

LETÍCIA PAIVA DE MATOS

**ALTERAÇÕES MORFOFISIOLÓGICAS EM *Cassia grandis* L. (Fabaceae)
CULTIVADAS EM REJEITO DE MINERAÇÃO DE FERRO**

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Biologia Celular e Estrutural, para obtenção do título de *Doctor Scientiae*.

Orientador: Juraci Alves de Oliveira

**VIÇOSA – MINAS GERAIS
2020**

**Ficha catalográfica elaborada pela Biblioteca Central da Universidade
Federal de Viçosa - Campus Viçosa**

T

M433a
2020

Matos, Leticia Paiva de, 1989-
Alterações morfofisiológicas em *Cassia grandis* L.
(Fabaceae) cultivadas em rejeito de mineração de ferro / Leticia
Paiva de Matos. – Viçosa, MG, 2020.
56 f. : il. (algumas color.) ; 29 cm.

Orientador: Juraci Alves de Oliveira.
Tese (doutorado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. *Cassia grandis* - Fisiologia. 2. Fitorremediação.
3. Metais pesados. 4. Revegetação. I. Universidade Federal de
Viçosa. Departamento de Biologia Geral. Programa de
Pós-Graduação em Biologia Celular e Estrutural. II. Título.

CDD 22. ed. 583.46

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APROVADA: 18 de fevereiro de 2020.

Assentimento:



Leticia Paiva de Matos
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Juraci Alves de Oliveira
Orientador

Ao Pedro, meu companheiro de
vida, dedico.

AGRADECIMENTOS

A Deus, pela vida. Ao Pedro, por me apoiar em minhas decisões, não me deixar desistir nos momentos em que me faltou coragem e por ser minha inspiração de ser humano. À minha mãe Elaine, minha inspiração diária para ser uma mulher forte, meu porto seguro e meu amor. Ao meu pai, pelo carinho e cuidado nos nossos bons momentos. Ao meu irmão, Luís Paulo e minhas sobrinhas, Nicolly e Sofia, por todo amor recebido e vida que trazem para mim. À toda minha família de Minas, em especial meus avós Luis e Lôra, e à minha família goiana, Edilson, Maria Divina e Camila, por todo amor e suporte. Essa conquista é nossa!

Ao professor Juraci, agradeço por ter me recebido tão bem em Viçosa, pela orientação e amizade durante o processo. À Taline pela ajuda no desenvolvimento da pesquisa. A todos os amigos do Laboratório de Biofísica Ambiental. Em especial, Daniel, Pedro e Vinícius (vocês já são mineiros de coração e carteirinha). Thainá, pela ajuda na fase final da tese. Aos meus irmãos, Heloísa e Claudio, nenhuma palavra seria suficiente para agradecer a parceria e lealdade. Então, fica registrado aqui, a gratidão por este reencontro. Esse doutorado foi mais especial por ter vocês do lado. Me lembrarei com carinho dos longos cafés com simpatia.

Aos professores, que cruzaram meu caminho, fazendo o doutorado ser mais leve. À Catarina, por ter me recebido em seu grupo e me ajudado em todo processo de construção deste trabalho. Ao Sebastián, pela ajuda nos experimentos, nas discussões e por ter se tornando um grande amigo. À Luzimar, por sempre estar disponível a ajudar, e pela paciência. À Mônica, minha orientadora da graduação ao mestrado, que sempre esteve do meu lado. E ao professor Eduardo Borges, por ter nos recebido em seu laboratório com tanto carinho.

A todos os técnicos e terceirizados que trabalham para nos dar o suporte necessário. Em especial Betinha, por todo carinho de sempre e Antônio Carlos, por me ajudar nos experimentos e ser sempre prestativo. Ao pessoal que trabalha no prédio da Biologia, em especial a Dona Maria, que sempre me recebia com um abraço.

À Ouro Preto, lugar onde sempre encontro refúgio. Rosa e Notre, por estarem sempre presentes em minha vida. À Paula, por ter sido meu braço direito no mestrado e doutorado. À Liliane, pela amizade e companheirismo desde o mestrado.

À todas as pessoas que me ajudaram ao longo do desenvolvimento de todo trabalho, em especial Mayara, Isabelle, Michel.

Por fim, e não menos importante, a Universidade Federal de Viçosa, ao Programa de Pós-graduação em Biologia Celular e Estrutural (BIOCEL), Universidade Federal de Ouro Preto e a Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), pelo suporte durante toda minha formação no ensino público. O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

RESUMO GERAL

MATOS, Letícia Paiva de, D.Sc., Universidade Federal de Viçosa, fevereiro de 2020. **Alterações morfofisiológicas em *Cassia grandis* L. (Fabaceae) cultivadas em rejeito de mineração de ferro.** Orientador: Juraci Alves de Oliveira

A atividade minerária gera impactos ambientais por diversos meios, como o acúmulo de metais pesados nos solos e na água. As técnicas de fitorremediação, são muito utilizadas e podem ter seu potencial ampliado com a associação a micro-organismos. Este trabalho avaliou o acúmulo de metais pesados e os efeitos sobre parâmetros morfofisiológicos de plântulas de *Cassia grandis*, associadas a fungos micorrízicos arbusculares (FMA), cultivadas em rejeito de mineração. Sementes de *C. grandis* foram germinadas em areia lavada e transplantadas para tubetes com substrato comercial, com ou sem FMA. Posteriormente, foram transferidas para vasos com rejeito de mineração e mantidas em casa de vegetação, por 4 meses, divididas em cinco grupos: substrato comercial sem inóculo (T₀), rejeito sem inóculo (T₁), rejeito e inóculo obtido de solo de floresta nativa (T₂), rejeito e inóculo obtido de área contaminada com rejeito (T₃), rejeito e inóculo obtido de área contaminada com rejeito com cultura de laboratório (T₄). Foram analisados os teores de metais e arsênio, atividade enzimática antioxidante, trocas gasosas, teores de pigmentos cloroplastídicos, de peróxido de hidrogênio, de malonaldeído e morfologia de folhas. Os elementos arsênio, cádmio e chumbo não foram detectados nos tecidos das plantas. Por outro lado, houve aumentos nas concentrações de ferro (Fe) e cobre (Cu) em folhas das plantas cultivadas com rejeitos de mineração em relação ao controle. O cultivo das plantas em substratos com rejeito de mineração resultou em aumento na atividade das enzimas antioxidantes e nos teores de antocianinas, e diminuição nos teores de clorofilas *a*, *b* e carotenoides. O aumento da atividade enzimática pode estar relacionado ao aumento da concentração de alguns metais pesados. Os parâmetros de trocas gasosas foram reduzidos e este fato está diretamente relacionado a alta compactação do rejeito, que impõe uma limitação hídrica as plantas. Nossos resultados indicam que o cultivo de *C. grandis* em rejeitos de mineração impõe danos fisiológicos e, a inoculação de FMA's, não resultou em ganhos positivos para a maioria dos parâmetros analisados. A limitação física ao desenvolvimento radicular foi o principal problema nos tratamentos contendo rejeitos. Entretanto, com o manejo adequado do solo, esta espécie pode ser utilizada para revegetação de locais contaminados por rejeito de mineração de ferro.

Palavras-chave: Fitorremediação. Metais Pesados. Fisiologia Vegetal. Revegetação.

GENERAL ABSTRACT

MATOS, Leticia Paiva de, D.Sc., Universidade Federal de Viçosa, February, 2020. **Morphophysiological's changes in *Cassia grandis* L. (Fabaceae) cultivated in mining tailings.** Advisor: Juraci Alves de Oliveira

The mining activity generates environmental impacts by several means, such as the accumulation of heavy metals in soils and water. Phytoremediation techniques are widely used and may have their potential expanded with microorganism's association. The purpose of this work was to evaluate the accumulation of heavy metals and the effects on morphophysiological parameters of *Cassia grandis* seedlings associated with arbuscular mycorrhizal fungi (AMF) grown in mining tailings. *C. grandis* seeds were germinated in washed sand and transplanted to tubes with commercial substrate with or without AMF. Subsequently, they were transferred to pots with mining tailings and kept at a greenhouse for 4 months, divided into five groups: commercial substrate without inoculum (T₀), tailings without inoculum (T₁), tailings and inoculum obtained from native forest soil (T₂), tailings and inoculum obtained from areas contaminated with tailings (T₃), tailings and inoculum obtained from areas contaminated with tailings, with laboratory culture (T₄). Metals and Arsenic contents, antioxidant enzyme activity, gas exchange, chloroplast pigment, hydrogen peroxide, malonaldehyde and leaf morphology contents were analyzed. Arsenic, cadmium and lead elements were not detected in the tissues of the plants. On the other hand, there were increases in iron concentrations (Fe) and copper (Cu) in leaves of the plants cultivated with mining tailings compared to the control. The cultivation of plants on substrates with mining tailings resulted in an increase in the activity of antioxidant enzymes also in anthocyanins levels, and the chlorophylls a, b and carotenoids levels decreased. The enzyme activity increase may be related to the increase in the concentration of some heavy metals. The gas exchange parameters were reduced which is directly related to the high compaction of the tailings, that imposes a water limitation on the plants. Our results indicate that the cultivation of *C. grandis* in mining tailings imposed physiological damage and the inoculation of AMF's did not result in positive gains for major analyzed parameters. Physical limitation to root development was the main problem in treatments containing tailings. However, with a proper soil management these species can be used to revegetate sites contaminated by iron mining tailings.

Keywords: Phytoremediation. Heavy Metals. Plant Physiology. Revegetation.

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INTRODUÇÃO GERAL

A atividade minerária gera, como subprodutos, resíduos que contém concentrações elevadas de vários metais pesados. No Brasil, por ser um polo produtor de minério de ferro gera, em consequência, enorme quantidade de rejeitos de mineração, os quais são, em sua maioria, dispostos em barragens de contenção. Embora sejam considerados eventos raros, nos últimos quatro anos o Brasil presenciou o rompimento de duas grandes barragens de rejeitos, sendo uma em 2015, na região de Mariana, e outra em 2019, em Brumadinho, Minas Gerais. Estes rejeitos causaram, e continuam causando, danos severos nas esferas aquática e terrestre, afetando todo o equilíbrio dos ecossistemas (Figueiredo et al. 2019; Thompson et al. 2020).

Os rejeitos oriundos da mineração, em sua maioria, possuem elevado teor de metais solubilizados, poucos nutrientes e, consequentemente, baixa atividade microbiana. Devido a este fato, torna-se mais difícil estabelecer uma cobertura vegetal nos solos onde este rejeito é acumulado (Chu et al. 2018).

Para a recuperação das áreas afetadas são necessárias ações em diferentes níveis de atuação para que a regeneração do ambiente ocorra de forma eficiente. Uma das possibilidades de mitigação e de recuperação dessas áreas é por meio do processo de fitorremediação. Para este processo, é necessária a escolha de espécies vegetais capazes de se adaptar e crescer neste tipo de ambiente. Neste contexto, a *Cassia grandis* surge como uma alternativa, visto que seu desenvolvimento em rejeito já foi estudado e obteve bons resultados (Guimarães et al. 2017).

As espécies leguminosas são reconhecidas pelo seu bom desenvolvimento e rendimento, bem como por sua capacidade de fazer associação com micro-organismos em suas raízes. Interações com micro-organismos, como fungos micorrízicos, protegem a planta dos contaminantes e promovem seu crescimento, aumentando a absorção de nutrientes pelas raízes e fixando o nitrogênio do ar (Scoriza e Correia, 2019).

Os fungos micorrízicos arbusculares (FMA) agem como uma expansão radicular e permitem uma maior eficiência na absorção de água e nutrientes (Girardin et al. 2019). Além disso, aumentam a tolerância das plantas pela imobilização de metais pesados nas raízes, sendo assim considerados excelentes bio-sorventes (Srivastava et al. 2015).

Plantas cultivadas em locais contaminados tem seu metabolismo afetado de modo geral, com o aumento na concentração de espécies reativas de oxigênio e, em consequência, aumento na atividade enzimática antioxidativa, na peroxidação lipídica e dos danos ao aparato fotossintético (Chu et al. 2018; Sinha et al. 2018).

Os objetivos propostos para esta pesquisa foram estudar o desenvolvimento de *Cassia grandis* cultivadas em rejeitos de mineração; analisar os parâmetros morfofisiológicos afetados e avaliar os benefícios advindos da associação das raízes com FMA's, isolados das áreas contaminadas com os rejeitos.

Esta pesquisa fez parte do projeto aprovado pela Fapemig (2016): Tolerância a metais pesados e efeitos ontogênicos em plantas: potencial para biorrecuperação de áreas afetadas por rejeitos de mineração Fapemig (APQ-01797-16), da Universidade Federal de Viçosa.

Article 1: Limitations to use of *Cassia grandis* L. in the revegetation of the areas impacted with mining tailings from Fundão dam

Water, Air and Soil Pollution

<https://doi.org/10.1007/s11270-020-04479-0>

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Acknowledgments

The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001, Fundação de Apoio à Pesquisa de Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support.

ABSTRACT

Revegetation is an alternative of recovery for contaminated areas by mining tailings. The objective of this study was to evaluate the physiological responses of *Cassia grandis* growing in iron mining tailings and the implications for revegetation purposes. Plants of *C. grandis* were grown for 4 months in substrate collected in affected areas by residues from Fundão dam and commercial substrate was used as control. Metal(oids) contents, antioxidant enzymatic activity, gas exchanges and chloroplast pigments contents were analyzed. Arsenic, cadmium and lead were not detected in plant tissues. On the other hand, there were increases in the contents of some elements, such as iron (Fe) and chromium (Cr), with marked reduction in the Pi levels, in plants cultivated with mining tailings compared to the control. The exposure to mining tailings increased the antioxidant enzymes activity and anthocyanins contents, whereas decreased chlorophyll a, b and carotenoid contents. The increase of enzymatic activity may be related to the increased concentration of toxic elements such as Cr. The photosynthetic rate (A), stomatal conductance (g_s), transpiration (E) and water use efficiency (WUE) have been reduced which is probably associated with the limitations of water availability caused by the high compaction of mining tailings. These physical limitations also may have caused deformations observed in the primary taproots of tailings-grown plants. Our results indicate that the cultivation of *C. grandis* in mining tailings imposes some physiological damage. Nevertheless, this plant species has the potential to establish and develop at locations affected by iron ore tailings with properly management.

Keywords: Ecotoxicology, Photosynthesis, Plant Physiology, Revegetation.

INTRODUCTION

Mining activities such as iron ore mining promotes the release of several toxic elements that can accumulate in ecosystems. Among these elements heavy metals have a great potential for contamination whereas it does not degrade and can be accumulated into environments, reaching the food chains and endangering the human health (Singh et al. 2016). Mining processes generate tailings as a result of the extraction and processing of minerals, which present small amounts of several elements including heavy metals (Adiansyah et al. 2015). Residues can accumulate in large amounts and when it is not disposed properly or due to accidents such as breaking dams, have the potential to pollute different ecosystems (Hatje et al. 2017, Queiroz et al. 2018).

The collapse of the Fundão dam located in Minas Gerais, Brazil, on November 5, 2015 released millions of m³ of iron mining tailings in the Rio Doce Basin leading to a huge environmental impact (Omachi et al. 2008). One year ago, another mining tailings dam broke up in Brumadinho, Minas Gerais, Brazil on January 25, 2019. This type of residue has a high pollution potential and can contribute to the release of several elements such as cadmium (Cd), lead (Pb), arsenic (As), iron (Fe), zinc (Zn), chrome (Cr), copper (Cu) and manganese (Mn) which can change the physical-chemical characteristics of the soil (Sun et al. 2018).

The remediation of an ecosystem contamination can occur by physical, chemical or biological processes either alone or combined. However, physical and chemical processes can drastically affect soil characteristics by negatively influencing the biota biodiversity of that environment (Chapman et al. 2019).

The recovery of affected sites by mining tailings involves many techniques in which phytoremediation/revegetation has emerged as an alternative for the stabilization and regeneration of these environments (Guo et al. 2014, Hosseini 2018). However, the majority of studies focus on a specific pollutant which limits its application to sites with several toxic agents as occurs in areas affected by mining tailings (Sarma 2011).

The exposal of plants to toxic concentrations of heavy metals leads to uncountable damages that are both structural and physiological changes negatively influencing the plants survival and the remediation processes of contaminated ecosystems. However, some species have strategies to reduce these negative impacts such as processes to immobilize the toxic element or by activating antioxidative defenses (Abercrombie et al. 2008). The analyses of these mechanisms are important to understand how plants

response to heavy metals stress, enabling greater efficiency in phytoremediation (Reis et al. 2019).

Tree species are known to have a high growth capacity in poor nutrient soils, high biomass accumulation, deep root systems, accumulation of contaminants and other characteristics that make it an important resource to remediation processes. However, there are few researches using native tree species from tropical and subtropical regions (Asensio et al. 2018). The leguminous *C. grandis* is suitable for the revegetation of mining areas due to its high nitrogen fixation capacity and growth in soils with low fertility (Guimarães et al. 2017). However, the cultivation in tecnosols change the photosynthetic, physiological, and biochemical parameters in plants (Chu et al. 2018).

Therefore, this research aim was to evaluate the physiological changes in *C. grandis* grown on mining tailings from Fundão dam and its revegetation possibilities in impacted sites.

MATERIALS AND METHODS

Plant material

The experiment was conducted at the Universidade Federal de Viçosa, Viçosa-MG, Brazil (20 ° 45'14 "S, 42 ° 52'55" W, height 650 m), from January to June 2018. The climate is classified according to Koppen and the Cwa type, as humid subtropical with the average annual temperature of 21.8 °C and 1,361 mm of annual precipitation average (Alvares et al. 2013). The plants were germinated and cultivated at a greenhouse with the ceiling and walls covered with low density polyethylene film. Seeds of *Cassia grandis* were immersed in H₂SO₄ concentrate, standing fully immersed for 15 min, washed with deionized water, germinated in sand and transplanted to tubes containing commercial substrate. After thirty days cultivation, five seedlings (with approximately 20 cm in length of shoot length) were transplanted to bags containing either 1000 cm³ of commercial substrate (control) or mining residue collected in Paracatu de Baixo nearby Gualaxo do Norte river, belonging to the Doce River basin which is an impacted area by iron mining tailing from the Fundão dam, Minas Gerais, Brazil, (20°18'16.1"S 43°13'50.1"W). These samples were kept for 4 months under greenhouse conditions (the seeds bags were irrigated daily until field capacity).

Metals determination

Plants were washed with deionized water and the roots were treated with DBC (Taylor and Crowder 1983) to remove the adsorbed iron oxides, then dried to a constant dry mass. About 100 mg of the dry mass were mineralized in 1.5 mL of nitric-perchloric acid mixture (2:1) until the complete organic matter and extract withdrawal used in the determination of Cr, Cu, Mn, Zn, Ca, K, Fe and Mg concentration by atomic absorption spectrophotometry (Shimadzu AA-6701F). For arsenic (As) determination a hydride vapor generator (HVG-1 Shimadzu) was used. Inorganic phosphorus was quantified by the colorimetric method.

Gas exchange determination

Photosynthetic rate (*A*), stomatal conductance (*g_s*) and transpiration (*E*) were measured with an infrared gas analyzer system (IRGA, LCpro-SD-ADC BioScientific Ltd.) on fully expanded leaves, using light intensity of 600 μmol m⁻² s⁻¹. The CO₂ concentration inside the leaf chamber, air humidity and temperature fluctuated according to ambient conditions. Data were collected from 7:00 to 10:00 at three-minute intervals. The measurement area of leaf chamber was calculated using ImageJ, resulting in 5.35 cm².

Pigment contents

The chlorophyll *a*, *b* and carotenoid contents were determined by dimethylsulfoxide (DMSO) method. The absorbance was determined by spectrophotometer (UV-Visible Quimis 0790U2M) and the values used to calculate the chlorophyll *a*, *b* and carotenoids levels (Wellburn 1994). Samples of 0.1 g fresh leaves were homogenized in 5 mL methanol-HCl 1 % (v/v) in ice bath and centrifuged at 10,000 *g* for 30 min at 4 °C. The total anthocyanin content was determined by the difference between the absorbance at 536 and 600 nm (Hodges et al. 1999) and the results expressed as mg g⁻¹ FW, as cyanidin-3-glucoside equivalents, by the coefficient molar extinction (26900 L cm⁻¹ mol⁻¹).

Extraction antioxidant enzymes

The superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and glutathione reductase (GR) were extracted from 0.3 g of fresh roots and leaves. The samples were macerated in liquid nitrogen, followed by addition of 2 mL extraction medium, filtration and centrifugation at 12,000 *g* for 15 min, at 4 °C. The supernatant was used as crude extract in the determination of the enzymatic activities. All extraction steps were performed at 4 °C. The extraction media were: a) 0.1 M potassium phosphate buffer pH 6.8, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 % (w/v) polyvinylpyrrolidone (PVPP), for SOD, CAT and POX; b) 2 mL of 0.1 M potassium phosphate buffer, pH 7.0, 1 mM EDTA (ethylene diamine tetraacetic acid) 0.02 % triton, 2 mM DTT (dithiothreitol), 1 mM PMSF and PVPP (polyvinyl pyrrolidone) 1 %, for GR (Carlberg Mannervik 1985). The protein contents of the enzyme extracts were determined with BSA as the standard to express enzyme activity (Waterborg and Matthews 1984).

Superoxide dismutase activity

The SOD activity was determined with the addition of 50 µL of the crude enzyme extract to 5 mL of reaction medium containing 50 mM sodium phosphate buffer pH 7.8 containing 13 mM methionine, p-nitro tetrazolium blue (NBT) 75 µM, 0.1 mM EDTA and 2 µM riboflavin. The reaction was conducted at 25 °C in a reaction chamber under 15 W fluorescent lamp illumination, kept inside a box. After 5 min exposure illumination was discontinued and the blue formazan, produced by NBT photoreduction, was measured by the absorbance at 560 nm (Giannopolitis and Ries 1977). A unit of SOD was defined as the amount of enzyme required to inhibit the NBT photoreduction by 50 % (Beauchamp and Fridovich 1971).

Peroxidase activity

The POX activity was determined with the addition of 0.1 mL of the crude enzyme extract to 4.9 mL of reaction medium containing 25 mM potassium phosphate buffer, pH 6.8, pyrogallol 20 mM and 20 mM H₂O₂ (Kar and Mishra 1976). Purpurogalin production was determined by increasing the absorbance during the first minute of reaction at 420 nm at 25 °C. The enzymatic activity was calculated using the molar extinction coefficient 2.47 mM L⁻¹ cm⁻¹ (Chance and Maehley 1955) and expressed in μmoles of H₂O₂ min⁻¹ mg⁻¹ protein.

Catalase activity

The CAT activity was determined with the addition of 0.1 mL of the crude enzyme extract to 2.9 mL of reaction medium containing 50 mM potassium phosphate buffer pH 7.0 and 12.5 mM H₂O₂ (Havir and Mchale 1987). The decrease in absorbance at 240 nm and 25 °C was measured in the first reaction minute. The enzymatic activity was calculated using the molar extinction coefficient 36 M⁻¹ cm⁻¹ (Anderson et al. 1995) and expressed in μmol H₂O₂ min⁻¹ mg⁻¹ protein.

Glutathione reductase activity

The GR activity was determined with the addition of 0.1 mL of the crude enzyme extract to 0.9 mL of reaction medium containing Tris-HCl 0,1 M, pH 7,5, GSSG 20 mM and NADPH 2 mM (Carlberg and Mannervik 1985). The decrease in absorbance at 340 nm and 30 °C was measured in the first reaction minute. The enzymatic activity was calculated using the molar extinction coefficient 6.22 mM⁻¹ cm⁻¹ and expressed in nmol of oxidized glutathione min⁻¹ mg⁻¹ protein.

Experimental design and statistical analysis

The experiments followed a completely randomized factorial design with 2 treatments and five replicates and the data were analyzed by software SAS 9.1 (SAS Institute Inc. 2004 NC, USA).

RESULTS AND DISCUSSION

In soils contaminated by mining tailings, the presence of extremely toxic metal(oids) such as Pb, Cd and As, is relatively common (Pistelli et al. 2017). Nevertheless, these elements were not detected in the tissues of plants grown in mining tailings collected in the region of Paracatu, indicating that these tailings do not have a relevant level of contamination of these metals(oids) (Table 1).

However, leaves presented increments by 932, 125, 63 and 220 % for Cr, Mn, Fe and Mg contents, respectively, and decrease by 54, 68, 45 and 74 % for Cu, Zn, K and Pi contents, respectively. In roots of tailing-grown plants, there was greater contents of Cr and Fe compared to the control, whereas the other elements, such as Mn, Zn, K, Pi and Mg, showed lower contents (Table 1).

The accumulation of Cr, Fe and Mn in leaves and Cr and Fe in roots may have contributed to the production of reactive oxygen species (ROS), triggering macromolecules damage and disruption of main metabolic pathways (Fernández et al. 2018). To protect cells against homeostatic disorders and damage from excessive ROS, induction of antioxidant enzyme activity acts as a general response to the toxic effects caused by heavy metals. This response to excess metals, may vary between plant species and different tissues (Sytar et al. 2013). The observed increase in the activity of antioxidant enzymes, SOD, CAT, POX and GR, in *C. grandis* may, therefore, occurred as a response to the stress caused by the mining tailings, especially by the increase in the concentration of some metals such as Cr (Figure 1).

The decrease of the Mn in roots and the accumulation in the shoots are related to its high mobility in the xylem, and consequent translocation to the leaves (Ducic and Polle 2005). In addition, the low levels of K, Zn and Pi observed, may have affected enzyme activities, energy status, osmotic balance and redox homeostasis (Blasco-Leon et al. 2018). Together, the toxic effects of some metals accumulation and nutritional starvation may lead to the disruption of normal cellular and ultimately the whole plant functions.

The contents of chlorophyll *a*, *b* and carotenoids were, respectively, 49, 49 and 17 %, lower and those of anthocyanins were 21 times higher in leaves plants grown on mining tailings (Figure 2).

The reduction in chlorophyll content may be due to inhibition of biosynthesis or accelerated degradation of this pigment (Kalaji et al. 2016). Decreasing chlorophyll content leads to decreased photosynthesis and therefore to plant growth.

The increase in anthocyanin contents may have been promoted by the reduction of Pi levels, as already observed in *Azolla caroliniana* (Adalberto et al. 2004) and *Alternanthera brasiliensis* (Henry et al. 2019). This effect occurs due to damage to the metabolism, promoted by Pi deficiency, which leads to gene expression related to anthocyanin synthesis, as part of the plants defense system against stress (Vasconcelos et al. 2018). The higher contents of anthocyanin are related to the ROS defense system, once these flavonoids also act as antioxidants. Thus, anthocyanin is effective in the H₂O₂ degradation which has a longer half-life and can diffuse into cellular tissues (Davies et al. 2018).

Photosynthesis (A), stomatal conductance (g_s), transpiration (E) and water use efficiency (WUE) were, respectively, 5.0; 5.6; 2.2 and 1.9 times lower in tailing-grown plants compared to control (Figure 2). The negative impacts on A, E, g_s, WUE and chloroplastidic pigments values demonstrates the high stress level imposed to the plants. The high particle density of the substrate from the affected areas, which exceeds the limit of 1.65 g cm⁻³ (personal information), and the consequent soil compaction reduces the water and nutrients availability leading to stomatal closure and lower CO₂ fixation. The decrease of photosynthesis rates causes lower photoassimilates production and consequently plant growth (Lahive et al. 2018).

Iron mining tailings typically consist of the finely crushed ore (Aubertin et al. 2002), with great compaction power and low hydraulic conductivity, directly influencing root development and promoting water deficit in plants (Guittonny-Larchevêque et al. 2016). This effect was observed in the experiment, with deformed primary taproots in plants grown directly in the tailings (Figure 3).

Root malformation, associated with low hydraulic conductivity of mining tailings, explain the deleterious effect, due to water deficit and stomatal closure, on gas exchange and photosynthetic metabolism, compromising plant development and its potential as a soil phytoremediator with these characteristics.

However, with the introduction of this species for phytoremediation of soils contaminated by iron mining tailings accompanied by previous management, soil decompression and nutritional supplementation, *C. grandis* may have good soil stabilization and recovery results. especially because of its capacity as a legume to make mycorrhizal associations with microorganisms capable of enriching the soil.

CONCLUSIONS

The *C. grandis* plants growing in mining tailings from Fundão Dam present no concentrations of Pb, Cd and As in tissues, indicating low chemical restriction for establishment of plants in affected areas. However, the morphophysiological aspects of plants are negatively influenced by mining tailings from Fundão Dam, especially due to soil physical characteristics that compromised root development and water availability, as well as low phosphorus availability. Thus, the use of this plant in soil recovery processes impacted by mining tailings must be accompanied by proper management, such as supplemental fertilization and reduced compaction in the root growth region.

Table 1: Contents of Cr, Cu, Mn, Zn, Pb, Cd, As, Ca, K, Fe, Mg and Pi, in leaves and roots of *C. grandis*, T₀ (control) and T₁ (treatment with tailings).

Element ($\mu\text{g g}^{-1}$ DW)	Leaves		Roots	
	T ₀	T ₁	T ₀	T ₁
Cr	1.78 b	24.05 a	11.95 b	29.33 a
Cu	44.03 a	21.46 b	97.75 a	69.37 a
Mn	76.74 b	157.37 a	38.16 a	26.36 b
Zn	34.18 a	28.52 b	10.80 b	12.67 a
Pb	nd	nd	nd	nd
Cd	nd	nd	nd	nd
As	nd	nd	nd	nd
(mg g⁻¹ DW)				
Ca	10.98 a	12.21 a	9.02 a	5.93 a
K	21.08 a	13.08 b	19.51 a	1.76 b
Fe	1.08 b	1.62 a	1.48 b	5.39 a
Pi	2.19 a	0.56 b	2.98 a	0.15 b
Mg	4.10 b	21.53 a	13.23 a	1.77 b

*Means followed by the same letter were not significantly different at 5% probability.

Nd-means no detected

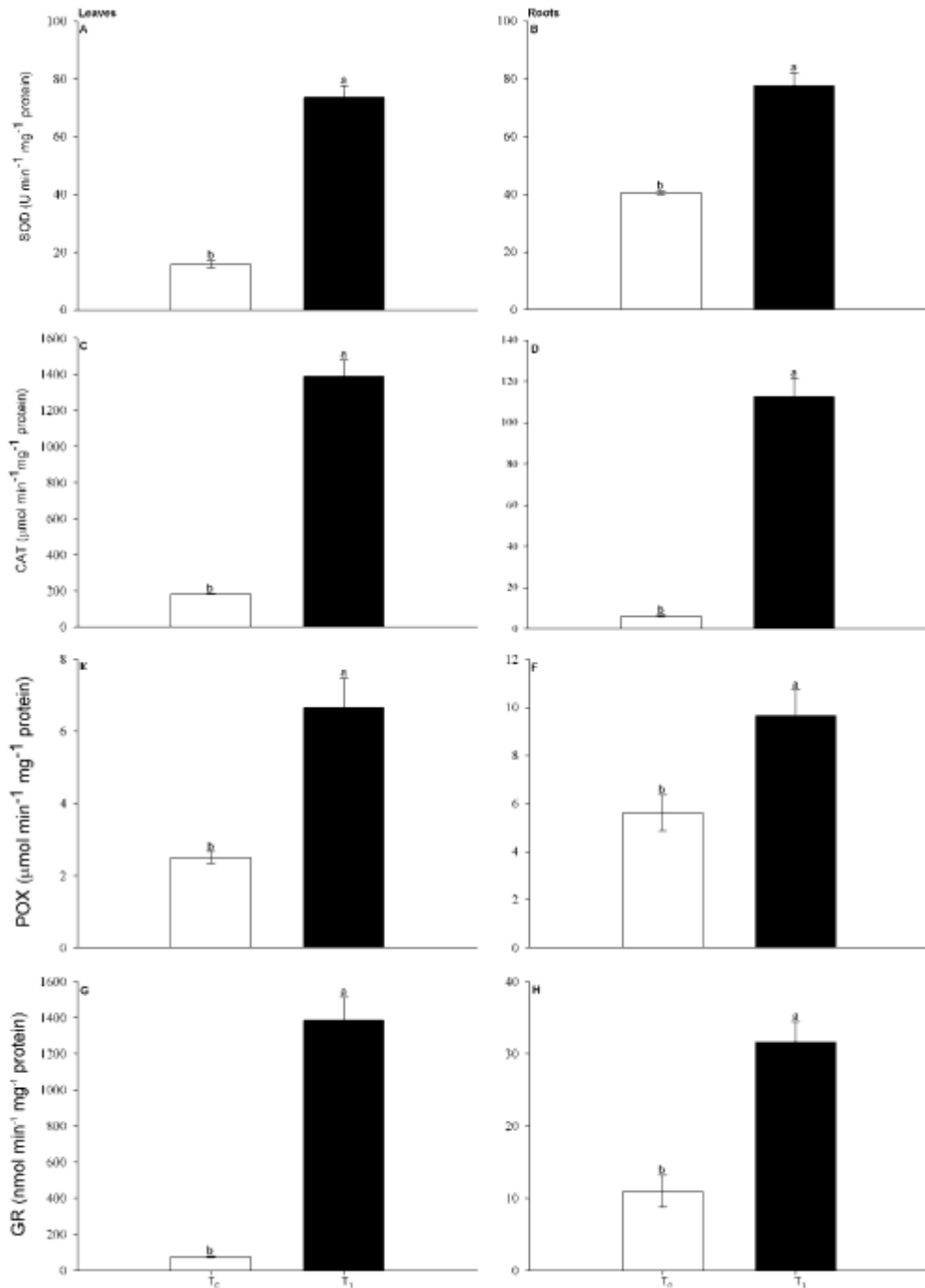


Figure 1: Antioxidant enzyme activity, SOD (A and B), CAT (C and D), POX (E and F) and GR (G and H) in leaves and roots of *C. grandis* in *C. grandis* grown in commercial substrate (T₀) and mining tailings (T₁). Means ± standard error followed by the same letter in each tissue of the plant were not significantly different at 5 % probability by T test.

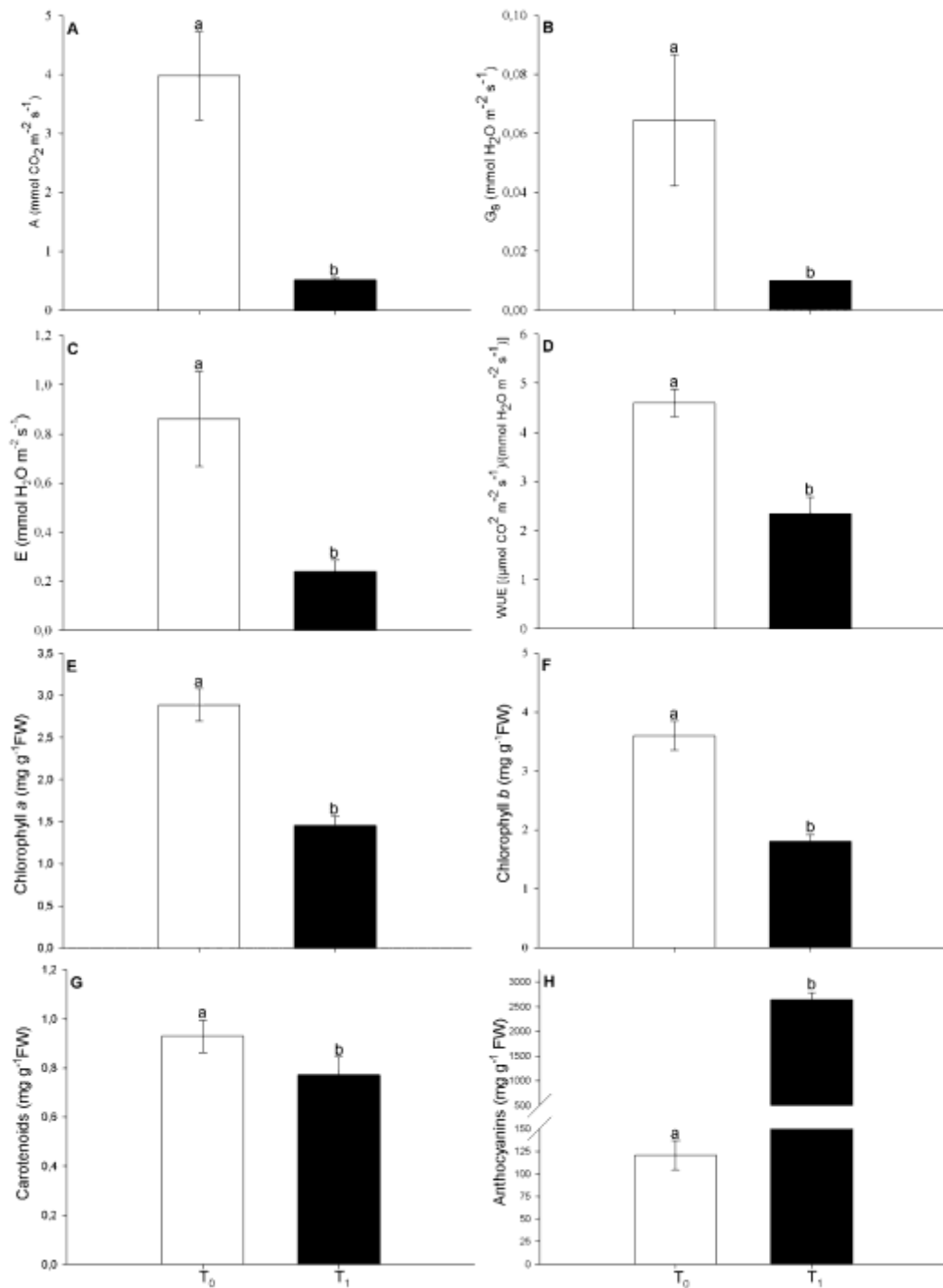


Figure 2: Rates of photosynthesis (A), stomatal conductance (B), transpiration (C) rates, water use efficiency (D) and contents of chlorophyll *a* (E), *b* (F), carotenoid (G) and anthocyanin (H) in *C. grandis* grown in commercial substrate (T₀) and mining tailings (T₁). Means ± standard error followed by the same letter were not significantly different at 5 % probability by T test.

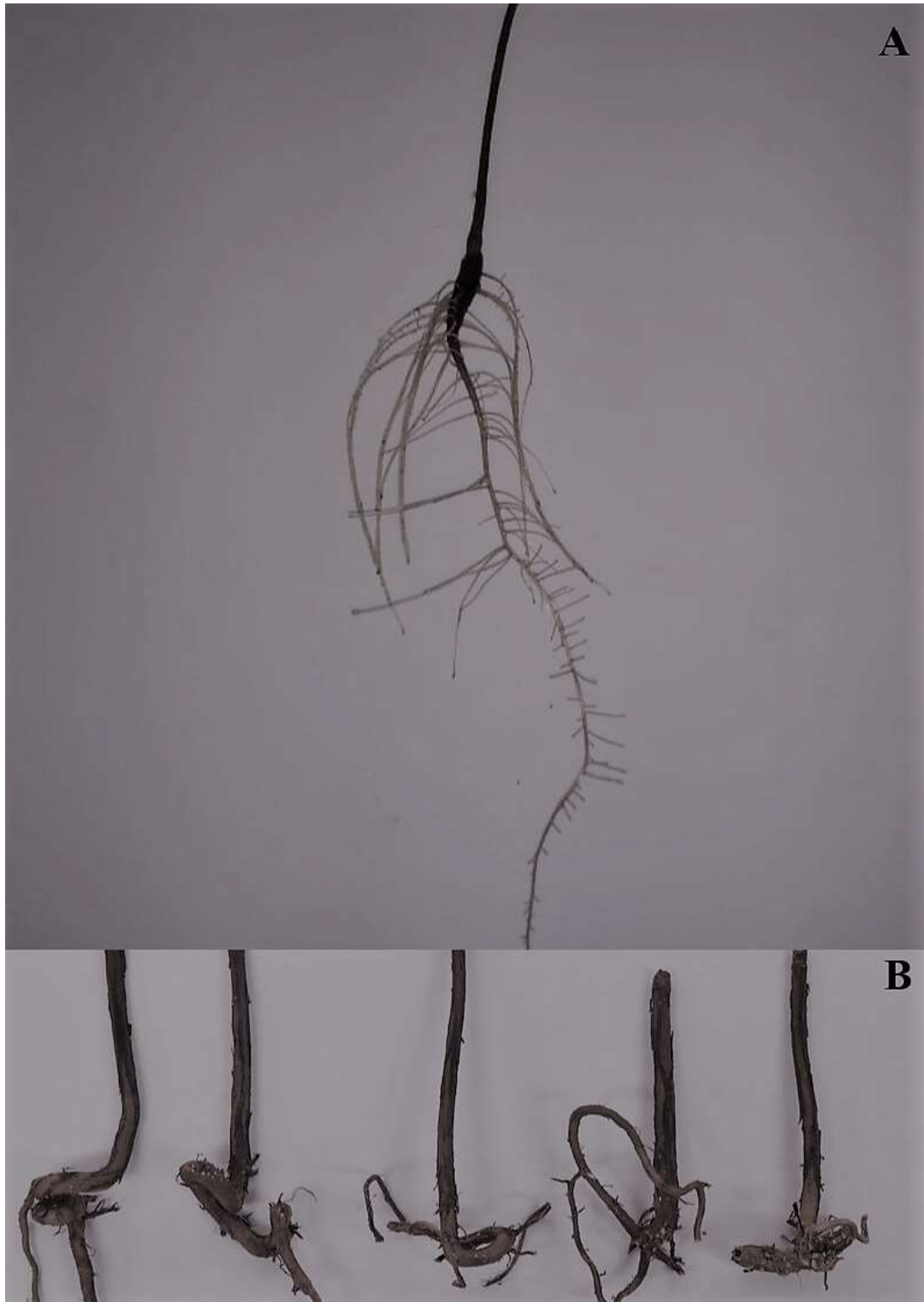


Figure 3: Morphological aspects of the roots of *C. grandis* subjected to commercial substrate (A) and mining tailings (B).

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Article 2: Could arbuscular mycorrhizal fungi improve morphophysiological's aspects of *Cassia grandis* grown in iron mining tailings?

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Acknowledgments

The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001, Fundação de Apoio à Pesquisa de Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support.

Formatado de acordo com as normas da revista “Environmental Monitoring and Assessment”

ABSTRACT

Arbuscular mycorrhizal fungi (AMF) can influence the survival and metabolism of plants grown in mining tailings. The objective was to evaluate the effects of AMF inoculation on the concentration of metals and physiological aspects of *Cassia grandis* seedlings growing in mining tailings. The *C. grandis* plants were grown in mining residue, with four treatments: T₁ (without inoculum), T₂ (with native forest inoculum), T₃ (with mining waste inoculum) and T₄ (with mining tailing inoculum and laboratory mix inoculum), for 4 months under greenhouse conditions. The anthocyanin and Cu contents was increased in the inoculated plants. On the other hand, there was a decrease in Cr concentration in leaves of the inoculated plants. The decrease in malondialdehyde levels observed in inoculated plants indicates lower membrane damage, when compared to non-inoculated. The hypertrophied cells found in all treatments are related to adaptation to stress, and the higher concentration of carbohydrates may be related to the inoculation of microorganisms. This accumulation can also be related to osmotic protection in response to water limitation, which was probably imposed by high density of particles from the mining tailing, which exceeds the limit of 3 g cm⁻³. Based on the results, the inoculation reduces the damage to the membrane, and even a low rate of photosynthesis, it continues to produce photoassimilates and causes morphological adaptations, as cell differentiation. But, is not possible to say that the inoculation process is effective in minimizing all the damages arising from the cultivation on mining tailings.

Keywords: Bioremediation, Heavy Metals, Plant Physiology, Oxidative Stress.

INTRODUCTION

Mining activity contributes to the release of various elements, such as arsenic (As), iron (Fe), manganese (Mn) and chromium (Cr) that may alter the physical and chemical soil characteristics (Guerra et al. 2017; Aires et al. 2018). In addition, mining tailings have few nutrients and low microbial activity, making it difficult to establish vegetation cover in contaminated soils (Grandlic et al. 2008).

The mining activity generates, as by-products, waste that contains high concentrations of various heavy metals. In Brazil, as a major producer of iron ore, it consequently generates an enormous amount of mining tailings, which are mostly disposed in containment dams. Although they are considered rare events, the rupture of these is considered a rare event, however, in the last four years, Brazil has witnessed the rupture of two large tailings dams, in the Mariana region (2015) and another in 2019, in Brumadinho, Minas Gerais. These tailings caused severe damage to the aquatic and terrestrial environments, affecting the entire balance of ecosystems. (Figueiredo et al. 2019; Thompson et al. 2020).

Among the recovery strategies of contaminated soils, phytoremediation has been highlighted as an alternative for stabilization and regeneration of these environments (Guo et al. 2014). However, it is not a simple process, since chemical, physical and biological characteristics may be limiting factors for the growth of the chosen species (Guimarães et al. 2017). The chemical factors, how the high concentration of metals available in the environment maybe are responsible for physiological and metabolic changes in plants, such as oxidative stress, decreased growth, photosynthetic rates and increase activity of antioxidant enzymes (Ayangbenro and Babalola 2017).

The tree species of *Cassia grandis* L., belonging to the Fabaceae family and subfamily Caesalpinioideae, appears as an alternative, since its tailings development has already been studied and obtained good results (Guimarães et al. 2017, Matos et al. 2020). Because it is a leguminous, it has the ability to associate with microorganisms in its roots and these interactions with them, such as mycorrhizal fungi, protect the plant from contaminants and promote its growth by increasing the absorption of nutrients by the roots and fixing nitrogen air in degraded soils (Scoriza and Correia 2019).

Mycorrhizal associations between plant roots and arbuscular mycorrhizal fungi (AMF) enhance soil exploitation through the hyphal network formed by the AMF that colonizes the roots, functioning as an extension of the root system, increasing water and nutrient uptake (Soares and Siqueira 2008; Douds Jr et al. 2010), showed positive impact

on gas exchange rates, in addition to tolerating biotic and abiotic stresses more efficiently (Asmelash et al. 2016; Chandrasekaran et al. 2019). The benefits of this association relate to the important role in ecosystem functioning and the importance of maintaining biodiversity (Van der Heijden and Hartmann 2016). The beneficial of AMF inoculation has already been observed in different plant species, even substrates containing tailings and trace elements (Leal et al. 2016, Stoffel et al. 2016, Wu et al. 2020).

The resistance of microorganisms to heavy metals, such as cadmium (Cd) and lead (Pb), and their effects on bioremediation potential has been the subject of studies (Hao et al. 2014). Inoculum can generally be isolated in nodules of some leguminous in contaminated sites (Teng et al. 2015), increasing the uptake of metals present in the medium (Hao et al. 2014). Plants inoculated with microorganisms previously isolated from contaminated soils resulted in increased plant growth and metal accumulation (Lampis 2015).

Thus, the objective of this study was to evaluate the effect of inoculation on physiology, anatomic characteristics and possibility to use *C. grandis* seedlings associated with AMF in mining tailings for revegetation.

MATERIALS AND METHODS

Plant material

The experiment was conducted in greenhouse conditions at the Universidade Federal de Viçosa, Viçosa-MG, Brazil (20 ° 45'14 "S, 42 ° 52'55" W, 650 m), from January to June 2018. The climate is classified as Cwa type, classified according to Koppen, humid subtropical, with average annual temperature of 21.8 °C and 1,361 mm of average annual precipitation (Alvares et al. 2013). Seeds of *Cassia grandis* were immersed in H₂SO₄ (98 %), for 15 min, washed with deionized water, germinated in sand, transplanted to tubes containing commercial substrate with 5 % vermiculite and 5 % sugarcane bagasse and addition of arbuscular mycorrhizal fungi (AMF), 10 % w/v from different inoculum (Table 1). After thirty days of cultivation, five seedlings (with approximately 20 cm of shoot length). Seedlings, with 20 cm of length, were transplanted to bags containing 1 kg of mining residue, collected in Paracatu de Baixo, Minas Gerais, Brazil, (20 ° 18'16.1 "S, 42 ° 52'55" W), pH 6,9 and 13,40 e 5,02 µg g⁻¹ Mn and Fe⁻¹ in exchangeable fraction and kept for 4 months under greenhouse conditions. The experimental design was completely randomized with four treatments and five replications, each experimental unit with one plant per bag.

Metals determination

Plants were washed with deionized water and roots were treated with DBC (Taylor and Crowder 1983) to remove iron oxides adsorbed and dried to constant dry mass. About 100 mg of the dry mass were mineralized in 1.5 mL of nitric-perchloric acid mixture (2:1) and extract used in the determination of Fe, Mn, Zn, Cu, Cr, Cd, Pb, Ca, K and Mg concentration by atomic absorption spectrophotometry (Shimadzu AA-6701F). For arsenic (As) determination a hydride vapor generator was used. The inorganic phosphate content in the supernatant was determined using the phospho-molybdate method (Lindeman 1958).

Photosynthesis determination

Photosynthetic rate (A), stomatal conductance (g_s) and transpiration (E) were measured with an infrared gas analyzer system (IRGA, LCpro-SD-ADC BioScientific Ltd.) on fully expanded leaves from the third node, using light intensity of $600 \mu\text{mol m}^{-2} \text{s}^{-1}$. The CO_2 concentration inside the leaf chamber, air humidity and temperature fluctuated according to ambient conditions. Data were collected from 7:00 to 10:00 at three-minute intervals. The measurement area of leaf chamber was calculated using ImageJ, resulting in 5.35 cm^2 .

Pigment contents

The chlorophyll a , b and carotenoid contents were determined by dimethylsulfoxide (DMSO) method. The absorbance was determined by spectrophotometer (UV-Visible Quimis 0790U2M) and the values used to calculate the chlorophyll a , b and carotenoids levels (Wellburn 1994). Samples of 0.1 g fresh leaves were homogenized in 5 mL methanol-HCl 1 % (v/v) in ice bath and centrifuged at 10,000 g for 30 min at 4 °C. The total anthocyanin content was determined by the difference between the absorbance at 536 and 600 nm (Hodges et al. 1999) and the results expressed as mg g^{-1} FW, as cyanidin-3-glucoside equivalents, by the coefficient molar extinction ($26900 \text{ L cm}^{-1} \text{ mol}^{-1}$).

Determination of antioxidant enzymes activity

The superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and glutathione reductase (GR) were extracted from 0.3 g of fresh roots and leaves. The samples were macerated in liquid nitrogen, followed by addition of 2 mL extraction medium, filtration and centrifugation at 12,000 g , 15 min, at 4 °C. The supernatant was used as crude extract in the determination of the enzymatic activities. All extraction steps were performed at 4 °C. The extraction media were: a) 0.1 M potassium phosphate buffer

pH 6.8, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethylsulfonic fluoride (PMSF) and 1 % (w/v) polyvinylpyrrolidone (PVPP), for SOD, CAT and POX; 2 mL of 0.1 M potassium phosphate buffer, pH 7.0, 1 mM EDTA (ethylene diamine tetraacetic acid) 0.02 % triton, 2 mM DTT (dithiothreitol), 1 mM PMSF and PVPP (polyvinyl pyrrolidone) 1 %, for GR (Carlberg and Mannervik 1985). The protein contents of the enzyme extracts were determined with BSA as the standard to express enzyme activity (Waterborg and Matthews 1984).

Superoxide dismutase activity

The SOD activity was determined with the addition of 50 μ L of the crude enzyme extract to 5 mL of reaction medium containing 50 mM sodium phosphate buffer pH 7.8 containing 13 mM methionine, p-nitro tetrazolium blue (NBT) 75 μ M, 0.1 mM EDTA and 2 μ M riboflavin. The reaction was conducted at 25 °C in a reaction chamber under 15 W fluorescent lamp illumination, kept inside a box. After 5 min exposure illumination was discontinued and the blue formazan, produced by NBT photoreduction, was measured by the absorbance at 560 nm (Giannopolitis and Ries 1977). A unit of SOD was defined as the amount of enzyme required to inhibit the NBT photoreduction by 50 % (Beauchamp and Fridovich 1971).

Peroxidase activity

The POX activity was determined with the addition of 0.1 mL of the crude enzyme extract to 4.9 mL of reaction medium containing 25 mM potassium phosphate buffer, pH 6.8, pyrogallol 20 mM and 20 mM H₂O₂ (Kar and Mishra 1976). Purpurogalin production was determined by increasing the absorbance during the first minute of reaction at 420 nm at 25 °C. The enzymatic activity was calculated using the molar extinction coefficient 2.47 mM L⁻¹ cm⁻¹ (Chance and Mchale 1955) and expressed in μ moles of H₂O₂ min⁻¹ mg⁻¹ protein.

Catalase activity

The CAT activity was determined with the addition of 0.1 mL of the crude enzyme extract to 2.9 mL of reaction medium containing 50 mM potassium phosphate buffer pH 7.0 and 12.5 mM H₂O₂ (Havir and Mchale 1987). The decrease in absorbance at 240 nm and 25 °C was measured in the first reaction minute. The enzymatic activity was calculated using the molar extinction coefficient 36 M⁻¹ cm⁻¹ (Anderson et al. 1995) and expressed in μ mol H₂O₂ min⁻¹ mg⁻¹ protein.

Glutathione reductase activity

The GR activity was determined with the addition of 0.1 mL of the crude enzyme extract to 0.9 mL of reaction medium containing Tris-HCl 0,1 M, pH 7,5, GSSG 20 mM and NADPH 2 mM (Carlberg and Mannervik 1985). The decrease in absorbance at 340 nm and 30 °C was measured in the first reaction minute. The enzymatic activity was calculated using the molar extinction coefficient $6.22 \text{ mM}^{-1} \text{ cm}^{-1}$ and expressed in nmol of oxidized glutathione $\text{min}^{-1} \text{ mg}^{-1}$ protein.

Hydrogen peroxide (H_2O_2) contents

Samples of 0.2 g fresh root and leaf mass were ground in liquid nitrogen and homogenized in 2.0 mL of 50 mM potassium phosphate buffer, pH 6.5, containing 1 mM hydroxylamine. After filtration, the homogenate was centrifuged at 10,000 g, for 15 min, at 4 °C, and the supernatant was collected (Kuo and Kao 2003). 50 μL aliquots of the supernatant were added to a reaction medium consisting of 100 μM $\text{FeNH}_4(\text{SO}_4)$, 25 mM sulfuric acid, 250 μM xylenol orange and 100 mM sorbitol in a final volume of 2 mL (Gay and Gebicki 2000). Samples were kept in the dark for 30 min and the absorbance determined at 560 nm. Color whites of the reagents and plant extracts were prepared in parallel and subtracted from the sample. The H_2O_2 contents in the samples were estimated based on calibration curve prepared with H_2O_2 standards and expressed in $\mu\text{mol g}^{-1}$ FW. Malondialdehyde analysis

Malondialdehyde (MDA) concentration was measured following the procedure of Hodges et al. (1999). Frozen leaf tissue was homogenized in 80 % cold ethanol and centrifuged to pellet debris. Different aliquots (1 ml each) of the supernatant were mixed either with 1 ml trichloroacetic acid (20 %) or with 1 ml trichloroacetic acid (20 %), containing 0.5 % thiobarbituric acid. Both mixtures were allowed to react in a water bath at 90 °C for 1 h. The samples were cooled in an ice bath and centrifuged. Absorbance of the supernatant was read at 440, 532 and 600 nm against a blank. MDA concentration was expressed in terms of nmol g^{-1} FW (Adhikari et al. 2020).

Microscopy analysis

Mature leaves from the third node with no visual symptoms of toxicity ($n=2$) were collected, fixed in FAA (formalin, acetic acid, 50 % ethanol, 1:1:18 by volume) and stored in 70 % ethanol (Johansen 1940). Fragments were dehydrated in ethylic series, included in methacrylate (Historesin, Leica Instruments) and cross-sectioned on an automatic advance rotary microtome. The sections were stained with 0.05 % toluidine blue O (pH 4.0) (O'Brien et al. 1964), and mounted on slides using synthetic resin (Permount, Fisher

Scientific, New Jersey, USA). Reaction with periodic acid - Schiff's reagent (PAS) with adequate control, for the detection of total carbohydrates (O'Brien and McCully, 1981). Scanned images were obtained under a microscope (model AX70 TRF, Olympus Optical) with U-Photo system, coupled to the digital photographic camera (model Spot Insightcolour 3.2.0, Diagnostic Instruments Inc.) and microcomputer with the image capture of Spot Basic program.

RESULTS

The height, diameter and height/diameter ratios of the plants subjected to treatments with inoculation did not differ from each other (Table 2). The concentration of Cu increased in leaves and roots, and those of Mn and Mg decreased in leaves when compared with T₁. Chromium contents decreased in leaves and increased in roots in treatments with inoculation, compared to T₁. No detection of Pb, Cd and As in plant tissues (Table 3).

Activities of SOD and GR was not influenced by the treatments. However, there was a reduction in CAT activity, in leaves and roots, and POX, in roots, of T₃ and T₄ (Figure 1). The inoculation promoted a reduction in the levels of MDA, in leaves and roots, and of H₂O₂, in leaves, and an increase in the levels of H₂O₂ in roots, when compared with T₁ (Figure 2).

The gas exchange rates in inoculated *C. grandis* did not differ between themselves and in relation to the control. The assimilation of CO₂ was very low, remained between 0.43 and 0.64 $\mu\text{mol m}^{-2} \text{s}^{-2}$. When analyzed as transpiration rates (E), it was observed that the T₂ is 62 % higher in relation to T₁. On the other hand, the T₁, T₃ and T₄ procedures were considered to be better in water use (WUE) (Figure 3).

The levels of chlorophyll *a*, *b* and carotenoids were not influenced by the treatments. Anthocyanin levels increased in treatments compared to T₁ (Figure 4).

The leaf of *C. grandis* has a uniseriate epidermis, with papillae abaxial face cells, with the presence of anthocyanin and uniseriate and multicellular trichomes. The leaf is hypostomatic. The epidermis of the leaf's adaxial face has square cells with a thin layer of cuticle. The mesophyll is formed by a layer of palisade parenchyma, with few intercellular spaces and three to four layers of spongy parenchyma. The vascular bundles are collateral with a fiber cap around them. Visually, plant exposure to mining tailings promoted alteration in the pattern of mesophyll division that caused cell hypertrophy in the palisade and spongy parenchyma in the leaves (Figure 5B-C) in comparison to

reference plants (Figure 5A). Occurred starch grains in the mesophyll cells, evidenced by reaction to Schiff, with a visual lower proportion in hypertrophied cells. The inoculated plants showed less thickness of the leaf mesophyll, especially in T4 (Figure 5 F-H).

DISCUSSION

When compared with *Cassia grandis* cultivated in commercial substrate, was observed increment in Cr, Cu, Mn and Mg concentrations in leaves, and Fe and Cr concentrations in roots. On the other hand, decreased of concentration of Zn, K, Pi in leaves, and Mn, Ca, K, Pi, Mg in roots (Matos et al. 2020). With the substrate used, composed of iron mining tailings, indicating that the plant absorbed a large amount of this element, which may have influenced the absorption of P and Mn (Jucoscki et al. 2016). All the elements cited, was important, in appropriate concentrations, for maintaining of plant nutrition. The AMF, which form associations with plant roots (Zhang et al. 2015), can influence the plant community (Lau and Lennon 2011), especially associated with leguminous species, allowing to increase the absorption of nutrients and water, as well as increasing tolerance to environments with high metals concentrations (Garcia et al. 2016).

The increase and reduction of Cr levels, respectively in roots and leaves, of plants inoculated in relation to T1, can indicates the influence of AMF in the absorption and accumulation of this metal. The charges on its surface makes it excellent biosorbents which can complex and immobilize elements in the roots of plants (Srivastava et al. 2015). Some unstable Cr states can be formed during their assimilation process, and these are responsible for the formation of free radicals, inducing oxidative stress (Sinha et al. 2018). Thus, the decrease in the translocation of metals to the aerial part, decreases the oxidative stress in these organs.

High concentrations of Cu observed in leaves and roots of inoculated plants can affect metabolism, due to its ability to catalyze reactions that form ROS, lipid peroxidation and damage the photosynthetic apparatus (Ayangbenro and Babalola 2017).

Usually, plants grown in mining tailings, have high antioxidative enzyme activity (Saadani et al. 2016, Delplace et al. 2020). However, in this study it was observed decrease in POX activity values in roots and CAT in roots and leaves, in inoculated plants, probably the was due to use at the other antioxidant mechanisms, such as the use of anthocyanins, also observed in other plants (Chu et al. 2018). The decreased in MDA

levels, observed in inoculated plants, indicates lower effects of lipid peroxidation in these plants, when compared to T₁, was observed in other studies (Pollastri et al. 2018).

Anthocyanins playing an important role in oxidant process, being used as hydrogen donors, inhibiting the superoxide anion (Talaat 2015). Thus, the presence of greater content of anthocyanin in plants inoculated, may indicate efficient antioxidant protection.

The metals Cr, Cu, Fe, Mn accumulation in plant leaves results in damage to the photosynthetic apparatus, influencing the carboxylation process (Santos et al. 2017), leading to a lower production of photoassimilates and decreased plant growth (Lahive et al. 2018). However, for the treatments analyzed, there was no difference in photosynthetic parameters expected in T₂, which presented reduction in the WUE rate and an increase in E, interestingly it would be expected that these parameters were affected, because high density of particles from the mining tailing, which exceeds the limit of 3 g cm⁻³ (Figueiredo et al. 2019) and restricting of the main root growth (Matos et al. 2020).

High compaction limited the availability of water and nutrients, leading to stomatal closure and reduced CO₂ fixation, since the healthy plants, growing in commercial substrate was observed a reduction of 90% approximately in the photosynthetic rate (Matos et al. 2020). These results may explain the no difference in photosynthetic parameters in T₂, which presented reduction in the WUE rate and an increase in E, due to were affected, because high density of particles.

The morphoanatomical damages identified, corroborate with all the other data obtained, indicating the presence of stress, to which the plants are subjected. Recognizing such damage caused by the response to stress, as well as the morphological and physiological characteristics affected, is fundamental for building a better performance analysis of tolerant genotypes in the field (Pandey et al. 2017). As hypertrophied cells found, they are related to adaptation to stress, as was observed in the leaves of native species of the Atlantic Forest (Andrade and Silva 2017).

The higher concentration of carbohydrates may be related in leaves of inoculated treatments to the inoculation and show that the vascular bundle is intact. Arbuscular mycorrhizal fungi stimulate ATPases of the root system, allowing a greater flow of carbohydrates allowing greater bi-directional transfer of nutrients and water (Pinos et al. 2019). This accumulation can also be related to osmotic protection, since the plant, when water is limited, can accumulate organic solutes and ions in order to maintain its hydration (Sourour et al. 2017).

Based on the results, AMF inoculation minimizes the stress generated by the cultivation in mining tailings, since reduces the damage to the membrane (MDA contents) and even with the plant with a low rate of photosynthesis, it continues to produce photoassimilates and causes morphological adaptations, as cell differentiation.

CONCLUSIONS

The data obtained on its physiological and anatomical changes are of great importance, since few studies have been carried out with this species grown in an area contaminated with iron mining tailings. The treatments were not presented differences among inoculated and non-inoculated plants. It's expected inoculation promotes advantages to the plants, however, in this experiment it was not possible to verify these benefits, probably, due to physical and chemical limitations presented by the mining tailings, on the root's development limitation.

Although some results obtained are satisfactory and make us believe that this plant could survive in such conditions, it was not possible to identify the benefits arising from the association of arbuscular mycorrhizal fungi with the root system of these plants.

Table 1: Inoculum prepared for colonization of *Cassia grandis* roots for later growth in mining tailing

Treatment	Inoculum
Treatment 1	Substrate without inoculum.
Treatment 2	Substrate prepared with vermiculite, sugarcane bagasse and native forest inoculum of arbuscular mycorrhizal fungi (1:1:1).
Treatment 3	Substrate prepared with vermiculite, sugarcane bagasse and tailings inoculum of arbuscular mycorrhizal fungi (1:1:1).
Treatment 4	Substrate prepared with vermiculite, sugarcane bagasse, tailing and laboratory microbial culture of inoculum arbuscular mycorrhizal fungi (1:1:1:1).

Table 2: Height (H), diameter (D) and ration H/D of *Cassia grandis* seedlings grown on mining tailing without inoculum (T₁), with native forest soil inoculum (T₂), tailing inoculum (T₃), tailing with laboratory microorganism culture (T₄)

Treatment	H (cm)	D (mm)	H/D
T ₁	8,10±0,91	1,93±0,22	4,25±0,39
T ₂	8,20±0,52	1,84±0,24	4,65±0,61
T ₃	7,20±0,45	1,84±0,16	4,05±0,56
T ₄	7,00±0,91	1,91±0,24	3,56±0,58

*Values are represented by means ± standard error of five repetitions. Means followed by the same letters do not differ by the Tukey's test ($p < 0,05$).

Table 3: Contents of Cr, Cu, Mn, Zn, Pb, Cd, As, Ca, K, Fe, Pi and Mg in leaves and roots of *Cassia grandis* grown in mining tailing without inoculum (T₁), with native forest soil inoculum (T₂), tailing inoculum (T₃), tailing with laboratory microorganism culture (T₄)

Leaves				
µg g⁻¹ DW	T₁	T₂	T₃	T₄
Cr	24.05±0.52	3.40±0.09	7.76±0.18	20.69±0.52
Cu	21.46±0.53	37.51±0.62	36.29±0.68	33.67±0.80
Mn	157.37±2.82	148.29±1.02	150.79±0.54	152.52±2.23
Zn	28.52±0.70	8.45±0.57	8.10±0.49	12.45±0.59
Pb	nd	nd	nd	nd
Cd	nd	nd	nd	nd
As	nd	nd	nd	nd
mg g⁻¹ DW				
Ca	12.21±0.38	13.76±0.32	13.85±0.25	12.48±0.72
K	13.08±0.57	10.99±0.46	12.20±0.38	12.05±0.61
Fe	1.62±0.10	1.51±0.07	1.39±0.13	1.66±0.08
Pi	0.56±0.03	0.63±0.04	0.57±0.05	0.68±0.03
Mg	21.53±0.39	17.13±0.22	13.97±0.75	12.48±0.54
Roots				
µg g⁻¹ DW	T₁	T₂	T₃	T₄
Cr	29.33±1.34	34.43±1.96	35.39±1.56	61.81±0.88
Cu	69.37±0.97	77.34±0.94	95.37±1.26	105.58±1.97
Mn	26.36±0.04	5.98±0.22	4.15±0.15	5.18±0.08
Zn	12.67±0.28	11.82±0.18	10.83±0.13	10.46±0.56
Pb	nd	nd	nd	nd
Cd	nd	nd	nd	nd
As	nd	nd	nd	nd
mg g⁻¹ DW				
Ca	5.93±0.37	7.83±0.11	6.67±0.16	7.06±0.20
K	1.76±0.03	2.65±0.16	2.43±0.08	2.47±0.12
Fe	5.39±0.11	4.88±0.15	4.80±0.28	4.76±0.41
Pi	0.15±0.01	0.15±0.01	0.15±0.01	0.12±0.01
Mg	1.77±0.09	1.81±0.03	1.99±0.05	2.70±0.12

*Values are represented by means ± standard error of five repetitions.

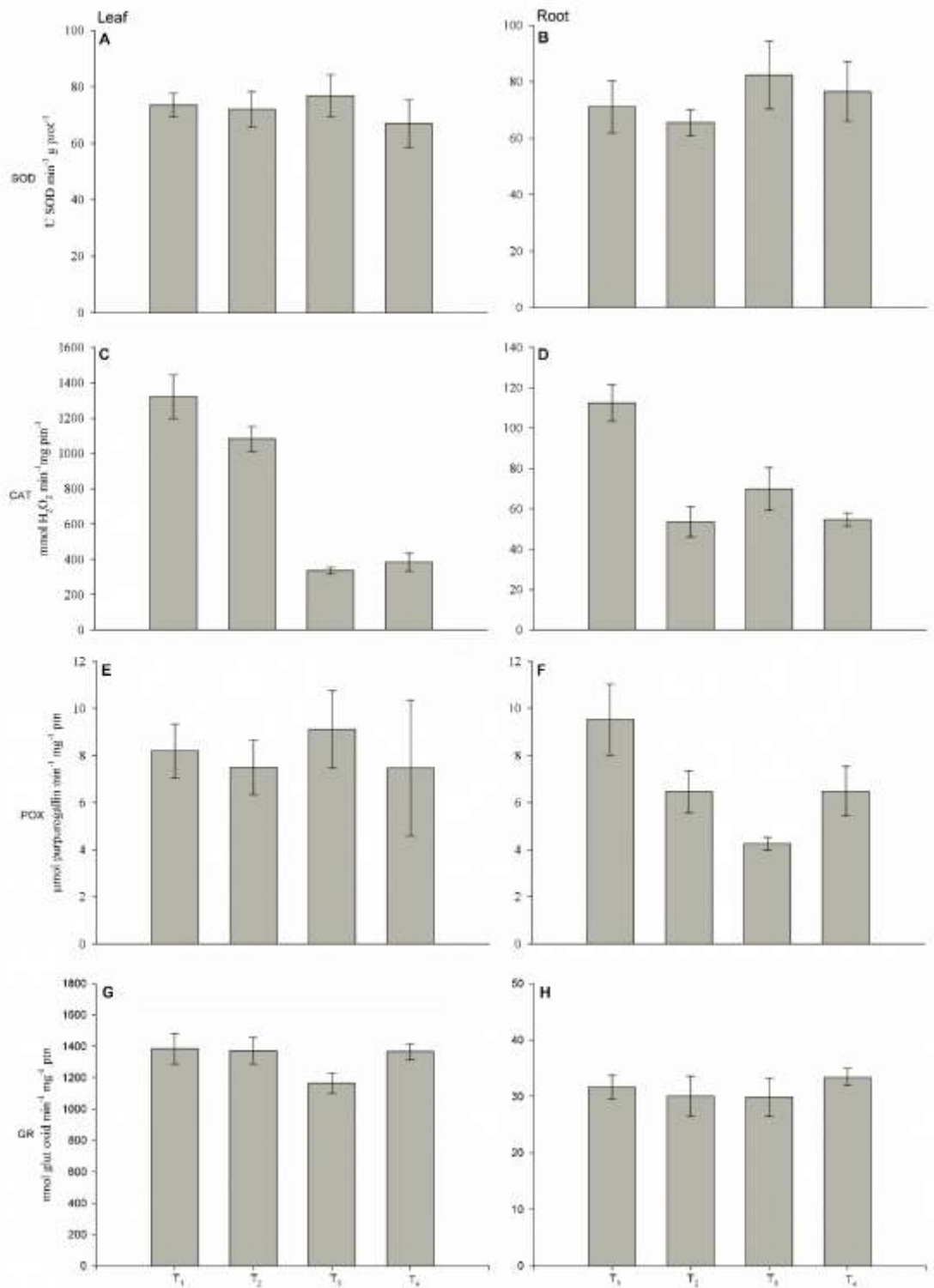


Figure 1: Antioxidant enzyme activity, SOD (A and B), CAT (C and D), POX (E and F) and GR (G and H) in leaves and roots of *Cassia grandis* grown on mining tailing without inoculum (T₁), native forest soil inoculum (T₂), tailing inoculum (T₃), tailing with laboratory microorganism culture (T₄).

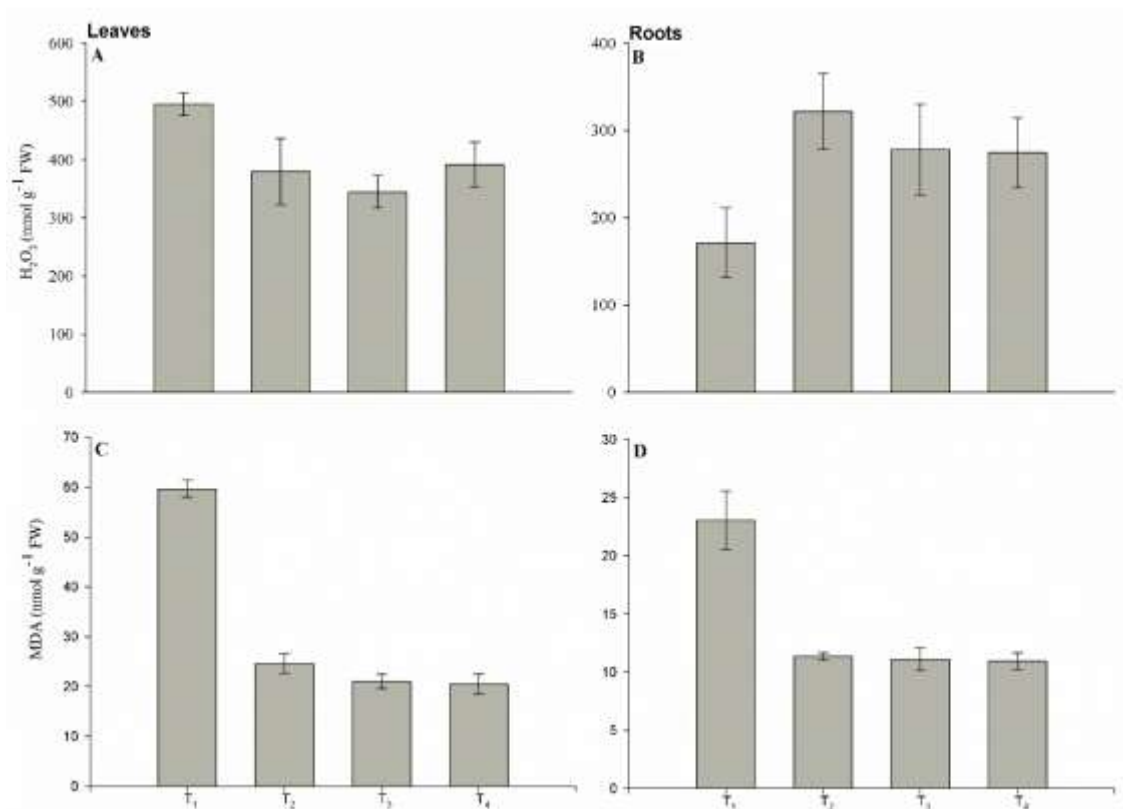


Figure 2: Hydrogen peroxide and MDA contents in leaves and roots in *Cassia grandis* grown on mining tailing without inoculum (T₁), native forest soil inoculum (T₂), tailing inoculum (T₃), tailing with laboratory microorganism culture (T₄).

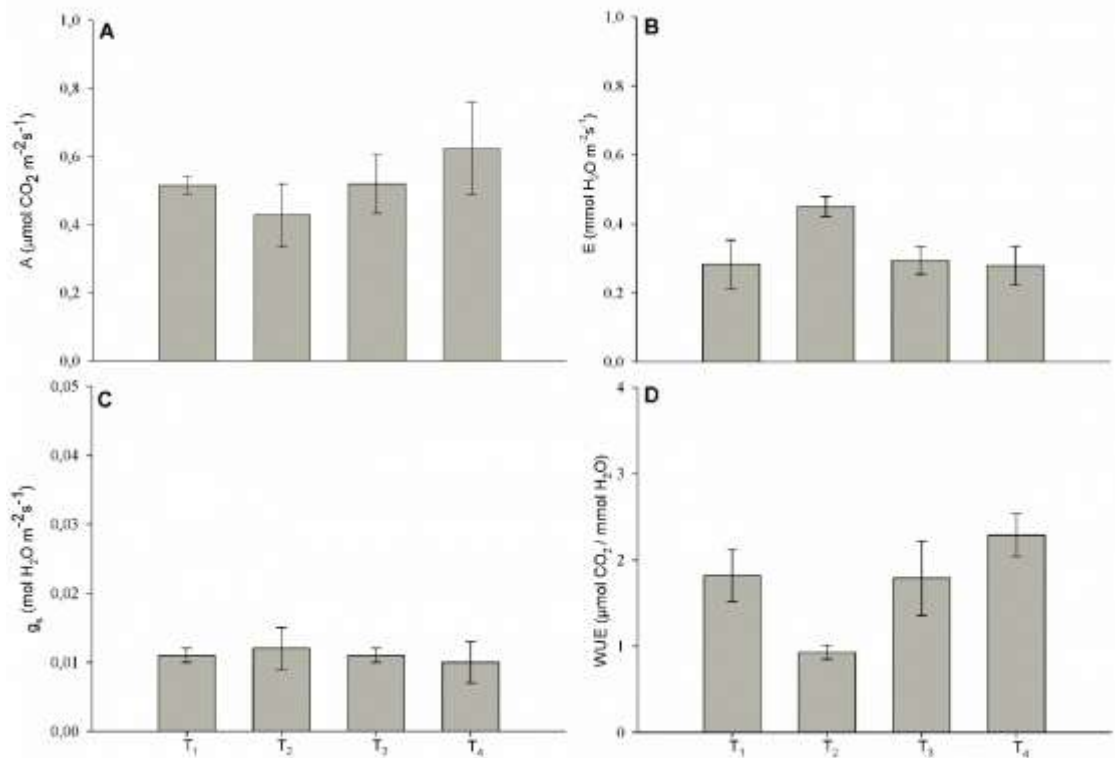


Figure 3: Photosynthesis (A), stomatal conductance (B), transpiration (C) rates, water use efficiency (D) in *Cassia grandis* grown on mining tailing without inoculum (T₁), with native forest soil inoculum (T₂), tailing inoculum (T₃), tailing with laboratory microorganism culture (T₄).

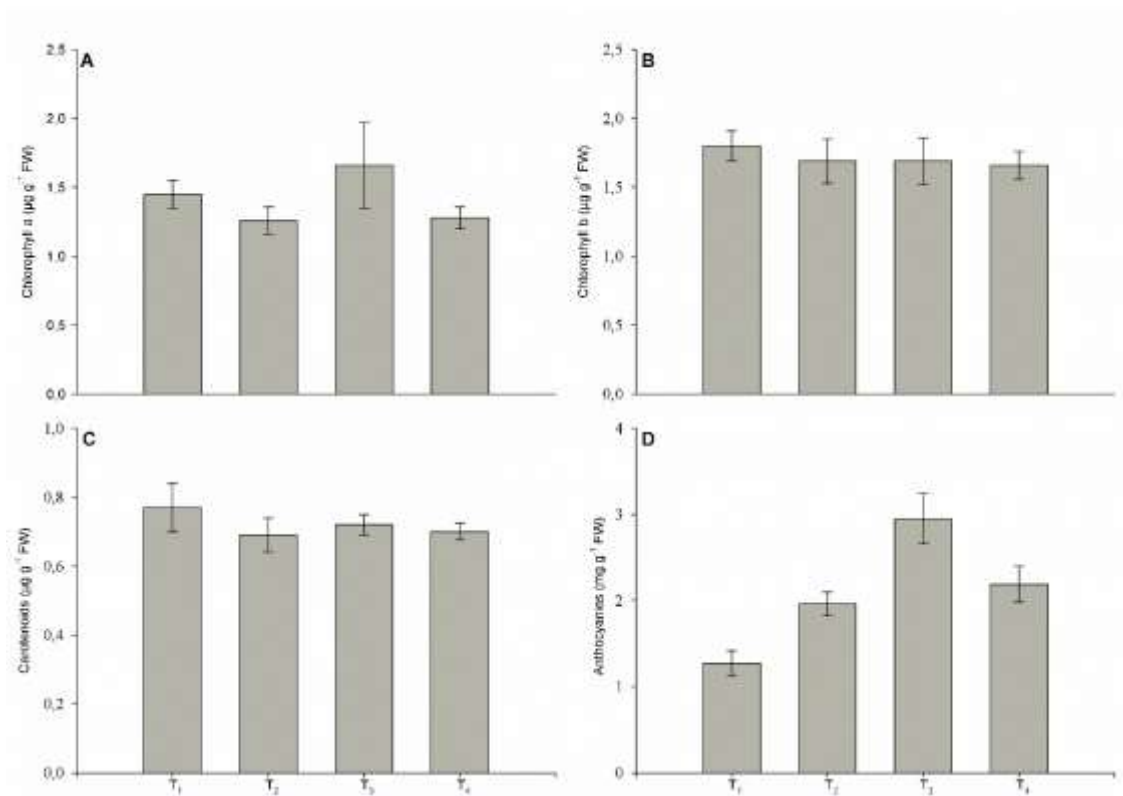


Figure 4: Chlorophyll a (A), b (B), carotenoids (C) and anthocyanins (D) contents in *Cassia grandis* grown on mining tailing without inoculum (T₁), native forest soil inoculum (T₂), tailing inoculum (T₃), tailing with laboratory microorganism culture (T₄).

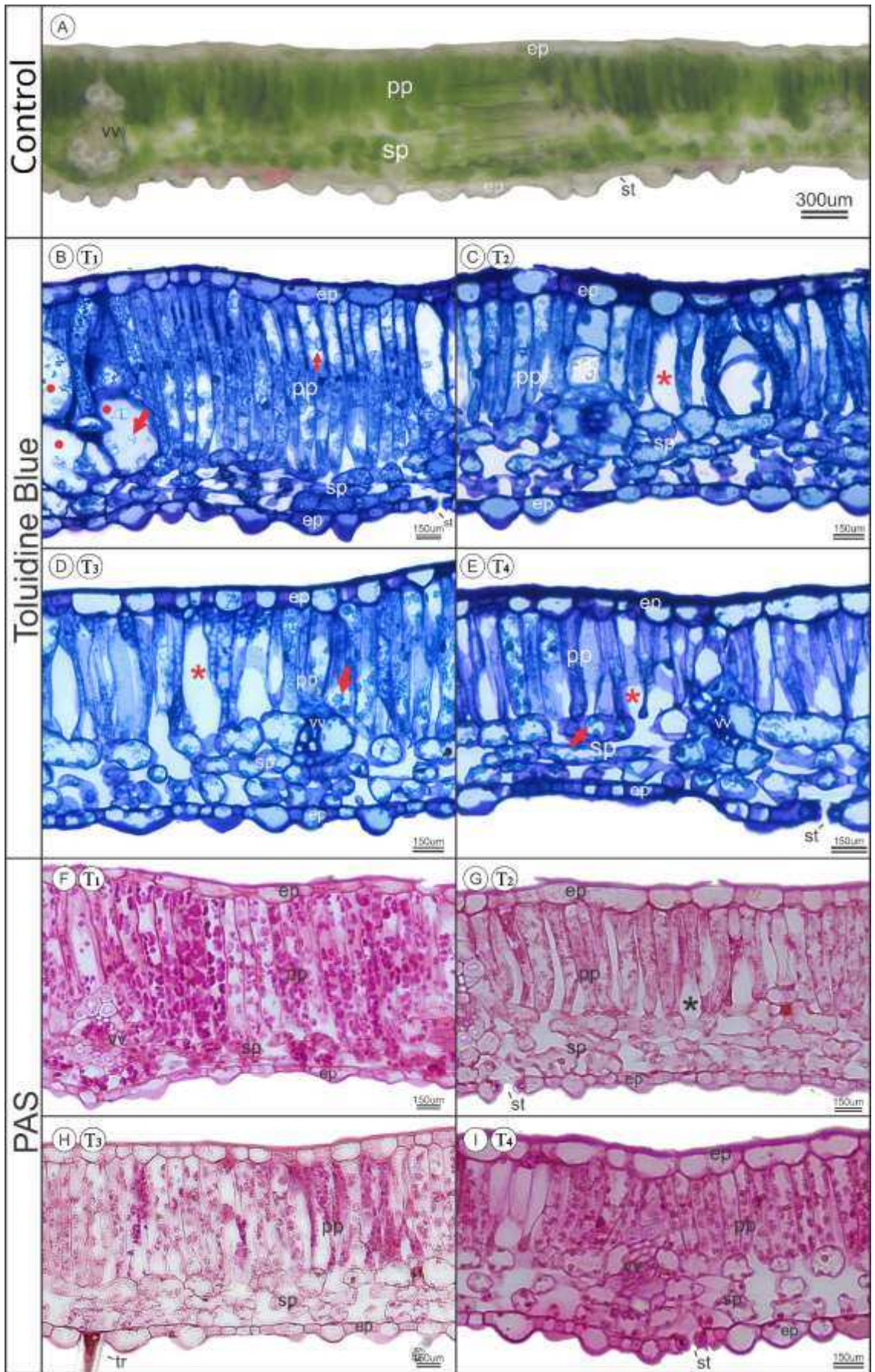


Figure 5: Leaf anatomy of *Cassia grandis* grown on commercial substrate (control), mining tailing without inoculum (B and F, T₁), with native forest soil inoculum (C and G, T₂), tailing inoculum (D and H, T₃) and tailing with laboratory microorganism culture (E and I, T₄). pp – palisade parenchyma; sp – spongy parenchyma; ep – epidermis; st – stomata; vv – vascular vessel; tr – trichome; * – hypertrophied cell; → starch granules.

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CONCLUSÃO GERAL

O processo de revegetação de locais que sofreram impactos ambientais, é de grande importância para o restabelecimento da biodiversidade e das características ambientais. No Brasil, dois grandes rompimentos de barragem de mineração, causaram grandes danos nas regiões afetadas (Mariana em 2015 e Brumadinho em 2019, ambas cidades localizadas no estado de Minas Gerais).

Neste contexto, a introdução de espécies nativas, com características comprovadas de biorremediação, pode ser uma alternativa eficaz, visto que este tipo de rejeito, possui em sua composição uma diversidade de metais. Apesar de já ter sido estudada para revegetação de rejeitos de mineração, a espécie *Cassia grandis*, possui poucos dados acerca de seu comportamento fisiológico e morfológico na literatura.

Os resultados obtidos mostram que, alta compactação do rejeito utilizado, foi o fato limitante do bom desenvolvimento desta espécie. As alterações morfológicas e fisiológicas nos fazem acreditar que com um manejo adequado do solo, *C. grandis* pode ser utilizada para o processo de revegetação destes ambientes.

Assim, a identificação as características fisiológicas e morfológicas e sua maior exploração, foi fundamental para entender o processo de adaptação ao estresse por esta espécie que ainda é pouco explorada e tem um grande potencial de desenvolvimento em ambientes não propícios ao mesmo.

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