JOBER CONDÉ EVANGELISTA FREITAS

ECOPHYSIOLOGICAL CONSTRAINTS AND STRATEGIES OF *MUCUNA PRURIENS* VAR. *UTILIS* (WALL. EX WIGHT) BAKER EX BURCK GROWN IN MINE TAILINGS FROM THE FUNDÃO DAM

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Thesis presented to the Federal University of Lavras, as part of the requirements of the Postgraduate Program in Plant Physiology, area of concentration in Plant Physiology, to obtain the title of Doctor.

Prof. Dr. Fernanda Carlota Nery
Advisor

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Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Freitas, Jober Condé Evangelista.
Ecophysiological constraints and strategies of *Mucuna pruriens* var. *utilis* (Wall. ex Wight) Baker ex Burck grown in mine tailings from the Fundão dam / Jober Condé Evangelista Freitas. - 2022.
39 p. : il.

Orientador(a): Fernanda Carlota Nery.
Coorientador(a): Paulo Henrique Pereira Peixoto.
Tese (doutorado) - Universidade Federal de Lavras, 2022.
Bibliografia.

1. Photosynthesis. 2. Mn toxicity. 3. Fe/Mn ratio. I. Nery, Fernanda Carlota. II. Peixoto, Paulo Henrique Pereira. III. Título.

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RESTRIÇÕES E ESTRATÉGIAS ECOFISIOLÓGICAS DE *MUCUNA PRURIENS* VAR. *UTILIS* (WALL. EX WIGHT) BAKER EX BURCK CULTIVADA EM REJEITO DE MINERAÇÃO DA BARRAGEM DO FUNDÃO

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This thesis is dedicated to all victims of the Fundão dam failure.

ACKNOWLEDGEMENTS

I would like to thank my husband (Igor), my best friends (Felipe and Matt), my two dogs (Sirius and Regulus), my mom (Maria), and my sister (Mariana) for always being by my side no matter what.

I sincerely thank all my friends (especially Aline, Marcella, Guilherme, Agostinho, Mateus, Lillian, and Dawyson) and colleagues from the Plant Physiology Sector for all the support and good moments.

I thank my friends/roommates in Lavras (Jeferson, Artur, and Carlos) for being the best people I would share a home with.

I would like to thank my advisor (Fernanda) and my co-advisor (Paulo) for the support, confidence in my potential, and friendship.

I am grateful to all the special and wonderful people that academia put in my way (especially Cristiano, Felipe, Nah, Nat, Bruna, Rogério, Leônidas, Fernando, Jemima, Igor, and Hugo).

I thank all board members.

I am grateful to the Federal University of Lavras, the Department of Biology and the Postgraduate Program in Plant Physiology.

Lastly, I would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES), Fulbright Commission, the West Virginia University, and Minas Gerais State Agency for Research and Development (FAPEMIG) for the fundings that allowed me to conclude my research and doctor’s degree.

"Workers of the world, unite!"
Karl Marx and Friedrich Engels

RESUMO

As atividades de mineração contribuem para a degradação ambiental de diferentes formas, como destruição física do meio ambiente, emissão de poeira contendo elementos tóxicos no ar, contaminação de solos e ambientes aquáticos por elementos tóxicos e colapsos de barragens. Em novembro de 2015, ocorreu o maior desastre mundial de mineração de ferro em Mariana, Minas Gerais/Brasil. Dadas as proporções do evento, os danos ambientais à Mata Atlântica naquela região foram inestimáveis. Uma vez que Fe e Mn são comumente encontrados no mesmo minério, o processo de mineração do Fe extrai a maior parte do Fe e descarta todos o conteúdo de Mn. De certa forma, mesmo que o Mn seja considerado inerte em termos de risco à saúde de plantas e animais, o alto teor desse elemento, como os observados em estudos sobre os rejeitos da barragem de Fundão, são preocupantes. Há até estudos relatando que altas concentrações desse micronutriente vegetal podem causar distúrbios fisiológicos nas plantas, o que pode impactar ainda mais o processo de recuperação em áreas impactadas por rejeitos via espécies exóticas selecionadas ou naturalmente por nativas. Buscou-se avaliar as alterações ecofisiológicas e fatores limitadores do crescimento na espécie nativa *Mucuna pruriens* cultivados em rejeitos. Cinco novos substratos de crescimento foram preparados misturando-se diferentes volumes de rejeitos de minério de ferro puros da barragem de Fundão com um substrato fértil do solo (0, 25, 50, 75 e 100% de rejeito/solo (v/v)). Avaliamos diferentes propriedades físico-químicas e de composição mineral dos substratos de crescimento. Nas plantas, também foram avaliados a composição mineral e traços fisiológicos como trocas gasosas, fluorescência da clorofila *a*, teor de carboidratos e prolina, massa seca da parte aérea e atividade da enzima dismutase do superóxido. Os resultados mais contrastantes foram observados comparando-se o tratamento controle ao tratamento com rejeito de minério de ferro puro, com uma tendência de resultados intermediários para os demais tratamentos (25-75% de rejeito). Quanto maior a concentração de rejeitos, mais prejudicado foi o crescimento. Mais do que apenas a baixa fertilidade do rejeito, essas tendências estavam relacionadas às altas concentrações de Mn encontradas nos substratos e nos órgãos dos vegetais avaliados. Observou-se também que a fotossíntese foi prejudicada por perturbações em parâmetros de fotoquímica e de trocas gasosas provavelmente devido aos altos níveis de Mn e ao desequilíbrio iônico associado que causa à razão Fe/Mn.

Palavras-chave: Fotossíntese. Toxicidade por Mn. Proporção Fe/Mn.

ABSTRACT

Mining activities contribute to environmental degradation in different ways, such as physical destruction of the environment, emission of dust containing toxic elements into the air, contamination of soils and aquatic environments by toxic elements, and dam collapses. In November 2015, the largest world's iron mining disaster occurred in Mariana, Minas Gerais, Brazil. Given the proportions of the event, the environmental damage to the Atlantic Rainforest in that region was inestimable. Given that Fe and Mn are commonly found in the same ore, the Fe mining process extracts most of the Fe and discards all the Mn. In a way, even if Mn is considered inert in terms of health risk to plants and animals, high contents of this element, such as those observed in studies on the tailings of the Fundão dam, are of concern. There are even studies reporting that high concentrations of this plant micronutrient may cause physiological disorders in plants, which can further impact the recovery process in areas impacted by tailings using selected exotic species or naturally by native ones. We aimed to evaluate ecophysiological changes and growth-limiting factors in the native species *Mucuna pruriens* grown on tailings. Five new growth substrates were prepared by mixing different volumes of pure iron ore tailings from Fundão dam with a fertile soil-substrate (0, 25, 50, 75, and 100% tailings/soil (v/v)). We evaluated different physicochemical properties and mineral composition of the growth substrates. In plants, we also evaluated mineral composition and physiological traits as gas exchange, chlorophyll *a* fluorescence, carbohydrate and proline contents, dry mass, and superoxide dismutase activity. The most contrasting results were observed comparing the treatment control to the treatment with pure iron ore tailings, with a trend of intermediary results for the other treatments (25-75% tailings). The higher the tailings concentration, the more impaired the growth. More than just to low tailings fertility, these trends were related to the high concentrations of Mn found in substrates and plant leaf tissues. We also observed that photosynthesis was impaired through disturbances in photochemical and gas exchange parameters probably due to the high levels of Mn and the associate ionic imbalance it causes to the Fe/Mn ratio.

Keywords: Photosynthesis. Mn toxicity. Fe/Mn ratio.

INDEX

1 INTRODUCTION

Mining activities contribute to environmental degradation in different ways, such as physical destruction of the environment, emission of dust containing toxic elements into the air, contamination of soils and aquatic environments by heavy metals, and dam collapses. Also, the world's biggest environmental disaster occurred in Brazil in November 2015, in which occurred the collapse of the Fundão tailings dam in the municipality of Mariana, Minas Gerais state (OMACHI et al., 2018). A huge amount of mud traveled through the surrounding area, contaminated waterways (especially the Rio Doce River), and reached the sea in the state of Espírito Santo (FELIZARDO et al., 2021). The loss of human life was relatively low, given the potential fatalities that tons of that mud could have caused if it had hit a more densely populated area (ISLAM; MURAKAMI, 2021). Given the proportions of the event, the environmental damage to the Atlantic Rainforest in that region was inestimable, since in a matter of minutes all the local fauna and flora were completely destroyed. In addition, dozens of families were left homeless.

One of the most important characteristics of the Fundão dam tailings is their low fertility for plant growth, given the low availability of mineral nutrients in their constitution and their physical-chemical attributes (CRUZ et al., 2020). The consensus in the literature is that the mine tailings that contaminated this entire area are inert in terms of metalloids and heavy metals toxicity. However, recent studies indicate that the high concentrations of Fe found in the mud have negatively affected the growth of plant species (CRUZ et al., 2020; SILVA et al., 2022). Another element found in large quantities in samples of these mining tailings is Mn since the ore containing the Fe is also rich in Mn (QUEIROZ et al., 2021). The mining process extracts most of the Fe and discards all the Mn in the dams. In a way, even if Mn is considered inert in terms of health risk to plants and animals, high contents of this element, such as those observed in studies on the tailings of the Fundão dam, are of concern. There are even studies reporting that high concentrations of this plant micronutrient may cause physiological disorders in plants, which can further impact the recovery process in areas impacted by tailings (SANTOS et al., 2017; HU; JINN, 2022).

Given the need for the recovery of areas degraded by the Fundão dam collapse, the planting of strategic species is essential for an accelerated forest succession process in the environment, as some scientific works are trying to do (CRUZ et al., 2020; ESTEVES et al., 2020; CRUZ et al., 2022; SILVA et al., 2022). However, understanding how native species respond to the limitations imposed on plant growth by the inherent infertility of the mud is fundamental for a better comprehension on the natural reclaiming process as well. Thus, the present work investigated the effects of the mud over *Mucuna pruriens* var. *utilis* (Wall ex Wight) Baker ex Burck as a model native species. This species was chosen because beyond being a native one, it is also a liana, a group of species that plays an important role in the early stages of ecological succession in degraded areas (ESTRADA-VILLEGAS et al., 2021). Thus, this study aimed to investigate the following question: What are the ecophysiological constraints and strategies of *M. pruriens* grown in the Fundão dam tailings compared to a fertile soil?

2 LITERATURE REVIEW

2.1 The Fundão Dam failure

Along with economic development, the demand for mineral products has increased globally in the past decades (LYU et al., 2019). However, this increased demand has negatively affected the environment with also increased extraction and processing of ores due to the environmental problems inherent to this kind of activity. In addition, the tailings that are generated by mining activities are disposed of in the form of a slurry in tailings dams. Due to the enormous volume of mining tailings that are stored inside such dams, incidents involving dam failure cause environmental and human life losses across the globe. A total of 366 dam failures occurred worldwide between 1915 and 2020, and this number is underestimated as many cases are never reported (ISLAM; MURAKAMI, 2021).

Incidents of this type can occur due to natural causes (such as earthquakes) but most stem from managers taking risks to overburden tailings dams, thereby increasing productivity and cutting costs added to the mining process (ARMSTRONG; PETTER; PETTER., 2021). Modern technologies exist for monitoring tailings dams and preventing accidents, but most mining companies are still in the process of deploying these resources. Recent tailings dam failures cases include those in Baia Mare and Baia Borsa (Romania), in 2000, San Marcelino Zambales (Philippines) in 2002, Karamken (Russia) in 2009, Ajka (Hungary) in, 2010; Padcal (Philippines) in 2012, Mount Polley (Canada) in 2014, Mariana (Brazil) in 2015, Luoyang Xiangjiang Wanji (China) in 2016, Vedanta (India) in 2017, Brumadinho (Brazil) in 2019, and Tieli (China) in 2020 (ISLAM; MURAKAMI, 2021).

In the past decade, two serious iron ore dam failures caused enormous socio-environmental impacts in areas of the Brazilian Atlantic Rainforest and also in distant regions such as estuarine sites (FELIZARDO et al., 2021). In November 2015, world's largest iron mining disaster occurred in the municipality of Mariana, Minas Gerais state (MG), resulting in deaths, several homeless people, and inestimable environmental damage to the Rio Doce river watershed due to the rupture of the Fundão dam (OMACHI et al., 2018). More than 50 million m^3 of iron ore mine tailings affected an area of the Atlantic Rainforest estimated at 457.6 ha (OMACHI et al., 2018). In January 2019, the second dam failure occurred in the municipality of Brumadinho, MG, causing less environmental damage but a higher number of deaths (SILVA ROTTA et al., 2020). Furthermore, there are at least 17 other mine tailings dams at imminent risk of collapse in MG (BOTELHO et al., 2021), which would again cause the same kind of impact.

Recent studies have confirmed that the Fundão dam tailings have non-toxic properties due to the low concentration of hazardous elements within their composition (CRUZ et al., 2020; ESTEVES et al., 2020; CRUZ et al., 2022; SILVA et al., 2022). However, nutrients necessary for plant growth were also found in low concentrations. Besides that, mine tailings tend to have other properties that are not ideal for plant growth such as low cation exchange capacity, low organic matter content, and small particle size, all because of the mining techniques used to extract minerals from soil (CRUZ et al., 2020; ESTEVES et al., 2020; CRUZ et al., 2022; SILVA et al., 2022). Thus, in addition to the drastic change in the landscape due to the destruction caused by the impact of a huge amount of mine tailings over the vegetation, a nutrient-poor and thick crust of a substrate named Technosol (given its technogenic elaboration process and physicochemical characteristics) covered the impacted sites (SCHAEFER et al., 2016), impairing plant growth.

Controversially, in terms of human health, a recent biomonitoring study in three communities of the Espírito Santo state shown a higher risk to the health of people living in the surrounding areas impacted by the Fundão dam failure, mainly due to the high levels of Al, As, Hg, and Ni (PAULELLI et al., 2022). The authors also declare that further studies are necessary to assess the potential toxics effects associated with the presence of the mud in the environment. Besides that, another study revealed that the dam failure also increased the ratio of mental disorders (like depression, anxiety, suicide tendencies, and so on) in the population surrounding Mariana's area, especially over the younger population (NEVES et al., 2018).

2.2 Plant growth in the Technosol

According to Nunes and colleagues (2022), some studies focused on recovering areas impacted by the failure of the Fundão dam were carried out but, in practical terms, the environmental problems are still there and no phytoremediation approach has been applied efficiently to solve them. The authors suggested phytoremediation strategies pointing out also the need for multidisciplinary approaches. In addition, Silva and colleagues (2022) suggested that more greenhouse studies evaluating the growth of native species at different proportions in a mixture between soil and mine tailings are needed.

The list of species already used in studies on the effects of the Fundão Tailings on plant growth includes mainly grasses (*Chrysopogon zizanioides*, *Cymbopogon citratus*, *Cymbopogon winterianus*, *Pennisetum glaucum*, *Sorghum bicolor*, *Zea mays*, and *Brachiaria decumbens*) (ZAGO et al., 2019; ESTEVES at al., 2020; SILVA et al., 2022) and tree species (*Cassia grandis*, *Albizia polycephala*, *Cybistax antisyphilitica*, *Handroanthus heptaphyllus*, *Handroanthus impetiginosus*, *Peltophorum dubium*, *Bowdichia virgilioides*, and *Dictyoloma vandellianum*) (CRUZ et al., 2020; MATOS et al., 2020, CRUZ et al., 2022). None of those grasses are native to that region and they are suitable only in studies to find mechanical insights on how the tailings affect plant growth. This is because the use of exotic grasses for reclamation of degraded areas results in dominance by those plants, impairing forest succession as native species are not able to colonize those areas (SANTOS et al., 2021). In this sense, reforestation techniques are more appropriate, but they need to follow specific protocols for tropical forests due to the frequent heavy rainfall which can result in landslides and uprooting of seedlings, difficulting reforestation efforts, especially in seriously degraded areas where soil conditions are not ideal for many plant species to grow, such as sites affected by mining activities (ZHAO et al., 2021).

2.3 *Mucuna pruriens*

The botanical family Fabaceae, order Fabales, includes about 751 genera, 19500 species, and is the third largest plant family (CHRISTENHUSZ; BYNG, 2016). In Brazil, 2534 species belonging to this family have been cataloged, of which 560 species belong to the Atlantic Rainforest domain in the state of Minas Gerais (LIMA et al., 2015). Within the species of this family, there are individuals with food, agronomic, medicinal, and environmental importance (PATHANIA et al., 2020).

The potential use of legumes to recover areas impacted by human activities is especially reported in the literature because they can grow on harsh sites due to their ability to get into symbiosis with rhizobia bacteria that fix atmospheric N and make it available to legumes, improving the process of land restoration and colonization by plants in degraded areas (NUNES et al., 2022). Another advantage of using legumes to recover those areas is that when a plant dies, its body becomes green manure which will increase the availability of nutrients in the soil (especially N) (ROCHA et al., 2020). Also, the incorporation of organic matter into the soil increases its fertility and microbiome biodiversity over time, which is a key factor in allowing a greater number of plant species to grow together on a site (ZHOU et al., 2022).

*M. prurien*s (also cited as *Mucuna aterrima*) is a legume best known for its medicinal properties due to the presence of (L-Dopa) 3, 4-dihydroxy-L-phenylalanine in its beans. This molecule has registered activities in the treatment of cancers, hypertension, and Parkinson's disease, and has antioxidant, anti-inflammatory, and antimicrobial properties (PATHANIA et al., 2020). This liana species can be found in many parts of the globe, especially in tropical regions (PATHANIA et al., 2020), and, in Brazil, it can be found in the phytogeographic domains of the Amazon Rainforest, Caatinga, Central Brazilian Savanna, and Atlantic Rainforest, the latter including the municipality of Mariana (MARA SANTANA, 2022; MOURA, 2022). This species has been included in the list of plants suggested to recover areas impacted by mining activities of Sn (LONGO; RIBEIRO; DE MELO, 2011) and also sand and pebble as a green manure in nutrient cycling strategies to increase N availability during the reforestation (ROCHA et al., 2020), being both areas of the Amazon Rainforest. Thus, both of those works demonstrated the ability of *M. pruriens* to grow in harsh environments and its potential to contribute to the reclaiming process of areas impacted by mining activities. Besides that, studies have shown that *M. pruriens* can be nodulated by rhizobacteria that fix atmospheric N and it also improves the rate of colonization by arbuscular mycorrhizal fungi (HOUNGNANDAN et al., 2000; SALEEM et al., 2018). In addition, lianas play an important role in plant biomass in tropical forests, especially those in early succession stages (ESTRADA-VILLEGAS et al., 2021). However, their influence on plant community and density decreases as the forest ages and trees begin to be dominant, shading out lianas and then naturally developing the species succession process (ESTRADA-VILLEGAS et al., 2021).

3 GENERAL CONSIDERATIONS

In summary, we observed that the main limiting factor for the growth of *M. pruriens* in soils covered by the tailings is probably the high concentration of Mn, which ends up stressing the plants by affecting its photosynthetic capacity.

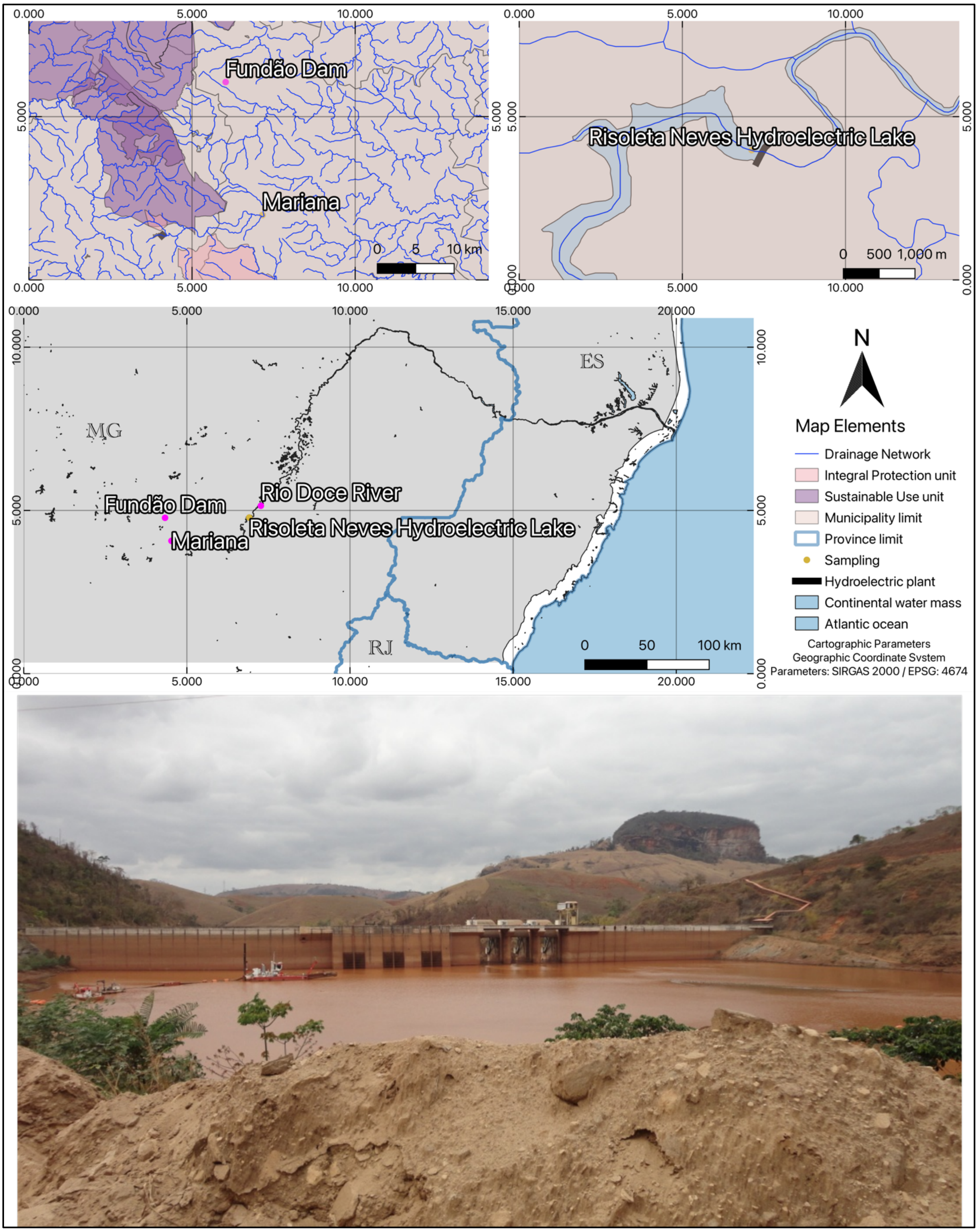
4 MATERIAL AND METHODS

4.1 Plant material, iron ore tailings sampling, and experimental conditions

The experiment was carried out in the Experimental Station of Plant Growth (coordinates 21°46'48.9"S 43°22'25.6"W, an altitude of 970 m) located at the Federal University of Juiz de Fora, MG, Brazil. Seeds of *M. pruriens* were germinated in Petri dishes covered with a layer of moistened filter paper. Five days after germination, three uniform seedlings were selected and transferred to 11 L polypropylene pots filled with different substrates as described below. The plants were grown in a greenhouse and watered properly. After 100 growth days, ecophysiological and biochemical analyses were performed.

The tailings samples were collected one year after the Fundão dam collapse (November 2016) from the mud dredged at the bottom of the Risoleta Neves Hydroelectric Lake (20°12'27.2"S 42°51'17.3"W), located ~90 km from the rupture site (FIGURE 1). The material was transported and stored in a place protected from light and moisture. Five new growth substrates were produced by mixing different volumes of pure iron ore tailings with a fertile substrate at the following values: 0 (control, fertile substrate), 25, 50, 75, and 100% (v/v). We targeted these percentages to verify the effects of Fundão dam tailings in a gradient to better explain our results. The fertile substrate consisted of a mixture of soil (latosol type, topsoil layer) and sand (1.5:1; v/v), supplemented with a chemical fertilizer composed of 12% N, 5% P_2O_5, 15% K_2O, 1% Ca, 1% Mg, 5% S, 0.2% Zn, 0.2% Fe, 0.05% Cu, 0.06% B, 0.08% Mn, and 0.005% Mo (1 g fertilizer/L substrate). An acidity corrector composed of 36% CaO and 9% MgO (2 g acidity corrector/L substrate) was also added to the mixture. Subsequently to substrate preparation, representative and random samples were obtained and analyzed.

Figure 1 – Map and picture of the region impacted by the Fundão dam collapse.



Source: Freitas (2022).

4.2 Physicochemical and mineral composition analyses of the substrate

All physicochemical substrate analyses (n=3) were performed following methodologies compiled and described by the Brazilian Agricultural Research Corporation (Embrapa) (SILVA, 2009; TEIXEIRA et al., 2017), compatible with current literature standards. We assessed particle (PD) and soil density (SD), cation exchange capacity (CEC), potential acidity (H+Al), and contents of organic matter (OM), clay, silt, and sand.

The extractors 1 M KCl (for Ca^{2+} and Mg^{2+}) and Mehlich-1 (Ni, Cr, Cd, Pb, Cu, Zn, Fe, Mn, P, K, and Na) were used in analyses of exchangeable-element content (SILVA, 2009; TEIXEIRA et al., 2017). Mineral bioavailability (n=3) was determined by colorimetry for P, flame photometry for K, and atomic absorption spectrophotometry for Ni, Cr, Cd, Pb, Cu, Ca, Mg, Zn, Fe, Mn, and Na. Total element content analyses consisted of quantifying the concentration values of N (sulfuric digestion and Kjeldahl method), and Ni, Cr, Cd, Pb, Cu, Zn, Fe, Mn, Hg, and As (nitric-perchloric digestion and inductively coupled plasma optical emission spectrometry; ICP-OES) (SILVA, 2009; TEIXEIRA et al., 2017).

4.3 Mineral analysis of plant tissues

After obtaining samples for the biochemical analyses, shoots were divided into stem and leaves, washed, and dried in a forced ventilation oven at 70 °C, until constant mass (n=3). The dry matter obtained was weighed and randomly sampled for the determination of plant nutrients in both stem and leaf tissues. For N, the sulfuric digestion method was performed and its content was assessed by the Kjeldahl method (SILVA, 2009). For P, K, Fe, Ca, Zn, Mg, Cu, and Mn, tissue samples were digested in a nitric-perchloric solution (3:1; v/v) and analyzed by the same method and standard reference materials used in soil samples (ICP-OES) (SILVA, 2009). The Fe/Mn ratio was calculated by dividing Fe content by Mn content.

4.4 Physiological analyses

Gas exchange analysis (n=6) of mucuna plants was performed using an infrared gas analyzer (IRGA model LI-6400XT, Li-Cor, Lincoln, NE, USA) between 8:00 and 12:00 h on young and fully expanded leaves with no phytopathogens infestation symptoms. The chamber was set to 1000 µmol m^{-2} s^{-1} photon flux density, 400 µmol mol^{-1} CO_2, and 25 °C. Thus, net photosynthesis (A), transpiration (E), stomatal conductance (g_s), and intercellular CO_2

concentration (*Ci*) were determined. In addition to those variables, water use efficiency (WUE; A/g_s) and carboxylation efficiency (CE; A/*Ci*) were also estimated. The maximum photochemical quantum yields of photosystem II (F_v/F_m; n=9) were assessed using a portable fluorometer (Handy PEA, Hansatech, Kings Lynn, UK) on leaves previously acclimated to dark for 30 min.

For the biochemical analyses, leaves with characteristics similar to those used in gas exchange analysis were collected and immediately processed in the laboratory. Protein extraction (n=5) followed Peixoto and colleagues (1999) and the protein content was determined as described by Lowry and colleagues (1951). Superoxide dismutase (SOD) activity was estimated in the protein extracts following Del Longo and colleagues (1993). The assay was conducted at 25 °C in a dark reaction chamber under the illumination of a 15 W fluorescent lamp and the biochemical reaction was started by illuminating the samples and terminated by shutting down the lamp after 4 min (GIANNOPOLITIS; RIES, 1977). One SOD unit was defined as the amount of enzyme required to inhibit nitroblue tetrazolium photoreduction by 50% (BEAUCHAMP; FRIDOVICH, 1971). Proline content (n=3) was evaluated by the method described by Bates and colleagues (1973), using acid ninhydrin as a reagent. The total soluble sugar (TSS; n=3) and starch contents (n=3) were determined according to DuBois and colleagues (1956).

4.5 Substrate scanning electron microscopy (SEM)

Images of the five different substrates were taken using an electron microscope (Quanta 250-FEI, Thermo Fisher Scientific). The samples were placed on stubs with carbon tape, metalized with gold and the microscope was set to operate at 30 kV. Image scans were obtained at 25x magnification in three fields.

4.6 Experimental design and statistical analysis

The experiment was carried out in a completely randomized design with five treatments (the substrates) and 10 biological replicates per treatment. However, all analyses were performed with different numbers of biological replicates, indicated in the paragraphs above as "(n=x)", where x is the number of biological replicates. Data were subject to analysis of variance (Anova) and Tukey's post-hoc test assuming a 5% probability of a Type I error. Residuals normality was evaluated by Q-Q plots. A Principal Component Analysis (PCA) was also performed. A linear regression model was fitted between A and g_s. All analyses and plots were performed in the software R (R CORE TEAM, 2020) using the packages *ggplot2*, *factoextra*, *cowplot*, *plyr*, and *agricolae*.

5 RESULTS

5.1 Substrate analysis

Overall, the most contrasting results were found when comparing the treatment control to the treatment with pure ore tailings for all the results, with the treatments between them (25%, 50%, and 75% tailings) presenting a general intermediary pattern. As for the chemical composition, we observed that increasing tailings proportion in the substrates decreased contents the content of P, K, Ca, Mg, Zn, Cr, and Na in their bioavailable forms to the plants, as well as for the total contents of N, Fe, Ni, Cd, Pb, Cu, Zn, Hg, Cr, and As (TABLE 1). No significant changes were found for the bioavailable forms of Cu, Ni, Cd, and Pb. However, the bioavailable forms of Fe and Mn increased in pure tailings and total Mn increased due to the increment of tailings in substrates.

Table 1 – Physicochemical properties and mineral contents of substrates. Means±standard deviations followed by the same letters do not differ from each other according to Tukey's test (p-value ≤0.05). H+Al, potential acidity; CEC, cation exchange capacity; OM, organic matter content; PD, particle density; SD, soil density.

Parameter	Tailings (%)				
	0	**25**	**50**	**75**	**100**
pH	6.3±0.1a	6.35±0.13a	6.06±0.68a	6.35±0.15a	6.03±0.45a
H+Al($cmol_c$/kg)	2.03±0.16a	1.66±0.26a	1.6±0.28ab	1.1±0.03bc	0.6±0.09c
CEC ($cmol_c$/kg)	11.27±0.8a	9.28±2.75a	7.38±2.32ab	4.49±1.2bc	1.28±0.4c
OM (dag/kg)	2.52±0.25a	2.46±0.03a	1.78±0.14b	1.08±0.11c	0.29±0.1d
Clay (dag/kg)	28.5±0.5a	22.50±0.5b	16.50±1.5c	12.5±0.5d	6±1e
Silt (dag/kg)	6.5±0.5e	14±0d	20.50±1.5c	27.5±0.5b	38±0a
Sand (dag/kg)	65±0a	63.5±0.5ab	63±0b	60±1c	56±1d
PD (g/cm^3)	2.65±0.06c	2.67±0.03c	2.93±0.06ab	2.88±0.05b	3.1±0.1a
SD (g/cm^3)	1.27±0.02d	1.38±0.02c	1.46±0.02b	1.46±0.02b	1.53±0.01a
			Bioavailable		
Ni (mg/kg)	1.26±0.2a	1.57±0.32a	2.28±1.26a	1.73±0.78a	2.02±1.44a
Cr (mg/kg)	3.11±0.83a	2.95±0.52a	2.49±0.56ab	1.37±0.64bc	0.17±0.24c
Cd (mg/kg)	0.81±0.58a	0.7±0.52a	0.51±0.38a	0.36±0.31a	0.17±0.12a
Pb (mg/kg)	0.58±0.67a	0.48±0.58a	0.76±0.59a	0.76±0.47a	0.43±0.34a

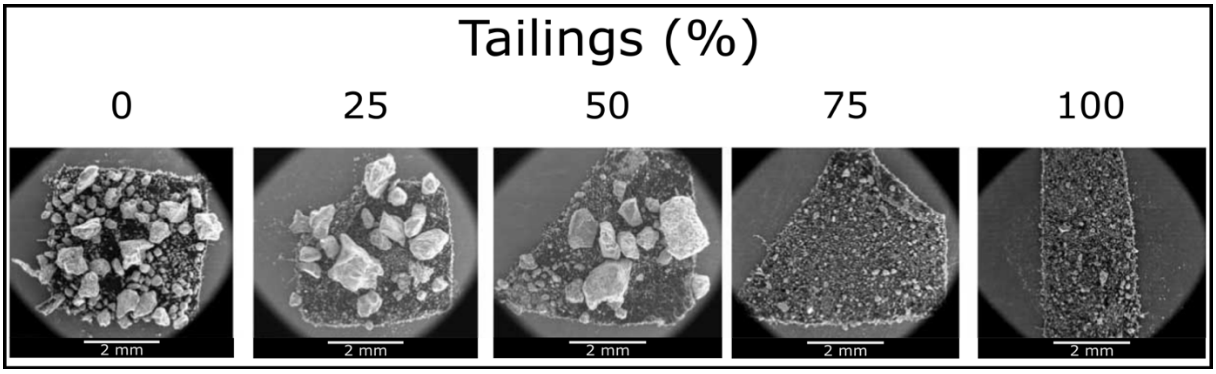
Cu (mg/kg)	2.9±0.99a	2.85±1.09a	2.49±1.07a	2.09±0.69a	1.51±0.34a
Zn (mg/kg)	21.06±5.95a	19.03±3.33a	13.84±3.36ab	7.51±2.23bc	3.11±1.26c
Fe (mg/kg)	66±23b	73.8±37.2b	104±46.1b	168.9±71.5b	337.4±119a
Mn (mg/kg)	54.82±19.18b	85.3±28.8b	113.8±48.2ab	113.3±40.7ab	161.3±25,7a
P (mg/kg)	31.87±1.21a	30.68±1.05a	22.59±4.25b	16±1.04c	8.08±1.45d
K (mg/kg)	369.5±74.6a	314.1±71.9a	246.9±61.7ab	114.65±12.43bc	22.21±0.31c
Ca ($cmol_c$/kg)	8.38±0.45a	6.88±2.3ab	5.52±2.04ab	3.43±0.99bc	1.02±0.37c
Mg ($cmol_c$/kg)	1.91±0.26a	1.56±0.44a	1.20±0.33ab	0.72±0.24bc	0.16±0.07c
Na (mg/kg)	24.41±3.01a	25.07±1.14a	21.12±1.14a	11.26±1.14b	0±0c
Total content					
N (dag/kg)	0.11±0.01a	0.08±0.01ab	0.06±0.01bc	0.06±0.01c	0.03±0.01d
Ni (mg/kg)	4.74±0.35b	6.53±0.26a	5.42±0.6ab	6.52±0.46a	2.45±0.56c
Cr (mg/kg)	29.65±2.75a	30.21±2.2a	20.69±1.76b	14.43±1.43c	5.29±0.9d
Cd (μg/kg)	10.5±1.38a	8.74±1.36ab	6.76±0.63bc	4.13±0.86cd	1.87±0.91d
Pb (mg/kg)	15.22±1.04a	14.59±2.77ab	9.52±0.71c	10.75±1.44bc	8.45±0.87c
Cu (mg/kg)	22.98±3.02a	20.53±1.29a	15.43±0.9b	10.25±0.86c	8.05±1.02c
Zn (mg/kg)	48.91±1.61a	46.98±1.84a	30.64±1.21b	12.32±1.1c	14.41±1.1c
Fe (mg/kg)	44943±8857a	41359±4972a	32893±2745a	17865±2146b	15613±3386b
Mn (mg/kg)	171.7±45.2c	199.9±18.4c	238.54±14.75bc	273.22±14.66ab	328.9±24.5a
Hg (μg/kg)	180.43±1.49a	165.66±4.83b	111.06±4.37c	84.2±3.47d	48.77±3.3e
As (μg/kg)	2884±731a	3059.5±64a	2435.7±68.1ab	1587.7±15.2bc	1243.6±6.94c

Source: Freitas (2022).

The physicochemical analyses of substrates also showed interesting results (TABLE 1). PD, SD, and silt content increased due to the increment of tailings in substrates. Differently, CEC, H+Al, and the contents of OM, clay, and sand decreased. No significant alterations were observed in the pH.

SEM photomicrographs of particles are shown in Figure 2. We observed reductions in particle size, smoothness, and agglomeration, in proportion to the increment of tailings in the substrates.

Figure 2 – Scanning electron microscopy photomicrographs showing substrate particles in each treatment. Magnification: 25x.

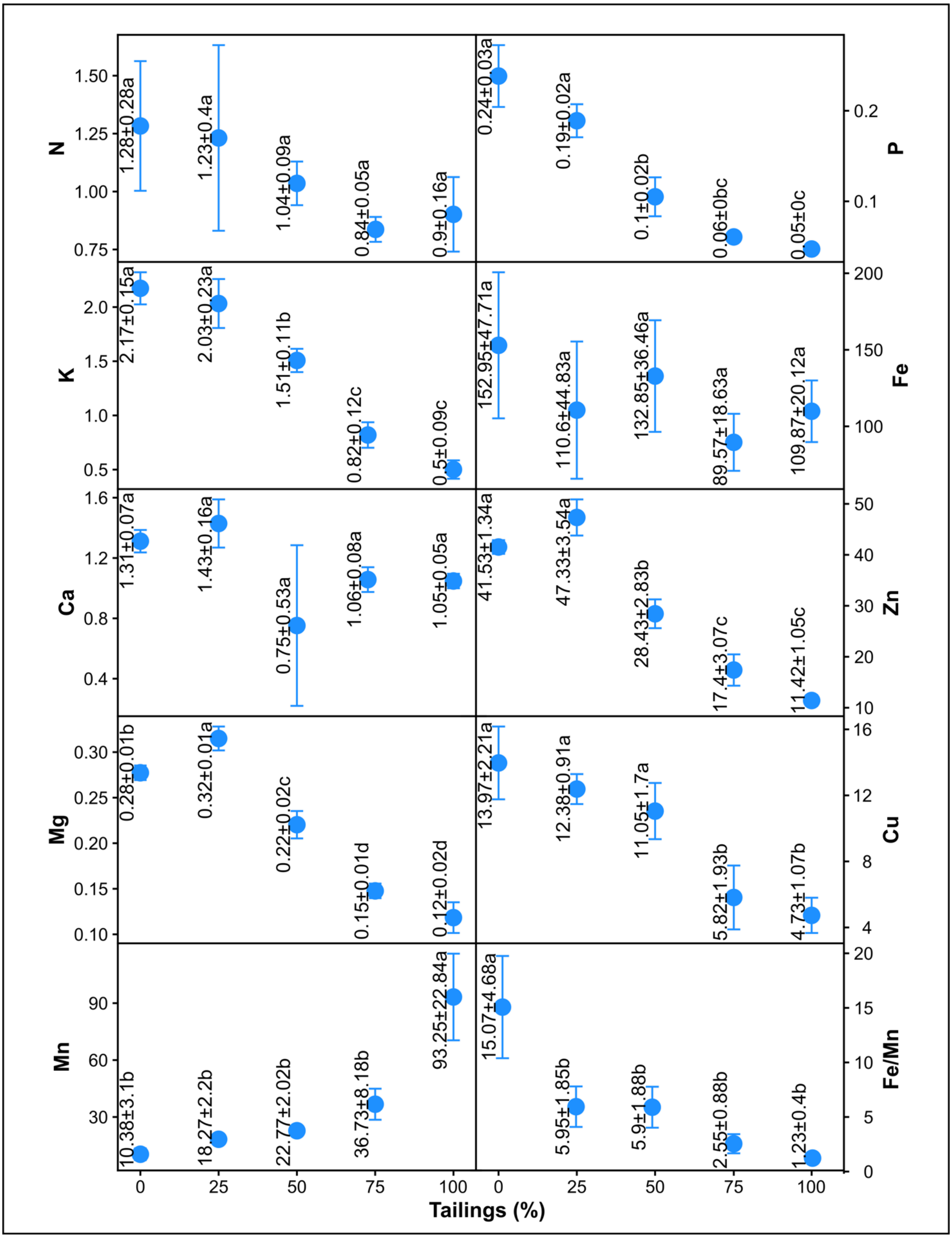


Source: Freitas (2022).

5.2 Plant analysis

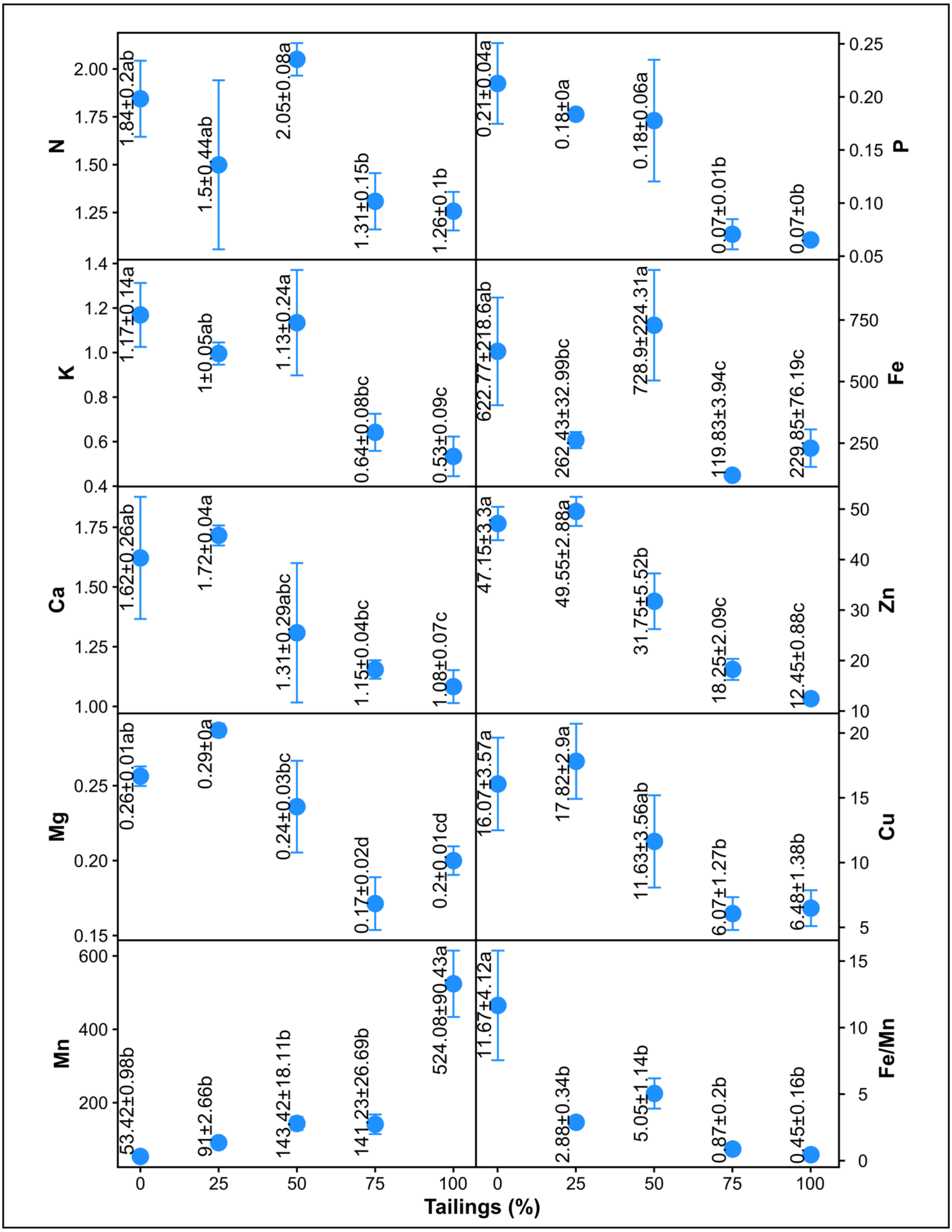
In general, nutritional analysis of stem tissues showed that K, Cu, Zn, Mg, and P contents decreased in response to the increment of tailings in the substrates, with no significant variations in N, Fe, and Ca contents (FIGURE 3). In contrast, Mn concentration increased in pure tailings. For leaf tissue analysis, we observed that the content of K, Ca, Mg, P, Zn, and Cu decreased due to the increment of tailings in the substrates (FIGURE 4). Mn was observed in the opposite pattern, increasing only in pure tailings, as the stem results also did. Besides that, the Fe/Mn ratio decreased similarly in all treatments with tailings in the substrates. On a multivariate level, PCA biplots (FIGURE 5) showed that the principal component 1 (PC 1) was the most explanatory, explaining ~70% of the total variation in the data for both leaf and stem tissues. Indeed, the treatments were better clustered when analyzed using that axis. It was also possible to observe that the Mn content was in opposition to all other minerals in both tissues and the treatments were aligned to the tailings content.

Figure 3 – Concentrations of nutrients and Fe/Mn ratios in the stems of mucuna plants grown in substrates with different Fundão dam tailings contents. N, P, K, Ca, and Mg were measured in dag/kg, and Fe, Zn, Cu, and Mn in mg/kg. Means±standard deviations followed by the same letters do not differ from each other according to Tukey's test (p-value ≤0.05).



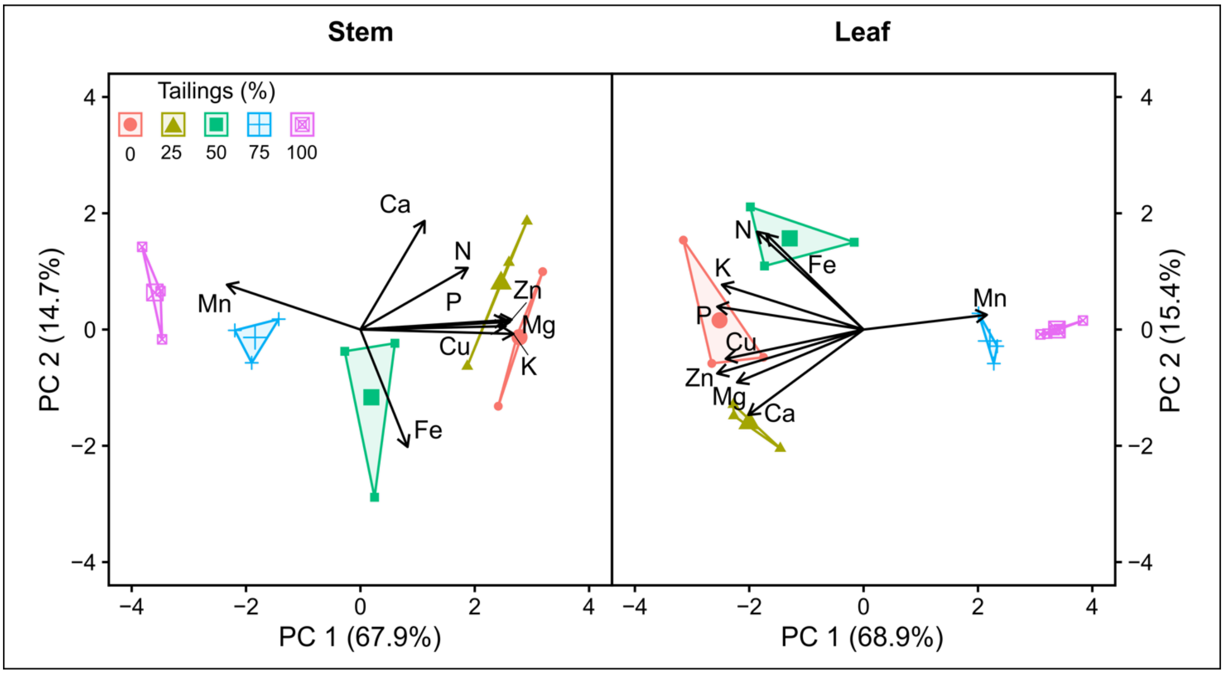
Source: Freitas (2022).

Figure 4 – Concentrations of nutrients and Fe/Mn ratios in the leaves of mucuna plants grown in substrates with different Fundão dam tailings contents. N, P, K, Ca, and Mg were measured in dag/kg, and Fe, Zn, Cu, and Mn in mg/kg. Means±standard deviations followed by the same letters do not differ from each other according to Tukey's test (p-value ≤0.05).



Source: Freitas (2022).

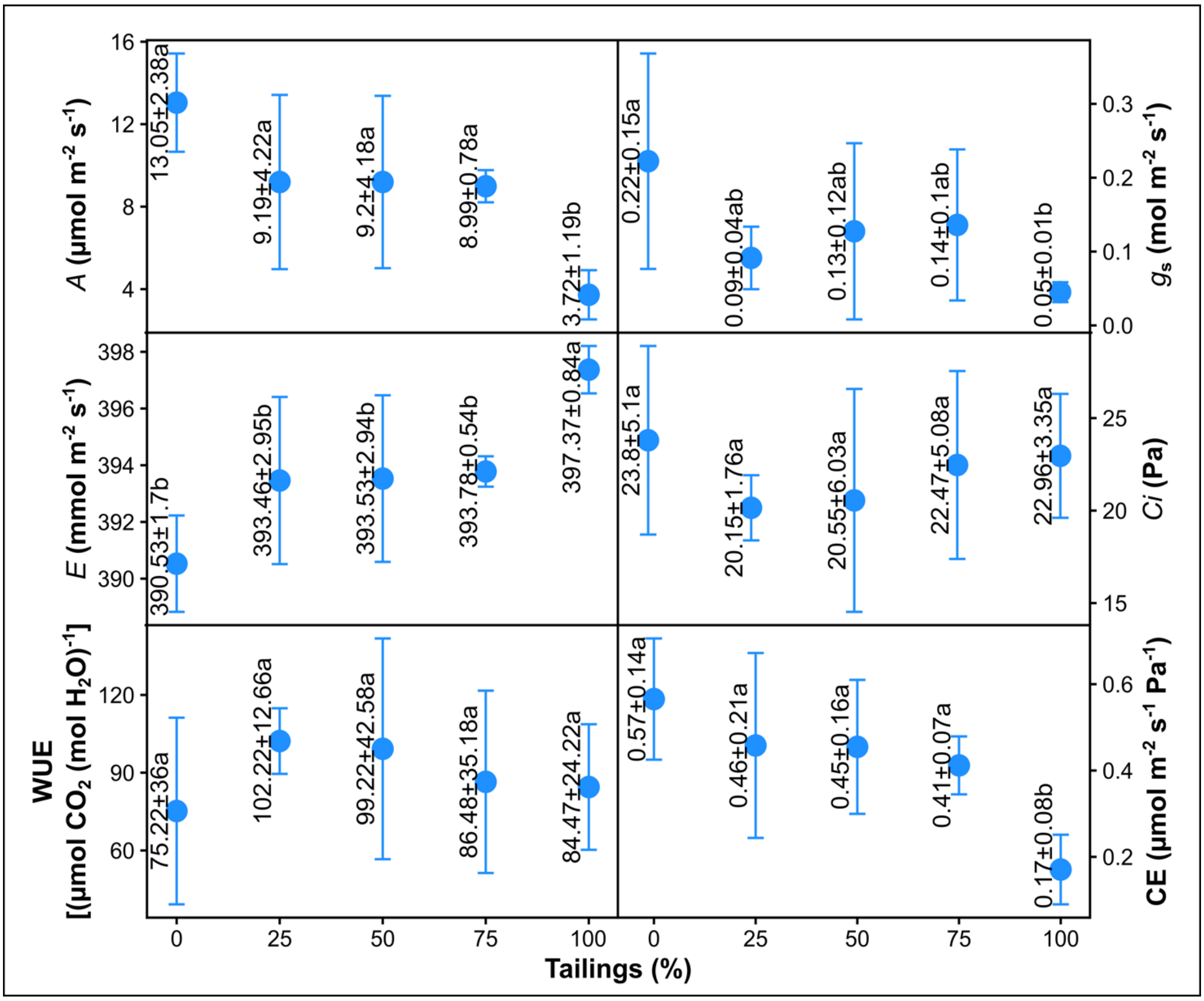
Figure 5 – PCA biplots of stem and leaf of mucuna plants grown in substrates with different Fundão dam tailings contents.



Source: Freitas (2022).

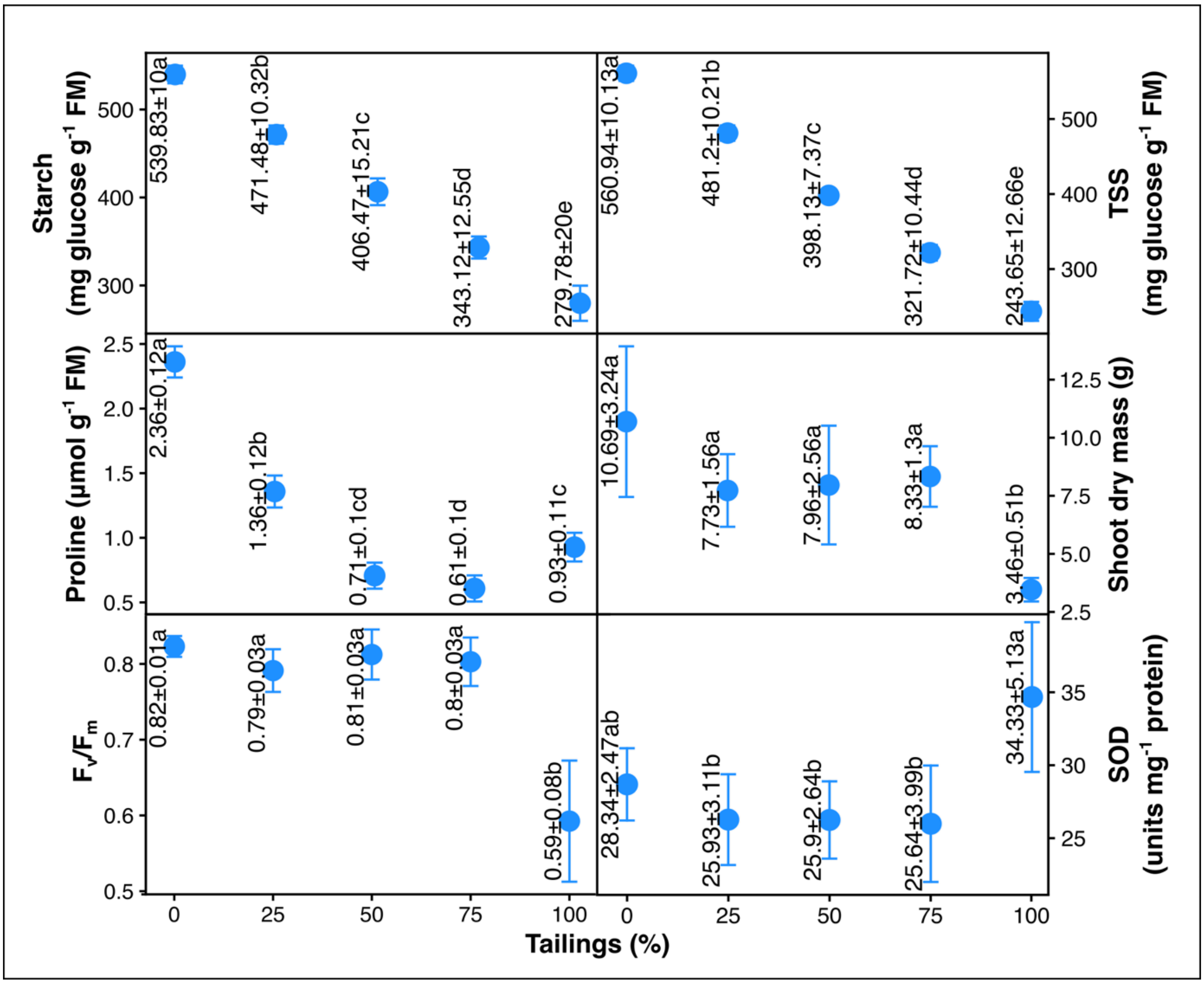
Regarding physiological traits, when differences between means were observed, the larger contrasts were also found comparing control plants to those grown in pure tailings. A, g_s, CE (FIGURE 6), F_v/F_m and dry mass (FIGURE 7) decreased only in pure tailings, while E (FIGURE 6) and SOD activity (FIGURE 7) increased. Decreases were also observed comparing proline, starch, and TSS contents in the plants grown on the substrates with more tailings to those of treatment control (FIGURE 7). No significant changes were observed for WUE and Ci (FIGURE 6). The fitted linear model between A and g_s resulted in the equation $A = 4.78 \cdot \ln(g_s) + 20.13$, $r^2 = 0.74$.

Figure 6 – Net photosynthesis (*A*), stomatal conductance (g_s), transpiration (*E*), CO_2 internal content (*Ci*), water use efficiency (WUE), and RUBISCO carboxylase efficiency (CE) of mucuna plants grown in substrates with different Fundão dam tailings contents. Means±standard deviations followed by the same letters do not differ from each other according to Tukey's test (p-value ≤0.05).



Source: Freitas (2022).

Figure 7 – Superoxide dismutase (SOD) activity, chlorophyll *a* fluorescence (F_v/F_m), contents of starch, total soluble sugars (TSS), protein, proline, and shoot dry mass of mucuna plants grown in substrates with different Fundão dam tailings contents. Means±standard deviations followed by the same letters do not differ from each other according to the Tukey's test (p-value ≤0.05). FM, fresh mass.



Source: Freitas (2022).

6 DISCUSSION

Similar to the results listed in previous studies, the levels of bioavailable nutrients found in pure tailings were low (TABLE 1), being the low fertility inherent to that kind of Technosol (ZAGO et al., 2019; CRUZ et al., 2020; ESTEVES et al., 2020). Thus, we noticed that high pH, low OM, and, consequently, CEC; low contents of macronutrients, and high contents of Fe and Mn are common characteristics of most of them compared to a fertile substrate (treatment control) and the reference soil from that region (SILVA et al., 2022).

Our results also showed that the treatments control and pure tailings had close pH values, similar to those reported by Andrade and colleagues (2018). Then, mixing them did not result in substrates with different pH. However, H+Al decreased due to the increment of tailings in the substrates. Indeed, soil buffering capacity is influenced by OM content in its composition and we also observed a decrease of OM in the substrates due to the increment of tailings. Additionally, the low OM and clay contents in the substrates decreased CEC as also described by Cruz and colleagues (2020). Thus, this is an important point to be highlighted, since low CEC directly implies low fertility.

The most likely cause of the Fundão dam failure involves excess water near the dam (MORGENSTERN et al., 2016). That occurs because the increment in the relative proportion of silt particles maximizes the risk of liquefaction due to the lubrication effect on fine particles, which drastically changes the behavior of tailings (HUANG; ZHAO, 2018; MORGENSTERN et al., 2016), making it more fluid. In fact, it was shown in Figure 2 and observed in the tailings treatments (25-75%) a trend of low particle and soil density, low clay content, and high silt and sand contents in pure tailings (TABLE 1), which contributes to increased soil compaction and low fertility as well.

Although the bioavailable levels of Fe and Mn in the pure tailings were higher than in the other treatments, this material did not have harmful levels of other important trace elements (Cr, Hg, Cd, Pb, and As) according to the Conselho Nacional do Meio Ambiente (National Environmental Council; Conama) legislation (CONAMA, 2009). The highest Mn content was found in pure tailings (TABLE 1) and it probably occurred because the ores used to mine Fe are also rich in Mn and it is commonly discarded during the Fe extraction process (ANDRADE et al., 2018). Thus, Mn and Fe were the only metals whose total contents were higher in pure tailings than in the treatment control. Despite that, there is no Conama regulation for Fe and Mn content thresholds in soil (CONAMA, 2009).

Nutrient analysis of leaf and stem tissues corroborated what was previously reported by Cruz and colleagues (2020): one of the main points for a plant to grow in tailings is the lower nutrient availability. Except for Mn, a trend of low concentration was observed in both tissues for most plant nutrients (FIGURE 3 and FIGURE 4) due to the increment of tailings in the substrate. This may disturb biological processes, as mineral nutrients are crucial for several physiological activities and are essential components of cellular components such as cell walls, membranes, pigments, enzymes, ATP, and so on, as well (MARSCHNER, 2012). Considering that a) treatment control plants were grown in fertile soil and showed superior traits; and b) the mine tailings have inherent low fertility, we assumed that nutrient contents found in the treatment control plants as the ideal concentrations for *M. pruriens* since there is no threshold available for this genus in the literature. Thus, we did not intend to discuss element-by-element deficiency in the present study because, overall, all element concentrations were found below treatment control concentration and many suppositions would be made on how this would impair *M. pruriens* growth. Additionally, no classic visual symptoms in leaves (e.g., necrosis, chlorosis, and so on) for any element relative to toxicity or deficiencies were observed (MARSCHNER, 2012).

The increase in Mn content in leaf and stem tissues due to the increment of tailings in the substrates contrasted with the results found for Fe, in which a content decrease was observed in the leaves but not in the stems. Since the bioavailable contents of Mn and Fe were higher in pure tailings (TABLE 1), the decrease in leaf Fe content in the treatments with 75% and 100% tailings was not associated with the lower concentration of this element in the substrates, unlike the other nutrients. Thus, we can suggest that the high Mn acquisition inhibited the acquisition of Fe in *M. pruriens* and possibly the other nutrients as well. At the shoot level, Mn toxicity in terms of Fe acquisition seems to be species-dependent (EL - JAOUAL; COX, 1998). It occurs via ion antagonism because the Mn bioavailable form is Mn^{2+}, a cationic configuration similar to Fe^{2+}, and also to Mg^{2+} and Ca^{2+} (MARSCHNER, 2012). However, not all Mn toxicity occurs due to Mn-induced Fe deficiency. For example, it can occur due to the formation of some Mn^{2+}-Rubisco molecules and since the CO_2/O_2 specificity of Mn^{2+}-Rubisco is ~20 times lower than Mg^{2+}-Rubisco, this might impair plant growth by increasing photorespiration (JORDAN; OGREN, 1983). Other edaphic conditions may impair Fe uptake, mainly the pH. This is because the bioavailable form of Fe is abundantly present at acid pH and conversely at alkaline levels. Given that the pH of all substrates was very similar, this does not appear to be the case. In this sense, this supports the hypothesis that Mn impaired the Fe translocation to leaves.

Queiroz and colleagues (2021) indicated that Mn is the main and harmful environmental contaminant of the Fundão dam tailings for both soil and water. According to Fernando and Lynch (2015), Mn toxicity in soybean can be observed at different levels, including antioxidant enzyme metabolism, gas exchange, and histological alterations, as also observed here and in other reports about plant growth in tailings (MATOS et al., 2020; ESTEVES et al., 2020). Hati and colleagues (1979) reported that in the leaves of cotton, wheat, soybean, and Kohno and Foy (1983) for common bean, the Mn toxicity occurs when Fe/Mn ratios were respectively 1.36, 1.40, 0.8, and < 1.5. In the present study, the Fe/Mn ratio in the leaves of plants grown in the pure tailings was 0.45 (FIGURE 3), which implies Mn toxicity. Moreover, PCA (FIGURE 5) also showed the high influence of Mn in plants by a) clustering and ordering them in relation to the treatments from 0 to 100% tailings and aligning the treatments with higher tailings concentrations (75 and 100%) in the direction of the arrow representing Mn; and b) showing its opposition to the other elements in leaf and stem tissues, similar to the results reported by Huang and colleagues (2020) for *Broussonetia papyrifera* (a promising species to be used in Mn phytoremediation efforts) comparing Fe and Mn contents as well. Thus, PCA also indicated that the low nutrient bioavailability and the high Mn concentration in substrates seemed to be the principal factors that negatively affected the growth of mucuna plants. This physiological behavior is the opposite observed in *Brachiaria decumbens* (SILVA et al., 2022), *Bowdichia virgilioides*, and *Dictyoloma vandellianum* (CRUZ et al., 2022) grown in tailings that had their growth impacted by high concentrations of bioavailable Fe. This is the first study reporting the negative effects of Mn found in the Fundão dam tailings on plant physiology.

The higher accumulation of Mn in shoots by *M. pruriens* in response to the increased Mn content in the environment indicates that this species is also a promising one to be further investigated for the use in Mn phytoextraction approaches, given that a similar accumulation trend (i.e., the more Mn in the environment, the more Mn is found in plant tissues) was also observed in *B. papyrifera* (HUANG et al., 2019b). The authors reported that in the treatment with the highest Mn content in the substrate (528.8 mg/kg), the Mn leaf concentration was 387 mg/kg and 80.58 mg/kg in the stems, while we observed (FIGURE 4) 524.08 mg/Kg Mn in the leaves and 93.25 mg/kg Mn in the stems (FIGURE 3) of plants grown in pure tailings (161.3 mg/kg Mn). In other words, *M. pruriens* accumulated more Mn in shoot than *B. papyrifera*. The soil naturally present in the area affected by the Fundão dam failure has greater fertility in comparison to the tailings but it is a harsh one for plants to grow (SILVA et al., 2022) and even like that mucuna plants area adapted to those conditions, being capable to grow properly and reproduce. What we do have more differentially in our experiments in comparison to that soil is the higher bioavailability of Mn, which further corroborates the hypothesis of Mn toxicity on *M. pruriens*, since this is an environmental novelty for which mucuna plants are not used to deal with and clearly impaired its growth.

Chlorophyll *a* fluorescence is a parameter widely assessed as a measure of plant stress in the literature because the photosystem II is sensitive to damage caused by the oxidative burst initiate by biotic and abiotic factors (CRUZ et al., 2020; ESTEVES et al., 2020; FREITAS et al., 2021). In our results (FIGURE 7), a significant decrease in F_v/F_m was observed for plants grown on pure tailings and no similar results were found when comparing our results to the previous studies with tailings, in which no (ESTEVES et al., 2020) or low (CRUZ et al., 2020) in F_v/F_m changes were observed. However, Chatzistathis and colleagues (2011) reported that olive plants under Mn toxicity showed negative correlations between Mn content and both F_v/F_m and Fe content in leaf tissues, similarly to our results. Thus, only the Mn concentration in pure tailings was sufficient to affect the maximum photochemical quantum yields of photosystem II, suggesting damage to the photosynthetic apparatus, similar to that reported by Millaleo and colleagues (2010) for *Arabidopsis thaliana* under Mn toxicity.

On gas exchange in *M. pruriens*, *A* and g_s decreased while *E* increased in pure tailings and no significant variation in *Ci* was noticed (FIGURE 6). That was similar to the results observed in soybean grown under high Mn concentration by Santos and colleagues (2017). The same authors also reported that low g_s implies low translocation of Mn to shoot through transpiration flow via xylem. Since less CO_2 will be available for photosynthesis due to stomata closure, *A* also decreased. However, due to the higher *E,* the Mn translocation and accumulation in leaves and stems remained high (FIGURE 4 and FIGURE 3, respectively). Santos and colleagues (2017) also observed that Mn toxicity decreased leaf tissue organization, characterizing a low stomatal opening. Besides that, Lidon (2002) working with rice observed that stomatal opening and stomatal length decreased as a result of Mn toxicity. The higher *E* followed by the lower g_s due to the increase of Mn content in the substrate probably occurred because the levels of compatible solutes as proline and TSS were also lower in the leaves of plants under higher Mn content treatments (FIGURE 7). No change in *Ci* was noticed comparing plants grown on pure tailings to those grown in the treatment control. This may have occurred because even with low g_s, the plants also showed low CE, thus characterizing low assimilation of CO_2 available in leaf tissue. Furthermore, some reports have shown that there is an impairment of photosynthesis due to degradation of the photosynthetic apparatus in plants grown under Mn toxicity (ROJAS-LILLO et al., 2014; WENG et al., 2013) in addition to an increase in photorespiration (JORDAN; OGREN, 1983). Finally, as *A* and g_s decreased similarly, no changes were observed in WUE and 74% of *A* variation was controlled by g_s as indicated by the linear model presented in the results section. In summary, we noticed that photosynthesis was impaired by stomatal closure and low photochemical efficiency due to the high content of Mn and the physiological constraints it drives to.

Since photosynthesis was limited, carbon metabolism and plant dry mass were also affected. Alongside less carbon fixation, the *de novo* biosynthesis of both TSS and starch decreased and it can be observed in the trend of our results (FIGURE 7). Since the uptake and fixation of CO_2 in the form of carbohydrate molecules is the main mechanism of plant biomass accumulation (LAL et al., 2022), the shoot dry mass was also affected, but only in pure tailings in a similar way as the photosynthetic parameters. In fact, in almost all research conducted using the Fundão dam tailings in which some kind of improvement in the substrate was made, higher plant dry mass was observed in the improved treatment (ZAGO et al., 2019; CRUZ et al., 2020).

Different kinds of stress can limit photosynthesis and consequently start a reactive oxygen species (ROS) burst due to an imbalance in electron transport, causing harmful oxidative stress that may damage cell structures (such as the photosystem II, as we assessed using F_v/F_m) and cause the death of cells, tissues, organs, and/or the whole plant (MITTLER, 2017). To cope with ROS, plants have evolved with a sophisticated antioxidant metabolism composed of, respectively, enzymatic and non-enzymatic ROS scavengers such as SOD and proline, in addition to many other biomolecules (DAS; ROYCHOUDHURY, 2014; MITTLER, 2017). SOD activity is essential for plant cells mainly in the chloroplasts, where an imbalance in electron transport can initiates the production of superoxide anions (GARCÍA-CAPARRÓS et al., 2021). Then, SOD efficiently converts the anion superoxide into hydrogen peroxide to be metabolized by catalase and/or ascorbate peroxidase (HUANG et al., 2019a). The changes in SOD activity results (FIGURE 7) were more significant in the pure tailings treatment compared to the other treatments, similarly to what was observed for the results of F_v/F_m (FIGURE 7). Also, Hu and Jinn (2022) reported that *Arabidopsis thaliana* increased MnSOD (an isoform of SOD found in the mitochondria that uses Mn as an enzymatic cofactor) activity in response to the increment of Mn in the substrate in stressed plants. Furthermore, they observed that higher Fe contents decreased MnSOD activity modulated by AtMTM1 and AtMTM2 ion carrier proteins, given that Fe compete with Mn for metal-binding sites in MnSOD. They also observed in a previous study that it occurs because the protein AtMTM1 (which carries Mn ions) has also a high affinity for Fe (HU et al., 2021). Thus, the increase in SOD activity along with the decrease of F_v/F_m reinforced the explanation on the deleterious effects the tailings on photosynthesis in mucuna plants similarly as reported by Cruz and colleagues (2020), and Esteves and colleagues (2020).

In our results, a decline in proline was noticed ins response to the increment of tailings in substrates (FIGURE 7). Taking into account that total N content can be used to estimate total/crude protein content by multiplying the values by a correction factor (commonly 6.25 to plants (SALO-VÄÄNÄNEN; KOIVISTOINEN, 1996; BIANCAROSA et al. 2017)), our N content results (FIGURE 3) indicated that the total content of proteins in leaves was affected in the treatments 75% and 100% tailings. Thus, such information may help to explain the decrease of proline in those treatments because all amino acids and proteins biosynthesis was compromised due to the N lack in the substrates.

7 CONCLUSIONS

We noticed that the Fundão dam tailings can impair *M. pruriens* growth and physiology, thus compromising the reclamation of the impacted land by this native species. Besides that, the most contrasting results were observed comparing plants and substrates of the treatment control to the treatment composed of pure tailings, with a trend towards intermediate results for the other treatments (25-75% tailings). We assigned these results to more than just the tailings, but more specifically to the high concentrations of Mn found in tailings and plant tissues, which negatively impacted on gas exchange traits. However, the effects of Mn toxicity on physiological parameters were not characterized and discussed in previous studies dealing with tailings, although some of them demonstrated high Mn content in tailings composition and other similar characteristics. Thus, we indicate that special attention should be paid to Mn in further studies because our results indicate it as a problematic environmental contaminant when available in high doses as similarly reported by other studies on Mn toxicity. To shed light on how tailings (and probably Mn toxicity) may affect plant growth, new perspectives should also include histological, metabolic, and molecular approaches, as well as relating the assimilation of Fe and Mn in roots, stems, and leaves.

BIBLIOGRAPHY

ANDRADE, G. F. et al. Agricultural use of Samarco's spilled mud assessed by rice cultivation: A promising residue use? **Chemosphere**, v. 193, p. 892–902, feb. 2018.

ARMSTRONG, M.; PETTER, R.; PETTER, C. Why have so many tailings dams failed in recent years? **Resources Policy**, v. 63, p. 101412, 1 out. 2019.

BATES, L. S. et al. Rapid determination of free proline for water-stress studies. **Plant and Soil 1973 39:1**, v. 39, n. 1, p. 205–207, ago. 1973.

BEAUCHAMP, C.; FRIDOVICH, I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. **Analytical Biochemistry**, v. 44, n. 1, p. 276–287, nov. 1971.

BIANCAROSA, I. et al. Amino acid composition, protein content, and nitrogen-to-protein conversion factors of 21 seaweed species from Norwegian waters. **Journal of Applied Phycology**, v. 29, n. 2, p. 1001–1009, apr. 2017.

BOTELHO, M. R. et al. Rompimento das barragens de Fundão e da Mina do Córrego do Feijão em Minas Gerais, Brasil: decisões organizacionais não tomadas e lições não aprendidas. **Revista Brasileira de Saúde Ocupacional**, v. 46, 2021.

CHATZISTATHIS, T. A. et al. Is chlorophyll fluorescence technique a useful tool to assess manganese deficiency and toxicity stress in olive plants? **Journal of Plant Nutrition**, v. 34, n. 1, p. 98–114, dec. 2010.

CHRISTENHUSZ, M. J. M.; BYNG, J. W. The number of known plants species in the world and its annual increase. **Phytotaxa**, v. 261, n. 3, p. 201–217–201–217, 20 maio 2016.

CONAMA - Conselho Nacional do Meio Ambiente. Resolução 420/2009. 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 atividade. **Diário Oficial da União**, 249, Brasil. 2009.

CRUZ, F. V. DA S. et al. Does Samarco's spilled mud impair the growth of native trees of the Atlantic Rainforest? **Ecotoxicology and Environmental Safety**, v. 189, p. 110021, feb. 2020.

CRUZ, F. V. DA S. et al. 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**, v. 29, n. 12, p. 18047–18062, mar. 2022.

DAS, K.; ROYCHOUDHURY, A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. **Frontiers in Environmental Science**, v. 2, dec. 2014.

DEL LONGO, O. T. et al. Antioxidant defences under hyperoxygenic and hyperosmotic conditions in leaves of two lines of maize with differential sensitivity to drought. **Plant and Cell Physiology**, v. 34, n. 7, p. 1023–1028, oct. 1993.

DUBOIS, M. et al. Colorimetric method for determination of sugars and related substances. **Analytical Chemistry**, v. 28, n. 3, p. 350–356, 1 mar. 1956.

EL-JAOUAL, T.; COX, D. A. Manganese toxicity in plants. **Journal of Plant Nutrition**, v. 21, n. 2, p. 353–386, feb. 1998.

ESTEVES, G. DE F. et al. Do tailings from the Mariana, MG (Brazil), disaster affect the initial development of millet, maize, and sorghum? **Environmental Science and Pollution Research**, v. 27, n. 31, p. 38662–38673, nov. 2020.

ESTRADA-VILLEGAS, S. et al. Lianas do not reduce tree biomass accumulation in young successional tropical dry forests. **Oecologia**, v. 195, n. 4, p. 1019–1029, apr. 2021.

FELIZARDO, J. P. et al. Sources of sedimentary organic matter and assessment of heavy-metal levels in estuarine sediments after Fundão dam breach. **Estuarine, Coastal and Shelf Science**, v. 261, p. 107507, oct. 2021.

FERNANDO, D. R.; LYNCH, J. P. Manganese phytotoxicity: new light on an old problem. **Annals of Botany**, v. 116, n. 3, p. 313–319, sep. 2015.

FREITAS, J. C. E. et al. Does the water regime differentially modulate the responses to water stress in *Lippia alba* (Verbenaceae) genotypes with different ploidy levels? **Industrial Crops and Products**, v. 160, p. 113137, 1 fev. 2021.

GARCÍA-CAPARRÓS, P. et al. Oxidative Stress and Antioxidant Metabolism under Adverse Environmental Conditions: a Review. **Botanical Review**, v. 87, n. 4, p. 421–466, 1 dez. 2021.

GIANNOPOLITIS, C. N.; RIES, S. K. Superoxide Dismutases I: Occurrence in Higher Plants. **Plant Physiology**, v. 59, n. 2, p. 309–314, feb. 1977.

HATI, N. et al. Liming of Acid Soil: II—Effect on plant available manganese and iron. **Journal of the Indian Society of Soil Science**, v. 27, n. 4, p. 394–398, 1979.

HOUNGNANDAN, P. et al. Response of *Mucuna pruriens* to symbiotic nitrogen fixation by rhizobia following inoculation in farmers' fields in the derived savanna of Benin. **Biology and Fertility of Soils 2000 30:5**, v. 30, n. 5, p. 558–565, 2000.

HU, S. H. et al. Significance of AtMTM1 and AtMTM2 for mitochondrial MnSOD activation in Arabidopsis. **Frontiers in Plant Science**, v. 12, p. 1629, aug. 2021.

HU, S. H.; JINN, T. L. Impacts of Mn, Fe, and oxidative stressors on MnSOD activation by AtMTM1 and AtMTM2 in Arabidopsis. **Plants 2022, Vol. 11, Page 619**, v. 11, n. 5, p. 619, feb. 2022.

HUANG, H. et al. Mechanisms of ROS regulation of plant development and stress responses. **Frontiers in Plant Science**, v. 10, p. 800, may 2019a.

HUANG, H. et al. Physiological responses of *Broussonetia papyrifera* to manganese stress, a candidate plant for phytoremediation. **Ecotoxicology and Environmental Safety**, v. 181, p. 18–25, oct. 2019b.

HUANG, H. et al. Improvement of manganese phytoremediation by *Broussonetia papyrifera* with two plant growth promoting (PGP) *Bacillus* species. **Chemosphere**, v. 260, p. 127614, dec. 2020.

HUANG, Y.; ZHAO, L. The effects of small particles on soil seismic liquefaction resistance: current findings and future challenges. **Natural Hazards**, v. 92, n. 1, p. 567–579, 14 may 2018.

ISLAM, K.; MURAKAMI, S. Global-scale impact analysis of mine tailings dam failures: 1915–2020. **Global Environmental Change**, v. 70, p. 102361, 1 set. 2021.

JORDAN, D. B.; OGREN, W. L. Species variation in kinetic properties of ribulose 1,5-bisphosphate carboxylase/oxygenase. **Archives of Biochemistry and Biophysics**, v. 227, n. 2, p. 425–433, dec. 1983.

KOHNO, Y.; FOY, C. D. Manganese toxicity in bush bean as affected by concentration of manganese and iron in the nutrient solution. **Journal of Plant Nutrition**, v. 6, n. 5, p. 363–386, may 1983.

LIDON, F. C. Rice plant structural changes by addition of excess manganese. **Journal of Plant Nutrition**, v. 25, n. 2, p. 287–296, feb. 2002.

LAL, M. K. et al. From source to sink: mechanistic insight of photoassimilates synthesis and partitioning under high temperature and elevated [CO_2]. **Plant Molecular Biology 2022**, v. 1, p. 1–20, 24 maio 2022.

LIMA, H. C. de et al. Fabaceae in Lista de espécies da flora do Brasil. **Jardim Botânico do Rio de Janeiro.** 2015.

LONGO, R. M.; RIBEIRO, A. Í.; DE MELO, W. J. Uso da adubação verde na recuperação de solos degradados por mineração na floresta amazônica. **Bragantia**, v. 70, n. 1, p. 139–146, 2011.

LOWRY, O. H. et al. Protein measurement with the Folin phenol reagent. **The Journal of biological chemistry**, v. 193, n. 1, p. 265–75, nov. 1951.

LYU, Z. et al. A Comprehensive Review on Reasons for Tailings Dam Failures Based on Case History. **Advances in Civil Engineering**, v. 2019, 2019.

MARA SANTANA, R. Funed-Pol - Coleção de lâminas de grãos de pólen. Version 1.91. **Fundação Ezequiel Dias**. Occurrence dataset. 2022.

MARSCHNER, P. **Marschner's Mineral Nutrition of Higher Plants**. Third Edition. Elsevier. 2012.

MATOS, L. P. et al. Limitations to Use of *Cassia grandis* L. in the revegetation of the areas impacted with mining tailings from Fundão dam. **Water, Air, & Soil Pollution**, v. 231, n. 3, p. 127, mar. 2020.

MILLALEO, R. et al. Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. **Journal of soil science and plant nutrition**, v. 10, n. 4, p. 470–481, 2010.

MITTLER, R. ROS are good. **Trends in Plant Science**, v. 22, n. 1, p. 11–19, jan. 2017.

MORGENSTERN, N. R. et al. Fundão tailings dam review panel report on the immediate causes of the failure of the Fundão dam. **Cleary Gottlieb Steen & Hamilton LLP, New York**, 2016.

MOURA, T. M. *Mucuna pruriens*. **Flora e Funga do Brasil**. Jardim Botânico do Rio de Janeiro. 2022.

NEVES, M. C. L. et al. **Pesquisa sobre a saúde mental das famílias atingidas pelo rompimento da barragem de Fundão em Mariana**. Belo Horizonte: Corpus. 2018.

NUNES, F. C. et al. Phytoremediation strategies for rehabilitation of soils affected by red mud: the mariana tailing dam collapse (Minas Gerais, Brazil). **Eurasian Soil Science**, v. 55, n. 5, p. 673–685, may 2022.

OMACHI, C. Y. et al. Atlantic Forest loss caused by the world´s largest tailing dam collapse (Fundão Dam, Mariana, Brazil). **Remote Sensing Applications: Society and Environment**, v. 12, p. 30–34, nov. 2018.

PATHANIA, R. et al. An assessment of potential nutritive and medicinal properties of *Mucuna pruriens*: a natural food legume. **3 Biotech**, v. 10, n. 6, p. 1–15, 1 jun. 2020.

PAULELLI, A. C. C. et al. Fundão tailings dam failure in Brazil: Evidence of a population exposed to high levels of Al, As, Hg, and Ni after a human biomonitoring study. **Environmental Research**, v. 205, p. 112524, apr. 2022.

PEIXOTO, P. H. P. et al. Aluminum effects on lipid peroxidation and on the activities of enzymes of oxidative metabolism in sorghum. **Revista Brasileira de Fisiologoia Vegetal**, v. 11, n. 3, p. 137–143, 1999.

QUEIROZ, H. M. et al. Manganese: The overlooked contaminant in the world largest mine tailings dam collapse. **Environment International**, v. 146, p. 106284, jan. 2021.

R CORE TEAM. R: A language and environment for statistical computing. **R Foundation for Statistical Computing**, Vienna, Austria, 2020.

ROCHA, R. et al. Effects of the planting of legume species and soil conditions on the recovery of a sand and pebble mining area. **Land Degradation & Development**, v. 32, n. 4, p. 1695–1705, feb. 2021.

ROJAS-LILLO, Y. et al. Manganese toxicity and UV-B radiation differentially influence the physiology and biochemistry of highbush blueberry (*Vaccinium corymbosum*) cultivars. **Functional Plant Biology**, v. 41, n. 2, p. 156, 2014.

SAALTINK, R. M. et al. Plant-specific effects of iron-toxicity in wetlands. **Plant and Soil**, v. 416, n. 1–2, p. 83–96, jul. 2017.

SALEEM, A. R. et al. Drought response of *Mucuna pruriens* (L.) DC. inoculated with ACC deaminase and IAA producing rhizobacteria. **PLOS ONE**, v. 13, n. 2, p. e0191218, feb. 2018.

SALO-VÄÄNÄNEN, P. P.; KOIVISTOINEN, P. E. Determination of protein in foods: comparison of net protein and crude protein (N × 6.25) values. **Food Chemistry**, v. 57, n. 1, p. 27–31, sep. 1996.

SANTOS, E. F. et al. Physiological highlights of manganese toxicity symptoms in soybean plants: Mn toxicity responses. **Plant Physiology and Biochemistry**, v. 113, p. 6–19, apr. 2017.

SANTOS, F. M. et al. Recovering ecosystem functions through the management of regenerating community in agroforestry and plantations with Khaya spp. in the Atlantic Forest, Brazil. **Forest Ecology and Management**, v. 482, p. 118854, feb. 2021.

SCHAEFER, C. E. G. R. et al. Paisagens de Lama: Os Tecnossolos para recuperação ambiental de áreas afetadas pelo desastre da barragem do Fundão, em Mariana. In: **A Ciência do solo e o desastre de Mariana**. Sociedade Brasileira de Ciência do Solo, Viçosa, p. 44, 2016.

SILVA, D. R. et al. Why is *Brachiaria decumbens* Stapf. a common species in the mining tailings of the Fundão dam in Minas Gerais, Brazil? **Environmental Science and Pollution Research**, v. 29, n. 52, p. 79168–79183, nov. 2022.

SILVA, F. C. (2009) Manual de análises químicas de solos, plantas e fertilizantes. Embrapa Informação Tecnológica, Brasília. 2009.

SILVA ROTTA, L. H. et al. The 2019 Brumadinho tailings dam collapse: Possible cause and impacts of the worst human and environmental disaster in Brazil. **International Journal of Applied Earth Observation and Geoinformation**, v. 90, p. 102119, aug. 2020.

TEIXEIRA, P. C. et al. **Manual de métodos de análise de solo**. Embrapa. 2017.

WENG, X.-Y. et al. Characteristics of the hyperaccumulator plant *Phytolacca acinosa* (Phytolaccaceae) in response to excess manganese. **Journal of Plant Nutrition**, v. 36, n. 9, p. 1355–1365, jul. 2013.

ZAGO, V. C. P.; DAS DORES, N. C.; WATTS, B. A. Strategy for phytomanagement in an area affected by iron ore dam rupture: A study case in Minas Gerais State, Brazil. **Environmental Pollution**, v. 249, p. 1029–1037, jun. 2019.

ZHAO, Y. et al. Tropical rainforest successional processes can facilitate successfully recovery of extremely degraded tropical forest ecosystems following intensive mining operations. **Frontiers in Environmental Science**, v. 9, p. 183, jun. 2021.

ZHOU, J. et al. Mycorrhizal and rhizobial interactions influence model grassland plant community structure and productivity. **Mycorrhiza**, v. 32, n. 1, p. 15–32, jan. 2022.