.92$, $p < 0.0001$; Fig. 2b).

The chlorophyll content in *D. costata* was 22.4% higher in T75 compared to T100 and did not differ from T0 in the 10th week. However, in the 19th week, the chlorophyll of *D. costata* in T25 and T0 did not differ from T75 and was 18% higher compared to T50 and T100, which also did not differ from T75. In the 29th week, the chlorophyll of *D. costata* in T75 and T50 differed from T25, while T100 did not differ. Additionally, the highest values of chlorophyll were found for T100, T75, and T50 treatments, and these were 18% higher than those for plants under T0 ($\chi^2_{\text{treatment*week}} = 169.96$, $p < 0.001$; Fig. 2c; Table S7). In *P. dubium*, the chlorophyll content was 18.6% higher in T25 and T0 compared to T100, T75, and T50 in the 10th week. In the 19th week, the NBI of *P. dubium* was 27.5% higher in T0 compared to the other treatments, which did not differ from each other. In the 29th week, the chlorophyll of *P. dubium* in T0 was 18.5% higher compared to the other treatments, which did not differ from each other ($\chi^2_{\text{treatment*week}} = 59.68$, $p < 0.001$; Fig. 2d).

The flavonoid content in *D. costata* were around 22% higher under T0 compared to the other treatments, which did not differ from each other in the 10th and 19th weeks. In the 29th week, the flavonoid amounts of *D. costata* under T25 and T0 were 34.8% higher compared to T100 and T75 ($\chi^2_{\text{treatment*week}} = 199.07$, $p < 0.001$; Fig. 2e; Table S7). However, the flavonoid content in *P. dubium* increased by 17.5% after the 10th week and decreased by 4.9% after the 19th week of development in all treatments ($\chi^2_{\text{week}} = 757.18$, $p < 0.001$, Fig. 2f). In the 29th week, the flavonoid content was higher in T0 compared to the T100 and T75 treatments in *P. dubium* ($\chi^2_{\text{treatment*week}} = 34.94$, $p < 0.001$).

No interaction was detected between treatment and week for the parameters g_s, A, and E for either species ($p > 0.05$, Fig. 3a-f). The g_s in *D. costata* increased by 16% after the 10th week of development and decreased by 56% after the 19th week, across all treatments ($\chi^2_{\text{week}} = 51.25$, $p < 0.001$, Fig. 3a; Table S7). Similarly, the g_s in *P. dubium* increased by 3.3% after the 10th week and decreased by 58.5% after the 19th week, across all treatments ($\chi^2_{\text{week}} = 158.64$, $p < 0.001$, Fig. 3b). The A in *D. costata* increased by 8% after the 10th week of development but decreased by 30.2% after the 19th week for all treatments ($\chi^2_{\text{week}} = 41.05$, $p < 0.001$, Fig. 3c). On the other hand, the A in *P. dubium* decreased by 10.3% after the 10th week and by 44.9% after the 19th week for all treatments ($\chi^2_{\text{week}} = 300.06$, $p < 0.001$, Fig. 3d). The E in *D. costata* decreased by 14.5% after the 10th week and 8.9% after the 19th week for all treatments ($\chi^2_{\text{week}} = 16.42$, $p < 0.001$, Fig. 3e). Similarly, the E in *P. dubium* decreased by 6.9% after the 10th week and 27.4% after the 19th week for all treatments ($\chi^2_{\text{week}} = 103.70$, $p < 0.001$, Fig. 3f).

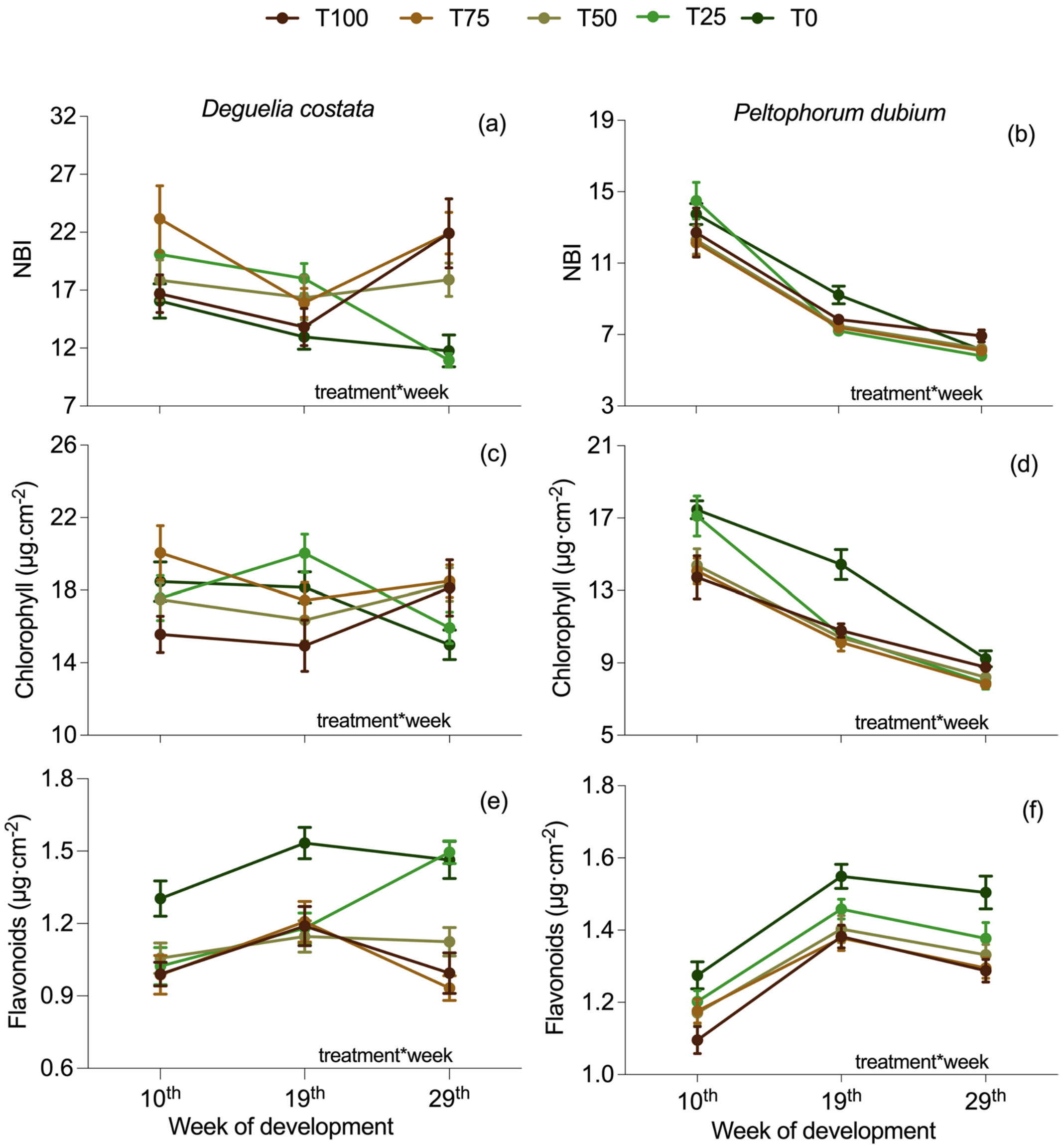


Fig. 2 Effect of increasing amounts of mining tailings on the physiological parameters in *Deguelia costata* and *Peltophorum dubium* grown in amended reference soils T100 (100% mining tailings), T75 (75% mining tailings), T50 (50% mining tailings), T25 (25% mining tailings); T0 (mining tailings-free reference soils). (a-b) Nitrogen balance index (NBI); (b-c) Chlorophyll; (d-e) Flavonoids. Data are the means ± SE ($n = 15$)

The amount of lipid peroxides was not affected by the treatments regardless of the plant species ($p > 0.05$, Fig. 4a-b). However, for *D. costata*, the activity of APX, CAT, and SOD enzymes under T100 and T75 treatments was approximately 50% higher compared to T0 and T25 ($\chi^2 = 36.772$, $p < 0.001$; $\chi^2 = 26.377$, $p < 0.001$; $\chi^2 = 54.942$, $p < 0.001$, Fig. 4c, e, g; Table S7). In the case of *P. dubium*, no significant differences were found for the APX activity among the treatments ($p > 0.05$, Fig. 4d). However, CAT activity was 10% higher in the T0, T100, and T75 treatments compared to T25 and T50 ($\chi^2 = 62.993$, $p < 0.014$, Fig. 4e). Additionally, it was observed that SOD activity was lower in the T50 treatment compared to T25 and T0, while

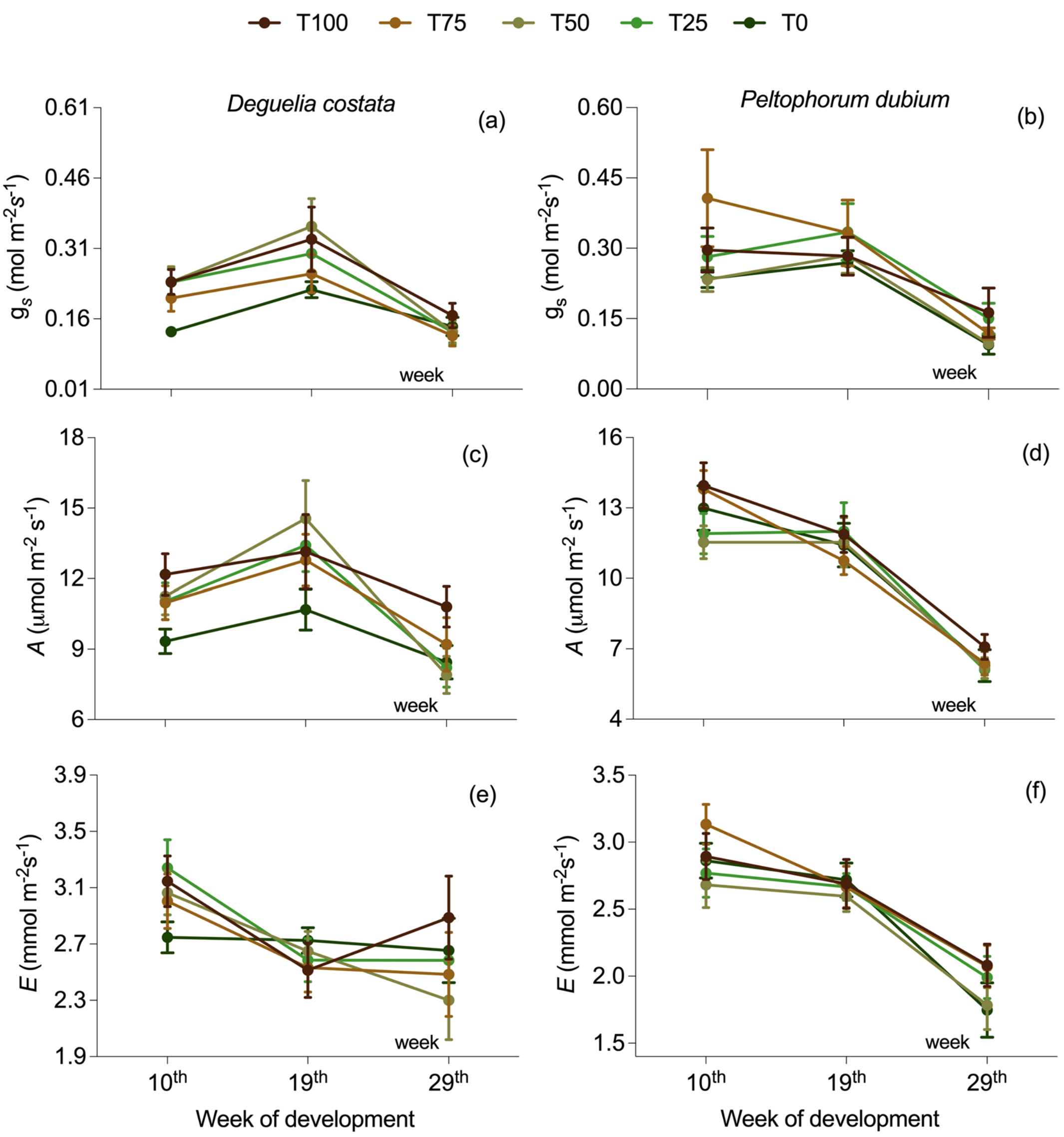


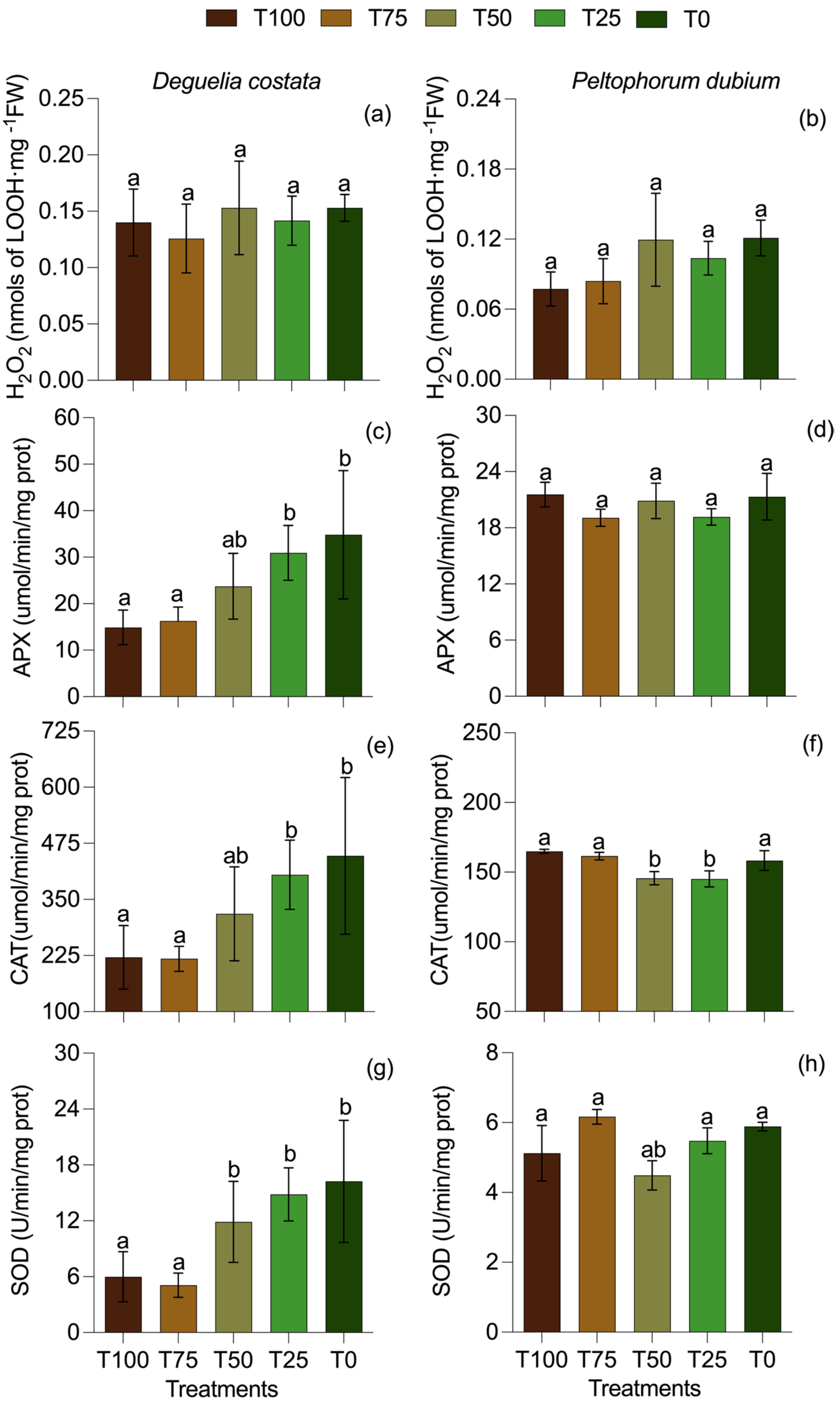
Fig. 3 Effect of increasing amounts of mining tailings on the physiological parameters in *Deguelia costata* and *Peltophorum dubium* grown in amended reference soils T100 (100% mining tailings), T75 (75% mining tailings), T50 (50% mining tailings), T25 (25% mining tailings); T0 (mining tailings-free reference soils). (a-b) Stomatal conductance (g_s); (c-d) Photosynthetic rate (A); (e–f) Transpiration rate (E). Data are the means ± SE (n = 15)

the latter did not differ from the T75 and T100 treatments ($\chi^2 = 17.005$, $p = 0.001$, Fig. 4f).

3.4 Seedling Development and Quality

The total dry mass for *D. costata* was higher in the T100 and T75 treatments compared to T50 and T25, with increases of 57.2% and 89.8%, respectively, compared to T0 ($F_{\text{treatment}} = 51.900$, $p < 0.001$, Fig. 5a; Table S7). As for the species *P. dubium*, the total dry mass was 31.2% higher in the T75 treatment compared to the T100, T50, and T25 treatments, and 55% higher compared to T0 ($F_{\text{Treatment}} = 24.332$, $p < 0.001$, Fig. 5b). In *D. costata*, the DQI in T100 was 57.7% higher than that of plants under T25 and 87.2% higher than in T0 and did not differ from T75

Fig. 4 Effect of increasing amounts of mining tailings on the physiological parameters in *Deguelia costata* and *Peltophorum dubium* grown in amended reference soils T100 (100% mining tailings), T75 (75% mining tailings), T50 (50% mining tailings), T25 (25% mining tailings); T0 (mining tailings-free reference soils). (a-b) hydrogen peroxide; (H_2O_2) (c-d) ascorbate peroxidase (APX); (e–f) catalase (CAT); (g-h) superoxide dismutase (SOD). Data are the means ± SE (n = 4)



(F treatment = 77.99, $p < 0.001$, Fig. 5c). The DQI of *P. dubium* in T75 was 30.7% higher than that of plants in T25 and 49.2% higher than in T0 and did not differ from T100 (F treatment = 27.848, $p < 0.001$, Fig. 5d).

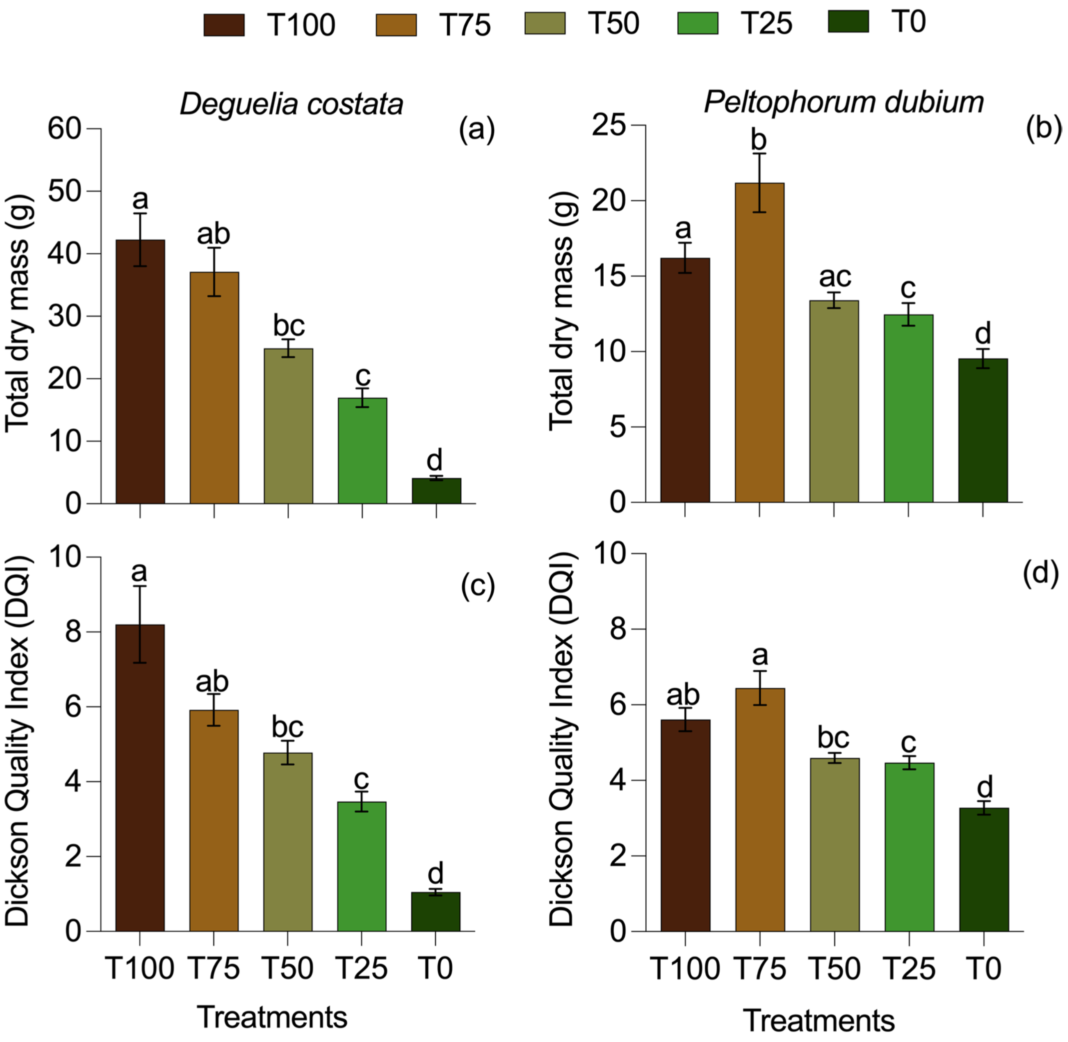


Fig. 5 Effect of increasing amounts of mining tailings on the seedling growth and quality in *Deguelia costata* and *Peltophorum dubium* grown in amended reference soils. T100 (100% mining tailings), T75 (75% mining tailings), T50 (50% mining tailings), T25 (25% mining tailings); T0 (mining tailings-free reference soils). (a-b) total dry mass and (c-d) Dickson Quality Index (DQI). Data are the means ± SE (n = 15)

3.5 Nutritional Analysis of Plant Material

The PCA for the leaf nutritional composition of *D. costata* explained 70.5% of the variability, with the first two principal components (PC1 and PC2) explaining 49.2% and 21.3%, respectively (Fig. 6a, Table S3 and S7). Concerning axis 1, *D. costata* plants under T100, T75, and T50 treatments, exhibited higher amounts of P, Ca, and K in the leaves. Conversely, *D. costata* plants in T0 treatment showed higher amounts of Na, S, and micronutrients (*e.g*., Mn, Fe, Zn, Cu, B) in the leaves, and lower amounts of nutrients pointing towards the opposite side (on the positive side of axis 1). Regarding axis 2, plants under T100 treatment had higher amounts of Cu, P, and Ca, and lower amounts of Mn and K, while plants cultivated under T50 and T25 treatments showed the opposite trend (Fig. 6a). The PCA for nutrients of *D. costata* roots explained 76.1% of the data variability, with the first two principal components (PC1 and PC2) explaining 58.5% and 17.6%, respectively (Fig. 6b, Table S4 and S7). Regarding axis 1, *D. costata* plants under in T100, T50, and T25 treatments exhibited higher amounts of P, Mg,

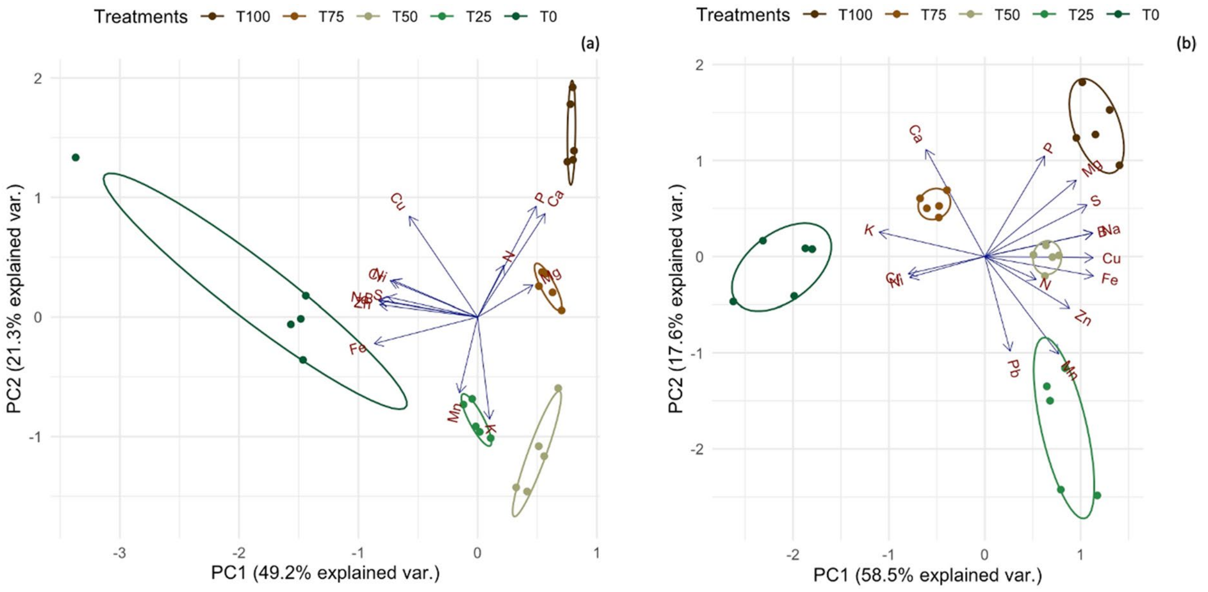


Fig. 6 Relationship between leaf and root nutrient concentrations of *Deguelia costata* grown in soils with increasing amounts of mining tailings (T100, 100% mining tailings; T75, 75% mining tailings; T50, 50% mining tailings; T25, 25% mining tailings; T0, mining tailings-free reference soils). (a) nutrient concentrations in the leaf, and (b) nutrient concentrations in the root (n = 5)

S, B, Na,—micronutrients (*e.g.,* Cu, Fe, Zn, and Mn) and the metal Pb in the roots. In contrast, *D. costata* plants in T0 treatment showed a higher amount of K in the roots. Regarding axis 2, plants under T100 and T75 treatments had higher amounts of Ca, P, and Mg, while plants under T25 treatment had higher amounts of Pb and Mn (Fig. 6b).

The PCA for nutrients of *P. dubium* leaves explained 74% of the data variability, with the first two principal components (PC1 and PC2) explaining 46.2% and 25.8%, respectively (Fig. 7a, Table S5 and S7). Regarding axis 1, *P. dubium* plants under T50 treatment had higher amounts of Mg, S, B, Ca, and Na, as well as the micronutrients Cu and Zn in the leaves. In contrast, *P. dubium* plants in T0 treatment had higher amounts of Ni in the leaves. As for axis 2, plants under T100 and T75 treatments had higher amounts of K and P in the leaves. Conversely, plants under T25 and T0 treatments had higher amounts of Fe and Mn in the leaves (Fig. 7a). The PCA for nutrients in *P. dubium* roots explained 75.2% of the data variability, with the first two principal components (PC1 and PC2) explaining 57.1% and 18.1%, respectively (Fig. 7b, Table S6 and S7). Regarding axis 1, *P. dubium* plants under T75 and T0 treatments had higher amounts of Na, macronutrients P, Mg, and S, and the micronutrients Fe, B, Mn, Zn, and Cu in the roots. As for axis 2, plants under T75 and T0 treatments had higher amounts of Ni and Cr in the roots. In contrast, plants under T100 and T50 treatments had higher amounts of Ca and Mg in the roots (Fig. 7b).

4 Discussion

4.1 Changes in Mining Tailings Quality Due to the Addition of Soil

In this study, mixing reference soil with mining tailings resulted in a composition that was less sandy and richer in clay. The physical characteristics of mining tailings, such as high density, particles packing, and elevated content of fine sand and silt, are detrimental to the establishment and development of plants (Cruz et al., 2020; de Pádua et al., 2021; Matos et al., 2020). The significant compaction of the mining tailings hinders root penetration, and the sandy texture reduces the water retention capacity of the soil (Schaefer et al., 2016). Additionally, plants grown at relatively lower amounts of mining tailings showed decreased

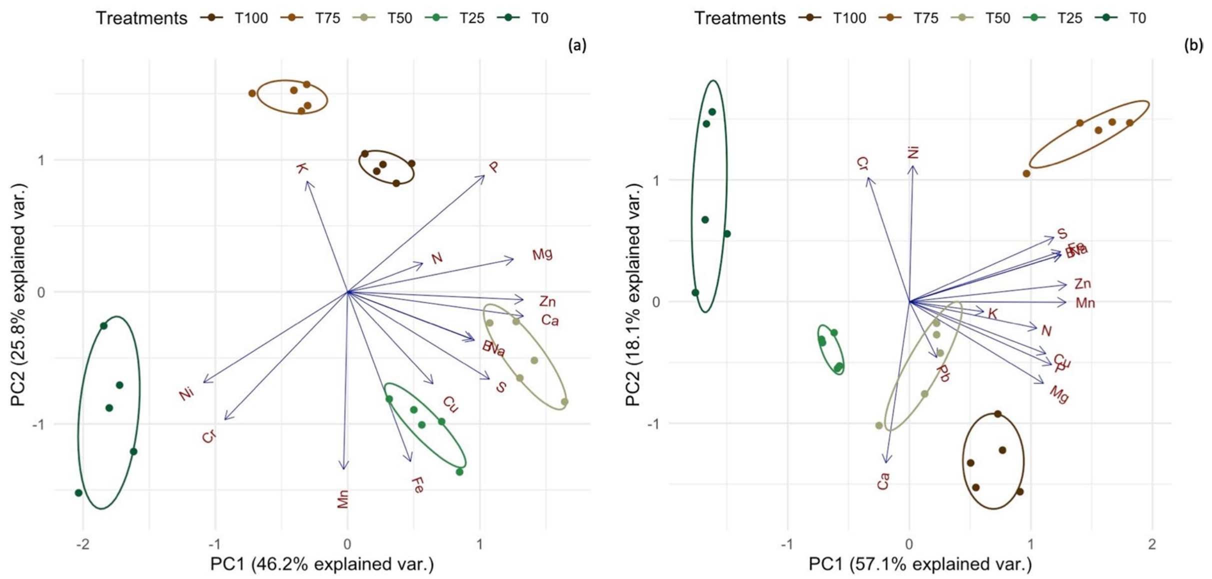


Fig. 7 Relationship between leaf and root nutrient concentrations of *Peltophorum dubium* grown in soils with increasing amounts of mining tailings (T100, 100% mining tailings; T75, 75% mining tailings; T50, 50% mining tailings; T25, 25% mining tailings; T0, mining tailings-free reference soils). (a) nutrient concentrations in the leaf, and (b) nutrient concentrations in the root (n = 5)

amounts of Cu, Mn, Fe, and Zn, which at high levels can be toxic to plants. Such a condition increased the OM and CEC in the substrates. However, the OM content in amended soil or reference soil solely (T0 = 32.5 g kg^{-1}) was still considered below the satisfactory reference value (40 g kg^{-1}) (Silva et al., 2022a). Although potentially toxic elements such as Pb, and Cr were detected at relatively high amounts in the mining tailings, such metals remained below the toxicity limit for plants according to the Brazilian environmental legislation (CONAMA, 2009). Contrary to our expectations, the amounts of the macronutrients P, K, Ca, and Mg decreased gradually with the addition of the reference soil. In other words, these nutrients were found at higher concentrations in the mining tailings compared to the reference soil used in this study. It is important to note that the soil underwent modification, which could lead to changes in the composition of vegetative species at the site (e.g., Figueredo et al., 2022). In addition, the mining tailings have distinct chemical properties compared to the reference soils, and when mixed, it can result in a complex matrix that can affect nutrients' bioavailability and heavy metals' mobility (Almeida et al., 2018; Ferreira et al., 2021; Queiroz et al., 2018). Finally, the addition of reference soil in the mining tailings, made the substrates more acidic. The decrease in the soil pH is well-known to improve the bioavailability of certain metals to plants, such as Mn and Fe, while potentially impairing the absorption of Ca, Mg, and K (Scarpa et al., 2022; Silva et al., 2022b).

4.2 The Amendment of Reference Soil With Increasing Amounts of Mining Tailings Does Not Affect Seed Germination

The presence of mining tailings in the reference soil did not affect the germination percentage of the native species *D. costata* and *P. dubium*. Seeds germinated both under higher and lower amounts of mining tailings regardless of the species studied. These findings are consistent with previous studies carried out with other tree species (Cruz et al., 2020; Scarpa et al., 2022) and native forage plants (Esteves et al., 2020). The lack of significant impact of mining tailings on seed germination may be attributed to the controlled environment of the greenhouse, in which essential factors like water, light, and temperature were carefully regulated. These ideal conditions ensured that the amounts of mining tailings used did not hinder the germination process. It is important to note that the conditions imposed by the tailings in the field may differ from those encountered by the plants in our greenhouse study. Additional challenges exist in the field, including water scarcity, herbivory, and other abiotic and biotic stresses. For instance, Schaefer et al. (2016) observed in the field that the formation of a surface seal called "hard-setting" can take place due to the characteristics of the tailings, which would hinder germination and root penetration.

4.3 The Addition of Reference Soil Into Mining Tailings Did Not Improve Plant Growth

The higher NBI and chlorophyll values observed in the T100 and T75 treatments may have contributed to a greater biomass gain and improved seedling quality at the end of the experiment. However, there were no significant changes observed in the values of gs, *A*, and *E*. Regarding *P. dubium*, the increase in OM content in the T0 and T25 treatments seemed to have contributed to higher NBI and chlorophyll values during the 10th and 19th weeks. Nevertheless, these changes did not have a positive impact on the values of gs, *A*, and *E* and the subsequent biomass gain and seedling quality. Conversely, individuals of *P. dubium* thrived under optimal development conditions in the T75 treatment. The enhanced growth of *D. costata* (under T100 and T75) and *P. dubium* (under T75), treatments with higher amounts of mining tailings, can be attributed to the higher uptake of macronutrients, such as P, K, Ca, Mg, and S, present in the mining tailings utilized in this investigation. Moreover, the pH of the mining tailings initially 6.89) and later 4.94 upon mixture with reference soil likely facilitated the establishment of a substrate with pH ranges that were more favourable for optimal plant growth, particularly for *P. dubium*. According to Msimbira and Smith (2020), the optimal soil pH range for plant growth typically falls between 5.5 to 6.5. In substrates with higher amounts of mining tailings, the pH may have favoured the reduction of bioavailability of potentially toxic metals, thereby benefiting plant development (Guerinot & Yi, 1994; Sharma & Nagpal, 2018). *Deguelia costata* exhibited higher levels of P, Ca, Mg, and S in the leaves grown under substrates with higher amounts of mining tailings, which inherently contained elevated levels of these macronutrients. In contrast, *P. dubium* was able to maintain higher amounts of K and Ca in the T75 treatment. These macronutrients are responsible for activating enzymes, maintaining osmotic balance in cells during photosynthesis, regulating stomatal movement, and influencing the transport of water, nutrients, and sugars (Hawkesford et al., 2022; Prajapati & Modi, 2012). Although nutrient content in plant tissues is contingent upon species-specific characteristics and requirements, it is closely associated with nutrient availability in the soil (Fritioff & Greger, 2003; Shtangeeva et al., 2009).

The limited growth observed in the T0 treatments may be attributed to increased metal uptake by plants due to soil acidity and poor soil quality. In acidic soils, metals tend to become more soluble in water, making them more available for root absorption with potentially toxic effects on plants (Esteves et al., 2020). In our study, we observed higher amounts of Ni and Cr in the leaves and roots of individuals in the T0 treatment, for both species. The higher retention of Ni in these substrates is likely due to the clayed nature of the soils, which possess a high cation exchange capacity and tend to retain more nickel on their surfaces (Galdos et al., 2004). Furthermore, the amount of bioavailable Ni and Cr tends to increase as pH decreases, as observed in the T0 treatment (Galdos et al., 2004; Kim et al., 2017). Elevated amounts of Cr can cause severe damage to plant development by interfering with chloroplast synthesis and the photosynthetic process (Shahid et al., 2017; Zornoza et al., 1999). There is a lack of literature establishing appropriate reference values for macro and micronutrients in leaves and roots of *D. costata* and *P. dubium*, which would allow for the assessment of the nutritional status of these species. Furthermore, the presence of Al was detected in the T0 and T25 treatments, which could be responsible for the poor development of the species, particularly in the T0 treatment. Aluminum (Al) tends to form less available and insoluble complexes with phosphorus (P) in soils and plant roots, resulting in a deficiency of P that hinders plant growth (Eekhout et al., 2017). Additionally, Al exerts inhibitory effects on root respiration, interferes with enzymes involved in polysaccharide deposition in cell walls, reduces cytokinin biosynthesis and transport, and modifies the structure and function of plasma membranes. These alterations significantly impact the absorption, transport, and utilization of various elements such as Ca, Mg, P, and

K, as well as the uptake of water by plants (Foy et al., 1978; Kochian et al., 2004).

Deguelia costata and *P. dubium* accumulated relatively high amounts of Fe in the roots compared to the leaves. This is a typical characteristic of tolerant plants, which tend to restrict the translocation of metals to the shoot and primarily accumulate them in the roots (Esringü et al., 2014; Gilberti et al., 2014). This selective accumulation strategy in the roots helps to avoid damage caused by excess metals in the shoot, which is more sensitive and performs critical functions such as photosynthesis. However, the amounts of Fe in the leaves of both species cultivated under T0, T25, and T50 treatments remained below the phytotoxic threshold considered for most plants (>500 mg Fe kg^{-1}; Pugh et al., 2002; Broadley et al., 2012). In contrast, higher amounts of Mn were detected in leaves compared to the roots, indicating that these plant species are capable of coping with higher levels of Mn. Notably, clear reference values for Mn in many plant species are currently unavailable.

It was anticipated that higher amounts of mining tailings would induce oxidative stress in plants. However, no changes in the amounts of lipid peroxides (quantified as H_2O_2 equivalents) were detected for both species. A study conducted by Caetano et al. (2022) revealed similar results, showing that mining residues from the Fundão dam rupture did not induce toxicity in *Cenostigma pluviosum* plants. Conversely, the increase in the activity of antioxidant enzymes such as APX, CAT, and SOD in the T0, T25 treatments may indicate a compensatory mechanism to maintain the levels of H_2O_2 in plant cells (Foyer & Noctor, 2005). The variations in antioxidant enzyme activity can be attributed to the specific characteristics of each species. It is possible that *D. costata* possesses a more efficient antioxidant system, leading to a more pronounced response regarding the activity of APX, CAT, and SOD compared to *P. dubium*. These differences may be influenced by the differential expression of genes related to antioxidant activity or by post-translational regulation of the enzymes (Shahabivand et al., 2016). Additionally, both *D. costata* and *P. dubium* in the T0 and T25 treatments maintained elevated levels of flavonoids in their leaves throughout the experiment, indicating that this could be a mechanism of alleviating stress and achieve a trade-off between defense and growth, particularly under T0 treatment. The biosynthesis of flavonoids can be triggered by stressing conditions as such secondary metabolites are powerful antioxidants (Kumar & Pandey, 2013). Overall, the results suggest that the amendment of a reference soil with mining tailings did not induce oxidative stress in *D. costata* and *P. dubium* during the initial stage of development and growth.

5 Conclusions

The findings unequivocally demonstrate that *D. costata* and *P. dubium* exhibited remarkable resilience by successfully thriving and growing even in highly contaminated environments (up to 100% mining tailings). As a result, both *D. costata* and *P. dubium* prove to be highly suitable candidates for the restoration of areas devastated by iron mining activities. Given their promising attributes, it is imperative to prioritize the utilization of these native species, along with other native plants, over exotic species that may compete with and disrupt the natural succession process. However, to ensure successful restoration, it is crucial to conduct further research encompassing various aspects, including monitoring the developmental stages of these plants, assessing seed viability and production, evaluating their ability to establish within the ecological community, and assessing their resilience to environmental stresses.

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Author Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Bárbara Dias Araújo, Renata A. Maia, Milton Barbosa, and Thamara F. Silva. Bárbara Dias Araújo and Renata A. Maia wrote the first draft, while Milton Barbosa, Luzia Valentina Modolo, Daniel Negreiros, and Geraldo Wilson Fernandes contributed to the final version of the manuscript. All authors read and approved the final manuscript.

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Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent for publication By submitting this manuscript, all authors explicitly agree to the publication of this manuscript in its current or revised form in any journal or other publication medium. All authors have reviewed and approved the final manuscript and have agreed to be accountable for all aspects of the work. All authors also confirm that this manuscript is original, has not been previously published, and is not under consideration for publication elsewhere.

Conflict of Interest The authors declare no competing interests.

References

Adiansyah, J. S., Rosano, M., Vink, S., & Keir, G. (2015). A framework for a sustainable approach to mine tailings management: Disposal strategies. *Journal of Cleaner Production, 108*, 1050–1062. https://doi.org/10.1016/j.jclepro.2015.07.139

Almeida, C. A., Oliveira, A. F., Pacheco, A. A., Lopes, R. P., Neves, A. A., & Queiroz, M. E. L. R. (2018). Characterization and evaluation of sorption potential of the iron mine waste after Samarco dam disaster in Doce River basin–Brazil. *Chemosphere, 209*, 411–420. https://doi.org/10.1016/j.chemosphere.2018.06.071

Andrade, G. F., Paniz, F. P., Martins, A. C., Jr., Rocha, B. A., da Silva Lobato, A. K., Rodrigues, J. L., Cardoso-Gustavson, P., Masuda, H. P., & Batista, B. L. (2018). Agricultural use of Samarco's spilled mud assessed by rice cultivation: A promising residue use? *Chemosphere, 193*, 892–902. https://doi.org/10.1016/j.chemosphere.2017.11.099

Araújo, B. D., Maia, R., Arantes-Garcia, L., Oki, Y., Negreiros, D., Assis, I. R., & Fernandes, G. W. (2022). Aftershocks of the Samarco disaster: Diminished growth and increased metal content of *Raphanus sativus* cultivated in soil with mining tailings. *Acta Scientiarum. Biological Sciences, 44*, e59175. https://doi.org/10.4025/actascibiolsci.v44i1.59175

Balestrin, D., Martins, S. V., Schoorl, J. M., Lopes, A. T., & Andrade, C. F. (2019). Phytosociological study to define restoration measures in a mined area in Minas Gerais, Brazil. *Ecological Engineering, 135*, 8–16. https://doi.org/10.1016/j.ecoleng.2019.04.023

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software, 67*, 1–48. https://doi.org/10.48550/arXiv.1406.583

Broadley, M., Brown, P., Çakmak, İ., Rengel, Z., & Zhao, F. (2012). Function of nutrients: Micronutrients. In P. Marschner (Ed.), *Marschner's mineral nutrition of higher plants* (3rd ed., pp. 191–248). Academic Press. https://doi.org/10.1016/B978-0-12-384905-2.00007-8

Caetano, A. L., de Pádua, M. P., Polo, M., Pasqual, M., & Pereira, F. J. (2022). Growth, anatomy, and gas exchange of Cenostigma pluviosum cultivated under reduced water levels in iron mining tailings. *Journal of Soils and Sediments, 22*, 381–391. https://doi.org/10.1007/s11368-021-03060-4

Campanharo, I. F., Martins, S. V., Villa, P. M., Kruschewsky, G. C., Dias, A. A., & Nabeta, F. (2021). Forest restoration methods, seasonality, and penetration resistance do not influence aboveground biomass stock on mining tailings in Mariana, Brazil. *Anais Da Academia Brasileira De Ciências, 93*, 1–13. https://doi.org/10.1590/0001-3765202120201209

CONAMA. (2009). Resolução No. 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. https://cetesb.sp.gov.br/areas-contaminadas/wp-content/uploads/sites/17/2017/09/resolucao-conama-420-2009-gerenciamento-de-acs.pdf Accessed on February 23, 2023.

Cordeiro, J., Gomes, A. R., Santos, C. H. B., Rigobelo, E. C., Baptista, M. B., Moura, P. M., & Scotti, M. R. (2022). Rehabilitation of the Doce River Basin after the Fundão dam collapse: What has been done, what can be done and what should be done? *River Research and Applications, 38*, 194–208. https://doi.org/10.1002/rra.3894

Crawley, J. M. (2013). *The R Book*. United Kingdom: John Wiley & Sons Ltd.

Cruz, F. V. S., Gomes, M. P., Bicalho, E. M., & Garcia, Q. S. (2022). Fertilization assures mineral nutrition but does not overcome the effects of Fe accumulation in plants grown in iron ore tailings. *Environmental Science and Pollution Research, 29*, 18047–18062. https://doi.org/10.1007/s11356-021-16989

Cruz, F. V. S., Gomes, M. P., Bicalho, E. M., Torre, D., & Garcia, Q. S. (2020). Does Samarco's spilled mud impair the growth of native trees of the Atlantic Rainforest? *Ecotoxicology and Environmental Safety, 189*, 1–12. https://doi.org/10.1016/j.ecoenv.2019.110021

da Silva, C. J., Fontes, E. P. B., & Modolo, L. V. (2017). Salinity-induced accumulation of endogenous H_2S and NO is associated with modulation of the antioxidant and redox defense systems in *Nicotiana tabacum* L. cv. *Havana.*

Plant Science, 256, 148–159. https://doi.org/10.1016/j.plantsci.2016.12.011

de Pádua, M. P., Caetano, A. L., Polo, M., Pasqual, M., & Pereira, F. J. (2021). Ecophysiological responses of *Copaifera langsdorffii* grown in mining tailings under lower water availability. *Water, Air, & Soil Pollution, 232*, 1–12. https://doi.org/10.1007/s11270-021-05037-y

DeLong, J. M., Prange, R. K., Hodges, D. M., Forney, C. F., Bishop, M. C., & Quilliam, M. (2002). Using a modified ferrous oxidation− xylenol orange (FOX) assay for detection of lipid hydroperoxides in plant tissue. *Journal of Agricultural and Food Chemistry, 50*(2), 248–254. https://doi.org/10.1021/jf0106695

Dickson, A., Leaf, A. L., & Hosner, J. F. (1960). Quality appraisal of white spruce and white pine seedling stock in nurseries. *Forestry Chronicle, 36*, 10–13. https://doi.org/10.5558/tfc36010-1

Donadio, N. M. M., & Demattê, M. E. S. P. (2000). Morfologia de frutos, sementes, e plântulas de canafístula (*Peltophorum dubium* (Spreng.) Taub.) e jacarandá-da-Bahia *(Dalbergia nigra* (Vell.) Fr. All. ex Benth.) - Fabaceae. *Revista Brasileira De Sementes, 22*, 64–73.

Duarte, E. B., Neves, M. A., de Oliveira, F. B., Martins, M. E., de Oliveira, C. H. R., Burak, D. L., Orlando, M. T. D., & Rangel, C. V. G. T. (2021). Trace metals in Rio Doce sediments before and after the collapse of the Fundão iron ore tailing dam, Southeastern Brazil. *Chemosphere, 262*, 1–9. https://doi.org/10.1016/j.chemosphere.2020.127879

Eekhout, T., Larsen, P., & De Veylder, L. (2017). Modification of DNA checkpoints to confer aluminum tolerance. *Trends in Plant Science, 22*, 102–105. https://doi.org/10.1016/j.tplants.2016.12.003

Esringü, A., Turan, M., Güneş, A., & Karaman, M. R. (2014). Roles of *Bacillus megaterium* in remediation of boron, lead, and cadmium from contaminated soil. *Communications in Soil Science and Plant Analysis, 45*, 1741–1759. https://doi.org/10.1080/00103624.2013.875194

Esteves, G. F., Souza, K. R. D., Bressanin, L. A., Andrade, P. C. C., Júnior, V. V., Reis, P. E., ... de Souza, T. C. (2020). Vermicompost improves maize, millet, and sorghum growth in iron mine tailings. *Journal of Environmental Management, 264*, 1–11. https://doi.org/10.1016/j.jenvman.2020.110468

Fernandes, G. W., Goulart, F. F., Ranieri, B. D., Coelho, M. S., Dales, K., Boesche, N., ... Soares-Filho, B. (2016a). Deep into the mud: Ecological and socio-economic impacts of the dam breach in Mariana, Brazil. *Nature Conservation, 14*, 35–45. https://doi.org/10.1016/j.ncon.2016.10.003

Fernandes, G. W., Toma, T. S., Angrisano, P., & Overbeck, G. (2016b). Challenges in the restoration of quartzitic and ironstone rupestrian grasslands. In G. W. Fernandes (Ed.), *Ecology and conservation of mountaintop grasslands in Brazil* (pp. 449–477). Springer.

Fernandes, G. W., & Ribeiro, S. P. (2017). Deadly conflicts: Mining, people, and conservation. *Perspectives in Ecology and Conservation, 3*(15), 141–144.

Fernandes, G. W., Maia, R. A., Arantes-Garcia, L., Pereira, E. G., Oki, Y., França, M. G., ... Barbosa, M. (2022). Deep capillary impact of a psyllid gall on its host ecophysiology, architecture, and performance. *Trees, 36*, 1193–1206. https://doi.org/10.1007/s00468-022-02280-6

Ferreira, G. W. D., Ribeiro, B. T., Weindorf, D. C., Teixeira, B. I., Chakraborty, S., Li, B., ... & Scolforo, J. R. S. (2021). Assessment of iron-rich tailings via portable X-ray fluorescence spectrometry: the Mariana dam disaster, southeast Brazil. Environmental Monitoring and Assessment, 193, 1–19. https://doi.org/10.1007/s10661-021-08982-7

Figueiredo, J. C. G., de Ávila, M. A., Souza, C. S., Neves, J. G. S., Tolentino, G. S., Oki, Y., ... & Nunes, Y. R. F. (2022). Relationship of woody species composition with edaphic characteristics in threatened riparian Atlantic Forest remnants in the upper Rio Doce basin, Brazil. *Nordic Journal of Botany, 2022*, 1–10. https://doi.org/10.1111/njb.03679

Figueiredo, J. C. G., Negreiros, D., Ramos L., Paiva, D. C, Oki, Y., Justino, W. S., ... & Fernandes, G. W. (2024). Reference sites of threatened riverine Atlantic forest in upper Rio Doce watershed. *Nature Conservation Research, 9*, 58–71.

Foy, C. D., Chaney, R. L., & White, M. C. (1978). The physiology of metal toxicity in plants. *Annual Review of Plant Physiology, 29*, 511–566. https://doi.org/10.1146/annurev.pp.29.060178.002455

Foyer, C. H., & Noctor, G. (2005). Oxidant and antioxidant signaling in plants: A re-evaluation of the concept of oxidative stress in a physiological context. *Plant, Cell & Environment, 28*, 1056–1071. https://doi.org/10.1111/j.1365-3040.2005.01327.x

Freitas, C. M., & Silva, M. A. (2019). Work accidents which become disasters: Mine tailing dam failures in Brazil. *Revista Brasileira de Medicina do Trabalho, 17*, 21–29. https://doi.org/10.5327/Z1679443520190405

Fritioff, Å., & Greger, M. (2003). Aquatic and terrestrial plant species with potential to remove heavy metals from stormwater. *International Journal of Phytoremediation, 5*, 211–224. https://doi.org/10.1080/713779221

Galdos, M. V., de Maria, I. C., & Camargo, O. A. (2004). Atributos químicos e produção de milho em um Latossolo Vermelho eutroférrico tratado com lodo de esgoto. *Revista Brasileira De Ciência Do Solo, 28*, 569–577. https://doi.org/10.1590/S0100-06832004000300017

Garcia, L. C., Ribeiro, D. B., Roque, F. O., Ochoa-Quintero, J. M., & Laurance, W. F. (2017). Brazil's worst mining disaster: Corporations must be compelled to pay the actual environmental costs. *Ecological Applications, 27*, 5–9. https://doi.org/10.1002/eap.1461

Gei, M., Rozendaal, D. M., Poorter, L., Bongers, F., Sprent, J. I., Garner, M. D., ... & Powers, J. S. (2018). Legume abundance along successional and rainfall gradients in Neotropical forests. *Nature Ecology & Evolution, 2*, 1104–1111.

Giannopolitis, C. N., & Ries, S. K. (1977). Superoxide dismutases: I. *Occurrence in Higher Plants. Plant Physiology, 59*, 309–314. https://doi.org/10.1104/pp.59.2.309

Gilberti, L., Menezes, A., Rodrigues, A. C., Fernandes, G. W., Berbara, R. L. L., & Marota, H. B. (2014). Effects of arsenic on the growth, uptake, and distribution of nutrients in the tropical species *Baccharis dracunculifolia* DC (Asteraceae). *Journal of Toxicology Science, 2014*, 1–27.

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry, 48*, 909–930.

Goulas, Y., Cerovic, Z. G., Cartelat, A., & Moya, I. (2004). Dualex: A New instrument for field measurements of epidermal ultraviolet absorbance by chlorophyll fluorescence. *Applied Optics, 43*, 4488–4496. https://doi.org/10.1364/AO.43.004488

Grotz, N., & Guerinot, M. L. (2006). Molecular aspects of Cu, Fe, and Zn homeostasis in plants. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1763,* 595–608. https://doi.org/10.1016/j.bbamcr.2006.05.014

Guerinot, M. L., & Yi, Y. (1994). Iron: Nutritious, noxious, and not readily available. *Plant Physiology, 104*, 815–820. https://doi.org/10.1104/pp.104.3.815

Hartig, F. (2022). *DHARMa: Residual diagnostics for hierarchical (multiLevel /mixed) regression models.* R package version 0.4.5. https://CRAN.R-project.org/package=DHARMa

Hatje, V., Pedreira, R. M., de Rezende, C. E., Schettini, C. A. F., de Souza, G. C., Marin, D. C., & Hackspacher, P. C. (2017). The environmental impacts of one of the largest tailing dam failures worldwide. *Scientific Reports, 7*, 1–13. https://doi.org/10.1038/s41598-017-11143-x

Hawkesford, M. J., Cakmak, I., Coskun, D., De Kok, L. J., Lamber, H., Schjoerring, J. K., & White, P. J. (2022). Functions of macronutrients. In R. Z. Rengel, I. Cakmak, & P. J. White (Eds.), *Marschner's Mineral Nutrition of Plants* (4th ed., pp. 201–281). Academic press.

Helene, L. C. F., Dall'Agnol, R. F., Delamuta, R. J. M., & Hungria, M. (2019). *Mesorhizobium atlanticum* sp. nov., a new nitrogen-fixing species from soils of the Brazilian Atlantic Forest biome. *International Journal of Systematic and Evolutionary Microbiology, 69*, 1800–1806. https://doi.org/10.1099/ijsem.0.003397

Huang, G., Ding, C., Guo, F., Li, X., Zhang, T., & Wang, X. (2017). Underlying mechanisms and effects of hydrated lime and selenium application on cadmium uptake by rice (*Oryza sativa* L.) seedlings. *Environmental Science and Pollution Research, 24*, 18926–18935. https://doi.org/10.1007/s11356-017-9573-5

Kim, H. S., Kim, K. R., Kim, W. I., Owens, G., & Kim, K. W. (2017). Influence of road proximity on the concentrations of heavy metals in Korean Urban Agricultural Soils and Crops. *Archives of Environmental Contamination and Toxicology, 72*, 260–268. https://doi.org/10.1007/s00244-016-0344-y

Kobayashi, T., & Nishizawa, N. K. (2012). Iron uptake, translocation, and regulation in higher plants. *Annual Review of Plant Biology, 63*, 131–152. https://doi.org/10.1146/annurev-arplant-042811-105522

Kochian, L. V., Hoekenga, O. A., & Pineros, M. A. (2004). How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. *Annual Review of Plant Biology, 55*, 459–493. https://doi.org/10.1146/annurev.arplant.55.031903.141655

Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: An Overview. *The Scientific World Journal, 2013*, 1–16. https://doi.org/10.1155/2013/162750

Lana-Costa, J., da Silva, M. M., Martins, A. O., Guarnier, J. C., Silva Junior, R. O., Silva, D. M., Nunes-Nesi, A., & Araújo, W. L. (2021). Biochemical and physiological aspects of restinga herbaceous plants tolerance to iron ore tailing plume along the coastal region of Espírito Santo, Brazil. *Environmental and Experimental Botany, 191*, 1–10. https://doi.org/10.1016/j.envexpbot.2021.104618

Lenth, R. (2022). *emmeans: Estimated marginal means, AKA least-squares means.* R package version 1.8.1-1. https://CRAN.R-project.org/package=emmeans

Lorenzi, H. (2016). *Árvores Brasileiras: Manual de Identificação e Cultivo de Plantas Arbóreas Nativas do Brasil.* Editora Plantarum.

Marta-Almeida, M., Mendes, R., Amorim, F. N., Cirano, M., & Dias, J. M. (2016). Fundão dam collapse: Oceanic dispersion of River Doce after the greatest Brazilian environmental accident. *Marine Pollution Bulletin, 112*, 359–364. https://doi.org/10.1016/j.marpolbul.2016.07.039

Matos, L. P., de Andrade, H. M., Marinato, C. S., Prado, I. G. O., Coelho, D. G., Montoya, S. G., ... & de Oliveira, J. A. (2020). Limitations to use of Cassia grandis L. in the revegetation of the areas impacted with mining tailings from Fundão Dam. Water, Air, & Soil Pollution, 231, 1–9. https://doi.org/10.1007/s11270-020-04479-0

Mehes-Smith, M., Nkongolo, K., & Cholewa, E. (2013). Coping mechanisms of plants to metal-contaminated soil. *Environmental Change & Sustainability, 54*, 53–90.

Mittler, R. (2002). Oxidative stress, antioxidants, and stress tolerance. *Trends in Plant Science, 7*, 405–410. https://doi.org/10.1016/S1360-1385(02)02312-9

Msimbira, L. A., & Smith, D. L. (2020). The roles of plant growth-promoting microbes in enhancing plant tolerance to acidity and alkalinity stresses. *Frontiers in Sustainable Food Systems, 4*, 106. https://doi.org/10.3389/fsufs.2020.00106

Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate–specific peroxidase in spinach chloroplast. *Plant Cell Physiology, 22*, 867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

Omachi, C. Y., Siani, S. M., Chagas, F. M., Mascagni, M. L., Cordeiro, M., Garcia, G. D., ... Thompson, F. L. (2018). Atlantic Forest loss caused by the world's largest tailing dam collapse (Fundão dam, Mariana, Brazil). *Remote Sensing Applications: Society and Environment, 12,* 20–34. https://doi.org/10.1016/j.rsase.2018.08.003

Pires, A. P., Rezende, C. L., Assad, E. D., Loyola, R., & Scarano, F. R. (2017). Forest restoration can increase the Rio Doce watershed resilience. *Perspectives in Ecology and Conservation, 15*, 187–193. https://doi.org/10.1016/j.pecon.2017.08.003

Prado, I. G. O., da Silva, M. C. S., Prado, D. G. O., Kemmelmeier, K., Pedrosa, B. G., da Silva, C. C., & Kasuya, M. C. M. (2019). Revegetation process increases the diversity of total and arbuscular mycorrhizal fungi in areas affected by the Fundão dam failure in Mariana, Brazil. *Applied Soil Ecology, 141*, 84–95. https://doi.org/10.1016/j.apsoil.2019.05.008

Prajapati, K., & Modi, H. A. (2012). The importance of potassium in plant growth–a review. *Indian Journal of Plant Sciences, 1*, 177–186.

Pugh, R. E., Dick, D. G., & Fredeen, A. L. (2002). Heavy metal (Pb, Zn, Cd, Fe, and Cu) contents of plant foliage near the Anvil Range lead/zinc mine, Faro, Yukon Territory. *Ecotoxicology and Environmental Safety, 52*, 273–279. https://doi.org/10.1006/eesa.2002.2201

Queiroz, H. M., Nóbrega, G. N., Ferreira, T. O., Almeida, L. S., Romero, T. B., Santaella, S. T., ... Otero, X. L.

(2018). The Samarco mine tailing disaster: A possible time-bomb for heavy metals contamination? *Science of the Total Environment, 637–638,* 498–506. https://doi.org/10.1016/j.scitotenv.2018.04.370

R Core Team. (2022). R: A language and environment for statistical computing.

Ramos, L., Negreiros, D., Ferreira, B. S. S., Figueiredo, J. C. G., Paiva, D. C., Oki, Y., ... & Fernandes, G. W. (2023) Strong relationships between soil and vegetation in reference ecosystems of a riparian Atlantic rainforest in the upper Doce River watershed, southeastern Brazil. *iForest-Biogeosciences and Forestry, 16*, 226–233. https://doi.org/10.3832/ifor4313-016

Rodriguez, R., & Redman, R. (2005). Balancing the generation and elimination of reactive oxygen species. *Proceedings of the National Academy of Sciences, 102*, 3175–3176. https://doi.org/10.1073/pnas.0500367102

Sáez-Plaza, P., Michałowski, T., Navas, M. J., Asuero, A. G., & Wybraniec, S. (2013). An overview of the Kjeldahl Method of nitrogen determination. Part I. Early history, chemistry of the procedure, and titrimetric finish. *Critical Reviews in Analytical Chemistry, 43*, 178–223. https://doi.org/10.1080/10408347.2012.751787

Santolin, C. V., Ciminelli, V. S., Nascentes, C. C., & Windmöller, C. C. (2015). Distribution and environmental impact evaluation of metals in sediments from the Doce River Basin, Brazil. *Environmental Earth Sciences, 74*, 1235–1248. https://doi.org/10.1007/s12665-015-4115-2

Scarpa, A. L. M., Cruz, Y. C., Duarte, V. P., Castro, E. M., Pasqual, M., Oliveira, J. P. V., & Pereira, F. J. (2022). Growth response, gas exchange, and leaf anatomy of *Handroanthus spp.* seedlings in mine tailings enriched with nutrient solution. *Journal of Soil Science and Plant Nutrition, 22*, 3774–3787. https://doi.org/10.1007/s42729-022-00926-5

Schaefer, C. E. G. R., Santos, E. E., Fernandes Filho, E. I., & Assis, I. R. (2016). Paisagens de lama: Os tecnosolos para recuperação ambiental de áreas afetadas pelo desastre da barragem de Fundão, em Mariana. *Revista Brasileira de Ciência do Solo, 42,* 18–23. https://www.sbcs.org.br/wp-content/uploads/2016/06/vol42num1.pdf

Segura, F. R., Nunes, E. A., Paniz, F. P., Paulelli, A. C. C., Rodrigues, G. B., Braga, G. U. L., ... Batista, B. L. (2016). Potential risks of the residue from Samarco's mine dam burst (Bento Rodrigues, Brazil). *Environmental Pollution, 218,* 813–825. https://doi.org/10.1016/j.envpol.2016.08.005

Seneme, A. M., Possamai, E., Vanzolini, S., & Martins, C. C. (2012). Germinação, qualidade sanitária e armazenamento de sementes de canafístula *(Peltophorum dubium*). *Revista Árvore, 36*, 01–06. https://doi.org/10.1590/S0100-67622012000100001

Shahabivand, S., Maivan, H. Z., Mahmoudi, E., Soltani, B. M., Sharifi, M., & Aliloo, A. A. (2016). Antioxidant activity and gene expression associated with cadmium toxicity in wheat affected by mycorrhizal fungus. *Zemdirbyste, 103*, 53–60.

Shahid, M., Shamshad, S., Rafiq, M., Khalid, S., Bibi, I., Niazi, N. K., ... Rashid, M. I. (2017). Chromium speciation, bioavailability, uptake, toxicity and detoxification in soil-plant system: A review. *Chemosphere, 178*, 513–533. https://doi.org/10.1016/j.chemosphere.2017.03.074

Sharma, A., & Nagpal, A. K. (2018). Soil amendments: A tool to reduce heavy metal uptake in crops for production of safe food. *Reviews in Environmental Science and Biotechnology, 17*, 187–203. https://doi.org/10.1007/s11157-017-9451-0

Shtangeeva, I., Alber, D., Bukalis, G., Stanik, B., & Zepezauer, F. (2009). Multivariate statistical analysis of nutrients and trace elements in plants and soil from northwestern Russia. *Plant and Soil, 322*, 219–228. https://doi.org/10.1007/s11104-009-9910-7

Silva Júnior, C. A., Coutinho, A. D., de Oliveira-Júnior, J. F., Teodoro, P. E., Lima, M., Shakir, M., ... Johann, J. A. (2018). Analysis of the impact on vegetation caused by abrupt deforestation via orbital sensor in the environmental disaster of Mariana, Brazil. *Land Use Policy, 76,* 10–20. https://doi.org/10.1016/j.landusepol.2018.04.019

Silva, A. P. V., Silva, A. O., Lima, F. R. D., Benedet, L., Jesus Franco, A., Souza, J. K., ... Carneiro, M. A. C. (2022a). Potentially toxic elements in iron mine tailings: Effects of reducing soil pH on available concentrations of toxic elements. *Environmental Research, 215,* 1–10. https://doi.org/10.1016/j.envres.2022.114321

Silva, J. G., Gomes, M. P., Pereira, E. G., Bicalho, E. M., & Garcia, Q. S. (2022b). Initial growth of *Peltophorum dubium* is affected by nitrogen source and manganese concentration. *Journal of Soil Science and Plant Nutrition, 22*, 201–211. https://doi.org/10.1007/s42729-021-00644-4

Soil Survey Staff. (1993). *Soil survey manual*. Washington, D.C.: USDA-SCS Government Printing Office.

Teixeira, P. C., Donagemma, G. K., Fontana, A., & Teixeira, W. G. (2017). *Manual de métodos de análise de solo*. Embrapa.

Temperton, V. M., Mwangi, P. N., Scherer-Lorenzen, M., Schmid, B., & Buchmann, N. (2007). Positive interactions between nitrogen-fixing legumes and four different neighboring species in a biodiversity experiment. *Oecologia, 151*, 190–205. https://doi.org/10.1007/s00442-006-0576-z

Toma, T. S. P., Overbeck, G. E., Mendonça, M. S., Jr., & Fernandes, G. W. (2023). Optimal references for ecological restoration: The need to protect references in the tropics. *Perspectives in Ecology and Conservation, 21*, 25–32. https://doi.org/10.1016/j.pecon.2023.01.003

World Mine Tailings Failures. (2019). *World mine tailings failures-from 1915.* World Mine Tailings Failures. https://worldminetailingsfailures.org/

Zornoza, P., Robles, S., & Martin, N. (1999). Alleviation of nickel toxicity by ammonium supply to sunflower plants. *Plant and Soil, 208*, 221–226. https://doi.org/10.1023/A:1004517414730