ANA LÍVIA MARTINS SCARPA

ECOPHYSIOLOGY AND ANATOMY OF TREE SPECIES WITH POTENTIAL FOR THE REFORESTATION OF AREAS IMPACTED BY MINING TAILINGS

LAVRAS – MG
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Orientador
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ECOFISIOLOGIA E ANATOMIA DE ESPÉCIES ARBÓREAS COM POTENCIAL PARA REFLORESTAMENTO DE ÁREAS IMPACTADAS POR REJEITOS DE MINERAÇÃO

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Botânica Aplicada, área de concentração em Estrutura e Funcionamento das plantas, para obtenção do título de Doutor.

APROVADA em 30 de julho 2021
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2021

À minha mãe Elizabeth, meu eterno amor, gratidão e saudade.

Dedico.

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"Por isso não tema, pois estou com você; não tenha medo, pois sou o seu Deus. Eu o fortalecerei e o ajudarei; eu o segurarei com a minha mão direita vitoriosa". (Isaías 41:10)

RESUMO

Cada vez mais, torna-se necessário o desenvolvimento de medidas que visem mitigar os impactos ambientais e sociais gerados por falhas em barragens de mineração. Estudos apontaram a falta de nutrientes minerais no rejeito de minério de ferro, característica que afeta diretamente o crescimento de mudas de espécies arbóreas. Entretanto, a identificação de espécies arbóreas nativas tolerantes à escassez de nutrientes minerais é vital para a revegetação de áreas degradadas por esses resíduos, bem como para o sucesso deste processo. Sabe-se que as espécies arbóreas *Schinus molle* e *Schinus terebinthifolius* são tolerantes a fatores ambientais adversos e metais pesados, enquanto *Handroanthus serratifolius* e *Handroanthus impetiginosus* são largamente utilizados em programas de recuperação, inclusive em áreas atingidas por rejeito de minério de ferro. Nesse contexto, o objetivo do trabalho foi avaliar a tolerância dessas espécies arbóreas a esse rejeito ou à escassez de nutrientes minerais, quanto à germinação de sementes, o estabelecimento, o crescimento inicial e a anatomia foliar de plântulas crescidas e desenvolvidas na lama de mineração provenientes do desastre do rompimento da barragem de Fundão em Mariana, estado de Minas Gerais, Brasil, acontecido em 2015. Além das respostas de crescimento, foram avaliadas as trocas gasosas e anatomia foliar de plantas de *Handroanthus* spp. após adição de solução nutritiva ao rejeito de mineração. Diante dessa pesquisa, foi possível contribuir com o cenário pós-desastre por rompimento de barragens de minério de ferro, com informações sobre o estabelecimento das espécies estudadas nessa condição.

Palavras-chave: *Schinus* spp.; *Handroanthus* spp.; Recuperação ambiental; Barragem de mineração; Mariana-MG.

ABSTRACT

It is increasingly necessary to develop measures aimed at mitigating the environmental and social impacts generated by failures in mining dams. Studies indicate that the lack of mineral nutrients in mining tailings directly affects the growth of tree species seedlings. However, the identification of native tree species tolerant to mineral nutrient scarcity is vital for the revegetation of areas degraded by these residues, as well as for the success of this process. The tree species *Schinus molle* and *Schinus terebinthifolius* are known to be tolerant to adverse environmental factors and heavy metals, while *Handroanthus serratifolius* and *Handroanthus impetiginosus* are widely used in recovery programs, including areas affected by iron ore tailings. In this context, the objective of the study was to evaluate the tolerance of these tree species to this residue or to the scarcity of minerals, regarding the germination of nutrients, establishment, initial growth and leaf anatomy of seedlings cultivated and developed in the tailings resulting from the collapse of the Fundão dam in Mariana, state of Minas Gerais, Brazil, in 2015. In addition to growth responses, gas exchange and leaf anatomy of *Handroanthus* spp. after addition of nutrient solution to mining tailings. In view of this research, it was possible to contribute to the post-disaster scenario due to the collapse of iron ore dams, with information on the establishment of the studied species in this condition.

Keywords: *Schinus* spp.; *Handroanthus* spp.; Environmental recovery; Iron ore dams; Mariana-MG.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

As atividades de mineração são essenciais para o ser humano, mas ao mesmo tempo, como outras atividades antrópicas, embora bem planejadas, podem gerar impactos ambientais (DONGMEI; CHANGQUN, 2008; SILVA-JUNIOR et al., 2018; QUADRA et al., 2019). O rompimento da barragem de Fundão, em Mariana, MG, afetou diretamente 863,7 ha de Áreas de Preservação Permanente (conforme definido pelo Código Florestal Federal) associadas aos cursos d'água devido ao alagamento dos rejeitos de minério (CARMO et al., 2017). Quase oito anos após o colapso, métodos de restauração ativa e passiva estão sendo aplicados na Mata Atlântica afetada e o reflorestamento desses ambientes requer uma atenção especial para garantir o restabelecimento da biodiversidade e dos serviços ecossistêmicos (CROUZEILLES et al., 2017; STUBLE; FICK; YOUNG et al., 2017; CAMPANHARO et al., 2020; MARTINS et al., 2020). Além disso, a identificação de espécies tolerantes à escassez de nutrientes minerais, bem como do conhecimento sobre a sua capacidade de germinação, crescimento inicial e estabelecimento nestas áreas é de suma importância para o sucesso na recuperação de áreas degradadas por rejeitos de mineração.

Solos contaminados por rejeito de minério de ferro geralmente apresentam baixo teor de carbono orgânico, contendo grandes quantidades de partículas finas e altas concentrações de alguns metais (WONG; TAM, 1977; HUDSON-EDWARDS et al., 2003). Nutrientes minerais fazem parte de compostos estruturais e de processos metabólicos indispensáveis para o crescimento e desenvolvimento vegetal adequado, enquanto a baixa fertilidade é uma das principais condições do solo que impõe estresses nutricionais às plantas (EPSTEIN; BLOOM, 2006). Alguns estudos apontaram a falta de nutrientes minerais no rejeito, característica que afeta diretamente o crescimento de mudas de espécies arbóreas (SEGURA et al., 2016, ANDRADE et al., 2018). Ações de plantio com espécies herbáceas e arbóreas exóticas foram feitas na mata ciliar da Bacia do Rio Doce sem o estudo adequado, o que pode gerar novos impactos na região (SILVA et al., 2015).

Schinus molle L. e *Schinus terebinthifolius* Raddi, ambas pertencentes à família Anacardiaceae, são espécies arbóreas nativas da América do Sul, mas introduzidas em diferentes regiões do planeta. Estas espécies são conhecidas por demonstrarem potencial para tolerar fatores ambientais adversos (PEREIRA et al., 2013; PEREIRA et al., 2016; PEREIRA et al., 2017). *Schinus* spp. são árvores com aplicações múltiplas, principalmente para arborização e recuperação de áreas degradadas. Em função de seu florescimento exuberante e de características arbóreas desejáveis *Handroanthus serratifolius* (Vahl) S. Grose e *Handroanthus impetiginosus* (Mart. ex DC.) Mattos são espécies pertencentes à família Bignoniaceae; consideradas de grande importância para o paisagismo, particularmente para a arborização de ruas, avenidas e, inclusive, para o reflorestamento em terrenos secos e pedregosos, bem como para recuperação de áreas degradadas (MAIA, 2004).

Desta forma, um dos objetivos deste trabalho foi avaliar quatro espécies arbóreas, sendo duas espécies de *Schinus* spp. e duas espécies de *Handroanthus* spp., cultivadas em resíduo de mineração durante a germinação e o crescimento inicial avaliando a biometria e modificações anatômicas foliares. Devido à composição do rejeito de mineração de ferro, também foram avaliadas as respostas de crescimento, baseados em aspectos fisiológicos e anatômicos de duas espécies de *Handroanthus* spp. quando adicionado solução nutritiva ao rejeito de mineração.

2 REFERENCIAL TEÓRICO

2.1 Desastres por rompimento de barragem de mineração

Produtos da indústria de mineração são amplamente utilizados na vida moderna, como por exemplo, em computadores, aviões, navios e jóias. Cada vez mais os recursos da mineração são uma importante fonte de renda em áreas de pobreza e desempenham um papel positivo no desenvolvimento econômico, além da geração de empregos para mais de 40 milhões de pessoas em todo o mundo (AZAPAGIK, 2004; KOSSOFF et al., 2014). Entretanto, com o avanço da ciência e da tecnologia, o rápido crescimento das atividades de mineração levou a um aumento na quantidade de rejeitos, que muitas vezes são armazenados em bacias de rejeitos (VILLAVICENCIO et al., 2014) . Rejeitos, que são produtos residuais no processo de beneficiamento, são geralmente armazenados na forma de lama (OZCAN; ULUSAY; ISIK, 2013; WEI et al., 2016). O objetivo de estabelecer uma barragem de rejeitos é armazenar rejeitos com segurança para proteger o ambiente natural de danos (INAM et al., 2011; SMUDA et al., 2014; XU; WANG, 2015; NAEINI; AKHTARPOUR, 2018). Uma vez que uma lagoa de rejeitos vaza, tem um grande impacto negativo na economia, nas propriedades vizinhas e na vida das pessoas (DRAVES; FOX, 1998; HUDSON-EDWARDS et al., 2003). Segundo Lyu e colaroradores (2019), em média, três das 3.500 barragens de rejeitos do mundo rompem a cada ano e o fracasso das barragens de rejeitos está intimamente relacionado ao estado da economia do país, visto que a proporção de rompimentos de barragens de rejeitos em países em desenvolvimento tem sido relativamente alta.

Conforme os dados levantados por Reis e colaboradores (2020), entre os anos de 1910 e 2019, foram identificados 250 eventos de rompimentos de barragens de mineração. Estes ocorreram principalmente na América do Norte, muitos com causas desconhecidas e se concentrando entre as décadas de 1960 e 1980. Com base na distribuição espaço-temporal dos eventos, os autores sugerem que houve certa migração da concentração dos casos de rompimento da Europa para a América do Sul e, sobretudo, para a Ásia, a partir do século XXI. Enquanto ao longo do século XX foram registrados 52 casos na Europa e apenas 17 na Ásia, somente a partir de 2010 já foram registrados 12 casos na Ásia, mas apenas dois na Europa (AZAM; LI, 2010; WISE URANIUM PROJECT, 2021). Entretanto, o número de falhas catastróficas em barragens de rejeitos de minas está aumentando globalmente e por serem uma das maiores estruturas projetadas do mundo deve ser priorizada a avaliação e a divulgação do risco de desastre combinado à ameaça situada (OWEN et al., 2020). Reis e coloboradores (2020) ainda descreveram os quatro casos de rompimentos de barragens de rejeitos de minério que ganharam grande destaque midiático e acadêmico pelas circunstâncias em que ocorreram, pelos danos gerados e pelas discussões sobre segurança de barragens que suscitaram, sendo elas: Barragem de Aznalcóllar, na Espanha; Barragem de Mount Polley, no Canadá, Barragem de Fundão e do Córrego do Feijão, ambas no Brasil.

2.2 O rompimento da barragem de Fundão e seus impactos

Aos cinco dias de novembro de 2015, a barragem da mina de ferro da Samarco (chamada de Fundão) derramou cerca de 50 a 60 milhões de m^3 de lama de rejeito de mineração no rio Gualaxo do Norte, um rio que pertence à Bacia do Rio Doce. Aproximadamente 15 km^2 foram inundados ao longo dos rios Gualaxo do Norte, Carmo e Doce, atingindo o Oceano Atlântico em 22 de novembro de 2015 (IBAMA, 2015; SEDRU, 2016). Com população estimada em 612 habitantes, o distrito de Bento Rodrigues, localizado na cidade mineira de Mariana, foi o primeiro a receber o impacto da onda de rejeitos. Foram relatadas 19 mortes e mais de 300.000 pessoas foram afetadas de alguma forma, pela destruição da sua vila e do seu território, pela perda dos seus meios de subsistência, do seu quadro de vida e das suas raízes territoriais ou, pelo menos, pela falta de água adequada ao consumo (SILVA; FERREIRA; SCOTT, 2015). Através do relatório técnico final do desastre, estudos concluiram que o colapso foi devido à liquefação, ou seja, quando os materiais sólidos (rejeitos arenosos) perdem sua resistência mecânica e apresentam comportamento fluido (MORGENSTERN et al., 2016). Além disso, foram detectados três eventos pequenos de choques sísmicos aproximadamente 1,5 h antes do colapso da barragem, apresentando magnitudes Richter regionais (mR) entre 1,4 e 2,6. Morgenstern e colaboradores (2016) sugerem ainda que embora esses movimentos fossem bastante pequenos e as incertezas associadas grandes, esses abalos provavelmente aceleraram o processo de falha que já estava bem avançado na Barragem de Fundão.

O Instituto Brasileiro de Meio Ambiente e Recursos Renováveis – IBAMA (2015) relatou e evidenciou "impactos agudos de contexto regional, entendidos como a destruição direta de ecossistemas, prejuízos à fauna, flora e socioeconômicos, que afetaram o equilíbrio da Bacia Hidrográfica do rio Doce, com desestruturação da resiliência do sistema". Para o órgão ambiental as florestas estabelecidas ao longo dos cursos d'água são vitais para o equilíbrio de todo o ecossistema e da biodiversidade local, especialmente em relação ao sistema hídrico:

> É inegável a importância ecológica de florestas ao longo de cursos d'água, com reflexos tanto para a manutenção da biodiversidade local como para as comunidades que com ela interagem de forma social e econômica. As vegetações nessas áreas atenuam a erosão do solo, regularizam os fluxos hídricos e impedem o processo de assoreamento dos cursos da água, dentre outras funções vitais. As Áres de Preservação Permanentes (APPs) e as áreas de reserva legal têm um papel fundamental no ciclo da bacia hidrológica como um todo (IBAMA, 2015, p. 7).

De acordo com Lopes (2016), a devastação pelo rejeito de mineração concentrou-se principalmente em matas ciliares remanescentes e no solo. A passagem da lama não somente destruiu e arrancou as árvores e a vegetação herbácea, mas levou ou soterrou a serrapilheira e seus bancos de sementes, devastando aproximadamente 1469 ha de vegetação natural (FERNANDES et al., 2016). Dessa forma, os ecossistemas atingidos pelo desastre tiveram seus processos de sucessão ecológica muito comprometidos (IBAMA, 2015). Deve-se ressaltar ainda, que além dos impactos gerados aos ecossistemas (aquáticos e terrestres), ocorreram mortes de peixes e a profunda contaminação do solo (SILVA; FERREIRA; SCOTT, 2015).

2.3 Composição dos resíduos de mineração

As barragens de rejeitos no Brasil surgiram em função das atividades minerárias, cerca de 300 anos atrás. O rejeito, conforme a NBR 13028 (ABNT, 2006) é conceituado como "todo e qualquer material não aproveitável economicamente, gerado durante o processo de beneficiamento de minérios". A mineralogia do corpo do minério, a eficiência do método de extração e do grau de intemperismo durante o armazenamento do rejeito definem as propriedades físicas e a composição química do rejeito oriundo da atividade mineradora (KOSSOFF et al., 2014). Solos contaminados por rejeito de minério de ferro geralmente apresentam baixo teor de carbono orgânico e de nutrientes básicos, contendo grandes quantidades de partículas finas e altas concentrações de alguns metais (WONG; TAM, 1977; HUDSON-EDWARDS et al., 2003).

Amostras de rejeito de minério proveniente da barragem de Fundão, coletadas pelo IBAMA (2015) dias após o ocorrido, apresentaram grande homogeneidade granulométrica com elevados teores de areia fina e silte que representavam 90% da fração de terra fina (< 2mm). Os teores de argila encontrados foram inferiores a 10%, característica que limita a capacidade de troca de cátions (IBAMA, 2015). Além disso, a composição granulométrica do rejeito apresentou adensamento intenso das partículas após a secagem. Essa característica permite uma rápida compactação do rejeito o que impede uma boa oxigenação das camadas inferiores do solo. Adicionalmente, o material tem elevados teores de minerais ferruginosos (hematita, magnesita e ilmenita) o que limita ainda mais a sua fertilidade. Ademais, a força do volume de resíduos lançado nos rios provavelmente revolveu e colocou em suspensão sedimentos do fundo dos cursos d'água que continham metais pesados e que podem lixivar ao longo do trajeto da lama (SEDRU, 2016). Análises do rejeito em estudos recentes demonstraram a presença de metais como o Ba, Pb, As, Fe, Sr, Mn, Al, porém os altos valores de pH da lama de rejeitos de mineração diminuem a disponibilidade de elementos potencialmente tóxicos para as plantas (SEGURA et al. 2016; ANDRADE et al., 2018; QUEIROZ et al., 2018; RIBEIRO JUNIOR et al., 2021; PÁDUA et al., 2021). Devido à pobreza da maioria desses materiais, constata-se que a adição de fontes de nutrientes em estéreis ou rejeitos da mineração é muito importante para o desenvolvimento das plantas no processo de revegetação, seja a fonte orgânica ou a mineral (TRINDADE; DIAS; JUCKSCH, 1997; SILVA et al. 2004).

2.4 Reflorestamento ou recuperação de áreas impactadas por resíduo de mineração

Globalmente, muitos locais de mineração estão localizados em áreas florestais e o reflorestamento ainda é considerado a melhor escolha para fornecer benefícios ecológicos e econômicos (PIETRZYKOWSKI, 2019). O objetivo amplo da restauração florestal é retornar ao solo a capacidade produtiva com um ecossistema composto por espécies nativas (GRANT; KOCH, 2007; ZIPPER et al., 2011). A restauração de paisagens florestais após graves pertubações oriundas da mineração apresenta desafios substanciais. O principal problema no reflorestamento de locais pós-mineração refere-se à condição degradada do crescimento das árvores da floresta variando de alta toxicidade proveniente de diferentes poluentes, falta de macro e micronutrientes, matéria orgânica e alterações do regime hídrico nos solos (BORISEV et al., 2018). O sucesso do plantio de espécies de árvores de rápido crescimento e alguns insumos tecnológicos para aumentar a fertilidade do solo se tornou a principal direção para mitigar os efeitos causados às florestas atingidas pela atividade mineradora (ADAMS et al., 2017; MACDONALD et al., 2015).

O uso de gramíneas, caracterizado por crescimento rápido e boa produção de brotos, tem mostrado resultados positivos nos estágios iniciais da reabilitação de ambientes relacionados às atividades de mineração (SANTOS et al., 2016; PEDROSO et al., 2018; RIBEIRO JUNIOR et al., 2021). Além de gramíneas, espécies arbóreas nativas capazes de se adaptar ao “novo solo” com condições limitantes são essenciais, e algumas características apresentadas por espécies de árvores podem ser determinantes para o sucesso ou falha na recuperação dessas áreas, tais como: ser resistente a pragas e doenças, facilidade de aquisição de propágulos, adaptabilidade, alta taxa de crescimento, eficiência na absorção e utilização de nutrientes e tolerância a metais pesados (ACCIOLY; SIQUEIRA, 2000; PEREIRA et al., 2017; ARAÚJO et al., 2018; CRUZ et al., 2020; PÁDUA et al., 2020). Além disso, têm sido relatados os benefícios da microbiota do solo associada às plantas para alcançar o sucesso em processos de revegetação (SANTOS et al., 2016; PEDROSO et al., 2018; RIBEIRO JUNIOR et al., 2021).

2.5 Efeito de resíduos de mineração na fisiologia e anatomia das plantas

Pang e colaboradores (2003) relataram que plantas de *Vetiveria zizanioides* encontradas em rejeitos de mineração de Guangdong, na China, reduziram a produção de biomassa, potencial de água na folha, conteúdo de clorofila e taxa fotossintética. Popa e Popa (2021) demonstraram que árvores de *Alnus alnobetula*, o amieiro verde, apresentaram maior taxa fotossintética, eficiência do uso da água e eficiência de carboxilação quando comparadas a outras espécies testadas e foi considerada a espécie mais bem adaptada e fotossinteticamente eficiente que colonizou naturalmente os depósitos de rejeitos nas montanhas Calimani, na Romênia. O efeito do rejeito de mineração da África do Sul reduziu parâmetros de crescimento de *Chloris gayana*, como a biomassa das plantas e altura do rebento, que foram revertidos pela aplicação de nutrição adicional (LUKASHE; MNKENI; MUPAMBWA, 2020).

Alguns estudos demonstram que a redução do comprimento do caule, o conteúdo de clorofila e a área foliar de algumas espécies de árvores são efeitos do rejeito de mineração do rompimento da barragem de Mariana (CRUZ et al., 2020). Pádua e colaboradores (2020) relataram que a redução da disponibilidade de água favoreceu o crescimento e características ecofisiológicas de *Copaifera langsdorffii*, bem como melhorou parâmetros estomáticos e tecidos mais espessos em rejeitos de mineração. O uso de características anatômicas das plantas pode ser importante para entender a tolerância da planta a estresses ambientais como a seca, metais pesados e deficiência ou excesso nutricional (PEREIRA et al, 2014; OLIVEIRA et al., 2018; CRUZ et al., 2020; SANTOS et al., 2015). Porém, os efeitos do rejeito de mineração nas características anatômicas de plantas com potencial para o reflorestamento são escassos na literatura.

2.6 Espécies utilizadas no trabalho

2.6.1 *Schinus molle* L.

Pertencente à família Anacardiaceae, a distribuição natural do gênero *Schinus* é limitada à América do Sul, com excessão de *Schinus molle* L. que possui distribuição do México à America do Sul. Segundo Silva-Luz e Pirani (2015), a distribuição natural de *S. molle* no Brasil é limitada aos estados do Paraná, Rio Grande do Sul e Santa Catarina, predominantemente no Pampa em vegetação de campo limpo. Entretanto, essa espécie pode ser encontrada em todo o Brasil como planta cultivada, utilizada na arborização urbana. *S. molle*, conhecida popularmente como aroeira-salsa é uma espécie arbórea com altura entre 3 e 15 metros e tronco curto com 25 a 35 cm de diâmetro, revestido por casca grossa e escamosa.

Suas folhas são compostas, sem estípulas, com 9-25 folíolos linear-lanceolados a lineares, subcoreáceos, glabros, com 3-8 cm de comprimento e de margens serreadas. As flores amareladas e pouco vistosas são reunidas em inflorescências e os frutos são drupas globosas e de coloração vermelha (BARKLEY, 1944). O sucesso de *S. molle* como planta exótica é atribuída a sua elevada tolerância à seca, às altas temperaturas, à competição por nutrientes e luz, bem como elevada taxa de crescimento e produção de biomassa (DEMELASH; TIGABU; ODEN, 2003; IPONGA; MILTON; RICHARDSON, 2008). As espécies do gênero *Schinus* são amplamente utilizadas em programas de restauração de áreas degradadas devido à suas características ecológicas e rusticidade (ARAÚJO; CASTRO; ALBUQUERQUE, 2007; IPONGA; MILTON; RICHARDSON, 2008), além do acúmulo de níveis elevados de metais pesados (DOGANLAR et al., 2021; PEREIRA et al., 2016)

2.6.2 *Schinus terebintifolius* Raddi

Schinus terebintifolius Raddi, também conhecida por aroeira-da-praia, aroeira, aroeira-vermelha, pimenta-rosa, peppertree é uma espécie pertencente a família Anacardiaceae. Embora mais frequente ao longo do litoral brasileiro desde Ceará até o Sul do país, *S. terebinthifolius* também é encontrado no interior do Brasil. Provavelmente abrange a maior parte da América do Sul e foi largamente introduzido como ornamental em outros países, entre eles os Estados Unidos. A árvore é mediana de 5 a 10 m de altura; o tronco pode chegar de 30 a 60 cm de diâmetro com casca grossa, mas é frequentemente menor em encostas e solos mais pobres (LORENZI; MATOS, 2008). Possuem folhas compostas com 3 a 10 pares de folíolos imparipinados, aromáticos medindo de 3 a 5 cm de comprimento por 2 a 3 cm de largura. Flores pequenas em panículas piramidais. Os frutos são drupas de um vermelho vivo, de 4 a 5 mm diâmetro, aromáticos, conferindo uma beleza notável à árvore (LORENZI; MATOS, 2008). Embora seja uma espécie pouco cultivada, a aroeira-pimenteira possui grande potencial para exploração econômica e ecológica (LENZI; ORTH, 2004). Com alta plasticidade ecológica pode ocupar diversos tipos de ambiente e formações vegetais (FLEIG; KLEIN, 1989).

2.6.3 *Handroanthus serratifolius* (Vahl) S. Grose

Handroanthus serratifolius (Vahl) S. Grose, popularmente conhecida como ipê, ipê-amarelo, ipê-do-cerrado, pau-d’arco, ipê-tabaco, ipê-pardo, pau-d’arco-amarelo, é uma espécie arbórea que pode atingir 25 m de altura e pertence à família Bignoniaceae (CARRERO et al., 2017; CORADIN et al., 2010). *H. serratifolius* apresenta folhas que se renovam anualmente. As flores são vistosas e de coloração amarela, e pela sua exuberância é altamente visível no local onde se encontra e seu florescimento ocorre de julho a setembro. O tronco do ipê-amarelo é cilíndrico e reto, podendo atingir de 20 a 90 cm de diâmetro e sua copa pode medir de 3 a 8 m de diâmetro. A casca é grossa, com espessura de 10 a 15 mm, de cor pardo-acinzentada (SILVA et al., 2011). O fruto é do tipo silíqua (MARTINELLI-SENEME et al., 2008), deiscentes e apresentam protuberâncias enrugadas (ALVES et al., 2013). Suas sementes não apresentam dormência, são consideradas ortodoxas, mas, podem perder a viabilidade em pouco tempo dependendo das condições que estejam armazenadas, são liberadas pelo vento e podem ser coletadas de agosto a início de outubro (SILVA et al., 2011; GONÇALVES, 2013). A espécie possui madeira de alta densidade e elevada durabilidade, sendo empregada na marcenaria, construções pesadas, estruturas externas e instrumentos musicais (CARRERO et al., 2017; CAMPOS FILHO; SARTORELLI, 2015). É, ainda, indicada para arborização urbana, reflorestamento e recuperação de áreas degradadas, principalmente, em solos salinos (ANDRADE, 2015; LOHMANN, 2012; PEREIRA; POLO, 2011).

2.6.4 *Handroanthus impetiginosus* (Mart. ex DC.) Mattos

A espécie *Handroanthus impetiginosus* (Mart. ex DC.) Mattos, conhecida popularmente como ipê-roxo é pertence à família Bignoniaceae (SOUZA; LORENZI, 2005). A espécie é nativa das Américas com ocorrência em todo Brasil, principalmente nos biomas Cerrado, Caatinga, Mata Atlântica, Pantanal e Amazônia (MAIA-SILVA et al., 2012; LOHMAN, 2015). O ipê-roxo é uma árvore de porte médio, até 35 m de altura e 90 cm de diâmetro, sendo as plantas decíduas e heliófilas, tolerantes à sombra enquanto juvenis (LORENZI, 2002). Possuem flores compostas, do tipo inflorescência, de cor roxa e odor suave, pouco pilosa e muito abundante. Suas flores produzem grande quantidade de néctar, atraindo vários polinizadores, sendo as abelhas os mais comuns. Possuem folhas de coloração verde-escura e o fruto é seco do tipo síliqua, deiscente e com sementes aladas, próprias para dispersão por anemocoria e com curto período de viabilidade em condições naturais (LORENZI, 2008; PANIZZA, 1997; LORENZI; MATOS, 2002). Sua madeira é muito procurada e de alto valor econômico. Possuem elevada densidade e durabilidade, sendo empregada na construção civil, como quilhas de navios, mourões, pontes e assoalhos, confecções de bengalas, além de produzir carvão de boa qualidade (PAULA; ALVES, 2007). Apresentam grande valor medicinal e ornamental, com ampla utilização na arborização urbana e na recomposição vegetal de áreas degradadas (POTT; POTT, 1994; LORENZI, 2008).

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SEGUNDA PARTE – ARTIGOS

ARTIGO 1 – SEED GERMINATION, INITIAL GROWTH AND LEAF ANATOMY OF SEEDLINGS OF FOUR TREE SPECIES IN MINE TAILINGS

MANUSCRIPT SUBMITTED TO SCIENCE OF THE TOTAL ENVIRONMENT

SEED GERMINATION, INITIAL GROWTH AND LEAF ANATOMY OF SEEDLINGS OF FOUR TREE SPECIES IN MINE TAILINGS

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ABSTRACT

The objective of this work was to test the tolerance of two species of *Schinus* spp. and two species of *Handroanthus* spp. cultivated in mining tailings from the rupture of the dam in the city of Mariana, Brazil. The seeds, later seedling, were cultivated in the mining waste and in sand in two experiments separately and the experimental design was completely randomized in a 2x3 factorial scheme, with six replications in each experiment. The substrates were maintained in field capacity. After 60 days of the establishment of the experiments, the germination data, biometric and anatomical analyzes of the leaves was evaluated, in addition to the characterization of macro and micronutrients of the tailings. The data were submitted to analysis of variance (ANOVA) and the comparison of means by the Scott-Knott test, for $p < 0.05$. The results of the present study demonstrate that the revegetation of the areas affected by these residues is feasible. The presence of macro and micronutrients may have favored some variables, such as the rate of emergence speed and percentage of germination, as well as in the growth of species, especially in *S. molle*, which showed greater growth capacity in mining waste. The species *S. terebinthifolius* also showed good results, but the species of *Handroanthus* showed greater sensitivity. There were no deformations in the anatomical structure of the leaves of the seedlings of any of the species studied, however the change in the growth capacity of the seedlings may be related to changes in the thickness of the leaf photosynthetic tissues, unfavorable as those observed in *Handroantus* spp. or favorable as observed in the species of the genus *Schinus* spp.

Key-words: Reforestation; Restoration; *Schinus* spp., *Handroanthus* spp., Mariana-MG

INTRODUCTION

The failure of tailings dams is a problem that affects many countries and regions worldwide. In 2015, the Fundão dam in Mariana, Minas Gerais, Brazil, burst, causing a disaster with wide socioenvironmental consequences. The tailings released by this event devastated large areas, destroying the native flora and fauna. This material is devoid of organic matter, causing unfavorable changes to the restoration of native flora (Brasil, 2015). Mine tailings may contain heavy metals and other pollutants (Souza, 2013; Pires et al., 2003; Pádua et al., 2021) that can be toxic to plants, preventing natural recovery. Environmental disasters of this kind have happened worldwide, with several cases reported in Europe (Rico et al., 2008) and Asia (Wei et al., 2013). In this context, reforestation is essential to promote the recovery of these areas, and this depends on the identification of tolerant species as well as knowledge about their ability to germinate and establish in these areas.

Reforestation is a way to accelerate succession processes and enable the return of fauna that promotes ecosystem services such as flower pollination and seed dispersal (Moreira et al., 2004). Trees are also important of these sites because of their metal removal capacity in addition to their large biomass and root system (Capuana, 2011). The efficiency of reforestation systems, however, is hampered by the lack of information on species with potential for use in these systems (Barbosa, 2000), as well as the lack of studies on the specific effects of mine tailings on species with use potential. As a result, seed germination and seedling establishment can be severely reduced in reforestation systems (Makhniova et al., 2019). Germination is one of the phases when the plant is most sensitive to heavy metals (Baroni et al., 2020). Studies on germination and seedling establishment of tree species are essential for the effective reforestation of regions degraded by mine tailings.

The species *Schinus molle* L. and *Schinus terebinthifolius* Raddi both belong to the family Anacardiaceae and are tree species native to South America but introduced to different regions of the planet. These species can tolerate adverse environmental factors (Pereira et al., 2013; Pereira et al. 2016a; Pereira et al., 2017). *Schinus* spp. are trees with multiple applications, such as ornamental, medicinal, and for timber, and can also be used for afforestation and recovery of degraded areas. They are evergreen, heliophile, pioneer trees, occurring in areas close to rivers and lakes and in dry and poor soils (Lorenzi, 2001).

Due to its exuberant flowering and desirable tree characteristics, *Handroanthus serratifolius* (Vahl) S.Grose and *Handroanthus impetiginosus* (Mart. ex DC.) Mattos are widely used in urban afforestation and reforestation. These species belong to the family Bignoniaceae and are considered highly important for landscaping, particularly for the afforestation of streets and avenues and even for reforestation in dry and rocky terrains, as well as for the recovery of degraded areas (Maia, 2004).

Here, we studied two species of *Schinus* and two species of *Handroanthus* because they have several characteristics that can contribute to the natural regeneration of degraded areas. We studied their tolerance to cultivation in mine tailings by evaluating their gemination, morphololological and anatomical changes during initial growth.

MATERIALS AND METHODS

Plant material and mine tailings

The seeds of the four species used (*Schinus molle*, *Schinus terebinthifolius*, *Handroanthus serratifolius* and *Handroanthus impetiginosus)* were collected from individuals present at the campus of the Universidade Federal de Lavras (21°13′27.1″S and 44°58′01.1″W) in the state of Minas Gerais, Brazil. The samples of mine tailings resulting from the spill that covers the affected areas in the region of Mariana, state of Minas Gerais, Brazil, were collected at approximately 4 km from the dam failure site (20°22′40″S 43°24′57″W) and transported to the Universidade Federal de Lavras. The samples were used to characterize the sludge regarding its chemical composition, as well as the presence of heavy metals (Table 1), according to the methods proposed by Claessen et al. (1997), in the Department of Soil Sciences, Universidade Federal de Lavras.

Table 1. Values of nutrients and pollutants and other characteristics of the sludge collected after the Fundão dam failure in Mariana, MG, Brazil.

Variable	Value
pH	7.8
K (mg L^{-1})	16.23
P (mg L^{-1})	11.88
Ca (cmolc L^{-1})	1.46
Mg (cmolc L^{-1})	0.10
Al (cmolc L^{-1})	0.04
H+Al (cmolc L^{-1})	0.62
SB (cmolc L^{-1})	1.60
t (cmolc L^{-1})	1.64
T (cmolc L^{-1})	2.22
Ni (µg kg^{-1})	1705.98
Cr (µg kg^{-1})	9473.26
Pb (µg kg^{-1})	4158.98
Zn (µg kg^{-1})	4.10
As (µg kg^{-1})	1425.51
Mn (mg kg^{-1})	562.37
Fe (mg kg^{-1})	55211.56

SB = sum of exchangeable bases; t = Effective Cation Exchange Capability; T = Cation Exchange Capacity at pH 7.0

Experimental design

After characterization, the mining sludge was sieved to obtain a homogeneous substrate for plant management. The experiments were conducted with two substrates: mine tailings (MT) and sand (SA), which were irrigated and maintained at field capacity, and water lost by evapotranspiration was replenished daily. Separate experiments were conducted for the species *Schinus* and *Handroanthus* because the plants within each genus are similar but differ significantly from the other genus in germination, anatomy, and growth. A completely randomized experimental design was used in a 2×3 factorial arrangement, with two substrates (washed sand and mine tailings) in the following combinations: Experiment 1 (*Schinus*): only *S. molle*, only *S. terebinthifolius*, and *S. molle* + *S. terebinthifolius*. Experiment 2

(*Handroanthus*): only *H. serratifolius*, only *H. impetiginosus*, and *H. serratifolius* + *H. impetiginosus.* In each experiment, six replicates were used (n = 36 for each experiment). The experimental plots differed by which variable was analyzed, as described below.

Seed germination

Each replicate consisted of a batch of 50 seeds (n = 36). When two species were used together (*S. molle* + *S. terebinthifolius* or *H. serratifolius* + *H. impetiginosus*), the lot had 25 seeds of each. The seeds were sterilized with 50% sodium hypochlorite and washed in distilled water twice. They were placed in 5-L pots containing 3L of the substrate (MT or SA) moistened to field capacity. The seeds were kept in a greenhouse for 60 days, and the water lost by evapotranspiration was replenished daily with deionized water.

The number of germinated seeds (emergence) was evaluated daily. The percentage emergence (E%) and the emergence rate index (ERI) were calculated at the end of 60 days, along with the total number of normal seedlings (NS). The calculations were performed according to the following equations: E% = [(number of emerged seedlings)/50]×100; ERI = $\sum$ (SE/t), where SE = number of seedlings that emerged at time t, and t = time in days.

Biometric analysis of seedlings

Three seedlings per replicate were collected for biometric analysis, and the mean measurements were used for each plot (n = 36). The seedlings were evaluated at the end of the experiment (60 days of age). The length of the longest root was measured with a digital caliper. The number of leaves and roots of each plant was counted, and the fresh weight of the roots and shoots was measured on an analytical balance (AY 220, Shimadzu, Japan).

Anatomical analysis

One seedling per replicate (n = 36) was collected, washed in running water, and fixed in a 70% solution of formaldehyde: glacial acetic acid:70% ethanol in the ratio of 0.5:0.5:9 for 72 hours. Then the samples were preserved in 70% ethanol until the date of analysis (Jensen, 1970 in Kraus and Arduin, 1997).

Cross-sections were obtained from the middle region of a leaflet located in the middle region of the leaves. To prepare the permanent slides, the samples were dehydrated in an ethanol series, infiltrated, and embedded in hydroxyethyl methacrylate according to the manufacturer's instructions (Leica Microsystems, Wetzlar, Germany). Sections were sliced by a semiautomatic rotary microtome 335 (Jinhua Yidi Medical Appliance CO., LTD, Zheiang, China), stained with 0.5% toluidine blue (O'Brien et al., 1964), and mounted with acrylic varnish. The slides were photographed under an Olympus CX31 microscope (Olympus, Tokyo, Japan) coupled to a digital camera. For each replicate, a slide was made, two sections and two fields were evaluated for each section, and the mean values were calculated for each replicate (n = 36).

ImageJ software was used to evaluate the images obtained for the following anatomical variables: thickness of the epidermal cells of the adaxial and abaxial surface, thickness of the subepidermal cells, thickness of the palisade parenchyma and thickness of the spongy parenchyma.

Statistical analysis

Using the statistical software Sisvar (Ferreira, 2011), the data were first tested for normality by the Shapiro-Wilk test. All data were normally distributed, so they were tested by analysis of variance, and the means were compared by the Scott-Knott test at $p<0.05$.

RESULTS

The interaction between the factors had a significant effect on the ERI of *Schinus* seedlings. The ERI was higher in *S. terebinthifolius* than in *S. molle* or in the combination of the species when the seeds were germinated in sand. However, the ERI increased in *S. molle* when grown in the mine tailings, causing the combination of the two species to also show an increase in this parameter (Table 2). Thus, the ERI of *S. terebinthifolius* was lower in mine tailings. For *Handroanthus* spp., all the seeds germinated in the sand had a higher ERI than those germinated in mine tailings (Table 2). When exposed to mine tailings, the two *Handroanthus* species had similar ERIs (Table 2), but *H. impetiginosus* seeds showed a higher ERI in the sand.

Table 2. Emergence rate index of tree species cultivated in mine tailings sludge resulting from the failure of the Fundão dam in Mariana, MG, Brazil. Data are mean ± standard deviation.

***Schinus* spp.** Emergence rate index (plants day^{-1})		
Species	**Sand**	**Mine tailings**
S. molle	0.61 ± 0.53 bB	8.13 ± 1.27 aA
S. terebinthifolius	3.76 ± 1.36 aA	4.65 ± 1.58 aB
S. molle + S. terebinthifolius	0.70 ± 0.21 bB	7.23 ± 2.13 aA
***Handroanthus* spp.** Emergence rate index (plants day^{-1})		
Species	**Sand**	**Mine tailings**
H. serratifolius	3.64 ± 1.06 aB	1.25 ± 0.97 bA
H. impetiginosus	5.45 ± 2.05 aA	0.03 ± 0.05bA
H. serratifolius + H. impetiginosus	3.84 ± 1.60 aB	0.98 ± 0.49 bA

Means followed by the same lowercase letter in the same row or the same uppercase letter in the same column do not differ by the Scott-Knott test at $p<0.05$.

The percentage of emergence for *Schinus* seeds showed no interaction between factors ($p>0.05$). This parameter was approximately 2.5 times higher in mine tailings than in sand (Table 3). The combination of *S. molle* and *S. terebinthifolius* seeds showed a higher germination percentage than either species germinated separately (Table 3). The interaction between factors had a significant effect on the percentage of germination in *Handroanthus* species. The percentage germination was higher in the sand in all *Handroanthus* groups than in the mine tailings sludge. In addition, *H. impetiginosus* presented lower germination in the sludge than *H. serratifolius,* leading to higher means when evaluating the two species together (Table 3).

Table 3. Percentage emergence of tree species cultivated in mine tailings resulting from the failure of the Fundão dam in Mariana, MG, Brazil. Data are mean ± standard deviation.

***Schinus* spp.**		
Substrate	**Emergence (%)**	
Sand	16.44 ± 15.95 b	
Mine tailings	40.11 ± 12.99 a	
Species	**Emergence (%)**	
S. molle	18.50 ± 17.38 b	
S. terebinthifolius	21.50 ± 11.49 b	
S. molle + *S. terebinthifolius*	44.83 ± 14.99 a	
***Handroanthus* spp.**		
Species	**Sand**	**Mine tailings**
H. serratifolius	39.67 ± 8.98 aA	28.33 ± 9.75 bA
H. impetiginosus	47.33 ± 11.43 aA	1.00 ± 1.67 bB
H. serratifolius + *H. impetiginosus*	45.33 ± 9.18 aA	21.00 ± 6.03 bA

Means followed by the same lowercase letter in the same row or the same uppercase letter in the same column do not differ by the Scott-Knott test at $p<0.05$.

For *Schinus* spp., there was an interaction between the factors for the number of roots produced per seedling. More roots were observed in *S. terebinthifolius* seedlings when cultivated alone in sand, but this species showed fewer roots when the seedlings were cultivated in mine tailings (Table 4). The mine tailings did not promote significant changes in the number of roots of *S. molle* or of the combination of the two *Schinus* species (Table 4). There was no interaction between the factors for the number of roots in *Handroanthus* seedlings. Overall, this genus had more roots when cultivated in the mine tailings. The comparison between species showed no significant differences (Table 4).

Table 4. Number of roots in seedlings of tree species cultivated in mine tailings sludge resulting from the failure of the Fundão dam in Mariana, MG, Brazil. Data are mean ± standard deviation.

***Schinus* spp.**		
Species	**Sand**	**Mine tailings**
S. molle	7.83 ± 1.33 aB	6.67 ± 2.94 aA
S. terebinthifolius	16.67 ± 4.13 aA	9.33 ± 2.73 bA
S. molle + S. terebinthifolius	11.33 ± 3.67 aB	11.67 ± 4.50 aA
***Handroanthus* spp.**		
Substrate	**Number of roots per plant**	
Sand	4.39 ± 13.77 b	
Mine tailings	18.89 ± 3.58 a	
Species	**Number of roots per plant**	
H. serratifolius	8.33 ± 13.77 a	
H. impetiginosus	14.83 ± 13.14 a	
H. serratifolius + H. impetiginosus	11.75 ± 14.42 a	

Means followed by the same lowercase letter in the same row or the same uppercase letter in the same column do not differ by the Scott-Knott test at $p<0.05$.

The plants of the *Schinus* species had the same number of leaves regardless of the treatment (Table 5). *Handroanthus* spp. had no significant differences in the number of leaves between sludge and sand, but *H. impetiginosus* showed fewer leaves than *H. serratifolius* in both substrates (Table 5).

Table 5. Number of leaves in seedlings of tree species cultivated in mine tailings sludge resulting from the failure of the Fundão dam in Mariana, MG, Brazil. Data are mean ± standard deviation.

***Schinus* spp.**	
Substrates	**Number of leaves per plant**
Sand	2.00 ± 0.01 a
Mine tailings	2.00 ± 0.02 a
Species	**Number of leaves per plant**
S. molle	2.00 ± 0.01 a
S. terebinthifolius	2.00 ± 0.01 a
S. molle + S. terebinthifolius	2.00 ± 0.03 a
***Handroanthus* spp.**	
Substrates	**Number of leaves per plant**
Sand	2.78 ± 1.00 a
Mine tailings	2.44 ± 0.85 a
Species	**Number of leaves per plant**
H. serratifolius	2.83 ± 1.03 a
H. impetiginosus	2.00 ± 0.01 b
H. serratifolius + H. impetiginosus	3.00 ± 1.04 a

The means followed by the same lowercase letter in the column do not differ by the Scott-Knott test at $p<0.05$.

In the *Schinus* spp., there was a higher length of the longest root (5.30 cm) in seedlings grown in mine tailings sludge and there was no significant difference between species (Table 6). Regarding the *Handroanthus* spp. seedlings, neither substrate nor species affected the length of the longest root (Table 6).

Table 6. Comparison of mean longest root length (cm) in seedlings cultivated in mine tailings sludge resulting from the failure of the Fundão dam in Mariana, MG, Brazil. Data are mean ± standard deviation.

***Schinus* spp.**	
Substrates	**Length of the longest root (cm)**
Sand	5.35 ± 1.97 a
Mine tailings	3.75 ± 1.39 b
Species	**Length of the longest root (cm)**
S. molle	4.89 ± 1.52 a
S. terebinthifolius	5.00 ± 1.36 a
S. molle+ S. terebinthifolius	3.77 ± 1.93 a
***Handroanthus* spp.**	
Substrates	**Length of the longest root (cm)**
Sand	28.58 ± 18.76 b
Mine tailings	53.03 ± 12.73 a
Species	**Length of the longest root (cm)**
H. serratifolius	47.01 ± 10.58 a
H. impetiginosus	39.41 ± 22.92 a
H. serratifolius +H. impetiginosus	36.00 ± 14.53 a

The means followed by the same lowercase letter in the column do not differ by the Scott-Knott test at $p<0.05$.

Regarding the total fresh weight, the *S. molle* seedlings had the highest values (77.87 g) regardless of the substrate (Table 7). In addition, the mine tailings sludge did not promote significant differences in this variable (Table 7). However, *Handroanthus* spp. cultivated in sand had a higher mean (0.96 g) total fresh weight than the seedlings cultivated in the mine tailings sludge (Table 7). A lower mean (0.58 g) for the total weight was also observed for *H. impetiginosus* seedlings regardless of the substrate (Table 7).

Table 7. Comparison of mean total fresh weight (g) of seedlings of the four tree species cultivated in mine tailings sludge resulting from the failure of the Fundão dam in Mariana, MG, Brazil. Data are mean ± standard deviation.

***Schinus* spp.**	
Substrates	**Total fresh weight (g)**
Sand	59.61 ± 15.36 a
Mine tailings	65.14 ± 22.60 a
Species	**Total fresh weight (g)**
S. molle	77.87 ± 24.60 a
S. terebinthifolius	55.54 ± 10.99 b
S. molle+ S. terebinthifolius	53.72 ± 7.64 b
***Handroanthus* spp.**	
Substrates	**Total fresh weight (g)**
Sand	0.96 ± 0.38 a
Mine tailings	0.70 ± 0.40 b
Species	**Total fresh weight (g)**
H. serratifolius	0.94 ± 0.35 a
H. impetiginosus	0.58 ± 0.20 b
H. serratifolius +H. impetiginosus	0.97 ± 0.51 a

The means followed by the same lowercase letter in the column do not differ by the Scott-Knott test at $p<0.05$.

Regarding the leaf anatomy of *Schinus* spp., plants grown in mine tailings sludge had a thicker epidermis of the adaxial face, thicker hypodermis cells, but thinner spongy parenchyma than plants grown in sand (Table 8).

Table 8. Anatomical characteristics of *Schinus* spp. cultivated in mine tailings sludge resulting from the failure of the Fundão dam in Mariana, MG, Brazil. Data are mean ± standard deviation.

Substrates	**AdE (µm)**	**HD (µm)**	**PP (µm)**	**SP (µm)**	**AbE (µm)**
Sand	10.71±1.81b	21.94±3.87b	16.81±2.6a	54.35±8.0a	8.45±1.3a
Mine tailings	13.26±1.84a	28.37±3.17a	15.87±3.09a	41.82±10.82b	8.87±1.58a
Species	**AdE (µm)**	**HD (µm)**	**PP (µm)**	**SP (µm)**	**AbE (µm)**
S. molle	11.97±2.72a	25.59±4.8a	16.87±3.27a	46.12±12.91a	8.97±1.41a
S. terebinthifolius	12.0±1.67a	24.72±4.91a	15.81±9.57a	50.05±9.57a	8.35±1.45a

Means followed by the same letter in the same column do not differ by the Scott-Knott test at $p<0.05$. AdE = adaxial epidermis thickness, AbE = abaxial epidermis thickness, HD = hypodermis thickness, PP = palisade parenchyma thickness, SP = spongy parenchyma thickness.

Regarding the leaf anatomy of the *Handroanthus* spp. (Fig. 2), the plants cultivated in the mine tailings sludge showed lower values than the plants grown in sand, except in the thickness of the epidermal cells of the abaxial surface (Table 9). There were no significant differences between species (Table 9).

Table 9. Anatomical characteristics of *Handroanthus* spp. cultivated in mine tailings sludge resulting from the failure of the Fundão dam in Mariana, MG, Brazil. Data are mean ± standard deviation.

Substrates	**AdE (µm)**	**PP (µm)**	**SP (µm)**	**AbE (µm)**
Sand	16.06 ± 2.79 a	36.77 ± 5.69 a	61.01 ± 13.96 a	13.48 ± 1.99 a
Mine tailings	11.95 ± 2.35 b	24.72 ± 3.74 b	36.25 ± 7.69 b	12.15 ± 3.71 a
Species	**AdE (µm)**	**PP (µm)**	**SP (µm)**	**AbE (µm)**
H. serratifolius	14.47 ± 3.74 a	32.12 ± 8.28 a	49.7 ± 15.31 a	13.32 ± 1.88 a
H. impetiginosus	13.54 ± 3.16 a	29.37 ± 7.26 a	47.56 ± 18.74 a	12.3 ± 3.82 a

Means followed by the same letter in the same column do not differ by the Scott-Knott test at $p<0.05$. AdE = adaxial epidermis thickness, PP = palisade parenchyma thickness, SP = spongy parenchyma thickness, AbE = abaxial epidermis thickness.

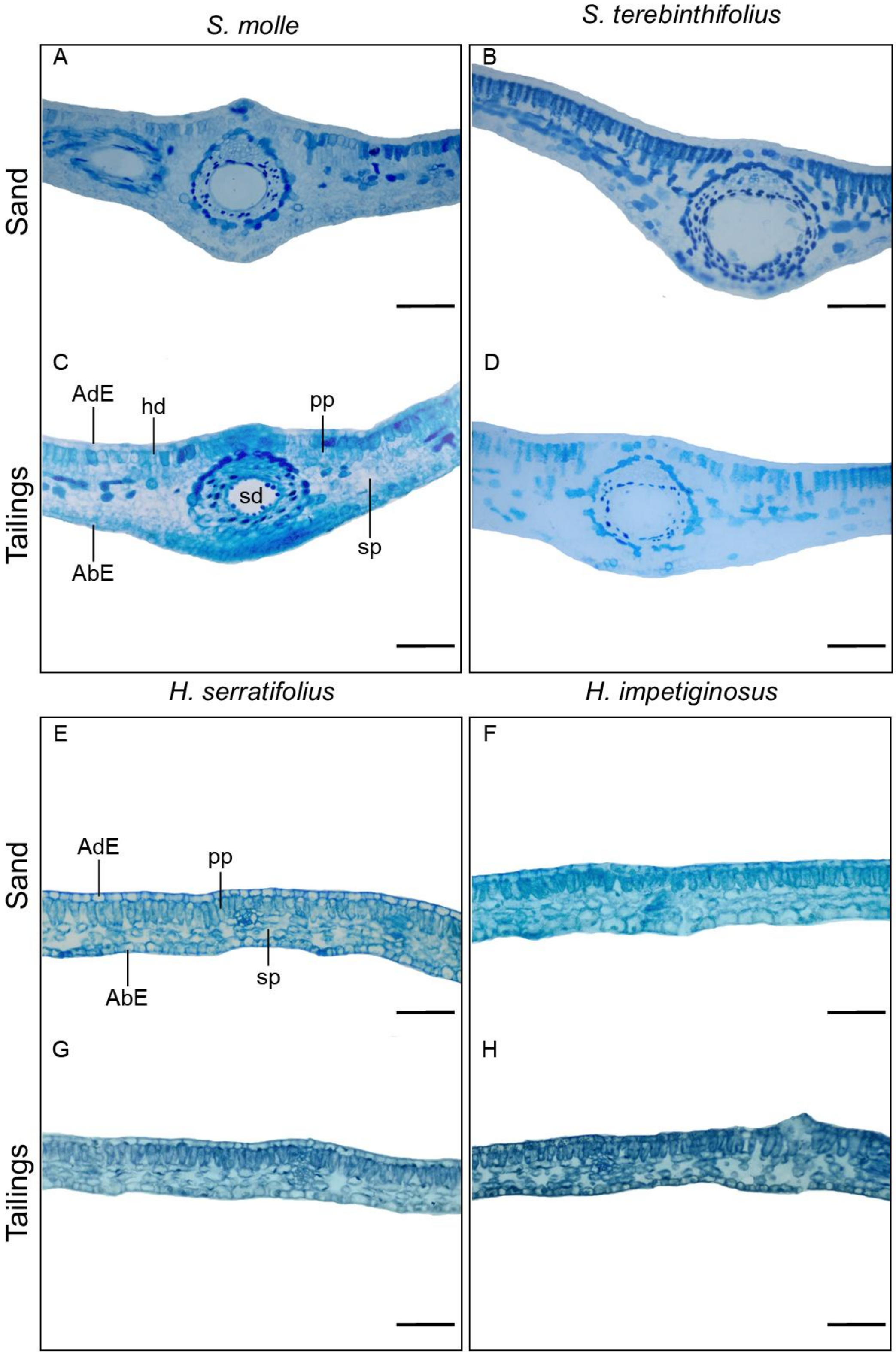

Fig 1. Cross-sections of *Schinus* spp. and *Handroanthus* spp. cultivated in washed sand and mine tailings. **sd** = secretory duct, **hd** = hypodermis, **AdE** = adaxial epidermis, **AbE** = abaxial epidermis, **pp** = palisade parenchyma, **sp** = spongy parenchyma. Bars: 100 µm.

DISCUSSION

The analysis performed on the sample of mine tailings showed the presence of macro- and micronutrients (Table 1). Toxic elements were also detected, but there was not enough toxicity to promote plant death, as all species studied survived without evident seedling mortality from the mine tailings. In addition, all plants were normal, without severe deformities that could prevent their development, in mine tailings. The macro and micronutrients and other parameters are within the ranges of values observed for soils of the affected region and in other locations (Kabata-Pendias, 2010; Segura et al., 2016). The presence of macro- and micronutrients may have favored some variables, such as the ERI and germination percentage, as well as the growth of species more tolerant to heavy metals, such as *S. molle* (Pereira et al., 2016; Ribeiro et al., 2019), in the mine tailings. However, it is important to note that mine tailings, even when they have the same origin, show variable chemical composition, as observed by Segura et al. (2016) and Andrade et al. (2018). Despite this variable chemical composition, the lack of lethality to seeds of the four species studied here demonstrates that sites with mine tailings from the Fundão dam can be effectively reforested through the introduction of species still in the seed stage that, after the revegetation process, establish themselves, producing new seeds that may germinate and consequently promote natural regeneration. The results of the present study therefore demonstrate that the revegetation of the areas affected by these tailings is viable.

The Pb, As, and Fe concentrations were quite high in the mine tailings of the Fundão dam (Table 1). High Pb and As concentrations can cause phytotoxicity in sensitive species (McBride, 1994; Oliver, 1997). According to Guerinot & Yi (1994), high Fe concentrations do not often cause damage to plants because most Fe is not bioavailable, in addition to being an important and essential micronutrient. However, in soils from where Fe ore is extracted, such as the Fundão dam region, the concentration of this element can increase significantly, reaching toxic levels for most plants (Audebert; Fofana, 2009; Sahrawat, 2004). Among the four species studied, *S. molle* showed the greatest tolerance to the conditions imposed by the mine tailings. In fact, it is tolerant to different toxic metals (Pereira et al. 2013; Pereira et al. 2016a; Pereira et al., 2017; Ribeiro et al. 2019). In the present study, its ability to grow in the contaminated tailings was evident. The species *S. terebinthifolius* also showed good results, while the *Handroanthus* species showed greater sensitivity. It is important to note that despite differences in germination and growth responses, all four species studied have potential for reforestation on tailings. The toxicity of the tailings may be related to the high but nonlethal concentrations of some elements, which was a favorable factor and only promoted growth reduction in more sensitive species.

As all species germinated and seedling growth was viable, the revegetation of the area affected by mine tailings was favored because the introduction of multiple species that were more or less tolerant to the tailings was possible. Jesus et al. (2016) reported that the combination of different plant species is necessary to ensure the success of reforestation and the environmental restoration of an area degraded by mineral exploration. The *Schinus* species were more tolerant than the *Handroanthus* species, but the possibility of introducing different species at the same time that will all have seed germination and seedling growth may be an important factor in these locations.

It is also important to note that the seeds of the tree species tested have potential for long-term storage and use, enabling long-term reforestation programs. *S. molle* has great potential in this sense because seeds can be stored for one year or more, and storage increases the germination percentage in this species by reducing the levels of essential oils present in the mesocarp that adhere to the seeds (Pereira et al., 2016b). The seeds of *S. terebinthifolius* can also be stored for one year or more, remaining viable even under different moisture conditions (Ribeiro et al., 2018). The seeds of the *Handroanthus* species are more sensitive to storage, which rapidly reduces seed vigor, germination, and seedling growth and reduces storage times (Souza et al., 2005). However, the studied species can be used in revegetation programs, and the introduction of seeds on mine tailings will depend on the characteristics of each species; the species may remain viable for long periods, as with *Schinus*, or for shorter periods, as with *Handroanthus*.

The germination parameters found in this experiment (percentage of emergence, time required for germination, ERI, etc.) are similar to those found in other studies of these species, demonstrating that there was no significant reduction promoted by mine tailings for the most tolerant species. The germination parameters of *S. molle* in this experiment were similar to those described in other studies (Pereira et al., 2017; Baroni et al., 2020), and the same was true of *S. terebinthifolius* (Ribeiro et al., 2018). The germination parameters of the two *Handroanthus* species differed from those in some published studies. For example, the germination percentage and ERI we found for *H. impetiginosus* were higher (da Silva et al., 2011; Santos et al., 2018). However, for *H. serratifolius*, the germination values obtained in the present experiment were similar to those found in the literature (de Oliveira et al., 2005). It is also important to note that the germination percentage, ERI, and other parameters vary greatly with environmental conditions. In this context, de Oliveira et al. (2005) reported similar values for *H. impetiginosus* to those found in the present study. Another aspect of the variations in these parameters is that germination for *Handroanthus* had contrasting values between sand and mine tailings. As the viability and vigor of *Handroanthus* seeds is greatly reduced over time (Souza et al. 2005) or by environmental factors (da Silva et al., 2011; de Oliveira et al., 2005; Santos et al., 2018), the low values found may be related to the natural vigor of the seeds used and not to the direct effect of mine tailings. The mine tailings were more limiting for the *Handroanthus* species than the *Schinus* species in terms of the germination parameters, and the literature shows that the *Schinus* species have greater vigor and stability in their germination parameters. This also justifies the use of separate experiments for these groups of trees and the comparison only between species of the same genus, as done here. In general, the values obtained for the germination parameters of *Schinus* and *Handroanthus* species are within the normal variation parameters published in other studies, demonstrating that these species are, in fact, viable for use in the reforestation of areas affected by mine tailings.

The seedling growth data show that the studied species have the potential to establish and grow in mine tailings, facilitating reforestation programs. Seedlings are very sensitive to heavy metal toxicity and reduce their biomass, root length, number of leaves, and other growth parameters when exposed to these pollutants (Ali et al., 2013; Anuradha and Rao, 2007). The presence of heavy metals such as Pb and other toxic elements such as As (Pereira et al., 2011) may have led to the reduced growth of *Handroanthus* seedlings in the mine tailings compared to when they were grown in the sand. However, the reduction in growth parameters in these species was not very pronounced, because the mine tailings reduced the total fresh weight by only approximately 20% and did not affect root length or number of leaves; in fact, the number of roots in *Handroanthus* was increased by the tailings. We can say, therefore, that despite noticeable toxicity, mine tailings do not promote a very intense reduction in seedling growth in *Handroanthus* species, enabling their use in reforestation. In addition, the *Schinus* species showed no reduction in seedling growth, and there was even an increase in some growth parameters in *S. molle*. Seedlings of the *S. molle* species show tolerance to high Cd concentrations and can produce higher biomass under contamination conditions because they can take advantage of the nutrients available in the substrate (Baroni et al., 2020). Due to the high tolerance to pollutants demonstrated by *S. molle*, its seedlings showed potential for greater growth because they can extract the macro- and micronutrients present in the mine tailings without suffering toxicity from this substrate. Therefore, the seedling growth data of the four tree species studied show that after seed germination, the viability of the reforestation system will be maintained since the seedlings show great potential for establishment and growth in mine tailings.

The leaf anatomy data of the studied species corroborate the growth and viability data of the seedlings under mine tailings. It should be noted that the anatomy data were not gathered for the species combinations because the anatomy is inherent to each species separately, and a treatment containing a combination of two different species could not generate only one data point. Thus, the results were generated from the comparison between the two species of each genus and the effect of mine tailings. The comparison of the anatomical parameters showed no significant differences for any of the variables when comparing *S. molle* with *S. terebinthifolius* or when comparing *H. impetiginosus* with *H. serratifolius* (Table 8, Table 9, and Fig. 1). The anatomical structures of the species studied are similar to those described in the literature for *S. molle* (Pereira et al., 2013; Pereira et al., 2016a; Pereira et al., 2017), *S. terebinthifolius* (Sabbi et al., 2010), *H. serratifolius*, and *H. impetiginosus* (da Silva et al., 2009). Thus, there were no anatomical deformities of the leaves of the seedlings of any of the species studied, confirming that they are viable for use in reforestation in mine tailings and that this substrate does not seem to have high enough toxicity to prevent the correct development of these tree species.

The quantitative changes in the leaf tissues of the seedlings help explain the differences in growth between the tree species studied under the effect of mine tailings. The phytotoxicity of the mine tailings was enough to reduce the leaf tissues of the two *Handroanthus* species compared to the seedlings grown in sand (Table 8). The lower thickness of photosynthetic tissues such as palisade and spongy parenchyma can reduce the photosynthetic rate and plant growth (Santos et al., 2015; Pereira et al. 2016a; Cruz et al., 2019). Therefore, the reduced growth of seedlings of the *Handroanthus* species in the mine tailings was related to the reduced thickness of the leaf photosynthetic tissues promoted by the phytotoxicity of the substrate. For seedlings of the genus *Schinus*, however, an opposite effect occurred: The leaf tissues of plants grown in the mine tailings were thicker, which favored a steady growth in *S. terebinthifolius* and even faster growth in *S. molle.* Thus, mine tailings can promote changes in the thickness of leaf tissues of seedlings of these tree species, and these changes can affect the growth and establishment of trees in the substrate as well as the success of restoration programs in such areas.

CONCLUSION

The tree species *S. molle*, *S. terebinthifolius*, *H. serratifolius*, and *H. impetiginosus* demonstrate germination capacity and adequate seedling growth, enabling their use in reforestation systems for areas impacted by mine tailings. The mine tailings from the Fundão dam failure in Mariana, state of Minas Gerais, Brazil, in 2015 exhibited a mild phytotoxic effect on seeds and seedlings of sensitive species but did not cause mortality. The toxicity of this material is related to the presence of heavy metals and other toxic elements. The change in seedling growth capacity of the tree species studied is related to changes that the mine tailings caused in the thickness of leaf photosynthetic tissues, which modulate the growth of these plants. The changes found may be unfavorable, as observed in *Handroanthus*, or favorable, as observed in the more tolerant genus *Schinus*.

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ARTIGO 2 – GROWTH RESPONSES, GAS EXCHANGE AND LEAF ANATOMY OF *Handroanthus* spp. IN MINING TAILINGS ENRICHED WITH NUTRIENT SOLUTION

MANUSCRIPT ELABORATE TO SCIENCE OF THE TOTAL ENVIRONMENT

GROWTH RESPONSES, GAS EXCHANGE AND LEAF ANATOMY OF *Handroanthus* spp. IN MINING TAILINGS ENRICHED WITH NUTRIENT SOLUTION

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ABSTRACT

The objective of this work was to evaluate the effect of nutrient solution application on growth, ecophysiological characteristics and leaf anatomical characteristics of *Handroanthus serratifolius* and *Handroanthus impetiginosus* cultivated in dam-breaking mining tailings in the city of Mariana, Brazil. Saplings of plants of the two species with 60 days of life were used and transplanted in the mining tailings kept in field capacity. The complete nutrient solution by Hoagland and Arnon (1950) was used with an ionic strength of 40% every 15 days, the height of the plants was measured weekly and at the end of the 60 days of the experiment, the effects of the addition of the nutrient solution on growth, gas exchange and leaf anatomy were evaluated. The experimental design was completely randomized in a 2x2 factorial scheme, with two species and two nutritional conditions: with nutrient solution (NS +) and without nutrient solution (NS-) with 7 replicates each treatment. Data were submitted to bilateral ANOVA for $p<0.05$. Mining tailings were characterized for the presence of macro and micronutrients and presented Al, Cd, Pb and Cr. The increase in the nutrient solution for mining tailings did not affect the growth of the evaluated plants, but increased the dry mass of *H. impetiginosus* plants. When the nutrient solution was applied, there was a higher photosynthetic rate of *H. impetiginosus* saplings compared to *H. serratifolius*, followed by higher stomatal conductance. Water use efficiency was higher in *H. impetiginosus* plants when compared to *H. serratifolius*. In addition, the diameter of the xylem cavities decreased as there was greater availability of nutrients for the plants. The *Handroanthus* species tested in this work showed potential for reforestation of areas impacted by iron mining tailings and the application of a nutrient solution favored the growth of *H. impetiginosus*. The increase in the growth capacity of *H. impetiginosus* plants is related to changes in the thickness of the leaf photosynthetic tissue and higher photosynthetic rate in mining tailings supplemented with nutrient solution. The application of nutrients in mining tailings depends on the tree species to be used in reforestation systems.

Key-words: Yellow ipe; Purple ipe; Reforestation; Fertilization.

INTRODUCTION

Large amounts of environmental pollutants come from the activity of mining industries (Lacaz et al., 2017) and most come from impacts caused by failures in mining waste dams, which have been reported to a global scale (Rico et. al., 2008). In November 2015 the Fundão dam, located in the municipality of Mariana in the state of Minas Gerais in Brazil collapsed promoting the biggest environmental disaster in the mining sector in the world in terms of the volume of waste dumped and the magnitude of the damage (Carmo et al., 2017). Its destructive effect negatively affected large areas and impacted the native fauna and flora (Silva Jr et al., 2018). Fundão dam wastes are comprised of iron mining tailings (Fernandes et al., 2016). Soils contaminated by iron ore tailings generally often show low carbon and nutrient concentrations containing large amounts of small particles and high rates of potentially toxic metals (Wong and Tam, 1977; Hudson-Edwards et al., 2003).

According to Epstein and Bloom (2006), because mineral nutrients are part of structural compounds and act in metabolic processes, these are essential for proper plant growth and development. As a consequence, low soil fertility is one of the main limitations that impose nutritional stress on plants (Epstein and Bloom, 2006). Some studies have shown the lack of mineral nutrients in mining tailings (Segura et al., 2016, Andrade et. al., 2018), a characteristic that damages the growth of saplings from tree species. Saplings must be in a sufficient amount and vigor to overcome the adversities that they will inevitably face in field conditions (Farias et al., 2007).

The genus *Handroanthus* Mattos consists, mainly, of tree species found in most Brazilian biomes, widely known for their showy pink, white or yellow flowers, which appear when trees are still devoid of foliage, in winter or early spring (Leite et al., 2018). Trees of the *Handroanthus* genus have ornamental potential and are widely used in reforestation systems (Lohmann, 2012). *Handroanthus serratifolius* (Vahl.) (yellow ipe) is a forest species of great relevance in several regions of Brazil for presenting potential for the timber industry and ornamental trees (Carvalho, 1994; Goulart et al., 2017). Its trees can reach 4 to 8 meters in height and present characteristics that qualify their use in the reforestation of degraded areas (Lorenzi et al., 2002; Santos et. al., 2009). *Handroanthus impetiginosus* (Mart. Ex DC.) Mattos, popularly known as purple ipe, is an arboreal species native to Brazil that can reach up to 35 meters in height, characterized by its dense wood and resistant to decomposition (Schulzea et al., 2008, Martins et al., 2012; Silva et al., 2015).

According to Silva et al. (2015), the use of exotic tree species in reforestation may generate new impacts in these sites. Mining tailings may fail or exceed the nutrient demand of plants used in the reforestation of impacted sites causing problems for sapling survival. Previous works showed the presence of macro and micronutrients in the iron mining tailings from Fundão dam (Pádua et al., 2021). Thus, the hypotheses of this work were: 1) iron mining tailings can provide sufficient macro and micronutrients for the growth of tree species with potential for reforestation and complementary fertilization is not necessary. 2) Responses to additional fertilization to mining tailings are related to anatomical and physiological traits and vary with species. Thus, the objective of this study was to test the effect of the application of nutrient solution in the growth, anatomical and ecophysiological traits of *Handroanthus serratifolius* and *H. impetiginosus* grown in iron mining tailings.

MATERIALS AND METHODS

Plant Material and Experimental Conditions

Samples of mining tailings were collected 4 km away from the Fundão Dam, in the Mariana city region, state of Minas Gerais, Brazil, (20º22'40 "S 43º24'57" W), transported and stored in the Universidade Federal de Lavras until the assembling of the experiments. To characterize the chemical composition and presence of heavy metals in the mining tailings (Table 1) was used the methodology proposed by Claessen and collaborators (1997). The calcium (Ca^{2+}), magnesium (Mg^{2+}) and exchangeable aluminum (Al^{3+}) was extracted with a 1.0 mol L^{-1} KCl solution; the contents of K and P available extracted with 0.025 mol L^{-1} H_2SO_4 + 0.05 mol L^{-1} HCl (Mehlich-1 extrator). The determination of micronutrients was performed using a chelating solution (DTPA) by spectrophotometry of atomic absorption (EAA). For toxic heavy metals (Cd, Pb and Cr) it was used nitroperchloric digestion (AOAC, 2005) combined with EAA, flame modality (Welz and Sperling, 1999). The pH at water-saturated conditions was determined using a portable soil pH meter. These analyzes were carried out at the Department of Soil Sciences at the Universidade Federal de Lavras.

Table 1. Nutrients and toxic elements detected in the mining

Macronutrients	**mg kg $^{-1}$**
P	134.75
Mg	89.75

K	125.1
Ca	2669.5
Micronutrients	**mg kg $^{-1}$**
Mn	481.38
Fe	39409.93
Zn	4.95
Cu	5.83
Na	44.9
Potentially toxic elements (PTEs)	**mg kg $^{-1}$**
Al	2981.42
Cr	10.66
Cd	2.74
Pb	4.92
Other characteristics	
pH	6.9

The saplings were obtained from seeds collected from *Handroanthus serratifolius* and *Handroanthus impetiginosus* individuals present at the Universidade Federal de Lavras. Seeds were sown in sand at maximum water holding conditions (water added to reach 25% of the substrate volume), water was replaced daily and nutrient solution was added fortnightly; 60-days-old saplings were then used in the experiments. The experiment was carried out in a greenhouse under a natural 12-hour photoperiod at temperature between 20ºC and 24 ºC. Mining tailings were sieved and then 1.8 L was added to 2.0 L pots. Saplings were then transferred to these tailings pots, which were kept at maximum water holding capacity which comprised of 30% of the total volume, or 0.6 L per vase. Water loss by evapotranspiration was replenished daily by weighing the pots and then replenishing the water to equal the weight of the previous day.

The nutrient solution proposed by Hoagland and Arnon (1950) at 40% of its ionic strength was used. The salts used as sources for nutrients are shown as follows: $NH_4H_2PO_4$, $Ca(NO_3)_2$, $Mg(NO_3)_2$, KNO_3, K_2SO_4, $FeSO_4.7H_2O$, H_2BO_3, $MnSO_4.H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$ and $H_2MoO_4.H_2O$. The nutrient solution was added to the mining tailings every 15 days.

The experimental design was completely randomized in a 2x2 factorial scheme, with two species (*Handroanthus serratifolius* and *Handroanthus impetiginosus*) and two nutritional conditions: with nutrient solution (NS+) and without nutrient solution (NS-). One plant from one pot was considered as a replicate, eight replicates were used for each treatment (n= 32).

Biometric analysis

Plant height was measured weekly until the end of the experiment using a ruler, height was considered from the soil surface to the height of the shoot apical meristem. Plants were collected 60 days after start of the experiment and weighed on an analytical balance (AY 220, Shidmadzu, Japan) to determine fresh mass and subsequently oven-dried at 60 ºC until constant mass (about 72 h) to determine total dry mass.

Gas Exchange Analyzes

Leaf gas exchanges were evaluated using an infrared gas analyzer (IRGA) model LI-6400XT (Li-COR Biosciences, Lincoln, Nebraska, USA). Measurements were performed on one leaf per plant at 60 days of experiment. The photosynthetically active radiation was fixed at 1000 µmol m^{-2} s^{-1} in the cuvette using the 6400-02B LED Light Source, under reference CO_2 concentration at 392.3 µmol air^{-1}, pump flow at 499.48 mol s^{-1}, leaf temperature at 20.32 ºC and vapor pressure deficit (VPD) at 0.91 kPa. The following parameters were obtained: stomatal conductance (g_s), transpiration rate (E) and net photosynthesis (Pn). The water use efficiency (WUE) was calculated by the Pn/E ratio. The chlorophyll content in the leaves was estimated with a SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan); measurements were taken at one leaf per plant at the leaf base, middle and tip; data was then averaged. Gas exchanges and chlorophyll content measurements were taken at the first fully developed composed leaf from the shoot apex at one leaflet per leaf.

Anatomical analysis

At the end of the experiment (60 days), the first fully developed leaf was sampled from each plant and fixed in a solution of formaldehyde, acetic acid and 70% ethanol (F.A.A.$_{70}$) by 72 hours and then stored in 70% ethanol (Kraus and Arduin 1997). Transversal sections were obtained with steel blades from the middle region of the leaf and cleared with 50% (V V^{-1}) sodium hypochlorite and washed in distilled water twice for 10 min. Sections were then stained with a solution of 1% of safranin (m V^{-1}) and 0.1% of astra blue (m V^{-1}) in the proportion of 3:7 (V V^{-1}); slides were further mounted with 50% glycerol (V V^{-1}) (Johansen, 1940). The slides were photographed under an Olympus CX31 microscope (Olympus, Tokyo, Japan). For each replicate one slide, two sections and two fields were evaluated; data was averaged to one replicate. The UTHSCSA ImageTool Software (University of Texas Health Science Center, San Antonio, Texas, USA) was used for image analysis. The following anatomical characteristics analyzed were: adaxial and abaxial epidermis thickness, palisade and spongy parenchyma thickness in the interveinal regional and the diameter of the xylem vessels and area of the vascular bundles in the midrib region.

Statistical analysis

Data were first checked for normal distribution by the Shapiro-Wilk test and then submitted to analysis of variance (two-way ANOVA) and the means were compared by the Scott-Knott test for $p<0.05$, using the Sisvar statistical software (Ferreira, 2011). All data showed normal distribution.

RESULTS

All plants survived in mining tailings independent of nutrition treatments (with or without nutrient solution). The results of the analysis of variance for the variables evaluated were summarized in Table 2.

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

Variable	***CV %***	***Mean square values***	***F test value***	***P-value***
Plant height (S)	30.8	13.029	3.839	0.0618
Plant height (N)	30.8	2.366	0.697	0.4119
Fresh mass (S)	20.2	0.093	0.647	0.4292
Fresh mass (N)	20.2	0.057	0.393	0.5367
Dry mass (S)	38.9	0.0841	1.613	0.0003[i]
Dry mass (N)	38.9	0.3906	7.492	0.0003[i]
Photosynthetic rate (S)	15.9	1.207	21.899	0.0005
Photosynthetic rate (N)	15.9	0.140	2.535	0.1373
Transpiration rate (S)	11.4	0.000127	0.006	0.9383
Transpiration rate (N)	11.4	0.009302	0.457	0.5119

Stomatal conductance (S)	32.7	7351587.042	2.894	<0.0001[i]
Stomatal conductance (N)	32.7	16195051.042	6.375	<0.0001[i]
Intercelullar CO_2 concentration (S)	21.9	44.657	3.762	0.0763
Intercelullar CO_2 concentration (N)	21.9	1.779	0.150	0.7055
Water use efficiency (S)	25.1	3.709	16.349	0.0016
Water use efficiency (N)	25.1	0.0487	0.215	0.6514
Chlorophyll (S)	23.7	17.26	0.652	0.4288
Chlorophyll (N)	23.7	0.36	0.014	0.9080
Adaxial epidermis thickness (S)	20.8	160.205	3.166	0.0778
Adaxial epidermis thickness (N)	20.8	17.587	0.348	0.5566
Palisade parenchyma thickness (S)	20.9	10773.7	34.560	<0.0001
Palisade parenchyma thickness (N)	20.9	7.038	0.023	0.8808
Spongy parenchyma thickness (S)	29.4	81986.96	50.387	<0.0001
Spongy parenchyma thickness (N)	29.4	3758.09	2.310	0.1313
Abaxial epidermis thickness (S)	22.9	100.983723	2.404	0.0155[i]
Abaxial epidermis thickness (N)	22.9	145.015259	3.452	0.0155[i]
Vascular bundle area (S)	31.0	376733932.786	0.046	0.8302
Vascular bundle area (N)	31.0	3.8	1.9	0.3417
Xylem vessel diameter (S)	27.4	571.195	8.710	0.0038[i]
Xylem vessel diameter (N)	27.4	106.333	1.621	0.0054[i]

P-value limit of the software is 0.0001, results lower than this limit are indicated as $p<0.0001$. (S) = Species (*H. serratifolius* and *H. impetiginosus*); (N) = Nutrient Solution (with or without). The variables that showed significant interaction to $p<0.05$, were indicated by ([i]).

The plant height did not showed interaction between the factors (Table 2). *Handroanthus serratifolius* and *H. impeginosus* did not show significant differences in their height (Fig. 1A); moreover, application of nutrient solution had no effect on this variable (Fig. 1B). The fresh mass also showed not significant interaction (Table 2) and there were not significant differences between species (Fig. 1C). The application of nutrient solution did not affect the fresh mass of *Handroanthus* species growing in mining tailings (Fig. 1D). The interaction between species and nutritional conditions showed significant for the dry mass (Table 2). The application of nutrient solution increased the dry mass of *H. impetiginosus* (Fig. 1E) though no significant effect was promoted in *H. serratifolius*. The dry mass of *H.*

serratifolius was greater than *H. impetiginosus* without the application of nutrient solution, however, the NS+ treatment increased the dry mass of the *H. impetiginosus* saplings as compared with *H. serratifolius* (Fig. 1E).

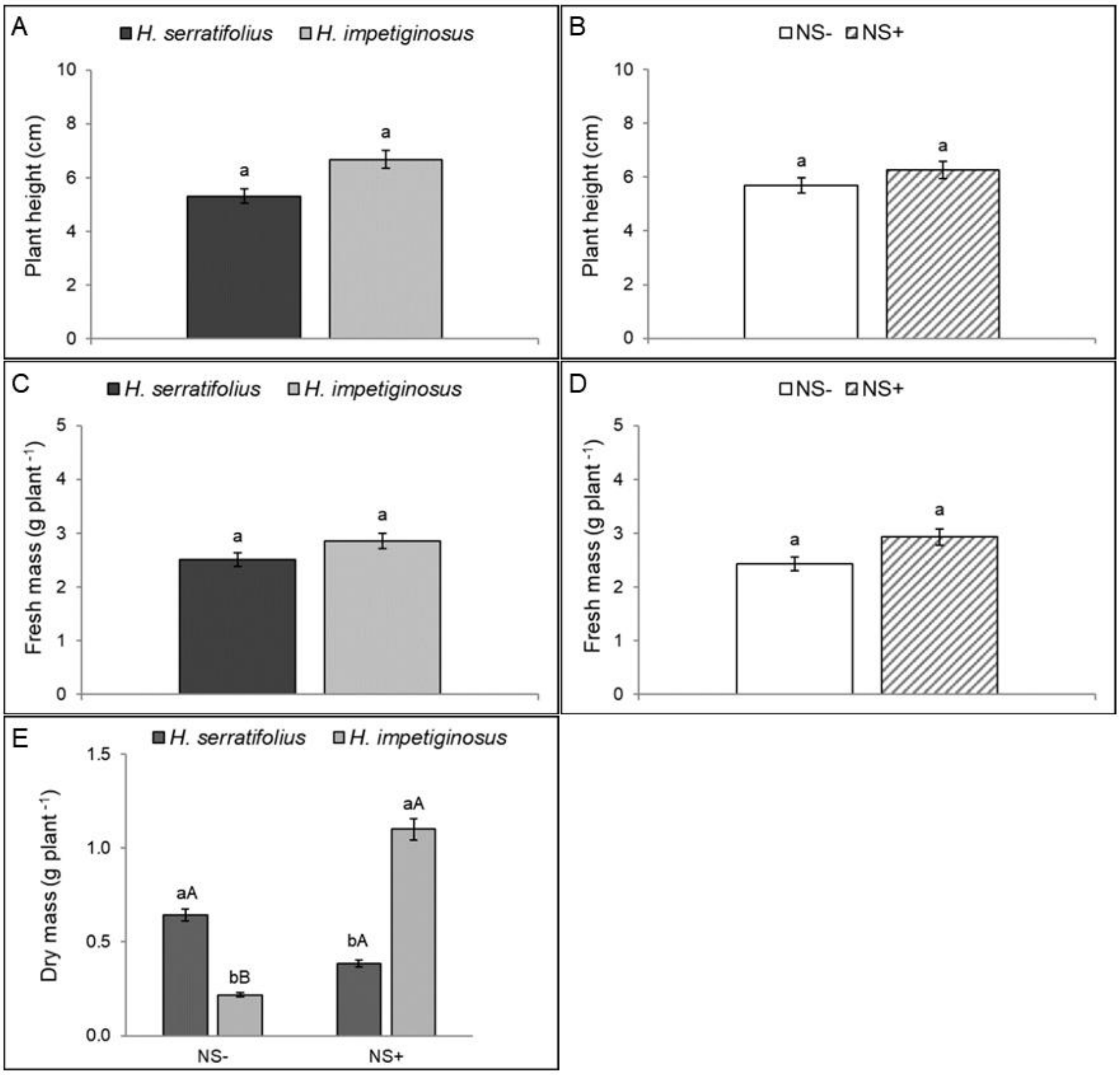

Fig. 1. Growth parameters of *H. serratifolius* and *H. impetiginosus* saplings grown in mining tailings under different nutritional conditions. Bars show standard errors. For graphs 1A-1D, means followed by the same letter do not differ by the Scott-Knott test at $p<0.05$. For the graph 1E, means followed by the same lowercase letters do not differ by the Scott-Knott test at $p < 0.05$ when comparing different species; in addition, means followed by the same uppercase letters do not differ by Scott-Knott test at $p <0.05$ when comparing the application of nutrient solution. NS-: without nutrient solution NS+: with nutrient solution.

The gas exchange parameters showed no interaction between species and the application of nutrient solution, except for the stomatal conductance (Table 2). The net photosynthesis of *H. impetiginosus* was approximately thrice the one from *H. serratifolius* (Fig. 2A); however, the application of nutrient solution promoted no significant differences for this variable (Fig. 2B). The transpiration showed no significant modification when comparing species (Fig. 2C) or the application of nutrient solution (Fig. 2D). In the absence of the nutrient solution, *H. serratifolius* showed higher stomatal conductance as compared with *H. impetiginosus*; however, application of nutrient solution reduced this parameter in *H. serratifolius* (Fig. 2E). The application of nutrient solution promotes no significant differences in stomatal conductance of *H. impetiginosus* but decreases this parameter in *H. serratifolius* leaves (Fig. 2E). Neither the species nor the application of nutrient solution promoted significant changes in the intercellular CO_2 concentration (Fig. 2F and 2H). The chlorophyll content showed no significant modifications between *Handroanthus* species or by the application of nutrient solution (Fig. 2G and 2I). The water use efficiency was higher in *H. impetiginosus* than in *H. serratifolius* (Fig. 2J), although no significant modification of this parameter was promoted by the application of nutrient solution (Fig. 2K).

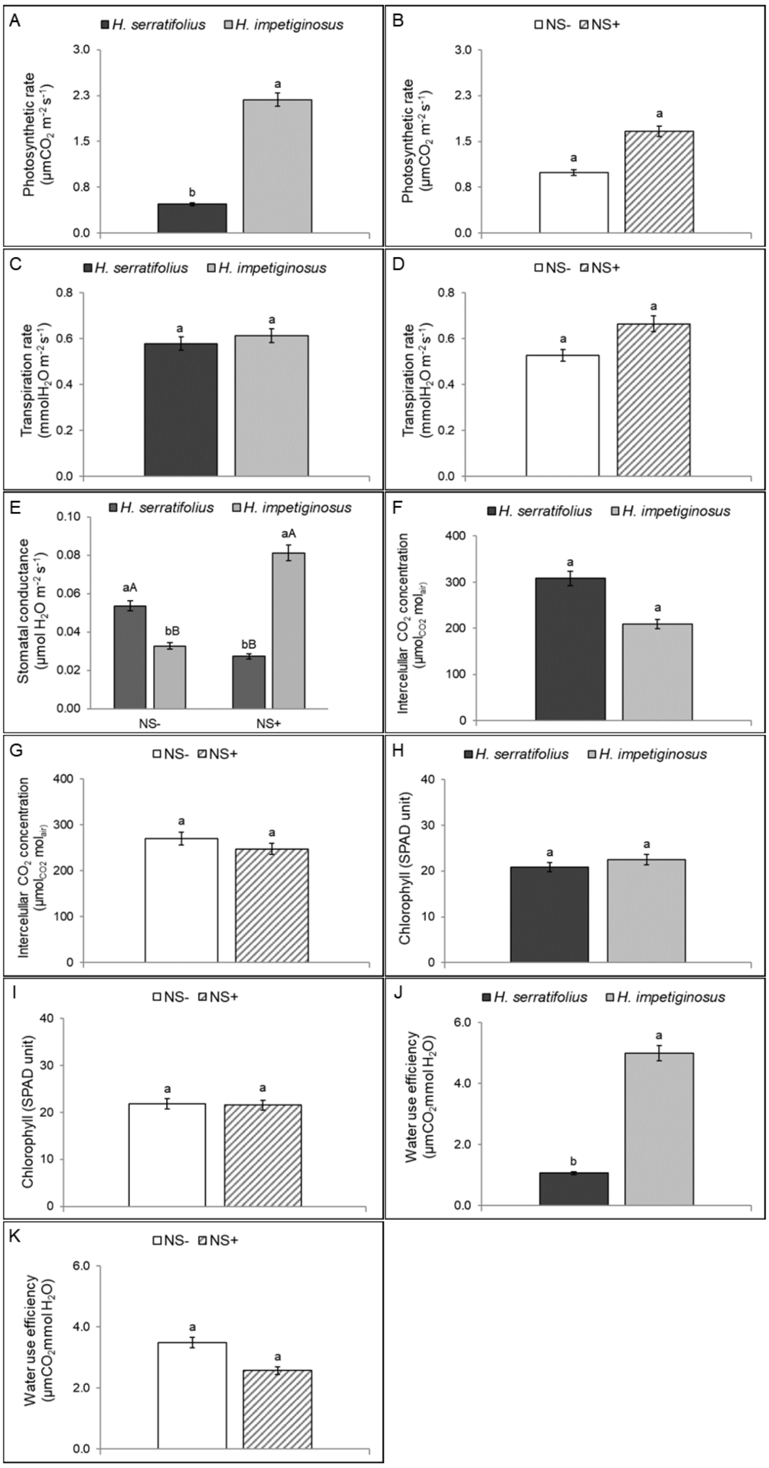

Fig. 2. Gas exchange parameters and chlorophyll content from *H. serratifolius* and *H. impetiginosus* grown in mining tailings under different nutritional conditions. Bars show standard errors. For graphs 2A-2D and 2F-2K, means followed by the same letter do not differ by the Scott-Knott test at $p<0.05$. For the graph 2E, means followed by the same lowercase letters do not differ by the Scott-Knott test at $p < 0.05$ when comparing different species; in addition, means followed by the same uppercase letters do not differ by Scott-

Knott test at $p < 0.05$ when comparing the application of nutrient solution. NS-: without nutrient solution NS+: with nutrient solution.

The leaf anatomy of *Handroanthus impetiginosus* and *Handroanthus serratifolius* is very similar (Fig. 4). In the interveinal region, leaves from both species show one-layered epidermis on adaxial and abaxial sides; epidermal cells are horizontally elongated in transversal section and the adaxial leaf side shows larger cells (Fig. 4). The mesophyll of both species is dorsiventral with palisade parenchyma on adaxial side and spongy parenchyma on the abaxial side (Fig 4). The palisade parenchyma shows one layer of vertically elongated cells with few intercellular spaces; the spongy parenchyma shows 2-3 cell layers with varied cell morphologies and plenty intercellular spaces (Fig. 4). Both leaves are hypostomatous as stomata are located only in the abaxial surface (Fig. 4). Collateral vascular bundles are found in the mesophyll containing xylem at the adaxial and phloem at abaxial side. Vascular bundles show groups of fibers surrounding its structure and, in the larger bundles, these fibers expand trending into the epidermis of leaf both sides (Fig. 4). In the midrib region, one-layered epidermis is found in both adaxial and abaxial sides and, in this region it, epidermis shows non-secretory trichomes (Fig. 4). Annelar collenchyma is found in both adaxial and abaxial sides of the midrib and it varies from two to three layers (Fig. 4). A single collateral bundle is found in the center of the midrib with xylem at adaxial and phloem at abaxial side (Fig. 4). The central vascular bundle is surrounded by sclerenchymal fibers which can completely involve the bundle or appear organized in clusters circling the bundle (Fig. 4). This variation depends on the distance from the leaf base as both species showed these two patterns. Internally to the collenchyma and circling the vascular bundle, layers of ground parenchyma are found (Fig. 4). This overall structure was not changed by the nutritional treatments (Fig. 4).

There was significant interaction between *Handroanthus* species and the application of nutrient solution for the abaxial epidermis thickness and xylem vessel diameter, whereas no other anatomical variable showed this significant interaction (Table 2). The adaxial epidermis showed no significant modifications between *Handroanthus* species or by the application of nutrient solution (Fig. 3A and 3B). The palisade parenchyma (Fig. 3C and 3D) and spongy parenchyma (Fig. 3E and 3F) were thicker in *H. impetiginosus* when compared with *H. serratifolius* (Fig. 3A-3F and Fig 4); however, these variables were not significantly modified by the application of nutrient solution (Fig. 3A-3F and Fig 4). *Handroanthus serratifolius* showed thinner abaxial epidermis when compared with *H. impetiginosus* in the absence of nutrient solution; no significant differences between species were found when nutrient solution was applied (Fig. 3G). The application of nutrient solution promoted no significant differences in the abaxial epidermis thickness of *H. impetiginosus* but increased this parameter in *H. serratifolius* leaves (Fig. 3G).

The xylem vessel diameter was smaller in the *H. serratifolius* leaves when compared with *H. impetiginosus* and under application of nutrient solution, but showed no significant differences in plants grown in the absence of nutrient solution (Fig. 3H and Fig. 4). The application of nutrient solution reduced the xylem vessel diameter of *H. serratifolius,* but promoted no significant differences in *H. impetiginosus* plants (Fig. 3H and Fig. 4). The vascular bundle area was not significantly affected by *Handroanthus* species (Fig. 3I and Fig. 4) and was not modified by the application of nutrient solution (Fig. 3J and Fig 4).

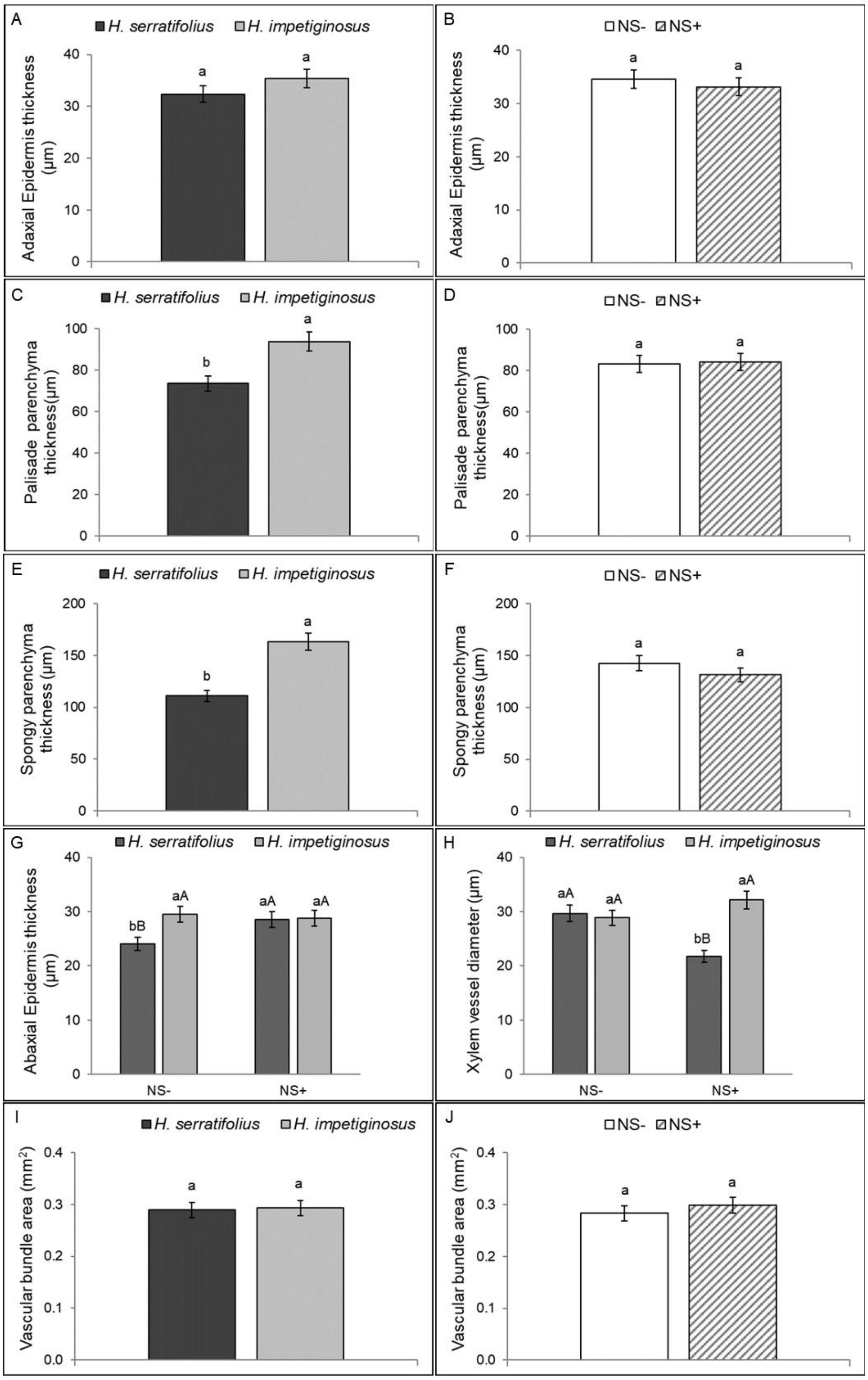

Fig. 3. Anatomical traits from *H. serratifolius* and *H. impetiginosus* grown in mining tailings under different nutritional conditions. Bars show standard errors. For graphs 2A-2D and 2F-2K, means followed by the same letter do not differ by the Scott-Knott test at $p<0.05$. For the graph 2E, means followed by the same lowercase letters do not differ by the Scott-Knott test at $p < 0.05$ when comparing different species; in addition, means followed by the same uppercase letters do not differ by Scott-Knott test at $p <0.05$ when comparing the application of nutrient solution. NS-: without nutrient solution NS+: with nutrient solution.

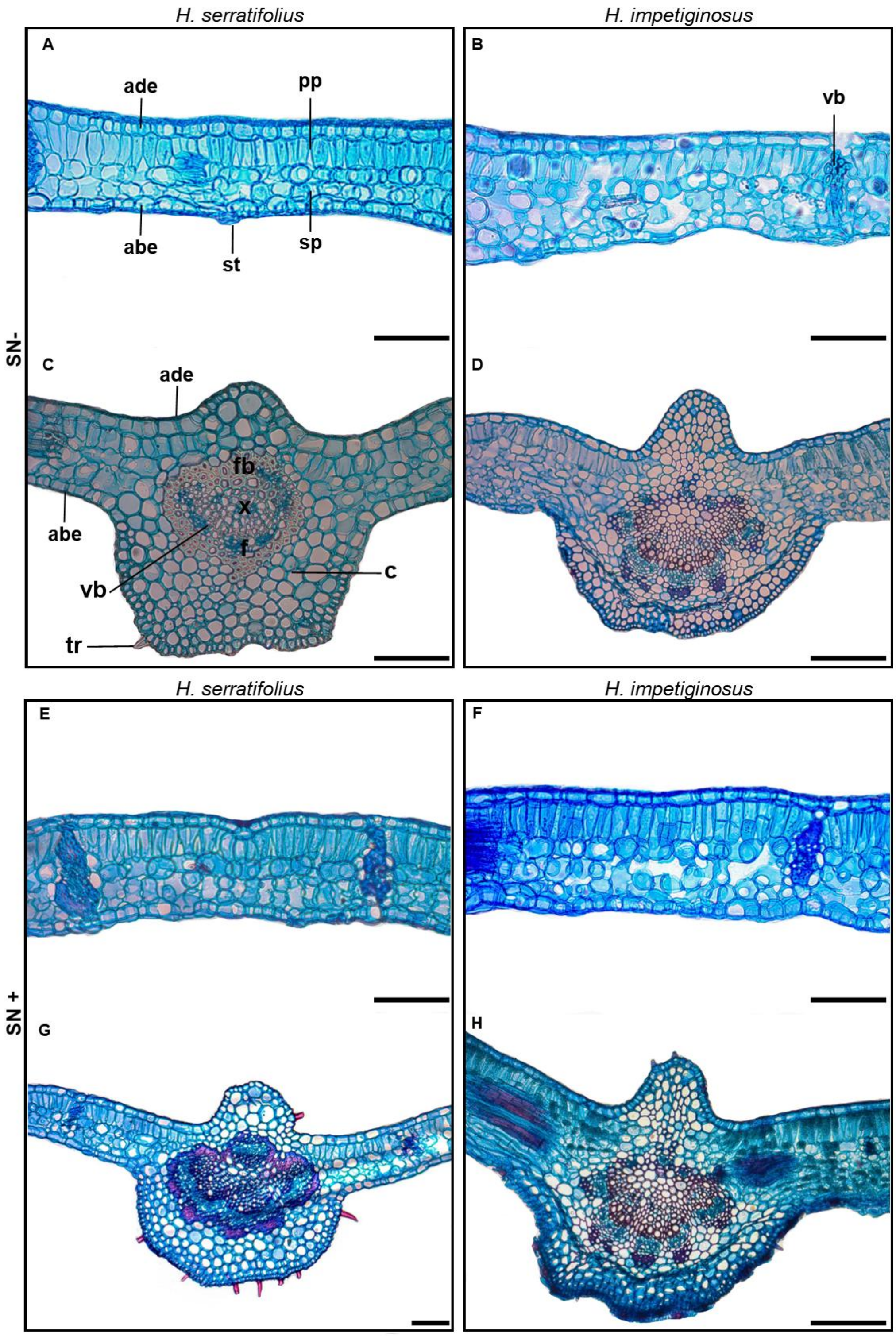

Fig. 4. Leaf anatomy of *Handroanthus* spp. grown in mining tailings under different nutritional conditions. Images A, B, E and F show the interveinal region and midrib images C, D, G and H) show the midrib region. **ade:** adaxial epidermis; **pp**: palisade parenchyma; **sp**: spongy parenchyma; **abe:** abaxial epidermis; **vb:** vascular bundle; **fb:** fibres; **x:** xylem; **f:** phloem; **c:** chlorenchyma; **t:** trichomes. NS-: without nutrient solution NS+: with nutrient solution. Bars: 100µm

DISCUSSION

The elemental analysis performed on the tailings sample detected the presence of macro and micronutrients, in addition to potentially toxic elements for plants. The presence of nutrients and potentially toxic elements in mining tailings was also detected in previous literature (Segura et al., 2016; SEDRU, 2016; Pádua et al., 2021). Despite the high concentrations of some elements in mining tailings, such as Fe, Al and Mn (Table 1), these may not fully available to plants (Guerinot and Yi, 1994) since mining tailings showed high pH value (Table 1). Another important factor to be highlighted is the tolerance of some species to high concentrations of potentially toxic elements, developing mechanisms and anatomical modifications to support this condition (Pereira et al., 2011; Ali et al., 2015; Baroni et al., 2020).

The presence of macro and micronutrients in the tailings was sufficient for the growth of *Handroanthus* species, as shown by growth results like the height and fresh mass of saplings since the application of nutrient solution did not increase these parameters. However, it is important to highlight that the use of nutrient solution increased the dry mass of *H. impetiginosus* plants that the necessity for addicional nutrition depends on species. Results shown that *H. serratifolius* can grow in mining tailings wihout nutritional complement, being viable for reforestation systems and favoring their efficiency even at early stages when there are little organic matter in the surface of the mining tailings. *Handroanthus impetiginosus* also survived in mining tailings and results also supports its use in reforestation systems although this species is favored by the application of additional nutrients and may show better development at later steps of succession, when more organic matter and nutrients may be available.

In this study, we observed higher photosynthetic rate of *H. impetiginosus* saplings compared to *H. serratifolius* and this may be related to a higher stomatal conductance, particularly when nutrient solution was applied and this higher photosynthesis resulted in increased dry mass production. The decrease in photosynthesis can result from the restriction of CO_2 uptake by the stomata when reduced stomatal conductance is registred (Souza et al., 2005; Rodriguéz-Gamir et al., 2011). Due to the higher photosynthetic rate, there was also an approximately 4-fold increase in the water use efficiency of *H. impetiginosus* plants under nutrient addition when compared to *H. serratifolius* under the same conditions (Fig. 2J).

Therefore, the plants of *H. impetiginosus* have greater growth potential as long as there is a greater input of nutrients in the mining tailings.

Some nutrients are directly linked to plant growth and potassium (K) is one of the most demanded macronutrients, playing important roles in several metabolic processes, such as enzymatic activation, respiration, photosynthesis and improving water balance (Lopes 1998; Prazeres et al. 2015). Potassium is directly linked to stomatal opening and closing, thus regulating the diffusion of CO_2 into mesophyll cells (Xu and Gosselin, 1994). Thus, K can interfere with the concentration of carbon in the substomatal cavity (Faquin and Andrade, 2004) regulating stomatal opening and conductance. In plants of *H. impetiginosus,* the aplication of a nutrient solution added significant supplement of K which may have favored the stomatal conductance and photosynthetic rate increasing the dry mass production of the species. These results indicate that *H. impetiginosus* saplings show a greater demand for nutrients when compared with *H. serratifolius* and growing in iron mining tailings. It is also noteworthy that the fertility of iron mining tailings can be considered low (IBAMA, 2015) due to the low cation availability and the lack of organic matter. Later successional stages increase the organic matter and fertility of soils as most of the forest carbon and organic matter stocks come from forest species (Machado et al., 2019). The *Handroanthus* species tested in this work show potential for reforestation of areas impacted by iron mining tailings, although they must be introduced in different stages. This may be true for other tree species and iron wastes from different regions, supporting the necessity to test the nutrient demand to each particular situation.

Soil macronutrient stocks for effective forest development need to be at least 10-15 mg Kg^{-1} of P, 100-175 mg Kg^{-1} of K, 150-300 mg Kg^{-1} of Ca and 28-33 mg Kg^{-1} of Mg (Bond, 2010); however, soil characteristics such as pH, organic matter content, redox potential, temperature, moisture and microbial activity influence the availability of nutrients (Moreira et al., 2017). Bittencourt et al. (2020) demonstrated that the absence of macronutrients can reduce the growth of *H. serratifolius* saplings, but here, the application of nutrient solution did not promote an effect on the growth of *H. serratifolius*, showing that the levels of nutrients found in iron mining tailings are sufficient for the development of this species (Table 1). For *H. impetiginosus* plants, Leite and collaborators (2019) reported that fertilized saplings significantly expanded their leaves and invested more in shoot growth, improving their rusticity and quality, corroborating the results presented here.

The leaf anatomical structure of *Handroanthus impetiginosus* and *Handroanthus serratifolius* as shown in the Figure 4 showed no evidences of toxicity since it are similar to the description of its structure in the literature, as shown by Silva et al. (2009). Results from leaf anatomical modifications of *Handroanthus* species growing in mining tailings support the gas exchange and growth results. The application of nutrient solution did not promote differences in the anatomical structure of the leaves of the *Handroanthus* species, except for the parameters in which there was an interaction between the factors. The higher photosynthetic rate of *H. impetiginosus* plants may be related to its thicker leaves as compared with *H. serratifolius,* regardless of the addition of nutrient solution. Many studies state that the thickness of photosynthetic tissues significantly increases the photosynthetic rate and plant growth (Santos et al., 2015; Pereira et al. 2016, Cruz et al., 2019). The thicker palisade and spongy parenchymas from leaves of *H. impetiginosus* may have favored the photosynthetic rate and growth when compared with *H. serratifolius*.

The diameter of the xylem pot is proportional to the plant's ability to capture water and nutrients (Tombesi et al., 2010; Díaz et al., 2018). Therefore, the xylem pot diameter of *H. serratifolius* plants was reduced in size when the nutrient solution was applied to mining tailings, while data for this parameter remained unchanged in *H. impetiginosus* plants, regardless of nutrient increment. Santos and collaborators (2015), observed similar results when testing different phosphorus concentrations for *Typha domingensis* Pers. plants, where the parameters of the xylem vessels in the vascular bundles on the adaxial side decreased with increasing concentration of P. Thus, the plants of *H. serratifolius* under higher nutrient input tends to reduce investment in xylem vessel diameter, confirming its lower nutrient demand compared to *H. impetiginosus* and can thrive in iron mining tailings without additional nutrient application.

CONCLUSION

The tree species *Handroanthus serratifolius* and *Handroanthus impetiginosus* showed potential to reforest areas impacted by iron mining tailings and can survive in mining tailings without additional application of nutrientes. Application of nutrient solution to iron mining tailings favored the growth of *H. impetiginosus* plants but have no effect in the growth of *H. serratifolius*. The increased growth capacity of *H. impetiginosus* plants is related to changes in the thickness of the leaf photosynthetic tissues and higher photosynthetic rate in mining tailings supplemented with nutrient solution. The benefits from the application of additional nutrients to iron mining tailings depend on the tree species to be used in reforestation systems.

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