UNIVERSIDADE FEDERAL DE ALFENAS

GISELE DE FÁTIMA ESTEVES

CRESCIMENTO E DESENVOLVIMENTO DE PLANTAS DE MILHETO, MILHO E SORGO EM REJEITOS DE MINERAÇÃO DO DESASTRE DE MARIANA – MG E O EFEITO BENÉFICO DA ADIÇÃO DE VERMICOMPOSTO

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Orientador: Prof. Dr. Thiago Corrêa de Souza
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GISELE DE FÁTIMA ESTEVES

"Crescimento e desenvolvimento de plantas de milheto, milho e sorgo em rejeitos de mineração do desastre de Mariana – MG e o efeito benéfico da adição de vermicomposto".

A Banca julgadora, abaixo assinada, aprova a Dissertação apresentada como parte dos requisitos para a obtenção do título de Mestre em Ciências Ambientais pela Universidade Federal de Alfenas. Área de Concentração: Ciências Ambientais.

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(Ayrton Senna)

RESUMO

Diante do acidente ocorrido em 2015 em Mariana- MG, houve uma grande preocupação em reestabilizar o ambiente, visto que o rejeito devastou uma extensa área. Desse modo, o presente estudo objetivou avaliar a germinação, crescimento e desenvolvimento iniciais de três espécies vegetais: milho DKB 390 (*Zea mays* L.), milheto BRS 1502 (*Pennisitum glaucum* L.) e sorgo BRS 332 (*Sorghum bicolor)*; assim como o efeito da adição de vermicomposto ao rejeito de mineração visando à promoção do crescimento dessas espécies. Assim foram realizados trêsI e II utilizou-se os seguintes tratamentos: 0T (100% sand), 25T (25% tailings + 75% sand); 50T (50 % tailings + 50% sand); 75T (75% tailings + 25% sand) and 100T (100% tailings). No experimento I foram avaliados a germinação e crescimento inicial, enquanto no experimento II os atributos de crescimento, eficiência fotossintética, acúmulo de metais e morfologia de raiz de plantas em estádio V3 foram avaliados. Observou-se que a germinação e o crescimento inicial das três espécies não sofrem influência do rejeito. No entanto, as plantas em estádio V3 apresentaram variações morfofisiológicas distintas entre as espécies, com alterações no crescimento, acúmulo de biomassa e morfologia das raízes. No experimento III utilizou-se os seguintes tratamentos: rejeito de mineração (Tailings), rejeito de mineração + vermicomposto na proporção (20g de húmus dm^{-3} rejeito) (Tailings + vermicopost) e um solo de referência (Soil). As avaliações se iniciaram quando as plantas estavam no estádio V3 e foram realizadas quinzenalmente, avaliando-se o crescimento, trocas gasosas e eficiência da clorofila. Ao final do experimento, foram avaliados o acúmulo de biomassa e de metal, e morfologia de raízes. A adição de vermicomposto favoreceu o incremento de biomassa seca de parte aérea e raiz nas espécies estudadas. Essas espécies apresentaram maiores comprimento, volume e diâmetro radiculares, favorecendo a absorção dos macronutrientes P, K, Mg e Ca e dos micros B, Fe e Zn. Também foram observadas maiores trocas gasosas e crescimento dessas plantas. Em síntese, o crescimento radicular é o principal responsável pelo sucesso das espécies cultivadas em rejeitos de mineração, independentemente do estágio de desenvolvimento em que se encontram.

Palavras-chave: Raízes de Plantas. Áreas Alagadas. Compactação. Fenômenos Fisiológicos Vegetais. Cereais.

ABSTRACT

Given the accident that occurred in 2015 in Mariana-MG, there was a great concern in restoring the environment, as the waste devastated an extensive area. In this way, this study aimed to evaluate the germination, and initial growth and development of three plant species: DKB 390 maize (*Zea mays* L.), BRS 1502 (*Pennisitum glaucum* L.) millet and BRS 332 (*Sorghum bicolor*) sorghum; as well as the effect of the addition of vermicompost to the mining tailings on the plant growth promotion. Thus, three experiments were conducted. The experiments I and II were performed using the following treatments: 0T (100% sand), 25T (25% tailings + 75% sand); 50T (50% tailings + 50% sand); 75T (75% tailings + 25% sand) and 100T (100% tailings). There were evaluated seed germination and initial growth in Experiment I, whereas in Experiment II there were evaluated plant growth, photosynthetic efficiency, metal accumulation and root morphology of plants in V3 developmental stage. It was observed that tailings did not affect seed germination and initial growth of all plant species. However, the morphophysiological changes of plants at V3 stage were different between species, with modifications in plant growth and biomass accumulation and root morphology. The experiment III was performed with the treatments: mining tailings (Tailings), mining tailings + vermicompost in proportion (20g of worm castings dm^{-3} tailings) (Tailings + vermicopost) and a reference soil (Soil). The evaluations started when the plants reached the V3 stage, and were performed biweekly, assessing plant growth, gas exchange and chlorophyll efficiency. At the end of the experiment, plant biomass, metal accumulation and root morphology were evaluated. The addition of vermicompost increased shoot and root dry biomass in all of the species. Those plants showed higher root length, volume and diameter, favoring the absorption of macronutrients P, K, Mg and Ca and micros B, Fe and Zn. It was also observed higher gas exchanges and improved plant growth. In summary, root growth is primarily responsible for the success of species grown in mining tailings, despite the plant developmental stage.

Key words: Plants Roots. Wetlands. soil compaction. Plant Physiological Phenomena. Grain.

SUMÁRIO

1 INTRODUÇÃO GERAL

Em Minas Gerais, nos últimos quatro anos, ocorreram os dois maiores acidentes da história da mineração do país. Em 2019, na cidade de Brumadinho, a barragem do córrego do Feijão se rompeu e ocasionou o maior acidente de trabalho da história do Brasil, deixando mais de 300 mortos. E em 2015, na cidade de Mariana, o considerado maior acidente socioambiental (OLIVEIRA; ROHLFS; GARCIA, 2019). O rompimento da barragem do fundão em Mariana -MG devastou mais de 663,2 km devido ao extravasamento de 50 milhões de metros cúbicos de rejeito, destruiu tudo que estava em seu caminho é infelizmente vidas humanas, proporcionando consequências imensuráveis desde Minas Gerais, até a foz do rio Doce, no Espírito Santo (LOPES, 2016).

Após o acidente vários estudos foram realizados tanto na água como para o solo. A água apresentou índices de metais acima dos permitidos pelo Conselho Nacional do Meio Ambiente (CONAMA) da mesma forma que o solo. No entanto, no solo é agravado pela alteração do pH e de sua estrutura física, os sedimentos que ficaram retidos nas margens dos rios contém metais, que podem sofrer erosões e lixiviações decorrentes das chuvas e, devido às partículas serem extremamente finas, ao ressecarem se tornam compactadas, prejudicando assim a reestabilização do ecossistema (HATJE et al., 2017; SEGURA et al., 2016).

Uma das atividades eficientes em recuperar áreas degradadas é o deslocamento de solos férteis para área contaminada, assim pode se resgatar o banco de sementes junto com a reestabilização da micro, meso e maro fauna (SILVA,CAMPAGNA;LIPP-NISSINEN, 2018), no entanto essa se torna inviável vista que é um processo oneroso devido à extensão atingida. Neste contexto, existe uma necessidade de estudos acerca de espécies que possam ser cultivadas na área, contribuindo com a extração e/ou mobilização desses elementos tóxicos e que favoreçam a descompactação devido a rusticidade do seu sistema radicular (ANJUM et al., 2016, ANJUM et al.,2017).

Uma opção são as gramíneas (Poáceas) que, além do seu interesse agrícola e econômico, se destacam pelo rápido crescimento e acúmulo de biomassa (SILVA et al., 2006). Nesse sentido, espécies como milheto e sorgo auxiliam na descompactação de solos (CALONEGO et al., 2011; HERRADA; LEANDRO; FERREIRA et al., 2017), assim como o milho, que sobrevive em solos compactados (GRZESIAK et al., 2014) e tem elevado potencial de translocação de Cu e Zn para a parte aérea (TAVARES;OLIVEIRA; SALGADO, 2014).

Ainda assim, só a utilização de gramínea seria um processo demorado, então se torna necessário melhorar a estrutura do rejeito de forma direta, visto em estudos anteriores que esse possui alteração no pH, deficiência de matéria orgânica e alta densidade (SEGURA et al., 2016). Assim, uma opção relativamente barata e eficiente é a areia, ela melhora a estrutura do solo assim como o vermicomposto, que possui elevadas concentrações de matéria orgânica e de ácidos húmicos que auxiliam na redução do pH do solo, melhoram o crescimento das plantas e auxiliam na disponibilidade e absorção dos nutrientes (ALBIACH et al., 2000). Dessa forma levanta-se a hipótese de que que a adição desses substratos ao rejeito de mineração ajudaria a mitigar os efeitos do rejeito nos genótipos de milho, milheto e sorgo e melhoraria o seu desenvolvimento.

2 REVISÃO BIBLIOGRÁFICA

2.1 O ACIDENTE E O IMPACTO DO REJEITO

A atividade mineradora é exercida no Brasil desde o século XVII e, neste período, já foram relatados diversos acidentes envolvendo mortes e contaminações ambientais. Em Minas Gerais ocorreram diversos acidentes envolvendo o desabamento de barragens como na cidade de Itabirito (1986) com o rompimento da barragem do grupo ITAMINAS, matando sete pessoas; em Nova Lima (2001) mineração Rio Verde causando a morte de cinco pessoas; em Miraí (2007) na mineração Rio Pomba Cataguases desalojando 4 mil moradores e afetando 4 municípios; em Congonhas (2008) uma falha no vertedouro da barragem da Companhia Siderúrgica Nacional inundou de lama parte da cidade; em Itabirito (2014) na mineração Herculano matando três pessoas.

Em 2015, o acidente que provocou o maior impacto socioambiental no Brasil ocorreu em Mariana-MG com o rompimento da barragem do Fundão, que continha rejeitos de mineração compostos principalmente por óxido de ferro e sílica (LACAZ; PORTO; PINHEIRO, 2017). O rejeito percorreu mais de 600 km ao longo do rio Doce e seus afluentes, resultando em 19 mortes, além dos inúmeros impactos ambientais. No início do ano de 2019, ocorreu o acidente de Brumadinho, onde a barragem do córrego do Feijão se rompeu, liberando cerca de 12 milhões de metros cúbicos de rejeito. O rejeito resultante do extravasamento percorreu mais de 100 km até atingir o rio Paraopeba, caracterizando o maior acidente de trabalho da história de mineração do país, em que mais de 300 vidas humanas foram perdidas, além da devastação da fauna e flora locais (FARIA, 2019).

O Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) em 2015 emitiu um Laudo Técnico Preliminar apontando que o impacto do rompimento da barragem do Fundão foi tão intenso nos
trechos do desastre que se torna impossível determinar um prazo para a reestabilização da flora e da fauna. O acidente ocasionou a perda lastimável de dezenove vidas humanas; o desalojamento de comunidades; restrições à pesca; alteração na qualidade e quantidade de água; falta de energia; destruição de 1.469 hectares de vegetação, incluindo Áreas de Preservação Permanente (APPs) da Mata Atlântica; impactou na fauna, flora e solo de toda extensão percorrida e também atingiu 3 rios (Gualaxo do Norte, Carmo e Rio Doce)

Devido a elevada concentração de sedimentos em suspensão nos cursos d'água, ocorreu o aumento da demanda bioquímica de oxigênio devido a diminuição da permeabilidade de luz, afetando assim todo ambiente aquático (STUTTER et al., 2017). O levantamento oceanográfico realizado pela Marinha do Brasil em 2015 também mostrou que os rejeitos ao alcançarem as águas do Rio Doce se dividiam em uma pluma superficial, e outra mais espessa, próxima ao fundo. Além da coloração alaranjada, essas plumas possuem partículas finas em suspensão, cuja quantidade decrescia gradualmente na medida em que se afastam da foz, foi observado que as condições metrológicas e oceanográficas interferem de forma diferente nas plumas o que pode resultar na dispersão dos rejeitos para áreas distintas.

O monitoramento continuou e comprovou a dispersão da pluma de sedimentos, e em 25 de setembro de 2017 foi emitido um relatório pelo Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) com os resultados do monitoramento por sobrevoos. Este indicou a incidência da mesma em toda a costa do Espírito Santo, extremo norte do Rio de Janeiro e extremo sul da Bahia, em quantidades e concentrações cumulativas, sendo comprovado por outros métodos de monitoramento, tais como satélite, isótopos e biogeoquímico (ICMBio, 2017).

O desastre na região de Mariana-MG ocasionou uma crise social aguda, tendo em vista a perturbação direta que provocou nas comunidades urbanas e rurais; aos agricultores, ribeirinhos, pescadores, indígenas e quilombolas que ali viviam e ainda usufruía dos recursos gerados pelos rios (FERREIRA; LEITE, 2015) e as maiores perdas de áreas foram nas categorias vegetação com redução de 11,99km^2 e das pastagens com redução de 4,81 km 2 (AIRES et al., 2018).

2.2 ANÁLISE DA ÁGUA E DO REJEITO

Um elemento químico em ambiente aquático pode ser afetado por diversos fatores como: pH, diluição, mudanças sazonais, ressuspensão de sedimentos entre outros (SEGURA et al., 2016). Análises realizadas em 2015 nos rios da região de Mariana-MG apresentavam violações aos padrões normativos. Várias amostras analisadas após a passagem da onda de rejeitos estavam contaminadas por, pelo menos, um elemento metálico o que evidencia a gravidade da situação (FELIPPE et al., 2016). Uma outra pesquisa realizada por Hatje et al.(2017), relata que as águas se encontram bem oxigenadas, porém com pH e temperatura elevados pela presença do rejeito. Nos sedimentos analisados foi observado que a porcentagem de ferro (Fe) biodisponível permaneceu relativamente constante e baixo próximo ao Rio Gualaxo do Norte (0,4%), aumentando ao longo do Rio Doce (10%) se elevando ainda mais próximo ao mar (16%), esse aumento gradual indica que o ferro foi mobilizado e torna-se mais biodisponível ao longo do trajeto. Ainda ressaltam que lama promoveu uma transformação do sistema fluvial, causando a degradação da planície inundando várias áreas.

Já o rejeito apresenta partículas de tamanhos variados de 1 a 0,002 mm, textura semelhante ao cimento quando seca e aspecto extremamente espesso e denso quando encharcado o que preocupa pela contaminação ao longo do tempo já que os sedimentos que ficaram retidos nas margens dos rios e dos reservatórios com as posteriores chuvas poderão ocasionar erosão, remoção e transporte destes sedimentos que contendo partículas com metais, prejudicando assim a estabilização de todo o ecossistema (HATJE et al., 2017; SEGURA et al., 2016). Na caracterização química, observou-se compostos inorgânicos e alguns elementos químicos (Ba, Pb, As, Fe, Sr, Mn, Al) com alto potencial de lixiviação (SEGURA et al., 2016). De maneira geral, a lama extravasada é quimicamente pobre, mas os problemas físicos são maiores que os químicos para a recuperação ambiental (SHAEFER et al., 2016; SILVA et al., 2017).

2.3 O REJEITO DE MARIANA E SEUS POTENCIAIS EFEITOS À SAÚDE HUMANA

Segundo o relatório emitido pela Fundação Bradesco (LEAL et al., 2015), devido à composição dos rejeitos de minérios, acredita-se que os solos da região de Mariana-MG se tornarão inférteis, pois a cobertura de lama que afetou o pH do solo, inviabilizará a vida dos microrganismos, deixando os solos pobres em matéria orgânica. Além de promover a desestruturação química dos solos, acarretando na lixiviação dos elementos químicos atingindo os lençóis freáticos. As atividades agrícolas e as pastagens ficarão impraticáveis devido a crosta espessa criada pelo ressecamento da lama. Vale ressaltar que as regiões classificadas como afloramento de rocha não sofreram alterações durante o período analisado (AIRES et al., 2018), no entanto a formação de um novo solo pode levar centenas de anos.

Os metais pesados são elementos que estão presentes naturalmente no ambiente, entretanto em grandes concentrações podem se tornar tóxicos. Nos seres humanos, isso ocorre devido à facilidade com que estes elementos são absorvidos pelo organismo e acabam acumulando em órgãos e tecidos, podendo atingir o sistema nervoso, órgãos hepáticos e sistema endócrino (MARTIN; GRISWOLD, 2009). Se a lama contendo os elementos (Ba, Pb, As, Fe, Sr, Mn, Al) citada por Segura et al. (2016), realmente lixiviar poderá ocasionar graves danos a todo ecossistema e principalmente aos seres humanos.

Intoxicações por bário (Ba) são raras, mas quando ocorrem, os sintomas são: vômitos, diarreia aquosa severa, cólicas abdominais, salivação e fraqueza. Devido à hipopotassemia profunda, pode ocorrer paradas respiratória e cardíaca, também, paralisia dos membros e músculos respiratórios com arreflexia, hipofosfatemia, acometimento visual, convulsões, rabdomiólise e insuficiência renal aguda; e depressão do sistema nervoso central (SNC), embora o indivíduo permaneça consciente, mesmo em estado grave (MARTIN; GRISWOLD, 2009).

Exposição prolongada ao chumbo (Pb) pode ocasionar fadiga, irritabilidade, insônia, anorexia e perda de peso, dores de cabeça, problemas na concentração, deslocamento do cálcio dos ossos evoluindo para uma osteoporose, mal funcionamento dos rins, do trato gastrointestinal, do sistema reprodutivo, lesões agudas ou crônicas do sistema nervoso e do sistema hematopoiético (OLSON, 2014).

Os casos de intoxicação por arsênio (As) se manifestam por fadiga, náusea, vômito, dor abdominal intensa, diarreia, sangramento no trato gastrointestinal, aparecimento de lesões e câncer de pele, bexiga e pulmão e doenças vasculares. Em casos mais graves, surgem efeitos cardiovasculares, hipotensão, taquicardia, arritmia, choque e morte (MARTIN; GRISWOLD, 2009; OLSON, 2014).

A intoxicação aguda pelo ferro (Fe) ocasiona diarreia, náuseas, vômitos e sangramento gastrointestinal, elevação da acidez do sangue, hiperventilação, redução na produção de urina, falência do fígado e dos rins, doenças cardiovasculares, doenças degenerativas cerebrais, desordens neurológicas, como demência (RIORDAN, 2002; ROBERTI et al., 2011).

O estrôncio (Sr) pertence a mesma família do cálcio (Ca) por isso se comporta de forma semelhante, o corpo humano tende a usá-lo da mesma forma. O elemento em si não é toxico o problema é seu isótopo Sr-90 que pode causar sérios problemas de saúde, entre eles câncer (SILVA et al., 2017).

O manganês (Mn) pode causar rigidez muscular, anorexia, salivação, deficiências neurológicas, transtornos comportamentais, doenças neuropsíquicas, embolia pulmonar e bronquite. Em casos graves pode, ocorre perda de expressão facial, rigidez, problemas no discurso, tremores, distonia, falhas nos reflexos e hipertonia muscular (OLSON, 2013; LEAL et al., 2015).

A intoxicação por alumínio (Al) ocorre principalmente pelas vias do trato gastrointestinal, pele e mucosa nasal. A poeira oriunda do metal causa irritação das vias aéreas, tosse, bronco espasmo e fibrose pulmonar. Após a absorção, o alumínio pode se concentrar nos ossos, glândulas paratireoides, rins e sistema nervoso central, agitação, confusão mental, convulsão, distúrbios da marcha e fala, alucinações auditivas e visuais (ANDRADE FILHO et al., 2013).

O acúmulo de Cr em solos ocorre principalmente por ações antropogênicas como: mineração, resíduos industriais, lodo de esgoto uso de pesticidas e fertilizantes, podendo ocorrer de formas naturais também como: erupções vulcânicas e intemperismo (NAGAJYOTI et al., 2010). É um elemento essencial para saúde humana, tendo um papel importante no metabolismo de glicose, gorduras e proteínas, no entanto a intoxicação com Cr pode causar: náuseas, diarreias, danos ao fígado e rim, hemorragias internas, dermatites e problemas respiratórios. Diversos estudos comprovaram que a exposição prolongada a grandes quantidades pode ocasionar câncer de pulmão (SILVA e PEDROZO, 2001; GUTTERRES et al., 2011)

Geralmente, os seres humanos são expostos a esses metais por ingestão, contato ou inalação, no entanto esses metais pesados são acumulados principalmente no solo e quanto maior acidez do solo mais solúveis e moveis eles se tornam, deixando-os mais fácil de serem absorvidos e acumulados por plantas que consequentemente os transferem para cadeia alimentar (MARTIN et al., 2009; ADREES et al., 2015),

2.4 O REJEITO E SEU EFEITO DE COMPACTAÇÃO

Uma das principais preocupações após o rompimento da barragem do Fundão foi a contaminação ambiental devido aos componentes do rejeito, estudos anteriores mostram a existência desses elementos químicos mais em baixo nível de toxidez (ANDRADE et al., 2018). No entanto analise desse material revelou que o rejeito é pobre em nutrientes e as suas propriedades físicas apresentam partículas coloidais muito pequenas (SEDRU, 2016; SEGURA et al., 2016), induzindo a compactação, assim, inibindo da restauração do ecossistema.

O efeito físico mais observado em solo compactados é o aumento da resistência da à penetração e da exploração das raízes, que alterar significativamente a arquitetura das mesmas, bem como as taxas de crescimento e desenvolvimento das plantas (BENIGNO et al., 2012).

A compactação do solo altera os espaços dos poros e, consequentemente, afeta a densidade, a porosidade e as propriedades hidráulicas do solo (MAHMOODLU, 2016). Os poros do solo são geralmente agrupados em duas categorias: micros que apresentam diâmetro inferiores a 0,06 mm e são importantes para a retenção de água, enquanto os macros tem diâmetro superior a 0,06 mm e influenciam na aeração e fluxo de água. Entretanto esses representam cerca de 74 a 100% do fluxo de água total do solo (ALAOUI e HELBLING, 2006), para que isso ocorra existem fatores que os beneficiem como: umidade do solo, textura, grau de compactação e conteúdo de matéria orgânica (LARSBO, 2011; MCGRATH; HINZ; SIVAPALAN, 2012).

2.5 FERTILIZANTES ORGÂNICOS

O uso de fertilizantes orgânicos auxilia na recuperação de solos degradados, pois além de fornecerem matéria orgânica e nutrientes, também melhoram a atividade microbiana, e a biodiversidade do solo, favorecendo os parâmetros físico-químicos e a ciclagem de nutrientes. (ALBIACH et al., 2000).

Entre os fertilizantes orgânicos o mais utilizado é o vermicomposto, que é um perfeito condicionador de solo pois aumenta a fertilidade do solo, favorece a absorção de Mg, Ca, P, K e nitratos, eleva a capacidade de troca de cátions (DEMIR 2019), facilita a atividade enzimática (protease, amilase, celulase e pectinase) e diminui a incidência de doenças de plantas, principalmente aquelas causadas por patógenos do solo devido a melhorar da atividade e biomassa microbiana (THIELE-BRUHN, 2012).

Assim, o vermicomposto pode ser o resultado de um reaproveitamento de resíduos que melhora a estrutura do solo, tornando-o mais poroso e permeável, diminuindo a resistência à penetração (AKSAKAL; SARI; ANGIN, 2016), o que influencia positivamente no crescimento vegetativo tanto de parte aérea como de raiz, (LAZCANO et al., 2009) o que faz com que a utilização desse material seja uma técnica Biotecnológica sustentável. Também foram relatados aumentos no número e a biomassa das flores (ARANCON et al., 2008; ATIYEH et al., 2002) e na produção de frutos (SINGH et al., 2008) em plantas de diferentes espécies cultivadas em substratos com adição de vermicomposto.

2.6 METAIS PESADOS E AS PLANTAS

A presença de metais pesados no solo pode inibir principalmente a absorção dos micronutrientes: Cu, Fe, Mn e Zn pelas raízes das plantas, devido ao mesmo sitio de ligação (ZHANG et al., 2014) e a sua mobilidade no solo depende: do pH, do potencial redox, da quantidade de matéria orgânica, dos minerais presentes na argila e da capacidade de troca catiônica (PEZZAROSSA; GORINI; PETRUZZELLI, 2011).

Os metais pesados de forma geral podem interferir na anatomia e morfologia da parte aérea e raízes, na absorção de nutrientes, na produção de metabólitos secundários, no balanço hídrico, nas trocas gasosas, no funcionamento dos cloroplastos e consequentemente inibição da biossíntese de clorofila, além de alterar as atividades enzimáticas das espécies vegetais (KUMAR et al., 2012).

A fotossíntese é um dos principais processos fisiológicos afetados pela presença dos metais: o Fe atua nas reações de transferência de elétrons (NADP) durante a fotossíntese (KERBAUY, 2008), é um dos componentes da ferredoxina e está envolvido na redução de nitrato (NO^{3-}) e sulfato ($SO_4{}^{2-}$) (FAQUIN, 2005), também participa da formação de algumas enzimas (catalases, peroxidases, citocromo oxidase e xantina oxidase).

O Fe em excesso nas plantas, prejudica o transporte fotossintético, induzindo ao estresse oxidativo (RAI; AGRAWAL; AGRAWAL, 2016), assim a formação de EROs, interfere diretamente nos processos metabólicos, incluindo fotoquímicos e obstruções bioquímicas da fotossíntese, consequentemente redução da taxa de assimilação de carbono (PEREIRA, 2013), onde pode ser observado o amarelecimento e, ou bronzeamento das folhas (SIQUEIRA-SILVA, 2012). Também causa desbalanço nutricional, reduzindo a absorção de alguns elementos essenciais como: P, Ca, K, Mg e Zn (AUDEBERT; FOFANA, 2009). Essa alteração nutricional se deve a formação da “placa de ferro” na superfície radicular (CHEN et al., 2006), que é constituídas de óxidos e hidróxidos de ferro, apresentam elevada capacidade para adsorver diferentes minerais (LIU et al., 2008), atuando como barreira à absorção de determinados nutrientes.

O Mn funciona principalmente como parte dos sistemas enzimáticos da planta, desempenhando papel primordial na ativação de enzimas na rota do ácido chiquímico (WILKINSON; OHKI, 1988). Faz parte de vários processos importantes como da fotólise da água e está envolvido em processos redox no sistema de transporte de elétrons da fotossíntese, conversão do nitrato para síntese de aminoácidos e proteínas. Também atua em várias enzimas da fase escura da fotossíntese, como a enzima málica e a carboxiquinase fosfoenolpirúvica (MALAVOLTA, 2006). Seu excesso reduz a taxa fotossintética líquida, devido à baixa assimilação de CO_2 e condutância estomática, pode causar desintegração dos cloroplastos o que reduz os níveis de clorofila a e b (RAI et al., 2016), assim é comum observar folhas jovens com manchas amarronzadas.

O excesso de Pb interfere na morfologia, crescimento e processos fotossintéticos das plantas, devido à distorção da estrutura do cloroplasto, deficiências de síntese de clorofila, desequilíbrio em plastoquinona, redução da absorção de minerais e obstrução do transporte de elétrons (POURRUT et al., 2011).

Já o Cr pode ser absorvido pelas plantas em suas duas formas: trivalente (Cr III) a qual é transportada através dos canais de íons; e a hexavalente (Cr VI) transportado pela forma ativa (SCHIAVON *et al.*, 2008). Em excesso afeta a absorção de nutrientes essenciais ao crescimento das plantas, devido a sua competição pelos transportadores de sulfatos (KLEIMAN et al., 1997), provocando cloroses nas folhas jovens e necroses em vários tecidos, podendo ser retido nas raízes onde acarretando diversos danos radiculares, que leva a inibição do crescimento (NEWMAN, 1997). Também afeta diretamente a fotossíntese em termos de fixação de CO_2, transporte de elétrons, fotofosforilação e atividades enzimáticas, interferindo nos parâmetros de trocas gasosas, assimilação de carbono, condutância estomática, carbono interno e evapotranspiração (RODRIGUEZ et al., 2012; VERNAY; GAUTHIER-MOUSSARD; HITMI, 2007).

Assim plantas sobre estresse por metais pesados podem alterar o processo de transferência de elétrons nas organelas celulares, condicionando à geração de espécies reativas de oxigênio (EROs), ocasionando peroxidação lipídica das membranas em todos os tecidos vegetais (FARMER, 2013). Como defesa aos danos causados pelas EROs, as plantas desenvolvem mecanismos antioxidantes, enzimáticos e não-enzimáticos, como por exemplo o aumento de prolina (GAJEWSKA et al., 2006). Observando os danos ocorridos nos parâmetros morfológicos e bioquímicos pode ser disser se a espécie vegetal é tolerante ao estresse por metais.

A fitorremediação é uma técnica barata que visa utilizar as características característica natural de espécies vegetais em acumular, remover, reduzir, degradar ou imobilizar contaminantes, presentes tanto no solo como água (PILON-SMITS, 2005). E a imobilização dos contaminantes in situ de metais diminui os riscos de contaminação ambiental e a vida humana (VAN HERWIJEN, 2007). Assim, várias famílias de plantas têm sido mencionadas na literatura como fitorremediadoras e, dentre elas, podemos citar: Caryophyllaceae, Cruciferae, Cyperaceae, Gramineae, Leguminosae e Chenopodiaceae (WHITE JR. et al., 2006).

2.7 AS ESPÉCIES ESTUDADAS

O uso de gramíneas para a fitorremediação com metais pesados tem crescido consideravelmente nos últimos anos, visto que essas plantas possuem rápido crescimento, sistema radicular profundo e elevada produção de biomassa (CHEN; ARORA, 2013; GILABEL et al., 2014; VAMERALI; BANDIERA; MOSCA, 2010). Suas raízes além de absorver metais também são uma excelente ferramenta para descompactar a estrutura do solo (PADMAPRIYA et al., 2016), criando novos macroporos devido à bioturbação e aceleração da secagem localizada do solo, o que pode resultar na formação de novos espaços (BOTTINELLI et al., 2014; SCHOLL, 2014) e favorecer a formação da microbiota.

O milho (*Zea mays* L.) é considerado uma das espécies mais importantes da economia brasileira e mundial, possui elevada produção de biomassa, aliada ao seu potencial de fitoestabilizador de Zn, Cd, Cu, Pb, Mn e As (KHAN et al., 2000; TAVARES et al., 2014). O seu sistema radicular é robusto apresentando uma raiz primária, com várias raízes seminais e nodais decorrentes de nós-tronco abaixo do solo (raízes da coroa) e nodos-tronco acima do solo (raízes chaves) e raízes laterais decorrentes desses eixos (HOCHHOLDINGER et al., 2004) o que as tornam plantas com alta capacidade de descompactar. É um dos cereais mais cultivados no Brasil, o plantio se faz em duas épocas: primeira safra (ou safra de verão) e segunda safra (ou safrinha). Ao longo das últimas décadas, tem-se verificado um decréscimo na área plantada no período da primeira safra, devido à concorrência com a soja, mas que tem sido compensado pelo aumento dos plantios na "safrinha". Sua produtividade se dá em função de vários fatores integrados, sendo os mais importantes a interceptação de radiação pelo dossel, eficiência metabólica, eficiência de translocação de foto assimilados para os grãos e a capacidade de dreno (EMBRAPA, 2015).

O milheto (*Pennisetum glaucum* (L.) R. Br.) é outra gramínea com alto potencial de tolerar metais tais como Cr, Cd e Cu (BAREEN et al., 2019) , suas raízes apresentam elevado comprimento e alta densidade o que favorece sua penetração em solos compactados, auxilia na descompacta e na ciclagem nutrientes (BOER et al., 2007). É considerado uma gramínea anual de verão, cespitosa, de crescimento ereto e que apresenta excelente produção de perfilho e vigorosa rebrota. Apresenta folhas com lâminas paralelinérveas e inflorescência na forma de panícula longa e contraída. Em comparação com o milho e o sorgo, requer mais calor para germinar e se estabelece de maneira uniforme. Nos últimos anos têm ocorrido um aumento das áreas plantadas devido a sua alta resistência à seca, adaptabilidade em solos com baixa fertilidade, elevada capacidade de extração de nutrientes, sistema radicular profundo e por ser uma planta com boa capacidade de produção de massa verde e seca (EMBRAPA, 2016).

O sorgo (*Sorghum bicolor* L.) possui crescimento rápido destacando-se pela rusticidade, grande produção de biomassa e elevada tolerância ao déficit hídrico (TOLENTINO et al., 2016) e a metais, tais como Ni e Mn (NAEINI; RAD, 2018). É uma planta autógama, com baixa taxa de fecundação cruzada. Nos últimos anos agrícolas apresentou um aumento expressivo de áreas plantadas. Isso principalmente, pelo alto potencial de produção de grãos e matéria seca, além da extraordinária capacidade fisiológica que permitem paralisar seu crescimento, ou diminuir o seu metabolismo, durante o estresse hídrico, e reiniciá-lo quando a água se torna disponível o que a favorece suportar os estresses ambientais. Sendo assim uma excelente opção para produção de grãos e forragem em situações de déficit hídrico e em solos de baixa fertilidade (EMBRAPA, 2015).

Existem relatos na literatura a respeito do uso de plantas das três espécies em estudos voltado para fitorremediação. Com isso, o potencial do milho para retirada de Cu, Zn, Pb e Cd de ambientes contaminados (XU et al., 2015), do milheto para tolerância ao Cd e Ni (ASOPA et al., 2017; GUPTA et al., 2017), assim como a tolerância do sorgo ao excesso de Ni, Mn e Cr (NAEINI; RAD, 2018; PADMAPRIYA et al., 2016; SERME et al., 2015) mostram o potencial de utilização dessas plantas para recuperação de áreas contaminadas, principalmente por apresentarem alternativas de uso que não sejam relacionadas à alimentação humana, como é o caso da geração de bioenergia.

3 JUSTIFICATIVA

É notória a preocupação ambiental com a região de Mariana- MG depois do desastre. Assim, devido a magnitude da área afetada, há um grande interesse em pesquisas para o desenvolvimento de tecnologias que visem o a recuperação dessas áreas contaminação com a lama, principalmente de maneira mais eficiente e barata (LIMA et al., 2010).

Na agricultura o uso de culturas de cobertura e adição de matéria orgânica é conhecido pela melhora que traz a estrutura dos solos, como a utilização de gramíneas devido à alta densidade radicular, o que propicia uma melhora nos atributos físicos do solo, assim como a areia que possui partículas maiores auxiliando na descompactação do solo. O milheto e o sorgo auxiliam na descompactação de solos (HERRADA; LEANDRO; FERREIRA, 2017; CALONEGO et al., 2011). Segundo Tavares et al. (2014) algumas plantas são eficientes no que diz respeito à acumulação de metais, tais como: milho (*Zea mays*). Na literatura existem relatos que espécies vegetais apresentam comportamento distinto quanto à forma de descompactação, absorção, distribuição e acúmulo desse elemento, podendo ocorrer variações mesmo entre variedades de uma mesma espécie, quando submetidas a condições similares de contaminação (ESTRELA; CHAVES; SILVA, 2018). Dessa maneira, são necessárias pesquisas para comprovar a eficiência em descompactação e a capacidade dessas plantas crescerem e se desenvolverem nessas condições. Também é importante a busca de alternativas que favoreçam o estabelecimento das espécies em condições de solos compactados.

4 OBJETIVO GERAL

Avaliar a germinação, crescimento e desenvolvimento iniciais de três espécies vegetais: milho DKB 390 (*Zea mays* L.), milheto BRS 1502 (*Pennisitum glaucum* L.) e sorgo BRS 332 (*Sorghum bicolor)*; assim como o efeito da adição de vermicomposto ao rejeito de mineração visando à promoção do crescimento dessas espécies.

4.1 OBJETIVOS ESPECÍFICOS

Desse modo os objetivos específicos desse trabalho são:

a) Avaliar a germinação e o crescimento inicial das espécies vegetais (milho, milheto e sorgo) sob rejeito de mineração;

b) Avaliar o desenvolvimento das três espécies (milho, milheto e sorgo) cultivadas em rejeito de mineração com adição de vermicomposto;

c) Verificar o papel do vermicomposto em promover o crescimento das plantas cultivadas em rejeito de mineração;

d) Avaliar as características morfofisiológicas de plantas das três espécies cultivadas em rejeitos de mineração.

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ARTIGO 1

Artigo submetido para Environmental Science and Pollution Research

Do tailings from the mariana-mg (brazil) disaster affect the initial development of millet, maize and sorghum?

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Abstract

The collapse of the Fundão dam, which occurred in Mariana-MG in 2015, resulted in the overflow of more than 50 million m^3 of tailings, leaving traces of destruction and immeasurable social and environmental consequences. Chemical and physical studies of the tailings have revealed that there are some chemical elements above the allowed by Brazilian regulations, as well as high compaction density, which restricts vegetation recovery in the place. Thus, this study aimed to analyze the effects of the tailings from the Fundão dam on the germination, initial growth and development of three plant species: millet, maize and sorghum. These genotypes were cultivated on substrates with five tailing availabilities: 0T (100% sand), 25T (25% tailings + 75% sand); 50T (50 % tailings + 50% sand); 75T (75% tailings + 25% sand) and 100T (100% tailings). In experiment I, germination and initial growth in seedlings were evaluated in these different substrates. In experiment II, growth attributes, photosynthetic efficiency (gas exchange and chlorophyll fluorescence), metal accumulation and plant root morphology of the three genotypes were evaluated in the vegetative stage of three fully expanded leaves (V3). Seedling germination and initial growth of the three species analyzed were not affected by the presence of the tailings. However, in plants at the V3 stage, morphophysiological response variations occurred among species, since, besides the growth and biomass accumulation, the morphological dynamics of the roots was altered. The variation in the amount of tailings in the substrate did not influence the absorption of iron, manganese and chromium by the studied plants. At the V3 stage, maize is more tolerant due to a more robust root system under the mining tailings and with lower morphological changes, in addition to greater water use efficiency.

Keywords: Poaceae, tailing sludge, heavy metals, photosynthesis, chlorophyll fluorescence, WinRhizo.

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1 Introduction

Mining activity has been practiced in Brazil since the 17th century and several accidents involving deaths and environmental contamination have been reported in this period. In the State of Minas Gerais, there were accidents involving dam collapse in Itabirito (1986), Nova Lima (2001), Miraí (2007), Cataguases (2007), Congonhas (2008), Itabirito (2014) (Beltrami et al. 2012). In Spain, in 1998, 5.5 million m3 of acid sludge spilled out, devastating an area of 46 km2 (Alastuey et al. 1999). Another case of great repercussion occurred in Hungary in 2010, when a dam collapsed, releasing 700,000 m3 of red sludge containing aluminum and leaving a trail of destruction in the environment (Gelencsér et al. 2011). However, this accident is considered 70 times lower in proportion than the tragedy that occurred in Mariana-MG in 2015 (Segura et al. 2016).

The disaster that occurred in 2015 due to the collapse of the Fundão dam (Samarco) in the municipality of Mariana-MG was the biggest accident recorded in the Brazilian mining history, with 50 million m3 of tailings, mainly consisting of iron and silica. The subdistrict of Bento Rodrigues was buried and the pollutants covered a length of 663.2 km, leaving a remnant of destruction from the interior of the state of Minas Gerais to the coast of the state of Espírito Santo. The accident struck the Rio Doce watershed and resulted in the loss of human life, displacement of communities, restrictions on fishing, changes in water quality and quantity, impacted the soil, fauna and flora, including Permanent Preservation Areas of Mata Atlântica (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – IBAMA, 2015).

An extension of 560 hectares resulted in tailings consisting of sludge and sand, which joined the local soil. Changes in soil pH, reduction in organic matter content, chemical disruption with leaching of the chemical elements, presence of metals and the creation of a thick crust created by sludge drying (compaction) hinder the natural revegetation process and plant succession in these tailings (Silva et al. 2017).

The tailings from the Fundão dam collapse also destroyed agricultural areas and pastures (IBAMA 2015). In this context, there is a need for studies on species that can be used for cultivation in the area that was affected by the dam collapse in the Mariana-MG region, contributing, mainly, to the agriculture that was strongly impacted. However, the response to stress from mining tailings or from any stress depends on its intensity and duration, as well as the species, genotype and plant development stage (Anjum et al. 2017; Anjum et al. 2016). Therefore, the knowledge about the influence of mining tailings on the cultivation of species of agricultural interest is crucial to initiate the resolution of socio-environmental problems of regions that go through this type of environmental disaster. Thus, it is necessary to address issues related to germination, as well as the survival and maintenance of plants at more advanced development stages. Therefore, the objective of this study was to verify the germination, initial growth and development of millet, maize and sorghum grown in mining tailings.

In agriculture, grasses (Poaceae) stand out due to the rapidity of growth and high root density, which favors an improvement in the soil physical attributes, contributing to the recovery of degraded areas (Silva, Fontes, Costa, & Alvarez, 2006). In this context, species such as millet and sorghum assist in soil decompaction (Calonego et al. 2011; Rivero Herrada et al. 2017). Maize, one of the most important cultivated species of the Brazilian and world economy, survives in compacted soils (Grzesiak et al. 2015) and also presents high production, allied to its translocation potential of Cu and Zn to the shoot (Tavares et al. 2014).

It was hypothesized that the germination and growth of millet, maize and sorghum plants are inhibited by increasing the content of mining tailings resulting from the collapse of the Fundão dam (Mariana-MG). In addition to plant germination and growth, biomass attributes and photosynthetic efficiency of plants (gas exchange and chlorophyll fluorescence) were evaluated. Due to the significant negative effect on roots, deeper/elaborate studies on root morphology were carried out. The heavy metals present in the tailings at proportions above that allowed by Brazilian environmental laws were also measured in the plants as a substitute analysis of the absorption of these metals by the plants in the field.

2 Material and Methods

2.1 Plant material, collection and physical and chemical analysis of tailings

The experiments were conducted at the Laboratory of Environmental Biotechnology and Genotoxicity, at Federal University of Alfenas (UNIFAL-MG). Seeds of three cereal species (Poaceae) were used in all experiments: the simple maize hybrid DKB390 (*Zea mays* L.), the sorghum hybrid BRS 332 (*Sorghum bicolor*) and the millet variety BRS 1502 (*Pennisetum glaucum* (L.) R. Br.). The seeds were supplied by Embrapa – Maize and Sorghum National Research Center, from Sete Lagoas-MG.

The tailings from the Samarco Fundão dam collapse were collected in the municipality of Mariana-MG at UTM 669690 West, 779984 South (Fig. S1). Subsequently, it was transported to the city of Alfenas-MG, where it was dried outdoors and sieved (4-mm mesh sieve), and then used in the experiments. Two experiments were carried out: the first consisted of germination test and initial seedling growth and the second of the morphophysiological analysis of fully expanded leaves at the V3 stage.

2.2 Experiment I: Germination test and initial growth of seedlings under tailings

The tests were conducted in transparent plastic "gerbox" boxes using substrates with five tailing availabilities: 0T (100% sand), 25T (25% tailings + 75% sand); 50T (50 % tailings + 50% sand); 75T (75% tailings + 25% sand) and 100T (100% tailings). The experiment was conducted in a B.O.D. chamber (Solab®) at 30°C±1, with a 12-hour photoperiod. Germination percentage (G%) and germination speed index (GSI) were evaluated in the first 48 hours of germination (Maguire 1962). Nine days after sowing, morphological evaluations were performed, such as root and shoot length, dry biomass of seedlings, and chlorophyll "a" fluorescence.

For each genotype, a completely randomized design wasused, with five replicates of 50 seeds for each treatment, totaling 25 boxes per species.

2.2.1 Morphological and growth parameters

Shoot and root length were evaluated with the aid of a digital caliper (King Tools - China). In order to evaluate dry biomass, the seedlings were placed in properly identified paper bags and kept in an air circulation oven (Nova Era 400 ND, Brazil), with a temperature of 60 ºC until constant weight, and then weighed in an analytical balance (Martes AY220 - Brazil).

2.2.2 Chlorophyll "a" fluorescence

The photosynthetic performance was obtained through a modulated fluorometer (FluorCAM Closed FC 800-C, PhotonSystems Instruments – PSI, Ltda, Czech Republic). For this analysis, the quenching protocols of the software itself were used (Oxborough 2004). The final leaflet emitted by the seedlings was standardized, which underwent a 20-minute adaptation in the dark, evaluating the maximum quantum yield of PSII (Qy_max, Fv/Fm). After a period of stable state adapted to light, the following parameters were evaluated: photochemical quenching (qP, (Fm_Lss - Ft_Lss)/(Fm_Lss - F0_Lss) and non-photochemical quenching (qN, 1-(F'm-F'0)/F'm).

2.3 Experiment II: Morphophysiological analyses of plants grown in mining tailings

In this experiment, the same mixtures of substrates of experiment I were used as treatments for the three plant species studied. Four seeds per pot of 500 cm^3 were sown and, after germination, thinning was carried out, maintaining two plants per pot. The plants were kept in a growth room at a temperature of 25ºC ± 2, with a 12-hour photoperiod, radiation of 15.17 W/m^2 and daily irrigation with distilled water (to maintain 80% of the maximum water retention capacity by the substrate), for a period of 22 days until reaching the vegetative stage V3. Since they are demanding crops, mainly for phosphorus, at stage V2, the plants received the application of complete Hoagland nutrient solution of (20 mL per pot) (Hoagland and Arnon 1950). For each species, the design was completely randomized, with five treatments and four replications. Each experimental plot consisted of two pots containing two plants.

At the V3 stage, growth evaluations, leaf gas exchange, chlorophyll "a" fluorescence and chlorophyll content were performed. The plants were collected for analysis of root morphology, determination of dry biomass and quantification of metals.

2.3.1 Morphological and growth parameters

The height of the plant culm was measured with the aid of a ruler. The plants were collected, separated into shoot and roots, washed, placed in brown paper bags properly identified and kept in an oven with air circulation (Nova Era 400 ND), at a temperature of 60 ºC until constant weight. Subsequently, they were weighed in an analytical balance (Martes AY220) to obtain root and shoot dry biomass. The root/shoot dry biomass was also determined.

2.3.2 Morphological characterization of the root system

The roots were duly collected, washed in running water and stored in pots containing 70% alcohol until the analyses were carried out using the software Winrhizo Pro 2007a (Regent Instruments, Sainte-Foy, QC, Canada). The following parameters were evaluated: root length (cm), mean root diameter (mm), surface area (cm2) and root volume (cm3). The roots were also categorized for length, surface area and volume in: fine roots (0 to 0.5 mm); medium roots (0.5 to 2.0 mm) and thick roots (above 2 mm). Indexes based on root morphological and dry biomass parameters were also determined: root length (CE – cm g-1), root fineness (FR – cm cm-3) and root tissue density (DTR – g cm-3) (De Souza et al. 2012).

2.3.3 Chlorophyll "a" fluorescence, chlorophyll content and leaf gas exchange

Chlorophyll "a" fluorescence was obtained through a fluorescence fluorimeter (FluorCAM Closed FC 800-C, PhotonSystems Instruments – PSI, Ltda, Czech Republic), using the same procedures and analyses described in Experiment 1. The first fully expanded leaf was standardized from the apex.

The chlorophyll content was measured by the SPAD index, determined in the first fully expanded leaf from the apex by a portable chlorophyll meter (SPAD – 502 Plus - Konica Minolta, Japan).

Gas exchange was evaluated through IRGA (Infra Red Gas Analyzer - LI-6400XT, LI-COR, United States) in the first fully expanded leaf from the apex. The analyses were performed in the morning, between 9 and 12 o'clock. The parameters analyzed were: net photosynthetic rate (A), stomatal conductance (gs), transpiration (E) and water use efficiency (WUE, given by A/E ratio). The CO_2 flow was 500 µmol mol-1 and the block temperature was 24 °C. The photosynthetically active radiation (PAR) used was 500 µmol m^{-2} s^{-1} for maize and 900 µmol m^{-2} s^{-1} for sorghum and millet. The PAR used was determined after the light curves for each species through IRGA.

2.3.4 Determination of heavy metals in plants

Heavy metals in the plants were determined from the dry biomass obtained at the end of the experiment. After drying, the plant material was ground and subjected to nitro-perchloric digestion (Association of the Official Agricultural Chemists 2005). The metals in plant tissues were quantified by atomic absorption spectrometry, flame modality (EAA/flame) (Welz and Sperling 1999). Due to the low amount of dry biomass, each analyzed sample consisted of a plant mixture with four replicates per treatment.

2.4 Data analysis

For the data analysis, the means and standard error were calculated for each parameter. The analysis of variance (ANOVA) was then used and, in case of significance ($p \leq 0.05$), the quantitative factors were submitted to regression analysis, where only significant trends ($p \leq 0.05$) with fit in the coefficient of determination ($R^2 > 0.6$) were indicated. The other factors were submitted to the Scott-Knott test ($p \leq 0.05$), using the Sisvar Software, version 5.6 (Federal University of Lavras, Brazil).

3 Results

3.1 Chemical and physical characterization of mining tailings

The chemical analysis of the tailings (Table S1) showed an alkaline pH (7.8), magnesium (Mg) and aluminum (Al) contents, with very low acidity and organic matter (OM). Calcium (Ca), potassium (K) and base saturation (SB) were low, but base saturation was above 70%, according to the recommendations of the 5th approximation (Guimarães et al. 1999). The granulometric analysis allows to classify it as a frank textural class, according to the Brazilian Soil Classification System (EMBRAPA 2006), with a density of 1.9 g cm-3. Most of the tailings is sand (47.9%) and silt (43.9%), and presents micropores in a larger quantity.

The analysis of metals (Table 1) in the tailings revealed the presence of nickel (Ni), lead (Pb), zinc (Zn), arsenic (As), chromium (Cr), manganese (Mn) and iron (Fe). Considering the Brazilian law, that is, the CONAMA Resolution 420/2009 (CONAMA 2009), only the elements Cr, Mn and Fe are at concentrations above the allowed values, either for industry or for agriculture.

3.2 Experiment I

The presence of tailings had no influence on the germination of millet (Fig. S2A and B), whereas for maize only under 50T, there was a reduction in germination percentage and germination speed index (GSI) (Fig. S2 D). In the case of sorghum, the highest percentages of germination were verified in 50T and 100T of tailings. On the other hand, GSI was promoted by the presence of tailings (Fig. S2 E and F).

In millet and sorghum (Fig. 1A and G), there was an increase in the height of the seedlings with the increase in the proportion of tailings. Maize showed lower seedling height only under 50T in relation to the other treatments (Fig. 1D). On the other hand, root length decreased due to the increase in the amount of tailings (Fig. 1B, E, H). Despite the variations in plant height and root length, the accumulation of dry biomass in millet and sorghum seedlings was not affected by the presence of tailings (Fig 1C and I). Maize, on the other hand, showed an increase in the dry biomass accumulation of seedlings with the increase in the proportion of tailings in the substrate (Fig. 1F).

For millet, no change was observed in the maximum quantum yield of PSII (Fv/Fm), photochemical quenching – qP and non-photochemical quenching – qN (Fig. S3 A-C). In maize, despite decreases or increases in these parameters, the treatment with 100 T was similar to treatment 0 T (Fig. S3 D-F). In sorghum, only qN decreased at proportions 75 T and 100 T in relation to the other treatments (Fig. S3 G-I).

3.3 Experiment II

The presence of tailings reduced the biomass of millet, maize and sorghum roots (Fig. 2A, D, G). The dry biomass of plant roots under 100 T reduced 45% for millet, 25% for maize and 60% for sorghum in relation to pure sand (Figure 2A, D, G). Rearding the shoot, the sorghum plants presented an increase in the accumulation of dry biomass of 10% in the 100 T treatment (Fig. 2H) and maize plants 87% in relation to 0 T (pure sand) (Fig. 2E). This response in relation to biomass accumulation shows that maize plants presented better performance under these cultivation conditions in relation to the other species. On the other hand, the dry biomass root/shoot ratio showed a small decrease at intermediate concentrations of millet tailings (Fig. 2C) but, in maize and sorghum, a decrease in the dry biomass root/shoot ratio was observed with the increase in the amount of tailings in the crop substrate (Fig. 2F, I).

The analysis of the root system of the plants showed a decrease in the root length of millet, maize and sorghum in the 100 T treatment in relation to the roots of the 0T treatment (Fig. 3A, E, I). On the other hand, there was an increase in the mean diameter of maize roots at the highest concentrations of tailings in relation to the roots of plants under T0 (pure sand) (Fig. 3F). In addition, there was a less drastic reduction in the surface area (25.6%) and in the volume (16.2%) of maize roots (Fig. 3G and H) than in millet (72.4% and 69.3%) (Fig. 3C and D) and sorghum (52.6% and 84.6%) (Fig. 3K and L) at the highest proportion of tailings relative to pure sand. It was observed that maize roots showed a greater trend of maintenance of diameter, surface area and volume with the increase in the amount of tailings, not suffering so drastically under such conditions (Fig. 3E-G).

In the indices that relate parameters of root morphology and dry biomass, it was observed, for millet, that the specific root length (SRL) and root fineness (RF) decreased in treatments 25T and 100T in relation to 0T (Fig. S4 A and C). For root tissue density (RTD), the opposite behavior was observed (Fig. S4 B). For maize, it was observed that SRL presented higher means in treatments 25 T and 50 T; however, the treatments with higher proportions of tailings were similar to the control (0T) (Fig. S4 D). Also for maize, RTD did not change and RF decreased at the highest proportions of tailings (Fig. S4 E and F). Regarding sorghum, there was no difference among treatments for any of these three attributes (Fig. S4 G-I).

Among the three species studied, maize presented a larger length, surface area and root volume, independent of diameter class and tailing treatment (Fig. S5 A-I). Comparing the 100T and 0T treatments, there was a reduction in very fine roots for the three species (Fig. S5 A) and in thick roots for maize (Fig. S5 G) with the increase in the proportion of tailings, while the roots were not altered (Fig. S5 D). The surface area of very fine, fine and thick roots in the three species showed the same length response, except for thick roots in maize, whose surface area did not reduce (Fig. S5 B, E, H). With regard to root volume, root radius decreased in very fine, fine and thick roots only in maize at 100 T (Fig. S5 C, F, I).

The presence of mining tailings did not alter the Fv/Fm of the species studied, except for 100 T of tailings in millet (Fig. 4A). The qN was not altered by the presence of tailings for maize. However, in millet and sorghum, qN was higher at the highest concentrations of tailings (Fig. 4B, F, J). In relation to qP, changes were observed only in sorghum plants that decreased in treatments with higher percentages of tailings (Fig. 4C, G, L). The spatial distribution in the leaves of the three species of Fv/Fm, qN and qP can be observed in Fig. S6, Fig. S7 and Fig. S8. The relative chlorophyll content (SPAD index) showed an increasing trend in maize and decrease in sorghum with the increase in the concentration of tailings (Fig. 4H and L), while in millet, a higher value was observed only in the 25 T treatment (Fig. 4D).

In relation to gas exchange, in the millet, the net photosynthetic rate (A) was reduced in plants under 25 T and 50T, increased in plants in 75 T and values similar to the control were observed in plants 100 T (Fig. 5A). Stomatal conductance (gs) and transpiration (E) did not change with the increase in the proportion of tailings in the substrate (Fig. 5B and C). In maize plants, there were variations in A. However, in the 100 T plants, the means were equal to the treatment with no tailings (0 T) (Fig. 5E). A decrease in gs and E was observed in maize with the increase in the proportion of tailings (Fig. 5F and G). In sorghum, there was an increase in the net photosynthetic rate and stomatal conductance, and there was no change in transpiration with the increase in tailing concentration (Fig. 5I, J, K). The water use efficiency behavior was similar in the three species, tending to increase with the increase in tailing concentration (Fig. 5D, H, L).

Regarding the presence of metals in the plants (Table 2), for Cr, with the increase in the concentrations of tailings (from 0 T to 100 T), there was a reduction of 75% in maize and 39% in sorghum, while there was no variation in millet. For Mn, an increase of 45% in millet and 8% in sorghum was observed, while for Fe, the increase was 26% in millet and 9% in sorghum. Maize showed a decrease of 12% in Mn concentration and a 4.5% increase in Fe in the tissues, showing its different behavior in relation to the other species.

4 Discussion

High concentrations of Fe, Mn and Cr were found in the tailings of the Mariana-MG disaster (in relation to the quantities allowed by the CONAMA Resolution 420/2009), corroborating the results observed in other studies carried out in the area of the disaster (Andrade et al. 2018; Hatje et al. 2017; Sedru 2016; Segura et al. 2016). However, it is necessary to consider questions related to soil characteristics, once the high pH (7.8) does not favor the availability of Mn and Fe for absorption by plants, since these metals are complexed in the form of oxides. Micronutrients are extremely sensitive to pH variations in the soil. In the case of iron, solubility can be reduced by up to 1000 times and manganese by up to 100 times by increasing one pH unit (Lindsay 1979). In this case, although availability may be reduced in the soil, leaching and the availability of these metals found altered when in contact with water is still worrying. In this context, the greater the distance from the collapse point of the Fundão dam where the leakage occurred with metals, the greater its availability in water courses (Hatje et al. 2017).

The descriptions of the mining tailings under study corroborate other studies carried out in the region regarding the chemical and physical characteristics of the tailings (Silva et al. 2016). However, even under high pH conditions, a high amount of exchangeable Mn was found and only iron and silicon oxides predominate (Silva et al. 2016). On the other hand, high amounts of Fe and Mn are characteristics common to the soils of this region, that is, the Iron Quadrangle in Minas Gerais (Carvalho-Filho 2008). In this context, the high concentrations of these metals in the tailings may not be a serious problem for the region, but may cause serious problems in areas of the Rio Doce Basin in the state of Espírito Santo, for example (Guerra et al. 2017). Still, it is also necessary to consider the passage of this element along the food chain.

The characteristic of the tailings was silt and sandy, attributes that facilitate the growth and elongation of fine roots faster than in soils with higher clay contents (Katerji et al. 2003). However, sandy soils with a density greater than 1.65 g cm-3 are considered compacted (Lesturgez et al. 1998), being limiting for root growth in the medium (Nunes et al. 2016). Thus, tailing compaction becomes another limiting factor to the crop, as well as the presence of metals which, despite not being in their available forms for absorption by the plants, can become so, once changes occur in pH, dilution, seasonal changes, particle resuspension, among others (Segura et al. 2016).

Little did the mining tailings inhibit the germination and GSI of the species studied. The success of germination in compacted or metal-based environments depends both on the characteristics of the tailings (such as pH, density) and on the species itself (Courtney and Mullen 2009). If, on the one hand, bauxite tailings differently affected the germination of plant species, on the other hand, soil compaction was not sufficient to negatively influence the germination of different grass species (Jorgensen et al. 2018). Thus, the responses observed for millet, maize and sorghum can also be results of plant breeding programs that contribute to the best physiological seed potential.

In spite of the reduction in the root length of the seedlings in response to the greater amount of tailings, there was no variation in the biomass of millet and sorghum seedlings, even occurring an increase in maize. It can be observed that, at the initial growth, the seedlings of the three species are not inhibited by the tailings. And maize can be highlighted as the species that was able to gain biomass against the tailings, giving signs of tolerance to the mining tailings from the initial growth stages. Seedlings capable of obtaining biomass allocation in the face of increased stress (mining tailings) may lead to plants with greater vigor and favor their future growth and development (Grzesiak et al. 2015). In contrast to that found in this study with maize, a reduction of up to 25% in the biomass of barley seedlings cultivated in the red sludge of an accident occurred in Hungary was observed (Ruyters et al. 2011).

The reduction in the root system in the three species may have occurred due to substrate/tailing compaction stress, since the main symptoms are the decrease in number, length and root thickness (Grzesiak et al. 2016). The absence of significant changes in photosynthetic efficiency in the seedlings (initial stages) of the three species under mining tailings can be explained by the lack of a fully formed photosynthetic apparatus at the time of analysis (Miguel et al. 2007).

Although seedling germination and initial growth have been little affected by the presence of mining tailings, when analyzing plants at the V3 development stage, large differences between species and significant morphophysiological variations are observed. Although the three species decreased root biomass with the increase in tailings, maize stands out with greater growth and tolerance, compared to millet and sorghum. The higher biomass data at early stages may explain this higher growth of maize at the V3 stage. The index known as root biomass by shoot ratio (R/S) indicates the direction of carbon allocation to the organs (Grzesiak et al. 2014). In all species, the largest investment was for the shoot than the root, whose reason was previously described: soil compaction that can prevent the root investment (Grzesiak 2009; Grzesiak et al. 2016).

Compacted soils can offer situations of lower water and oxygen availability for plants; thus, there is a trend of stomatal closure, reduction in the net photosynthetic rate and pigment content (Grzesiak et al. 2016; Saradadevi et al. 2016), and the changes are more evident in sensitive plants. The photosynthetic rate was increased in sorghum and not so much in the other species studied and this may show a superiority of this species against the tailings, but this increase could be only an adjustment of the photosynthetic apparatus, since there was a decrease in the chlorophyll content of sorghum.

It is worth noting that maize was the species that decreased stomatal conductance (and thus transpiration) on mining tailings, which may have contributed to water savings shown in water use efficiency (WUE). In addition to allowing survival in an environment with lower water availability (Grzesiak et al. 2016), this lower conductance and transpiration may also be an important strategy for maize to avoid absorbing the metals that may come via transpiratory flow, which can be demonstrated by reducing the absorption of chromium and manganese, as well as lower iron uptake by maize plants under 100% of tailings

(Table 2). Sorghum seems to respond to the presence of the tailings by a stomatal effect (greater stomatal conductance, leading to a higher photosynthetic rate). However, maize decreased its stomatal conductance without decreasing photosynthesis, evidencing higher biochemical (non-stomatal) efficiency, which could be due to an increase in carboxylation capacity, a greater activity of the carboxylic enzymes or a reduction in the conductance limitation by the mesophyll (Grassi and Magnani 2005).

Regarding chlorophyll a fluorescence, the value of its measurement is also in its relation with photosynthesis, since the light absorbed by plants that do not stimulate the production of carbohydrates is dissipated as heat or re-emitted as light in the form of fluorescence (Brestic and Zivcak 2013). In maize, sorghum and millet, there are studies that relate measurements of fluorescence and specific responses of genotypes due to stress (Lu et al. 2015; de Sousa et al. 2017; Guo et al. 2018). Plants under stress (heavy metals and soil compaction) tend to have lower values of maximum quantum yield of photosystem II (Fv/Fm) and photochemical dissipation (qP), besides higher non-photochemical dissipation values (Grzesiak, 2009). Thus, maize was the most efficient in the accumulation of dry biomass, since maize plants were the only ones that presented increase in shoot biomass in response to the increase in the proportion of tailings, and this fact may be related to the maintenance of photosynthetic efficiency and the increase in the SPAD index in these plants (Grzesiak 2009; Grzesiak et al. 2014; Lin, He, & Chen, 2016; Saradadevi et al., 2016), corroborating their higher biomass obtained under the tailings.

In this study, the negative impacts of the increase in tailings on root growth were evident. The root response of these plants and the physical characteristics of the tailings indicate the occurrence of substrate compaction since, besides the growth and accumulation of biomass, the morphological dynamics of the roots was altered.

The decrease in the length, surface area, root volume and even the attributes SRL and FR of maize were much lower than in the other species, when comparing the treatments with 100% of tailings. Greater growth of the root system by maize under the tailings results in better soil exploration and, consequently, greater absorption of water and nutrients, besides greater aeration. The smaller impact on FR and SRL indicates that, in maize, the plants under tailings invested in soil growth and exploration per carbon unit (Kramer-Walter et al. 2016) than in the other species, that is, growth with lower metabolic cost.

It is reported in the literature that plants under soil compaction can produce aerenchyma (Colombi and Walter 2016), air-filled tissue that favors root aeration and higher SRL, which could explain the higher root yield of maize under the tailings in this research. In maize, no change in root tissue density (DTR) implies that the species tended not to invest in the lignification and suberization of its roots and confirms the greater investment in soil exploration with the increase in tailings (Kramer-Walter et al. 2016). It is interesting to note that, in millet, SRL and FR felldecreased, which can be explained by the increase in DTR.

In general, an increase in root mean diameter was observed in the three species with increased tailings, an evident response in compacted soils (Tracy et al. 2012). However, the higher investment in fine roots by maize may have allowed greater absorption of nutrients and water, due to the limiting nutrient profile of the tailings and their density and porosity (Zou et al. 2001). Despite the higher uptake, these roots have a low useful life and limited exploration potential in more compacted soils (Tracy et al. 2012). However, they presented functionality in maize.

Millet, maize and sorghum plants absorbed amounts of Mn and Fe (Table 2) above the limits described in the literature, where the maximum predicted value of Mn ranges between 20-150 mg kg-1 and Fe between 20-250 mg kg-1 for maize and sorghum species (Guimarães et al. 1999). The amount of Cr absorved is acceptable for plants used for human nutrition (EFSA CONTAM 2014) Although they did not show toxicity symptoms by any of these metals, the metabolism of these plants may have undergone changes due to high concentrations of metals. In the case of maize, the reduction in stomatal conductance and transpiration rate probably contributed to the lower metal absorption in the 100T treatment.

5 Conclusion

Mining tailings do not influence the germination process and the initial growth of maize, millet and sorghum. On the other hand, plants at the V3 stage present lower shoot and root biomass, when cultivated under mining tailings. The lower plant growth is related to the reduction in root system growth, as well as changes in root morphology, characterized by the lower volume and root surface, besides a reduction in the amount of very fine roots. There is more investment in shoot growth.

At the V3 stage, maize is more tolerant than millet and sorghum grown in mining tailings. The physiological attributes related to the greater tolerance are smaller reductions in growth and smaller changes in the morphology of the root system, increase in mean root diameter and greater water use efficiency under mining tailings. Thus, the more robust root system of maize under mining tailings gives it greater tolerance to cultivation in mining tailings.

Compliance with Ethical Standards

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Figures and captions

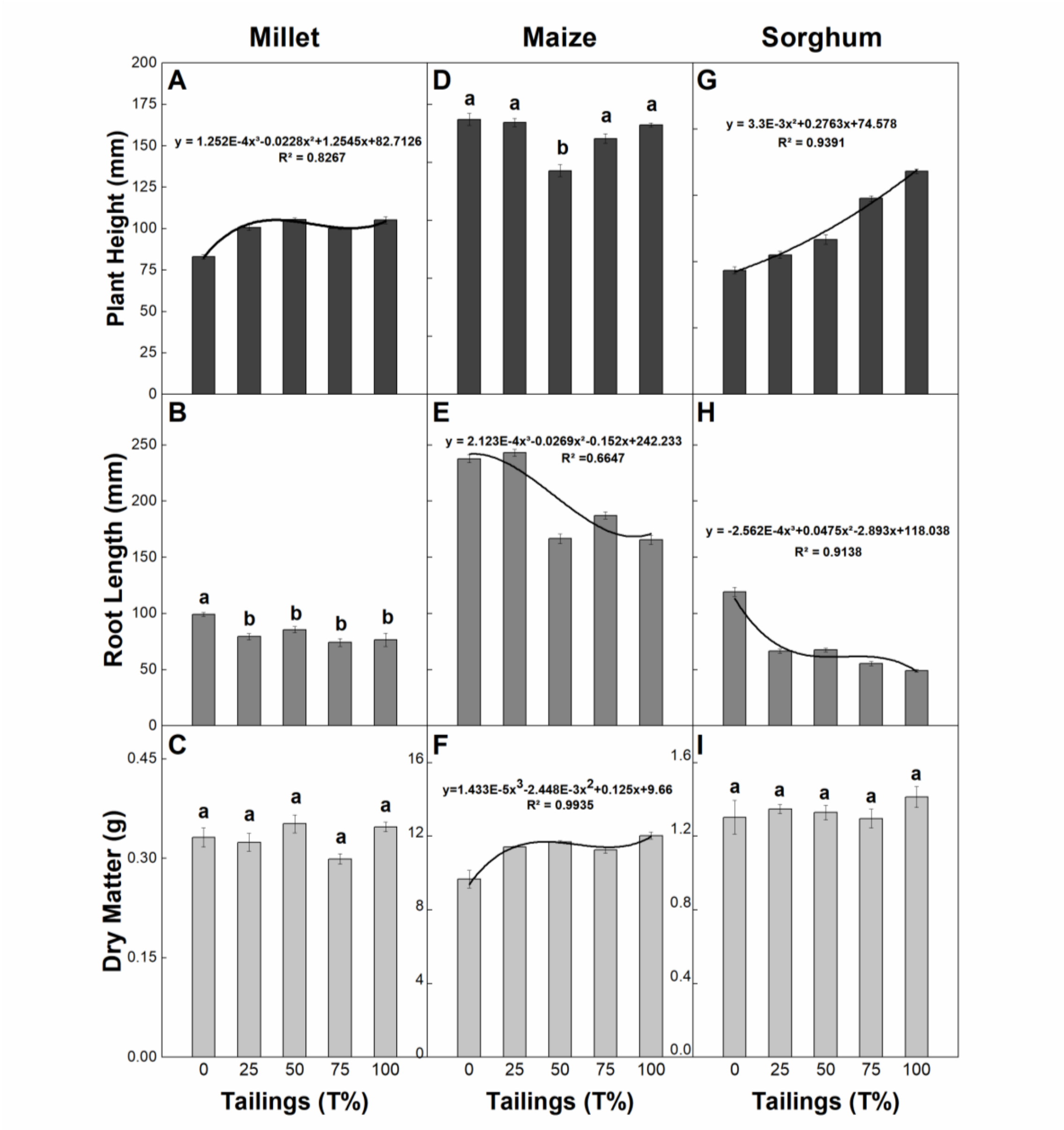

Fig. 1 Initial growth: seedling height (A, D and G); root length (B, E and H) and total dry biomass content of the seedlings (C, F and I) of millet, maize and sorghum, under different tailing availabilities: 0T (100% sand), 25T (25% tailings + 75% sand); 50T (50% tailings + 50% sand); 75T (75% tailings + 25% sand) and 100T (100% tailings). Among treatments, means followed by the same letter do not differ by the Scott-Knott test ($p \leq 0.05$). The bars correspond to the standard error of the mean of five replicates.

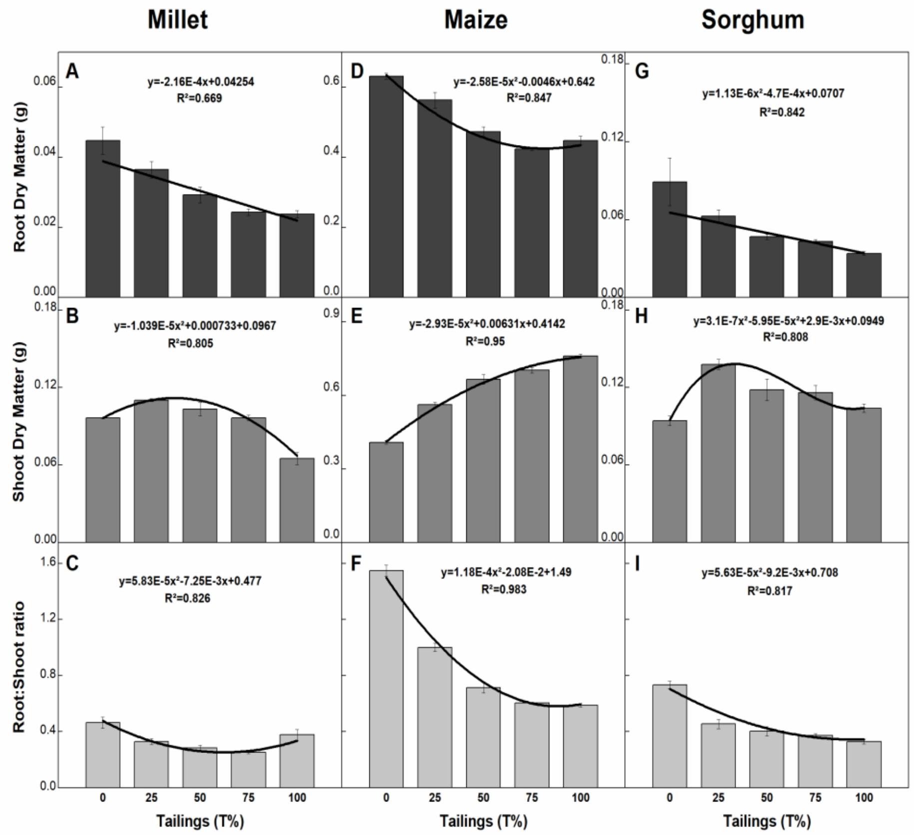

Fig. 2 Root (A, D and G) and shoot (B, E and H) dry biomass and root/shoot ratio (C, F, I) of millet, maize and sorghum plants at the V3 stage, under different tailing availabilities: 0T (100% sand), 25T (25% tailings + 75% sand); 50T (50% tailings + 50% sand); 75T (75% tailings + 25% sand) and 100T (100% tailings). The bars correspond to the standard error of the mean of four replicates.

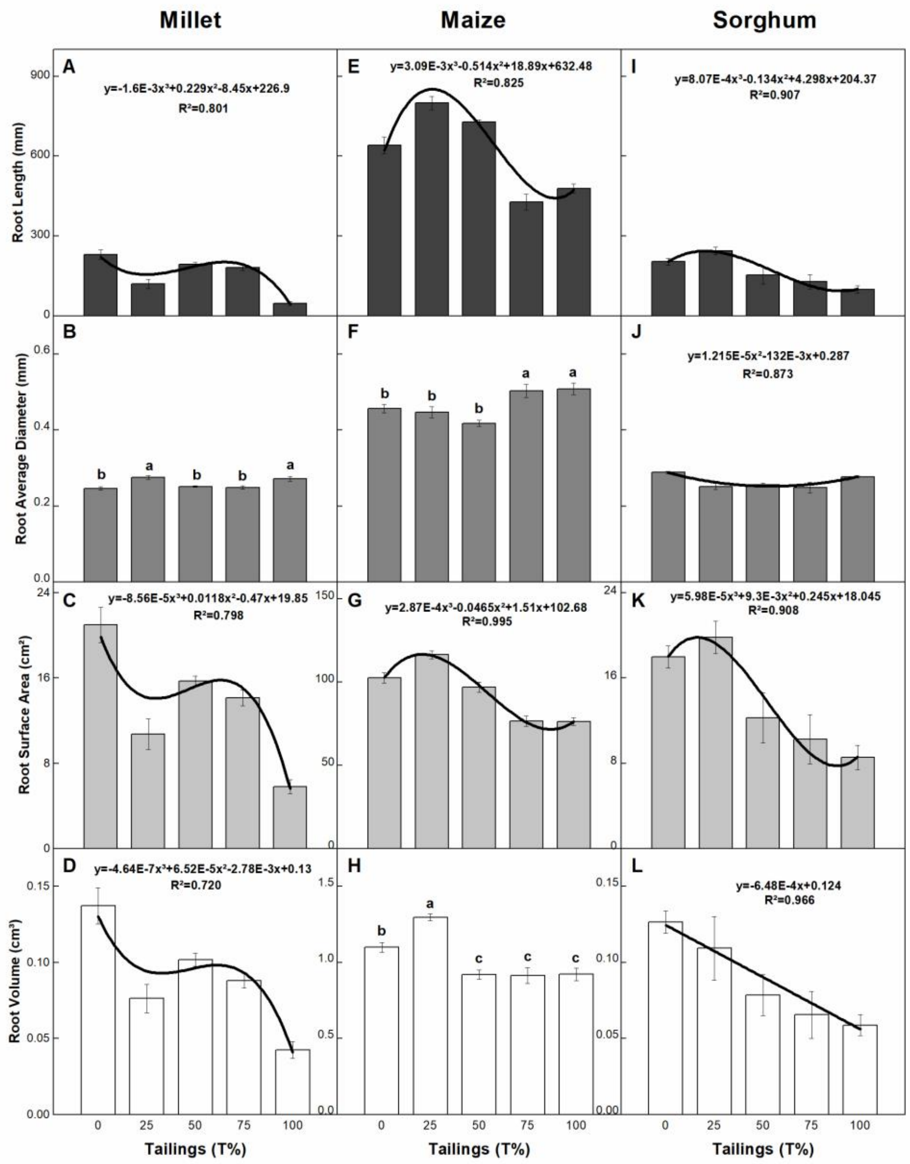

Fig. 3 Root length (A, E, I), mean diameter (B, F and J); surface area (C, G and K) and root volume (D, H e L) of millet, maize and sorghum plants at V3 stage, under different tailing availabilities: 0T (100% sand), 25T (25% tailings + 75% sand); 50T (50% tailings + 50% sand); 75T (75% tailings + 25% sand) and 100T (100% tailings). Means followed by the same letter do not differ by the Scott-Knott test ($p \leq 0.05$). The bars correspond to the standard error of the mean of four replicates.

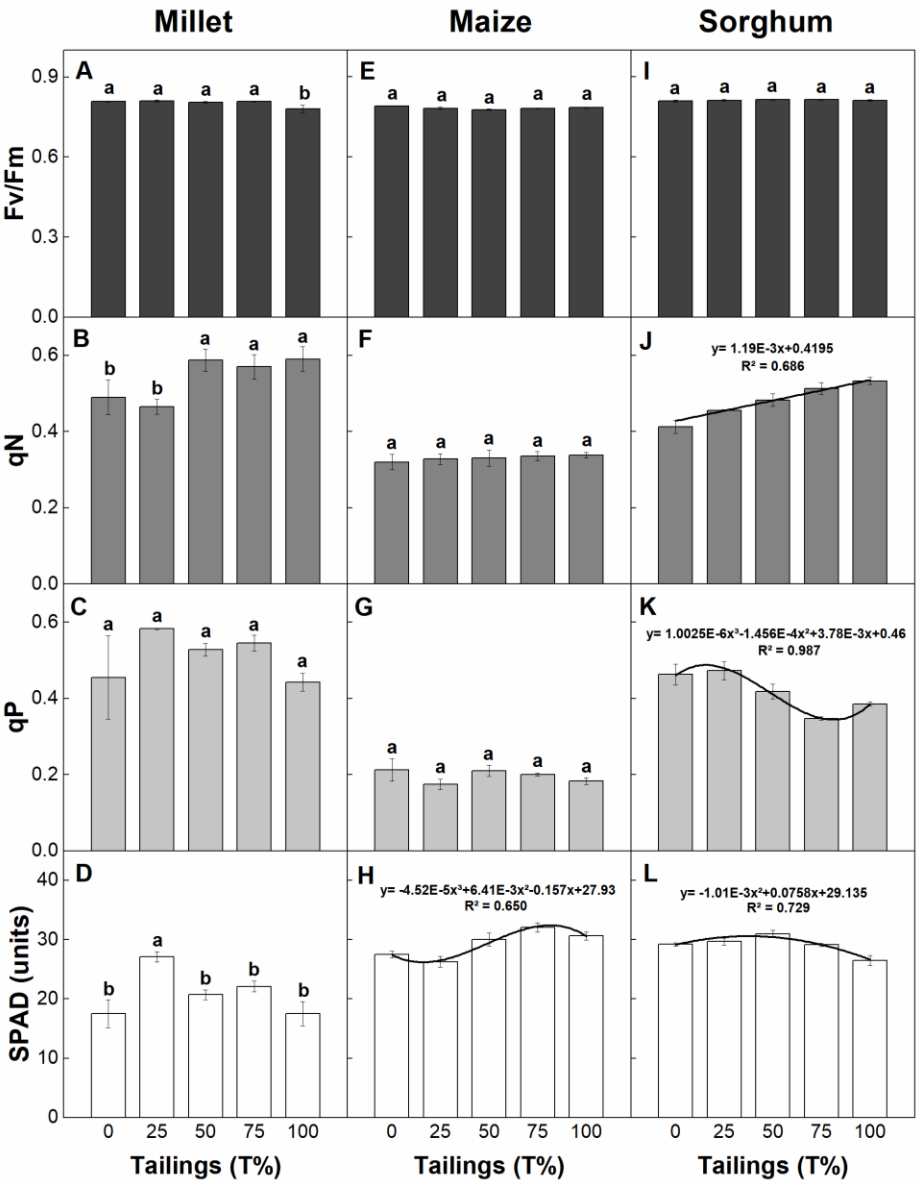

Fig. 4 Maximum quantum yield of photosystem II (Fv/Fm - A, E and I); non-photochemical quenching (qN - B, F and J); photochemical quenching (qP - C, G and K); and chlorophyll content by the SPAD index (D, H and L) of millet, maize and sorghum plants at V3 stage, under different tailing availabilities: 0T (100% sand), 25T (25% tailings + 75% sand); 50T (50% tailings + 50% sand); 75T (75% tailings + 25% sand) and 100T (100% tailings). Means followed by the same letter do not differ by the Scott-Knott test ($p \leq 0.05$). The bars correspond to the standard error of the mean of four replicates.

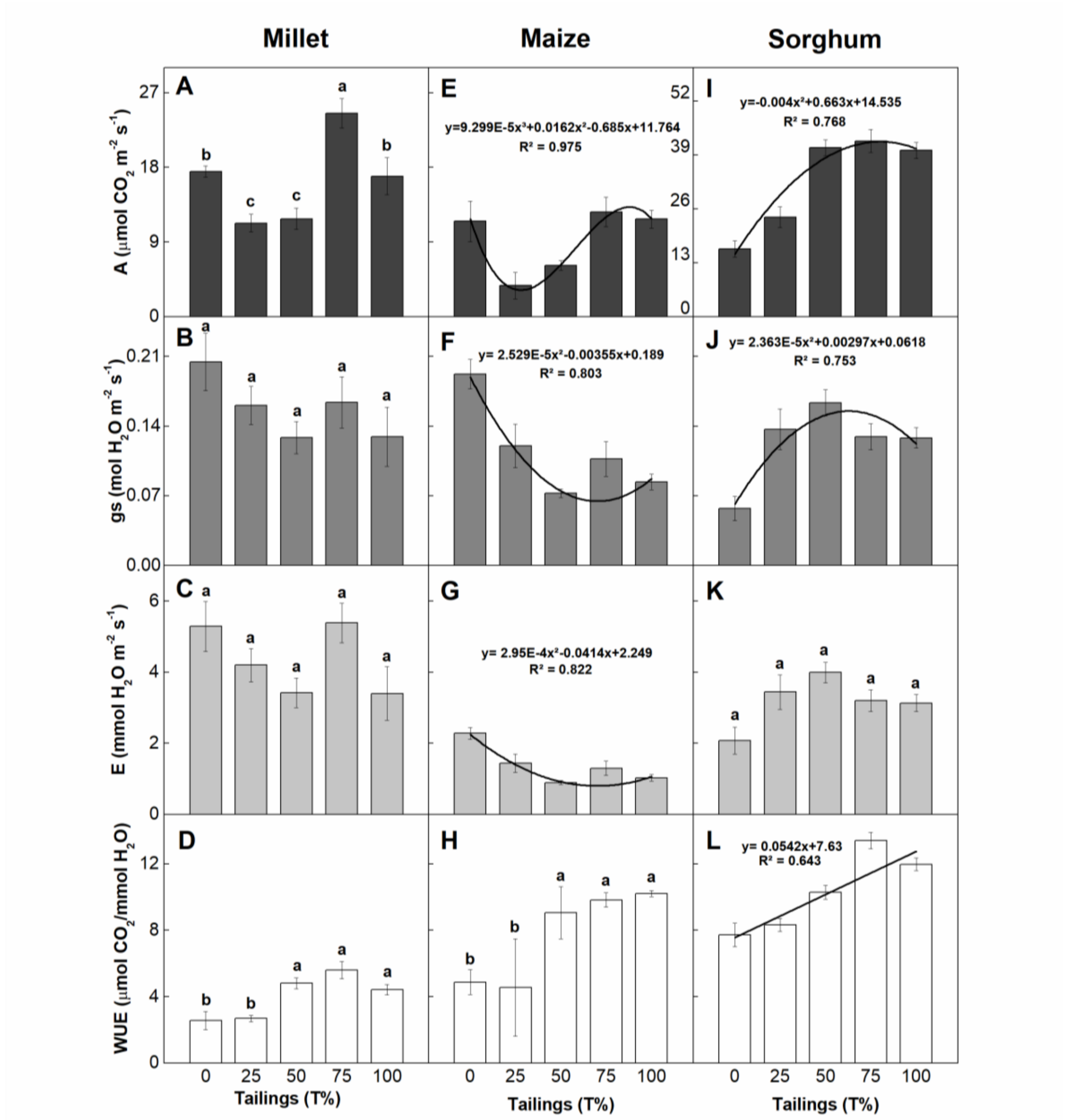

Fig. 5 Net photosynthetic rate (*A* - A, E and I); stomatal conductance (*gs* - B, F and J); transpiration (*E* - C, G and K) and water use efficiency (*WUE* - D, H and L) of millet, maize and sorghum plants at V3 stage, under different tailing availabilities: 0T (100% sand), 25T (25% tailings + 75% sand); 50T (50% tailings + 50% sand); 75T (75% tailings + 25% sand) and 100T (100% tailings). Means followed by the same letter do not differ by the Scott-Knott test ($p \leq 0.05$). The bars correspond to the standard error of the mean of four replicates.

Suplementary data

Do tailings from the Mariana-MG (Brazil) disaster affect the initial development of millet, maize and sorghum?

Environmental Science and Pollution Research

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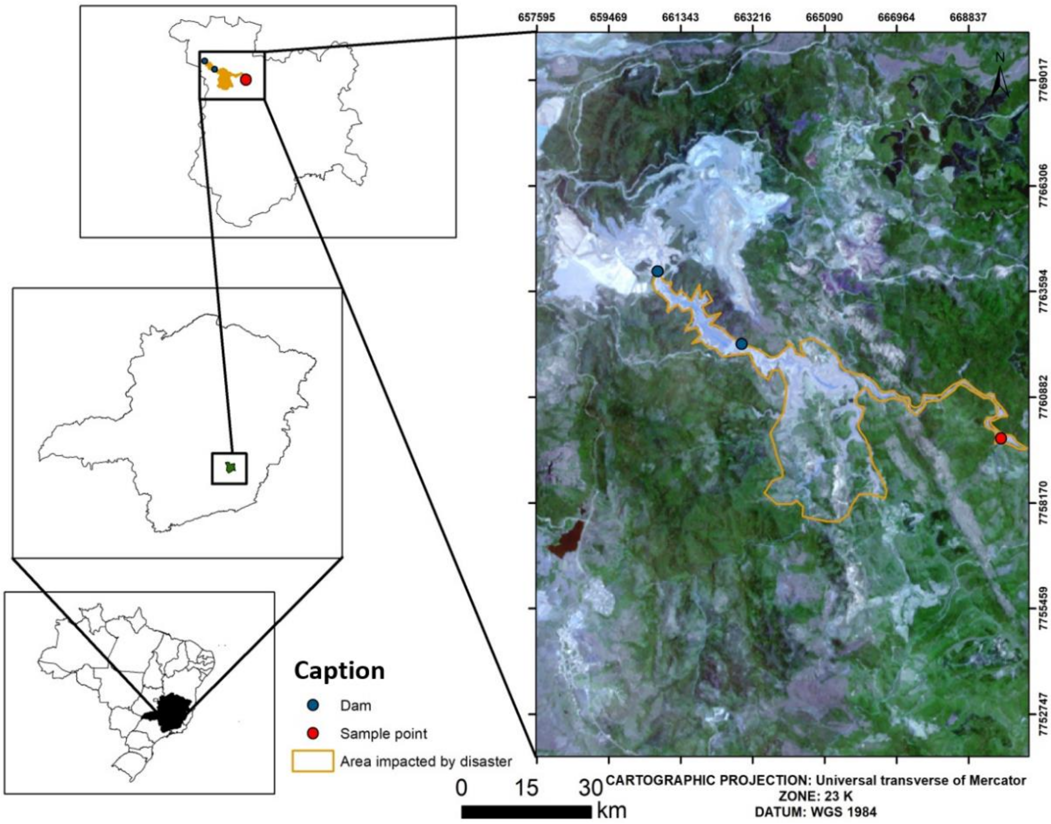

Fig. S1 An overview of the area of the Mariana-MG collapse and the collection point of the tailings after dam collapse.

Table S1 Chemical and physical analysis of the tailings collected in the city of Mariana-MG (UTM 669690 West, 779984 South), which was used for plant cultivation.

pH	K	P	Ca	Mg	Al	H+ Al	M.O	P-Rem	S.B	t	T	V	m
	-------mg dm^{-3}--------		--------------cmol$_c$ dm^{-3}-------------				---dag kg^{-1}---	mg/L	-------cmol$_c$ dm^{-3}-------			-------%-------	
7.8	16.23	11.88	1.46	0.10	0.04	0.62	0.27	43.35	1.60	1.64	2.22	72.14	2.44

Clay	Silt	Sand			Classification
< 0.002 mm	0.053- 0.002mm	Total	Thick 2.00 – 0.210 mm	Fine 0.210- 0.053 mm	Brazilian System SBCS
--(g kg^{-1})--					
81.67	439	479	61	418	Frank

Ds	Dens. Particles	VTP	Micropore	Macropore	U.SAT.
g cm^{-3}	Kg dm^{-3}	--%--			
1.90	2.53	47.33	31.6	16.73	37.67

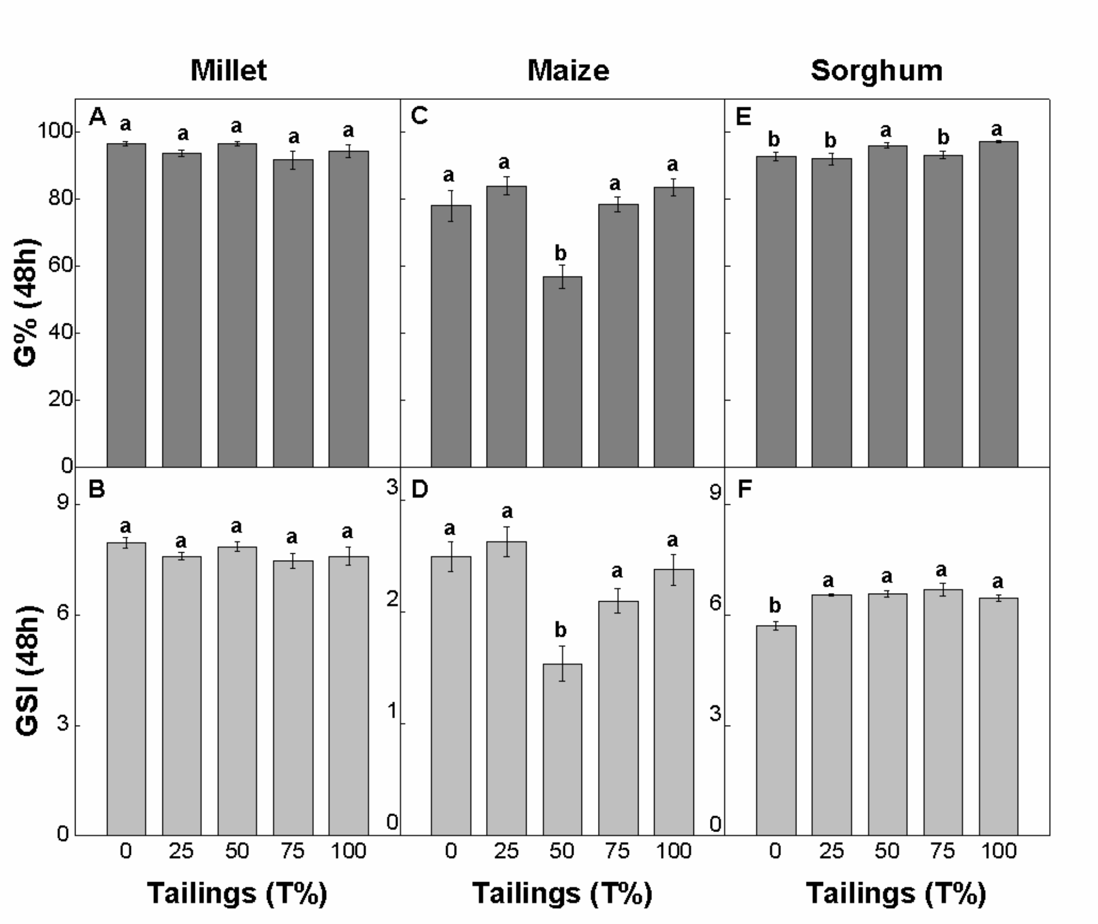

Fig. S2 Germination percentage (G% - A, C and E) and germination speed index (GSI - B, D and F) of millet, maize and sorghum, under different tailing availabilities: 0T (100% sand), 25T (25% tailings + 75% sand); 50T (50% tailings + 50% sand); 75T (75% tailings + 25% sand) and 100T (100% tailings). Among treatments, means followed by the same letter do not differ by the Scott-Knott test ($p \leq 0.05$). The bars correspond to the standard error of the mean of five replicates.

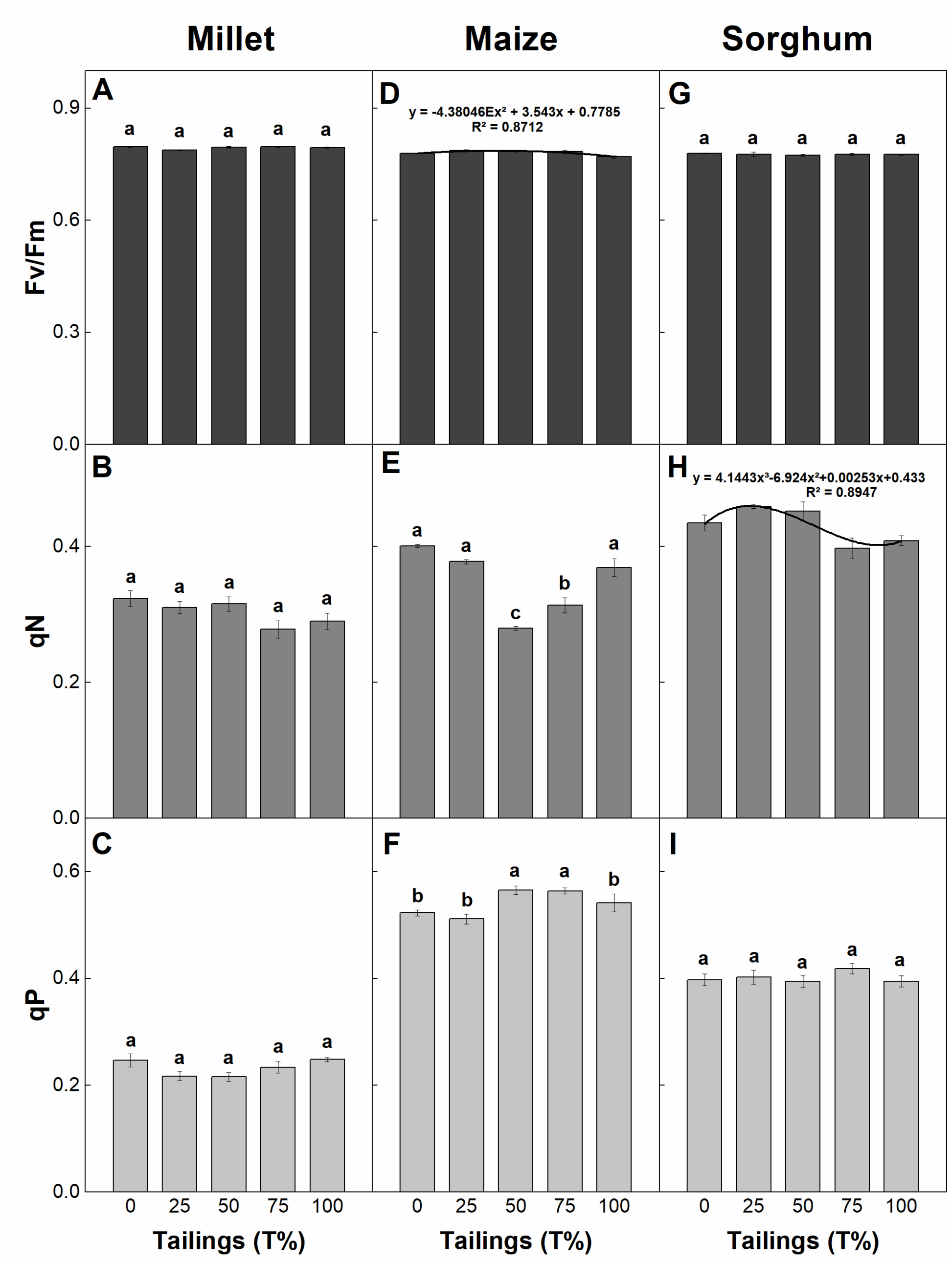

Fig. S3 Maximum quantum yield of PSII (Fv/Fm - A, D and G); non-photochemical dissipation (qN - B, E and H) and photochemical dissipation (qP - C, F e I) of millet, maize and sorghum, under different tailing availabilities: 0T (100% sand), 25T (25% tailings + 75% sand); 50T (50% tailings + 50% sand); 75T (75% tailings + 25% sand) and 100T (100% tailings). Among treatments, means followed by the same letter do not differ by the Scott-Knott test ($p \leq 0.05$). The bars correspond to the standard error of the mean of five replicates.

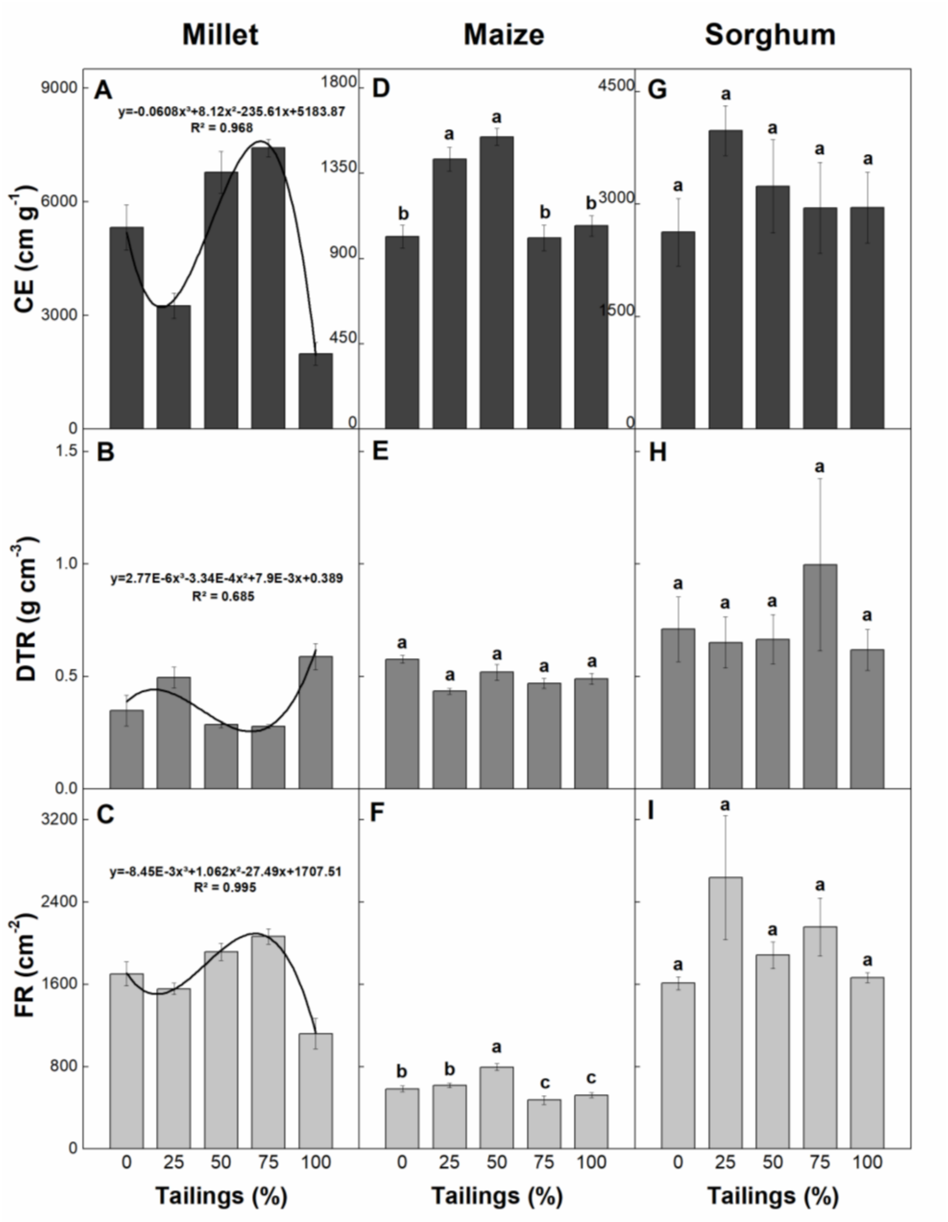

Fig. S4 Specific root length (SRL - A, D and G); root tissue density (RTD - B, E and H) and root fineness (RF - C, F e I) of millet, maize and sorghum plants at V3 stage, under different tailing availabilities: 0T (100% sand), 25T (25% tailings + 75% sand); 50T (50% tailings + 50% sand); 75T (75% tailings + 25% sand) and 100T (100% tailings). Means followed by the same letter do not differ by the Scott-Knott test ($p \leq 0.05$). The bars correspond to the standard error of the mean of four replicates.

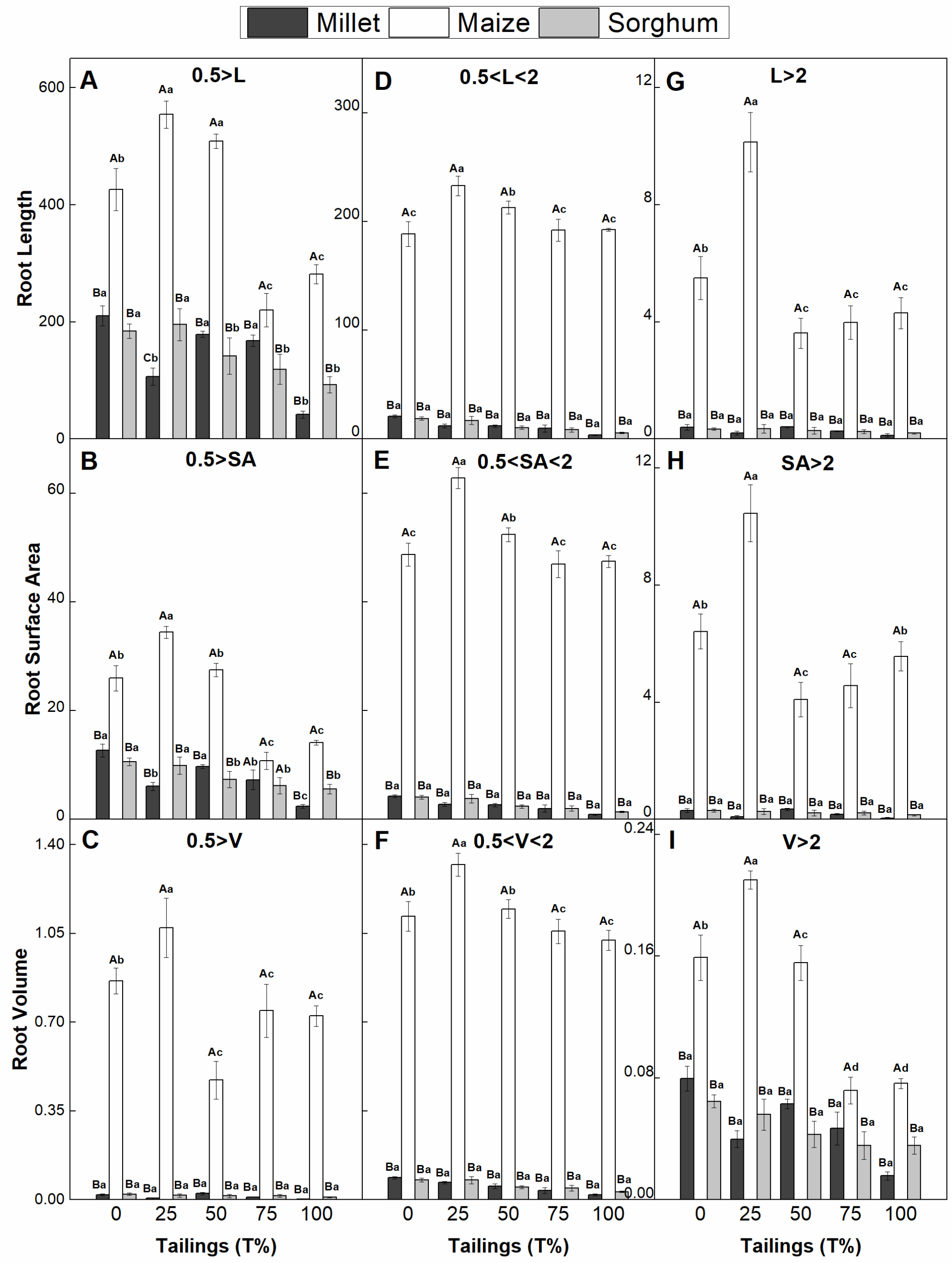

Fig. S5 Length of very fine (L<0.5 - A), fine (0.5<L<2 - D) and thick (L>2 - G) roots; Surface area of very fine (0.5>SA - B), fine (0.5<SA<2 - E) and thick (SA>2 - H) roots; and volume of very fine (0.5>V - C), fine (0.5<V<2 - F) and thick (V>2 - I) roots of millet, maize and sorghum plants at V3 stage, under different tailing availabilities: 0T (100% sand), 25T (25% tailings + 75% sand); 50T (50% tailings + 50% sand); 75T (75% tailings + 25% sand) and 100T (100% tailings). Means followed by the same letter do not differ by the Scott-Knott test ($p \leq 0.05$). Uppercase letters show the comparison of the species in each treatment, while lowercase letters compare the effect of the treatments within each species evaluated. The bars correspond to the standard error of the mean of four replicates.

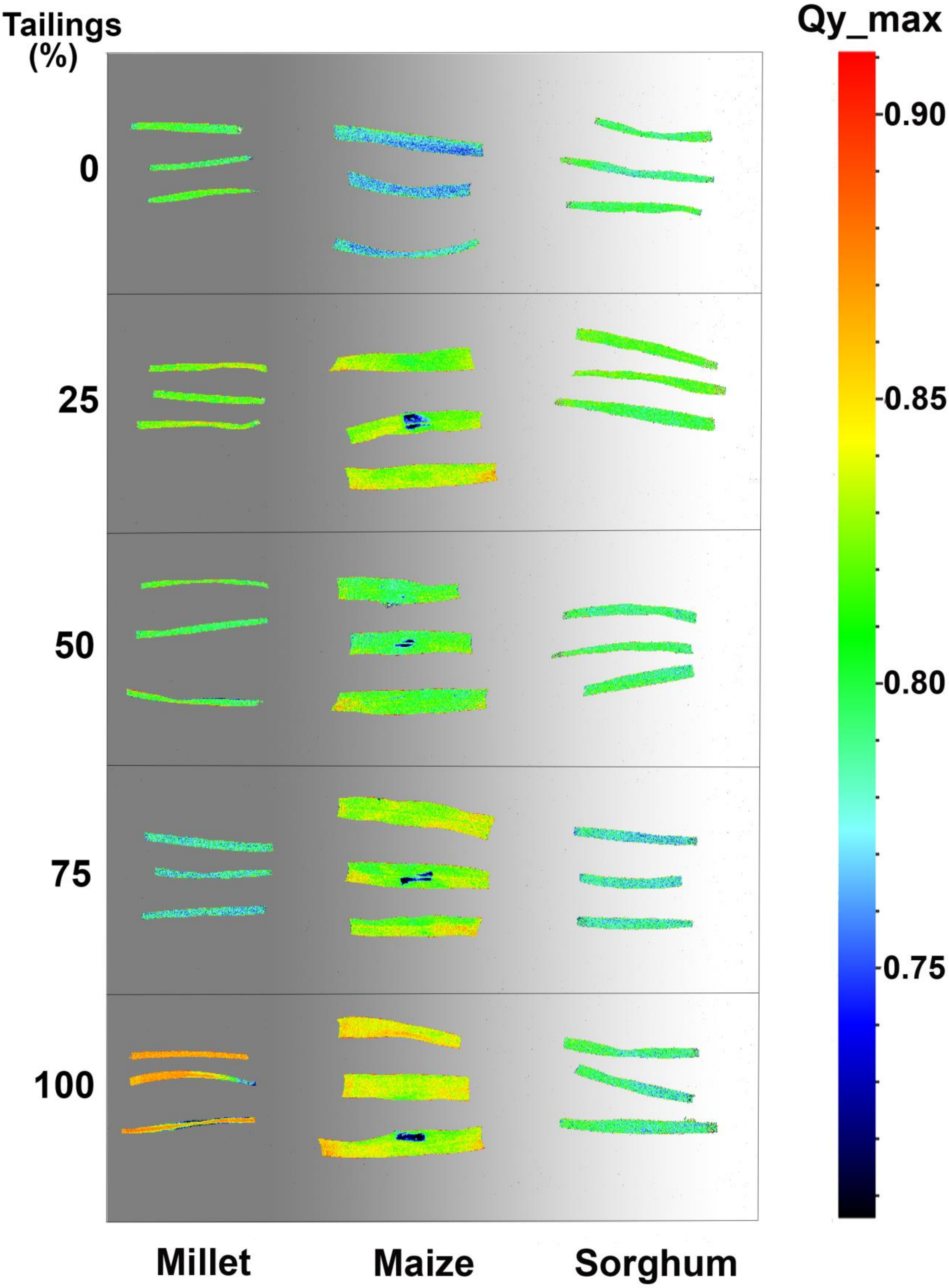

Fig. S6 Leaf distribution of the maximum quantum yield of photosystem II (Fv/Fm) of millet, maize and sorghum plants, at V3 stage, grown under different tailing availabilities: 0T (100% sand), 25T (25% tailings + 75% sand); 50T (50% tailings + 50% sand); 75T (75% tailings + 25% sand) and 100T (100% tailings).

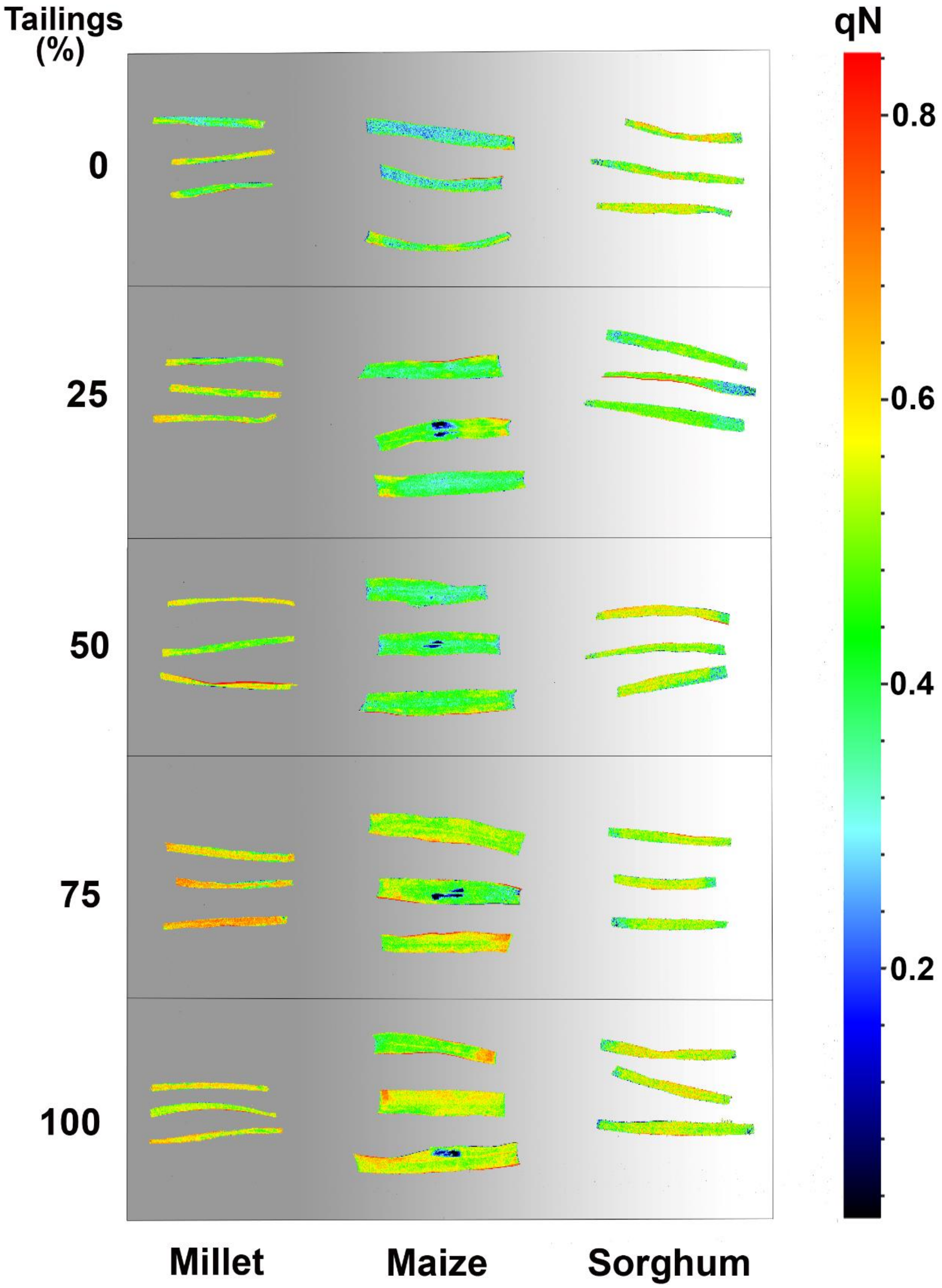

Fig. S7 Leaf distribution of the non-photochemical quenching of millet, maize and sorghum plants, at V3 stage, grown under different tailing availabilities: 0T (100% sand), 25T (25% tailings + 75% sand); 50T (50% tailings + 50% sand); 75T (75% tailings + 25% sand) and 100T (100% tailings).

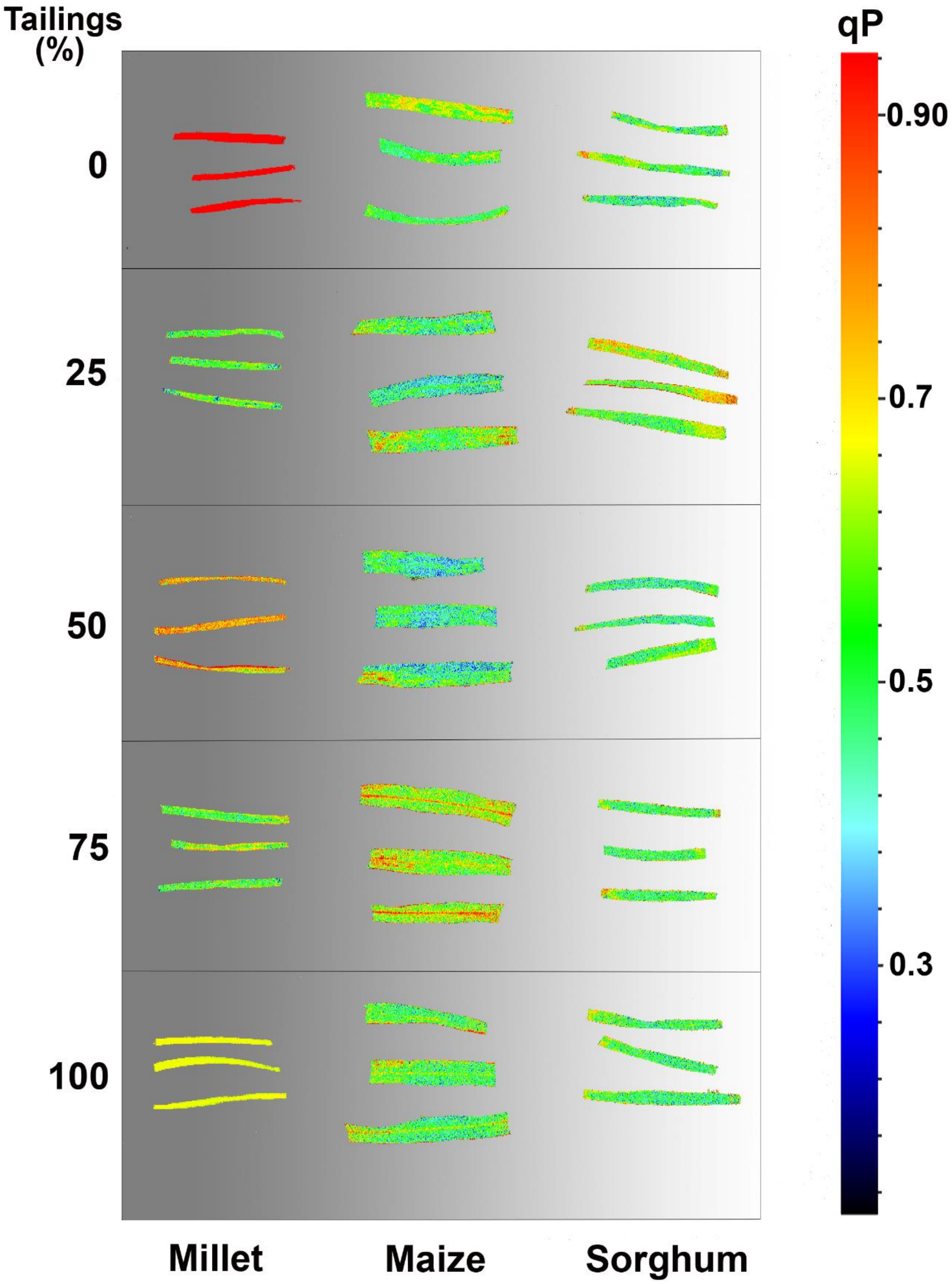

Fig. S8 Leaf distribution of the photochemical quenching of millet, maize and sorghum plants, at V3 stage, grown under different tailing availabilities: 0T (100% sand), 25T (25% tailings + 75% sand); 50T (50 % tailings + 50% sand); 75T (75% tailings + 25% sand) and 100T (100% tailings).

ARTIGO 2

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Vermicompost improves the growth of maize, millet and sorghum under iron mining tailings

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Abstract

The Fundão dam was designed to store iron mining tailings in the region of Mariana-MG and, with its rupture, there was an overflow of tailings, affecting the soil due to the formation of a thick crust resulting from drying (compaction), hindering the natural revegetation process. In this context, the use of organic fertilizers, including vermicompost, is an alternative to reduce the physical limitations of the soil, changing metal interactions. For this reason, the addition of vermicompost was implemented in iron mining tailings, aiming to verify morphological and physiological aspects of maize, millet and sorghum plants. The experiment was conducted in a greenhouse, using 6-dm^3 pots. The plants were submitted to three treatments: mining tailings, mining tailings + vermicompost and a reference soil. From the V3 stage onwards, biweekly growth, leaf gas exchange and chlorophyll fluorescence evaluations were performed, as well as, at the end of the experiment, dry biomass, metal, macro- and micronutrient quantification and root morphology. The tailings showed characteristics of substrates with physical limitation and low nutrient content, besides high concentrations of chromium, iron and manganese. The addition of vermicompost favored the increase in shoot and root dry biomass, increased length, volume, surface area and root diameter, favoring the absorption of macro- and micronutrients, reflecting in the growth of the studied species. In addition, vermicompost yielded greater investment in thick and very thick roots and, in general, plants showed no metal toxicity. Considering the characteristics of the studied tailings, it can be concluded that the vermicompost favors the growth of plant species and may be a viable alternative for the beginning of the recovery process of areas containing iron mining tailings.

Keywords: gas exchange, WinRhizo, root morphology, heavy metal, Poaceae, sludge.

1 INTRODUCTION

Mining has grown throughout the world (Oladipo et al., 2016) and, knowing the complexity of the activity and the lack of care by society, the negative effects on the environment tend to increase. In countries such as Brazil, accidents with dams have devastated the environment and worried authorities, who are seeking incentives for new techniques to correct the pollution generated. In addition, many remediation programs for soils contaminated with mining tailings have been costly and inefficient in depollution. At the Aznalcóllar mine (Spain), for example, after 20 years of the remediation program in the mine overflow areas, polluting waste is still present (García-Carmona et al., 2019).

In 2015 occurred the largest accident recorded in the mining history of Brazil, the collapse of the Fundão dam. The dam was designed to store iron mining tailings in the region of Mariana, state of Minas Gerias, Brazil, whose responsible is the company Samarco S/A. With the rupture of this dam, there was an overflow of 50 million cubic meters of tailings, mainly consisting of iron oxide and silica, leaving a trail of destruction. The tailings covered an area of 663.2 km, mixing with the soil, causing changes in soil pH, reduction in organic matter content, leaching of chemical elements, presence of metals, creating a thick crust due to tailings drying (compaction), thus hindering natural revegetation and plant succession in these areas (Silva et al., 2017).

Soil recovery in environments with mining tailings is complex and, particularly in the case of Mariana tailings, problems with compaction and heavy metals stand out. In the literature, cultivated plant species have been considered in decontamination processes due to the fast growing characteristics and large amount of biomass. Many cultivated species of Poaceae, Fabaceae and Brassicaceae families have high biomass yields that can compensate for the low concentration absorbed and translocated to tissues, making them similar or even better than hyperaccumulators (Vamerali et al., 2010). In addition, many of these cultivated plants have a strong increase in metal accumulation when fertilization and organic changes are performed (Sierra Aragón et al., 2019; Vamerali et al., 2010). Therefore, the use of vermicompost in this case is justified.

Regarding the physical change in the soil generated by the tailings, this process in the soil decreases the number of macropores as a result of the high density, which results in physical resistance to the roots, reduction in aeration and infiltration availability of water and nutrients and, consequently, increased runoff and erosive processes (Jimenez et al., 2008). Again, among the alternatives to minimize the effects of soil densification are the use of fertilization and organic changes, as well as the planting of species with a robust root system.

The vermicompost has greater maturity and stability than other compounds, has more humic and fulvic acids (humate) and calcium carbonate, which are constantly excreted by earthworm calciferous glands. The addition of vermicompost influences soil pH, which may affect the mobility and bioavailability of heavy metals, as well as improving plant growth, since vermicompost assists in nutrient absorption due to increased rooting, reducing physical soil limitations (Góes et al., 2011; Huang et al., 2016; Sharma and Nagpal, 2018).

Species such as maize, millet and sorghum assist in soil decompaction (Calonego et al., 2011; Rivero Herrada et al., 2017) and are part of metal phytoextraction programs through cultivated plants (De Boer et al., 2018; Oladipo et al., 2016; Tavares et al., 2014; Tolentino et al., 2016; Vamerali et al., 2010).

Thus, considering the following points: (1) Given the extent and severity of the trail left by the mining tailings from the disaster, where approximately 11.99 km^2 of vegetation and 4.81 km^2 of pasture were lost (Aires et al., 2018); (2) the destruction of agricultural areas (IBAMA, 2015); (3) the need for studies on species that can be used for the recovery of this native and agricultural environment; (4) The stress response to mining tailings depends on its intensity and duration, as well as species, genotype and plant development stage and the physicochemical conditions and soil management (Anjum et al., 2017, 2016; Sharma and Nagpal, 2018), it was hypothesized that the addition of vermicompost to mining tailings improves tailing conditions and, consequently, the development of cultivated plants. For this reason, the addition of vermicompost was implemented in iron mining tailings to verify morpho-physiological aspects of maize, millet and sorghum plants.

2 MATERIAL AND METHODS

2.1 Experimental conditions

The experiment was carried out in a greenhouse in Alfenas-MG (UTM 402117.38 East, 7627089.90 South), from December 2018 to April 2019. Mining tailings were collected in Mariana-MG (UTM 669690 West, 779984 South) and were transported to Alfenas-MG, where they were dried outdoors and sieved (4-mm sieve). The experiment was carried out in 6-dm^3 plastic pots, using three species of field crops: maize DKB 390 (*Zea mays* L.), millet BRS 1502 (*Pennisitum glaucum* L.) and sorghum BRS 332 (*Sorghum bicolor*).

The plants were grown on three substrate types: mining tailings (Tailings), mining tailings + vermicompost (20g dm^{-3} tailings) (Tailings + V) and a reference soil (Soil). A randomized block design was used, with three substrate types and three species, totaling 54 pots. Throughout the experimental period, the substrate was maintained at 70% of maximum water retention capacity. Pot irrigation was determined from their weighing on interleaved days. Vermicomposting was performed to obtain the vermicompost, which had cattle manure as its main source.

After a 45-day incubation period of tailings plus vermicompost, sowing was performed and four plants per pot were grown for a period of 50 days. Fertilization occurred according to the minimum demand of each species, according to the recommendations for each species. Three biweekly collections were performed C1 (stage V3), C2 (stage V6 for maize and V5 for millet and sorghum) and C3 (stage V7 for maize, R5 for millet and V6 for sorghum), where growth, leaf gas exchange, chlorophyll “a” fluorescence and relative chlorophyll content were evaluated. At the end of the experiment, the plants were collected for dry biomass determination, metal quantification and root morphology.

2.2 Characterization of substrates used

Composite samples were taken from the tailings and vermicompost and were submitted to physical, chemical and metal analysis to know the composition of these substrates used in the experiment.

2.3 Determination of macro- and micronutrients and chromium in plants

The plant material (roots and shoots), after drying in a forced circulation oven at 65 ºC, were sent for the determination of macro- and micronutrients, besides chromium. Macro- and micronutrients were determined according to the recommendations of (Malavolta et al., 1997), while chromium was quantified by atomic absorption, using an inductively-coupled argon plasma (ICP-OES). Nutrient and metal levels in plants were discussed and compared according to the Brazilian literature (Guimarães et al., 1999).

2.4 Biomass and growth parameters

Stem height and leaf area were measured biweekly with the aid of a measuring tape and a caliper, respectively. At the end of the experiment, the plants were collected, separated into shoots and roots, washed once in 0.5% hydrochloric acid solution and twice in distilled water, placed in properly identified brown paper bags and kept in a greenhouse with air circulation (Nova Era 400 ND - Brazil), at 65 ºC until constant weight. Subsequently, they were weighed on an analytical balance (Martes AY220 - Brazil) for the obtention of dry root and shoot biomass.

2.5 Leaf gas exchange, chlorophyll content and chlorophyll “a” fluorescence

Gas exchange was evaluated through IRGA (Infra Red Gas Analyzer - LI-6400XT, LI-COR, United States) in the last fully expanded leaf; the analyses were performed in the morning (between 9 and 12 o’clock). The parameters analyzed were: net photosynthetic rate (A), stomatal conductance (gs), transpiration (E) and water use efficiency (WUE, given by A/E ratio). The CO_2 flow was 500 µmol mol^{-1} and the block temperature was 30 °C. The photosynthetically active radiation (PAR) used was 1800 µmol m^{-2} s^{-1} for all crops, determined after the light curves for each species.

The chlorophyll content was measured in the last fully expanded leaf from the apex, by a portable chlorophyll meter (SPAD – 502 Plus - Konica Minolta, Japan).

Chlorophyll "a" fluorescence was obtained through a modulated Mini-PAM fluorometer (Heinz Walz, Effeltrich, Germany). In samples adapted in the dark, the maximum photosystem efficiency (PS II) was estimated by the Fv/Fm ratio. Photochemical quenching was calculated as qL = (Fm'-Fs)/(Fm'-Fo'), and non-photochemical quenching was calculated as NPQ = (Fm-Fm')/Fm'. The effective photochemical quantum yield (YII) = Fm'-Fs/Fm' = ΔF/Fm' was also calculated. Details of the procedures can be seen in (Dos Reis et al., 2019).

2.6 Morphological characterization of the root system

The roots were duly collected, washed in running water and stored in pots containing 70% ethanol until the analyses were carried out using the software Winrhizo Pro 2007a (Regent Instruments, Canada). The following parameters were evaluated: length (mm), surface area (mm^2), mean diameter (mm), root volume (mm^3) and root tissue density (RMDE – dry matter/root volume; g / mm^3).

2.7 Data analysis

For data analysis, the means and the standard error of the mean for each parameter were calculated. Subsequently, the analysis of variance (ANOVA) was performed, and the data were submitted to the Scott-Knott comparison test ($p \leq 0.05$) using the Sisvar software, version 5.6 (Federal University of Lavras, Brazil). For the parameters evaluated throughout the collections (growth, gas exchange, chlorophyll "a" fluorescence and chlorophyll content), the interaction between collections and treatments were also evaluated. For the parameters analyzed punctually (metal quantification, dry biomass and root morphology), the interaction between species and treatments was evaluated at the end of the experiment.

3 RESULTS AND DISCUSSION

3.1 Physicochemical characterization of substrates

The vermicompost used in this study (Table 1) showed the presence of cadmium (Cd), chromium (Cr), lead (Pb), copper (Co), iron (Fe), manganese (Mn), boron (B), zinc (Zn) sulfur (S), calcium (Ca), magnesium (Mg), organic carbon (C), nitrogen (N), potassium (K_2O) and phosphorus (P_2O_5). The pH was alkaline (8.6) and the humidity was 21.4. This analysis demonstrates the presence of macro- and micronutrients in the compound used, as well as the presence of some heavy metals. The quantities of metals are within tolerable limits for organic fertilizers in Brazil (MAPA - Ministry of Agriculture, Livestock and Supply, 2016).

Chemical and physical analysis of mining tailings (Table 2) showed very low alkaline pH (7.8), magnesium (Mg), aluminum (Al) and organic matter (OM) contents. Calcium (Ca), potassium (K) and cation exchange capacity (CEC) were low (Guimarães et al., 1999). The tailings showed mainly particles of sizes 0.2-0.05 mm and 0.05-0.002 mm, with a large amount of micropores and a density of 1.9 g cm^{-3}, showing great physical limitation potential. Compared to a study with the same type of tailings previously collected at another point of the disaster (Zago et al., 2019), an improvement in the physicochemical characteristics such as a greater presence of organic matter and a higher proportion of particles with a size smaller than 0.002 xx were observed in the tailings, yielding higher CEC and, consequently, greater availability of elements such as P, K, Ca and Mg for the plants. However, it is noteworthy that conditions are still unfavorable for plant cultivation, mainly due to the high pH, which reduces nutrient availability (Andrade et al., 2018).

Regarding the analysis of metals in the tailings, the presence of nickel (Ni), lead (Pb), zinc (Zn), arsenic (As), chromium (Cr), manganese (Mn) and iron (Fe) was verified. According to the Brazilian law, the elements Cr, Mn and Fe are at concentrations above the allowed values (CONAMA, 2009). High Fe and Mn contents are typical of the latosol that makes up the region, as is Al (Andrade et al., 2018). However, studies have found high concentrations of these elements in the tailings (Andrade et al., 2018; SEDRU, 2016; Segura et al., 2016a; Zago et al., 2019). This is due to the composition of Fe mining tailings, where Al and Mn are unwanted elements in the final mining process and are stored in tailings dams.

3.2 Determination of macro- and micronutrients and chromium in plants

The nutrient content in the tissues (shoots and roots) of the studied plants can be observed in the Supplementary Material (Table S1). There are elements above the reference values for tissue analysis interpretation, according to the Brazilian recommendations (Guimarães et al., 1999). For maize shoot, the concentrations of P and Ca in tailings, K in tailings + vermicompost, S in soil; Mn in the three treatments and Zn in tailings. For the root, Ca in soil and tailings; Cu in soil; Mn and Fe in the three treatments. For millet shoot, N, P, K, S and Zn in soil and tailings and Mn in the three treatments. In the root, Ca, Mn and Fe in the three treatments; Cu in soil and Zn in soil and tailings. For sorghum shoot, N, Mn, Zn and Fe in the three treatments; P in tailings and K in soil. Root, Mn, Zn and Fe in the three treatments and Cu in soil.

The observed behavior may be due to the fact that the vermicompost is known to increase soil fertility, increasing the availability of macronutrients P, K, Ca and Mg (Demir, 2019). Thus, the addition of vermicompost led to a greater nutrient absorption by plants which, consequently, improved their physiological performance. In maize, the application of poultry manure and sawdust mixtures to mining tailings improved the essential nutrients of the shoot and immobilized Zn, Mn, Cd, Cu and Pb on the substrate (Oladipo et al., 2016).

Observing only the nutrient content, it is not possible to observe large differences between treatments. However, the opposite occurs when considering nutrient accumulation in relation to plant biomass (Table 3). The addition of vermicompost to tailings (Tailings + V), compared to the treatment using only tailings, resulted in increased macronutrients in maize (P, K, Mg, S), millet (N, P, K, S) and sorghum (N, P, K, Ca, Mg, S) shoots, and also in maize (P, K, S), millet (P, K, Ca, Mg) and sorghum (N, P, K, Ca, Mg) roots.

For micronutrients, there was an increase in maize (B, Fe), millet (B, Fe), and sorghum (all evaluated micronutrients, B, Cu, Mn, Zn, Fe) shoot. In the roots of the studied plants, a greater accumulation of micronutrients was observed, highlighting B, Cu, Zn, Fe in maize, B, Cu, Mn, Fe in millet and B, Cu, Mn, Zn and Fe in sorghum. Chromium was found only in the roots of plants from the treatment tailings + vermicompost (Table 3).

Considering the reference values for tissue analysis in maize and sorghum (Guimarães et al., 1999), the plants presented lower values of macronutrients and Cu, besides excess micronutrients (Mn, Zn e Fe). Regarding millet, considering the values of macro- (Teixeira et al., 2005) and micronutrients (Teixeira et al., 2008) found in previous studies, higher values were observed for both, except for Fe in the shoot. The higher absorption of Mn, Zn and Fe is related to the presence of these micronutrients at high amounts in the tailings used in this experiment (Table 2).

Maize, millet and sorghum plants absorbed amounts of Cr, Fe and Mn (Table S1) above the limits described in the literature, where the maximum predicted value for Cr is 1.3 mg kg^{-1} (WHO, FAO, 1996), while Mn varies between 20-150 mg kg^{-1} and Fe between 20-250 mg kg^{-1} for tropical maize and sorghum species (Guimarães et al., 1999). It is noteworthy that the plants did not show visible toxicity symptoms by any of these metals. Tailings supplementation with organic matter has been related to lower Fe and Mn absorption by plants due to metal complexation with organic matter, resulting in lower bioavailability (Zago et al., 2019).

On the other hand, the addition of organic matter may be related to the higher metal availability to plants (Antoniadis and Alloway, 2003; Pittarello et al., 2017; Vamerali et al., 2010), as observed in this study. This may be associated with the greater presence of humic acids supplied by vermicompost, which may influence the bioavailability of metals and nutrients (due to complexation), in the activation of aquaporins and other metal transporters or the stimulation of root growth (Pittarello et al., 2017). Corroborating these results, the addition of organic compound increases the absorption of As by barley and wheat, even increasing the translocation of this metal to the shoots (González et al., 2019).

The potential of maize for phytoremediation of areas contaminated with chromium, lead and cadmium has already been described (Bashmakov et al., 2017; Figlioli et al., 2019; Martínez-Trujillo and Carreón-Abud, 2015), mainly playing a role in the removal of Cu, Zn, Pb, and Cd of sewage sludge (Xu et al., 2015). Although there is translocation to the shoots and even to the grains, in some cases the levels of metals found in the grains do not prevent their consumption, depending on local legislation (Xu et al., 2015). Millet is also considered to be tolerant to the excess of some heavy metals (Asopa et al., 2017; Gupta et al., 2017), and this tolerance is related to the lower metal translocation to the shoot. In the case of sorghum, the species is tolerant to excess Ni and Mn, with excess metal kept in the roots (Naeini and Rad, 2018; Serme et al., 2015). The performance of sorghum plants was also not altered by the presence of Cr in the substrate, demonstrating the tolerance of the species to the metal (Padmapriya et al., 2016). Applying sewage sludge to compacted soil improved soil characteristics, allowing for greater metal absorption along with better sorghum plant performance (Zuo et al., 2019). The amounts of metal found in the species of this study do not show the phytoextraction role of these plants in the tailings, even with the greater availability of these metals by the addition of vermicompost.

3.3 Biomass and growth parameters

Shoot and root dry biomass of maize, millet and sorghum was higher in the treatment with tailings + vermicompost, followed by soil and finally tailings (Figure 1A - C). When compared to plants grown in tailings, the addition of vermicompost to the tailings led to a 163.5% increase in shoot biomass and a 246% increase in root biomass in maize (Figure 1A); 432.8% in shoots and 900% in millet roots (Figure 1B) and 278% in shoots and 735% in sorghum roots (Figure 1C). The addition of vermicompost also yielded greater biomass accumulation in relation to plants grown in the reference soil (Figure 1).

In general, along the collections (C1 to C3), there was an increase in plant height (Figure 2 A-C) and leaf area (Figure 2D - E), but lower growth was observed in plants grown in mining tailings. On the other hand, analyzing the treatments, the addition of vermicompost to the tailings provided greater increase in height and leaf area of these plants (Figure 2; Supplementary Material Figure S1). This higher growth is probably due to increased substrate porosity, as vermicompost improves soil aggregates through the action of polysaccharide-producing microorganisms which deposit cementing agents between soil particles (Demir, 2019), improving water retention, aeration and drainage (Tomati and Galli, 1995).

The reduction in biomass of maize, millet and sorghum plants grown in tailings occurred in plants with lower macro- and micronutrient contents in shoots and roots, except for Mn and Zn in maize shoots. With the same tailings, rice was grown and 50% sludge resulted in decreased root growth and grain yield (Andrade et al., 2018). In both cases, the reduction in growth may be due to nutritional deficiency and the physical properties of the tailings. On the other hand, the addition of vermicompost increased the content of these nutrients in relation to plants grown in tailings. The organic matter present in vermicompost is the most significant factor for increasing the stability of these aggregates, indicating improvement in soil quality and recovery (Wick et al., 2014), reducing the physical limitations of the substrate on the root system (Liu et al., 2011). In this study, the addition of vermicompost favored the investment in root biomass of the three species, an important factor for tolerance to physical resistance of the substrate, an abiotic stress already pointed out in plants under iron mining tailings from the Mariana-MG disaster (Andrade et al., 2018).

3.4 Leaf gas exchange, chlorophyll content and chlorophyll “a” fluorescence

For maize, in the first collection (C1), the vermicompost increased gas exchange parameters (An, gs and E) and, along the other collections (C2 and C3), the most pronounced increase was observed in the tailings (with some exceptions) (Figure 3 A, D, G). In millet (Figure 3 B, E, H) and sorghum (Figure 2 C, F, I), in C1 and C2, the addition of vermicompost resulted in An, gs and E similar to that of the soil, but larger than in the tailings plants. In C3, for millet, the vermicompost yielded An similar to that of the soil but, for gs and E, there were no differences between treatments. In the case of sorghum, the gas exchange of plants under tailings + vermicompost was similar to that of plants under tailings. Water use efficiency (WUE) in maize was lower in the treatment with tailings in all collections (Figure 3 J). In millet, initially, the highest WUE was observed in the treatment with vermicompost + tailings and, in the following collections, the treatments showed no difference. (Figure 3 K). At first, sorghum had a higher WUE in the treatment with tailings; however, in the other collections, there was no difference between treatments (Figure 3 L).

Among the treatments, the addition of vermicompost led to a higher relative chlorophyll content in maize plants, compared to the plants of the tailings treatment, which always had lower contents (Figure 3 M). In millet, less chlorophyll production was observed in the tailings treatment in C1 and C2. However, in C3, the soil treatment was higher (Figure 3 N). In sorghum, a lower relative chlorophyll content was observed in the plants grown in tailings and the addition of vermicompost increased the content in all collections (Figure 3 O). Plants grown in compacted soils frequently have chlorosis due to low N uptake capacity and increased pH (Kobaissi et al., 2013), which changes the uptake dynamics of nutrients and other elements by plants. In maize and sorghum, especially in C3, a relationship was observed between the low chlorophyll content in the tailings and the increase in photosynthetic rate, showing a compensatory effect in these plants to compensate for the low pigment content.

Regarding the chlorophyll *a* fluorescence parameters, there was no difference between the treatments in the three plants studied (Supplementary Material Figure S2), and it is sufficient to highlight only among treatments, an increase in the effective quantum yield (YII) of millet with the addition of vermicompost in the tailings (Figure S2b). Contrary to what was observed in this study, plants grown in compacted soils show reduced electron transport through PS II, which can present structural lesions in their complexes, increasing chlorophyll fluorescence emission (Asch et al., 2009).

Not only does soil physical limitation affect root, but also shoot growth, since there is transmission of chemical stimuli from roots under stress to plant growth zones (Tardieu, 1994). As a result, lower root growth is related to lower water and nutrient absorption and, consequently, lower stomatal conductance (Grzesiak et al., 2013). This reduction in gas exchange was very marked in millet and sorghum, species in which there was also lower nutrient absorption, which corroborated the lower dry biomass and height of these plants grown in mining tailings. In contrast, although the cultivation in tailings did not reduce plant leaf area compared to those cultivated in soil, the addition of vermicompost also yielded an increase in the photosynthetic leaf area of these plants, especially in millet.

3. 5 Morphological characterization of the root system

The addition of vermicompost to the tailings (Tailings + V) resulted in greater length, surface area, volume and mean root diameter (Figure 4 A, B, C, E) than in the tailings treatment. In some cases, even higher values were observed in Tailings + V than those of plants grown in soil. The addition of vermicompost increased root tissue density (RTD) in relation to tailings for maize and millet, and the largest increment was observed in millet (Figure 4D). For maize and millet, the highest mean root diameter was found in the tailings + vermicompost treatment and, for sorghum, the smallest diameter was observed in the tailings treatment (Figure 4 E).

One of the main signs of soil physical limitation observed in plants is the reduction in root growth due to the high soil strength, coupled to the low availability of nitrogen and oxygen, which influences the reduction in height, diameter, leaf number, specific leaf area, epidermal cell thickness and cell wall (Grzesiak et al., 2013). Shallow root growth, decreasing number of axial and lateral roots, as well as lower root branch biomass, also occur (Colombi and Walter, 2016). This was observed in the roots of the three crops when cultivated in mining tailings. However, in maize, reductions in root length, volume and surface area under tailings occurred in smaller proportions than in the other species, compared to soil.

In general, in the three species studied, the addition of vermicompost led to an increase in length, surface area and volume in all root diameter classes in relation to the tailings treatment (Table 4). Even so, the largest increments were observed in the thick and very thick roots in the three species. The negative effect of tailings occurred in smaller proportions for maize roots than in the other species.

The addition of vermicompost favored root growth on surface, volume, diameter and dry biomass. Even so, much more significant increases were observed in millet and sorghum for all root classes analyzed. It is also found that the largest increases were observed in the thick and very thick root classes, with an increase of about 600% in thick and very thick millet roots and over 1000% in thick and very thick sorghum roots for length, volume and surface area. In the case of maize, the largest increases due to the addition of vermicompost were observed in very thick roots, greater than 100% (Table 4). Higher investment in thick and very thick roots, mainly for maize and millet, resulted in higher RTD in tailings + V treatment. The increase in RTD shows the investment in lignification and suberization of their roots, which allows greater survival of these plants in compacted soils (Kramer-Walter et al., 2016). On the other hand, in sorghum, whose investment in very thin roots was high, there was no increase in RTD in plants grown in tailings + V, compared to the treatment with only tailings, showing that these plants invested in greater substrate exploration through thinner roots (sensitive to compaction), while in other species the investment occurred in roots with larger diameter.

The formation of thick and very thick roots may be one of the strategies used by cultivars to mitigate the effects of the physical limitation of tailings, as thin root formation is costly in terms of carbon, oxygen and nitrogen due to high respiration rates and short shelf life (Pregitzer et al., 1998). Thus, the larger the root diameter of the species or the greater its ability to increase the root diameter when subjected to physical resistance, the greater the success of this species in compacted soils (Correa et al., 2019; Kolb et al., 2017).

The root growth pattern found in maize provides evidence of greater tolerance of this species to the physical limitation of mining tailings than the other genotypes. This can be verified by the smaller reductions in biomass, volume and surface area when it was grown in tailings, that is, maize roots remain more stable despite the greater physical limitation of the tailings. In the case of sorghum and millet, whose roots suffer drastic effects by the physical limitation of the tailings, the addition of vermicompost mitigates the deleterious effects of this limitation, increasing growth, especially in thick and very thick roots, which favor the survival of plants in compacted substrates. In addition to compaction due to the low water retention capacity of its particles (Shaefer et al., 2016; Silva et al., 2017), iron mining tailings are also characterized by the absence of organic compounds and low nutrient availability (Festin et al., 2019). Physical methods for the recovery of mining soils consist in the application of compounds that reduce erosion and improve the physical characteristics of the soil, reducing the physical limitations of the tailings and allowing minimum conditions for the revegetation of these areas. In this context, the addition of organic compounds, such as vermicompost, to mining tailings may be an alternative used for the revegetation of mining areas due to the physicochemical changes of the substrate, making it conducive to the establishment of new plant species (Festin et al., 2019).

Mining tailings generally have high amounts of heavy metals and, depending on the collection site, there may be variations in the metal and the amount available for absorption (Andrade et al., 2018; Segura et al., 2016b; Zago et al., 2019). Thus, it is necessary to consider that the absorption of metals by plants depends on the physicochemical characteristics of the soil such as pH, CEC, organic matter and amount of clay. In this context, the question arises about the use of these plant species for revegetation of contaminated tailings areas (Sharma and Nagpal, 2018). Several studies have been conducted in search of cereals that are grown in locations prone to metal bioavailability (Vamerali et al., 2010). Other studies show the increased efficiency of plant metal absorption in phytomanagement processes with the addition of manure, compost and vermicompost (Arthur et al., 2016; Sierra Aragón et al., 2019; Vamerali et al., 2010).

Given the above, considering the limitations for root growth of cereals grown in tailings, the application of vermicompost may be a viable alternative for the beginning of the recovery process of areas containing iron mining tailings. The introduction of species with robust and dense root growth allows the beginning of the soil recovery process so that it has the minimum conditions (physicochemical and biological) for the beginning of the natural revegetation process, for example, with native species of the region. In addition, the results shown in this study support even the restructuring of agriculture, which was also affected by the disaster, as the plants showed no signs of heavy metal toxicity.

4 CONCLUSION

The addition of vermicompost improves the physical conditions of mining tailings, acting as a soil conditioner and yielding an increase in shoot and root dry biomass, thus leading to a greater growth of the studied plant species. The main role of vermicompost in plants is to promote the growth of thick and very thick roots, which is one of its strategies for survival of species under physical limitation.

Vermicompost can be used in environments containing mining tailings to favor the growth of cultivated species.

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TABLES AND FIGURES

Table 1: Vermicompost analysis.

pH	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn	B	S	Pb	Cd	Cr	C	C/N	Humidity
-	%			g kg^{-1}		mg kg^{-1}									g kg^{-1}	-	%
8.6	1.4	2.8	1.5	44	12	214	28.4	523	309	127	9	29.7	27	80.4	20.6	14.7	21.4

Table 2: Chemical and physical analysis of mining tailings collected in the city of Mariana-MG (at point UTM 669690 West, 779984 South), used for growing plants.

pH	OM	P-Rem	P	K	Ca	Mg	Al	H+ Al	S.B.	CEC	T	m	v	Fe	Mn	Cr
	dag kg^{-1}	mg L^{-1}	mg dm^{-3}		cmol$_c$ dm^{-3}							%		g kg^{-1}		
7.8	0.27	43.4	11.9	16.2	1.5	0.1	0.04	0.6	1.6	1.6	2.2	2.4	72.1	55.2	0.6	9.5

Granulometry				Micropore	Macropore	PTV	U.SAT.	Soil density	Particle density
2-0.2 mm	0.2-0.05 mm	0.05-0.002 mm	< 0.002 mm	%				g cm^{-3}	kg dm^{-3}
61	418	439	81.6	31.6	16.7	48.3	37.7	1.9	2.5

PTV: total pore volume; SB: sum of bases; CEC: cation exchange capacity; OM: organic matter

Table 3: Nutrient and metal accumulation in shoots and roots of maize DKB 390, millet BRS 1502 and sorghum BRS 332.

	Identification	**N**	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Mn**	**Zn**	**Fe**	**Cr**
		---------(g plant^{-1})---------						**---------(mg plant^{-1})---------**					
Shoot	Maize soil	0.557 Aa*	0.057 Ab	0.474 Ab	0.116 Aa	0.107 Aa	0.062 Ab	0.289 Ab	0.122 Aa	7.397 Aa	1.779 Aa	5.300 Ab	-
	Maize Tailings	0.370 Ab	0.065 Ab	0.237 Ab	0.123 Aa	0.028 Ac	0.038 Ac	0.149 Ac	0.020 Ab	7.506 Aa	1.791 Aa	4.058 Ab	-
	Maize T+ V**	0.466 Ba	0.082 Aa	1.045 Aa	0.093 Aa	0.081 Ab	0.070 Aa	0.495 Aa	0.017 Ab	6.609 Ba	2.092 Ba	6.822 Aa	-
	Millet soil	0.647 Aa	0.057 Ab	0.574 Aa	0.101 Aa	0.054 Ba	0.048 Bb	0.236 Bb	0.177 Aa	6.262Aa	1.687 Aa	4.001 Ba	-
	Millet Tailings	0.236 Bc	0.029 Bc	0.201 Ab	0.040 Bb	0.015 Ab	0.019 Bc	0.094 Bc	0.052 Ab	2.494 Bb	0.686 Bb	1.386 Bb	-
	Millet T+V	0.390 Bb	0.090 Aa	0.636 Ba	0.091 Aa	0.070 Aa	0.059 Ba	0.319 Ba	0.027 Ab	6.126 Ba	2.012 Ba	5.753 Aa	-
	Sorghum soil	0.498 Bb	0.050 Ab	0.464 Aa	0.054 Bb	0.047 Bb	0.043 Cb	0.171Cb	0.169 Aa	6.658 Ab	2.028 Ab	2.982 Bb	-
	Sorghum Tailings	0.201 Bc	0.029 Bb	0.153 Ab	0.040 Bb	0.006 Ac	0.017 Bc	0.094 Bc	0.012Ac	3.369 Bb	0.911 Bc	1.913 Bb	-
	Sorghum T+V	0.598 Aa	0.082 Aa	0.560 Ba	0.069 Aa	0.063 Aa	0.055 Ba	0.271 Ba	0.070 Ab	10.372 Aa	3.064 Aa	5.628 Aa	-
Roots	Maize soil	0.181 Aa	0.022 Ab	0.099 Ab	0.065 Aa	0.027 Aa	-	0.162 Ab	0.543 Aa	2.891 Aa	0.844 Aa	15.399 Ab	-
	Maize Tailings	0.062 Ab	0.011 Ac	0.021 Ac	0.044 Ab	0.003 Ab	-	0.079 Ac	0.019 Ac	1.521 Ab	0.246 Ac	7.093 Ac	-
	Maize T+V	0.102 Ab	0.032 Aa	0.296 Aa	0.044 Bb	0.021 Aa	-	0.226 Aa	0.090 Ab	3.093 Aa	0.593 Ab	24.777 Aa	0.069 Aa
	Millet soil	0.077 Ba	0.011 Ba	0.060 Bb	0.031 Bb	0.013 Bb	-	0.081 Bb	0.194 Ba	0.813 Bb	0.627 Bb	6.928 Bb	-
	Millet Tailings	0.030 Aa	0.003 Bb	0.018 Ac	0.009 Bc	0.002 Ac	-	0.022 Bc	0.008 Ac	0.287 Bb	0.156 Aa	1.324 Bc	-
	Millet T+V	0.100 Aa	0.018 Ca	0.100 Ca	0.064 Aa	0.025 Aa	-	0.124 Ba	0.100 Ab	2.943 Aa	0.661 Ab	14.976 Ba	0.068 Aa
	Sorghum soil	0.077 Bb	0.009 Bb	0.083 Bb	0.015 Cb	0.008 Bb	-	0.069 Bb	0.188 Ba	1.215 Bb	0.413 Cb	5.411 Cb	-
	Sorghum Tailings	0.021 Ab	0.003 Bc	0.018 Ac	0.006 Bb	0.001 Ac	-	0.023 Bc	0.007 Ab	0.426 Bb	0.114 Ac	1.406 Bc	0.005 Ab
	Sorghum T+V	0.081 Aa	0.015 Ba	0.105 Ba	0.028 Ba	0.011 Ba	-	0.090 Ba	0.075 Aa	1.958 Aa	0.483 Aa	8.312 Ca	0.033 Aa

* Uppercase letters compare the means between crops and lowercase letters compare the means between treatments for each crop. Means followed by the same letter do not differ by the Scott-Knott test ($p \leq 0.05$). ** V=vermicompost

Table 4: Length, surface area and root volume, per diameter class, in maize DKB 390, millet BRS 1502 and sorghum BRS 332.

		Maize			Millet			Sorghum		
	Diameter classes	Soil	Tailings	Tailings +V**	Soil	Tailings	Tailings + V	Soil	Tailings	Tailings + V
Root length	Very thin	763.10 Aa*	427.66 Ab	532.66 Bb	536.45 Ba	164.17 Bc	410.17 Cb	616.50 Bb	252.38 Bc	1023.36 Aa
	Thin	575.84 Ab	400.03 Ac	698.92 Aa	241.41 Ba	75.16 Bb	218.37 Ca	279.37 Bb	87.46 Bc	546.23Ba
	Thick	162.50 Ab	124.77 Ac	217.81 Aa	45.07 Ca	7.16 Bb	51.47 Ca	66.71 Bb	7.18 Bc	108.76 Ba
	Very thick	67.11 Ab	44.53 Ac	92.37 Aa	16.42 Ba	2.63 Bb	19.52 Ca	22.01 Ba	2.18 Bb	30.54 Ba
Root surface área	Very thin	57.86 Aa	32.72 Ac	42.51 Bb	30.92 Ba	9.54 Bb	25.79 Ca	33.87 Bb	13.39 Bc	66.18 Aa
	Thin	175.92 Ab	129.03 Ac	221.13 Aa	72.74 Ba	20.92 Bb	68.19 Ba	90.97 Cb	25.25 Bc	165.40 Ba
	Thick	149.46 Ab	114.03 Ac	201.73 Aa	40.73 Ca	6.27 Bb	46.35 Ca	60.47 Bb	6.45 Bc	97.04 Ba
	Very thick	159.47 Ab	98.22 Ac	222.10 Aa	35.82 Ba	6.97 Bb	50.47 Ba	49.55 Ba	4.75 Bb	68.78 Ba
Root volume	Very thin	0.44 Aa	0.25 Ac	0.33 Bb	0.21 Ba	0.06 Bb	0.18 Ca	0.21 Bb	0.08 Bc	0.46 Aa
	Thin	5.05 Aa	3.88 Ac	6.52 Aa	2.05 Ca	0.54 Bb	2.00 Ca	2.75 Bb	0.66 Bc	4.71 Ba
	Thick	11.53 Ab	8.76 Ac	15.67 Aa	3.09 Ca	0.46 Bb	3.50 Ca	4.61 Bb	0.49 Bc	7.27 Ba
	Very thick	36.13 Ab	19.63 Ac	54.36 Aa	7.16 Ba	1.67 Bb	13.13 Ba	9.86 Ba	0.89 Bb	14.97 Ba

Very thin correspond to diameters between 0 and 0.5 mm; thin, between 0.5 and 2 mm; thick, between 2 and 4.5 mm and very thick, above 4.5 mm.

* Uppercase letters compare the means between crops and lowercase letters compare the means between treatments for each crop. Means followed by the same letter do not differ by the Scott-Knott test ($p \leq 0.05$).

**V=vermicompost

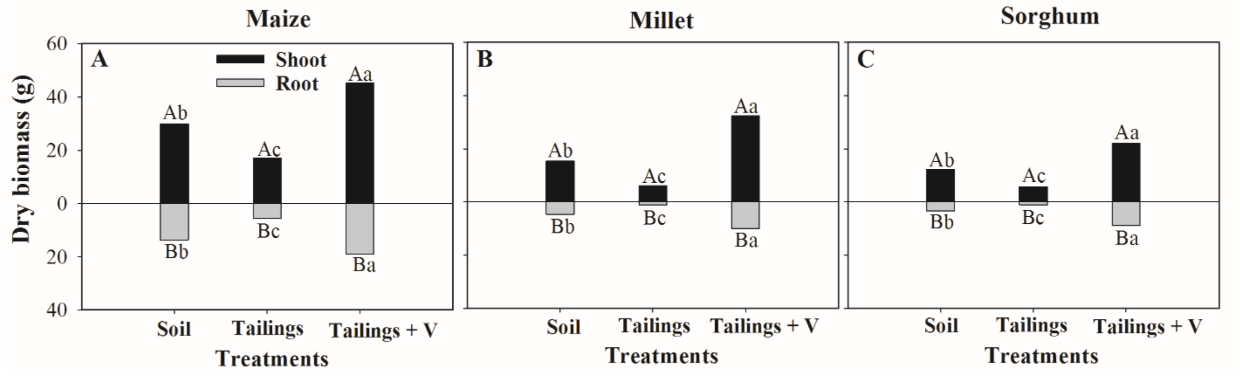

Figure 1: Shoot and root dry biomass of maize (A), millet (B) and sorghum (C) (stage V7 for maize, R5 for millet and V6 for sorghum), grown on different substrates: Soil, mining tailings and mining tailings + vermicompost. Uppercase letters compare parts of the plant and lowercase letters compare treatments. Means followed by the same letter do not differ by the Scott-Knott test ($p \leq 0.05$).

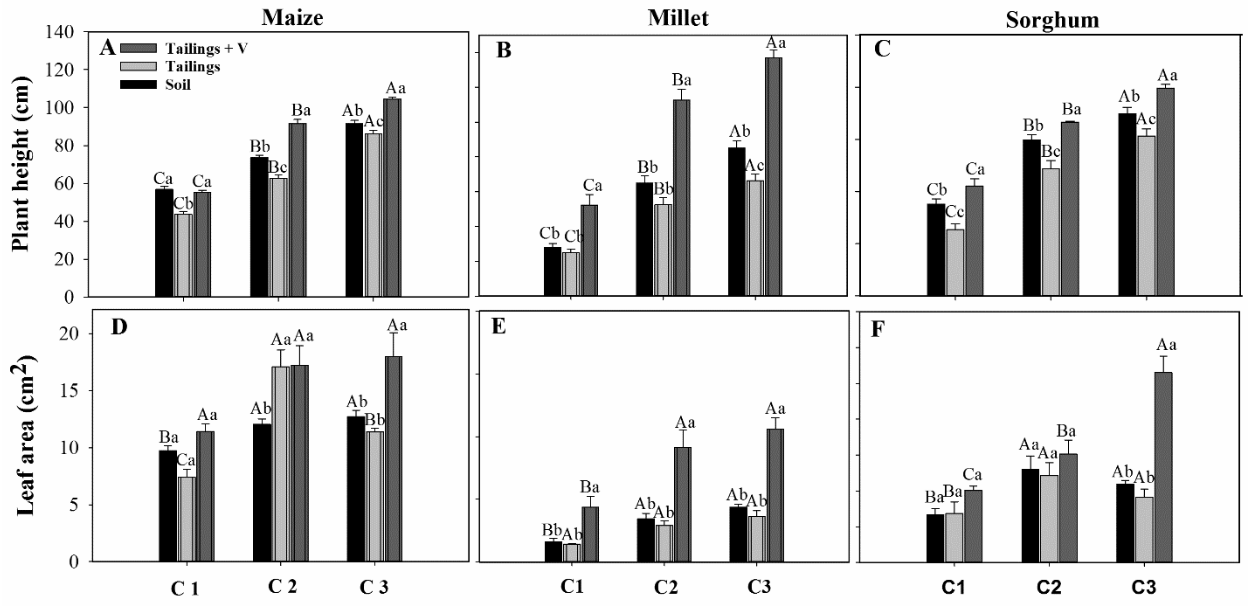

Figure 2: Height (A - C) and leaf area (D - F) of maize, millet and sorghum plants in three collection seasons (C1 to C3): C1(stage V3), C2 (stage V6 for maize and V5 for millet and sorghum) and C3 (stage V7 for maize, R5 for millet and V6 for sorghum). Different substrates were used: Soil, mining tailings and mining tailings + vermicompost. Uppercase letters compare the means between collections and lowercase letters compare the means between treatments within each collection. Means followed by the same letter do not differ by the Scott-Knott test ($p \leq 0.05$). The bars correspond to the standard error of the mean of five replications.

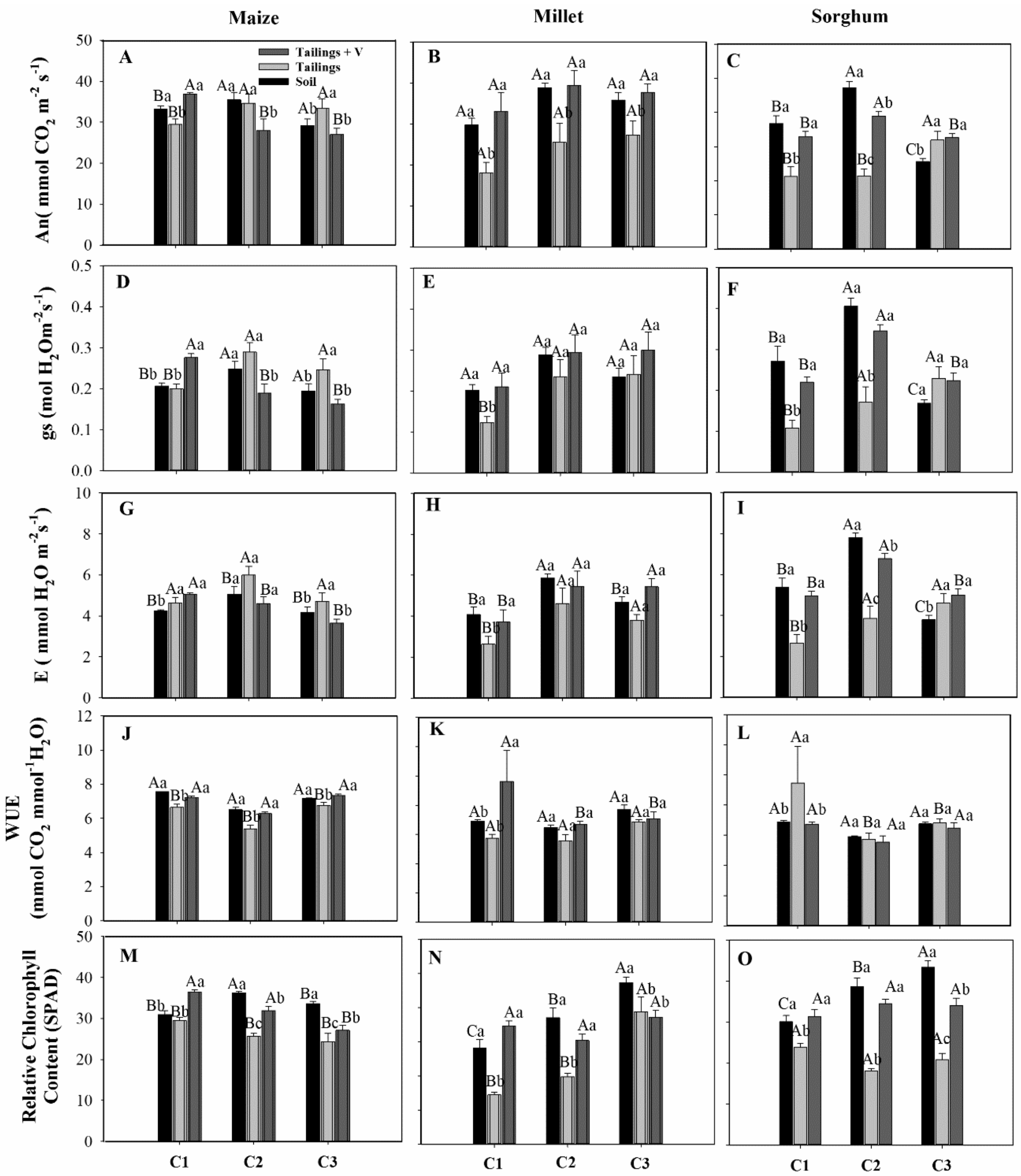

Figure 3: Net photosynthetic rate An (A-C); stomatal conductance gs (D-F); transpiration E (G-I); water use efficiency WUE (J-L) and chlorophyll content by SPAD index (M-O) of maize, millet and sorghum plants in three collection seasons (C1 to C3): C1(stage V3), C2 (stage V6 for maize and V5 for millet and sorghum) and C3 (stage V7 for maize, R5 for millet and V6 for sorghum). Different substrates were used: Soil, mining tailings and mining tailings + vermicompost. Uppercase letters compare the means between collections and lowercase letters compare the treatments within each collection. Means followed by the same letter do not differ by the Scott-Knott test ($p \leq 0.05$). The bars correspond to the standard error of the mean of five replications.

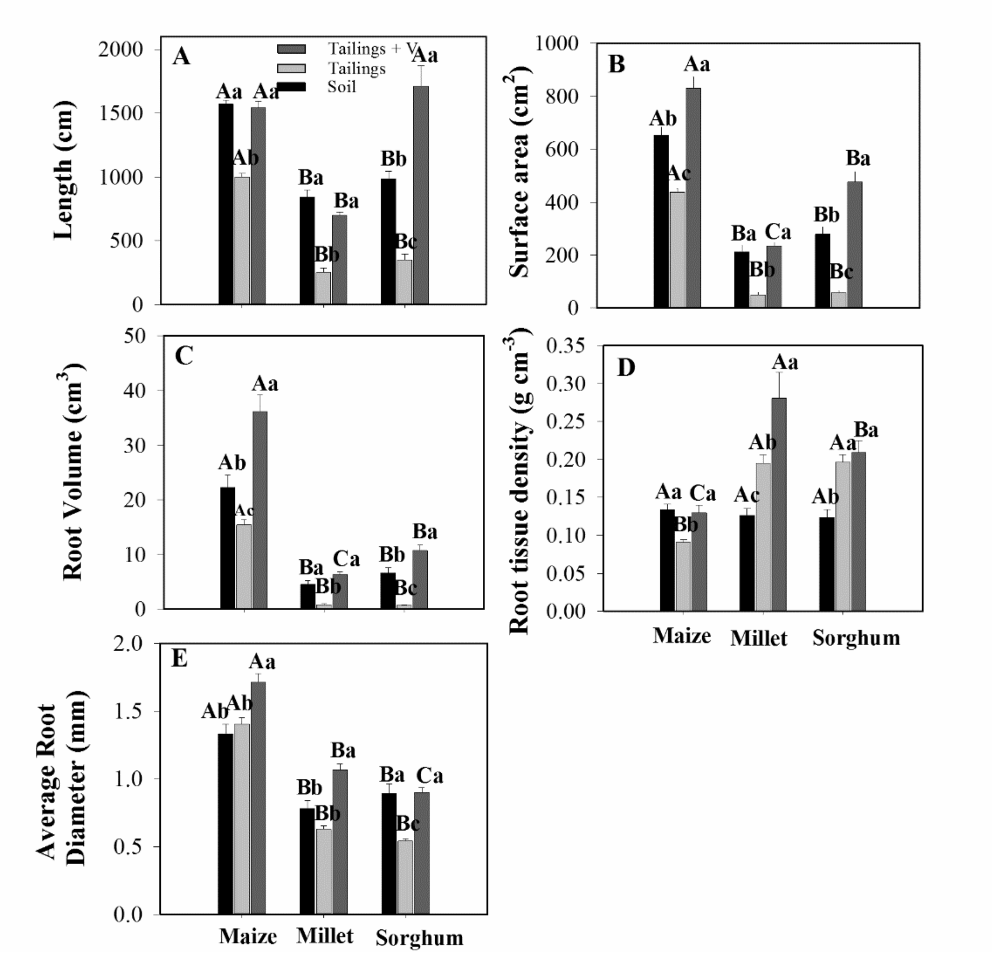

Figure 4: Root length (A); surface area (B); root volume (C) root volume; root tissue density RTD (D); mean root diameter (E), of maize, millet and sorghum plants, (stage V7 for maize, R5 for millet and V6 for sorghum). Different substrates were used: Soil, mining tailings and mining tailings + vermicompost. Uppercase letters compare the means between cultivars and lowercase letters compare the means between treatments. Means followed by the same letter do not differ by the Scott-Knott test ($p \leq 0.05$). The bars correspond to the standard error of the mean of five replications.

SUPPLEMETARY DATA

Table S1: Nutritional analysis in shoots and roots of maize DKB 390, millet BRS 1502 and sorghum BRS 332.

	Identification	N	P	K	Ca	Mg	S	B	Cu	Mn	Zn	Fe	Cr
		(g kg^{-1})						(mg kg^{-1})					
Shoot	Maize soil	18.9 Ba	1.95 Bb	16.07 Ba	3.94 Bb	3.63 Aa	2.11 Ba	9.80 Ba	4.14 Ba	250.93 Bb	60.35 Bb	179.78 Aa	-
	Maize Tailings	21.18 Ca	3.70 Ba	13.58 Ba	7.06 Aa	1.62 Bb	2.17 Ca	8.51 Ba	1.14 Ba	429.67 Aa	102.52 Aa	232.30 Aa	-
	Maize T+V**	10.53 Bb	1.85 Bb	23.60 Aa	2.10 Ab	1.83 Ab	1.58 Bb	11.19 Ba	0.38 Aa	149.25 Bb	47.24 Bb	154.07 Ba	-
	Millet soil	39.99 Aa	3.53 Ab	35.50 Aa	6.26 Aa	3.33 Aa	2.99 Aa	14.60 Aa	10.93 Aa	387.27 Aa	104.35 Aa	247.43 Aa	-
	Millet Tailings	41.61 Aa	5.06 Aa	35.43 Aa	7.07 Aa	2.62 Aa	3.27 Aa	16.62 Aa	9.12 Aa	420.71 Aa	121.12 Aa	244.80 Aa	-
	Millet T+V	11.86 Bb	2.75 Bb	19.34 Ab	2.77 Ab	2.12 Aa	1.80 Bb	9.70 Bb	0.83 Ab	186.30 Bb	61.18 Bb	174.98 Ba	-
	Sorghum soil	33.76 Aa	3.42 Aa	31.46 Aa	3.64 Bb	3.21 Aa	2.91 Aa	11.58 Ba	11.45 Aa	451.40 Aa	137.50 Aa	202.15 Aa	-
	Sorghum Tailings	32.24 Aa	4.69 Aa	24.51 Aa	6.43 Aa	0.92 Bb	2.75 Ba	15.04 Aa	1.87 Bb	540.81 Aa	146.30 Aa	307.14 Aa	-
	Sorghum T+V	29.67 Aa	4.05 Aa	27.81 Aa	3.44 Ab	3.11 Aa	2.71 Aa	13.46 Aa	3.45 Ab	514.72 Aa	152.05 Aa	279.30 Aa	-
Roots	Maize soil	11.95 Aa	1.46 Aa	6.55 Cb	4.26 Ab	1.79 Aa	-	10.70 Aa	35.77 Aa	190.62 Aa	55.64 Ba	1015.31 Bb	-
	Maize Tailings	9.17 Ca	1.59 Ba	3.11 Ba	6.54 Ba	0.38 Cc	-	11.73 Ba	2.88 Ab	226.45 Ba	36.59 Cb	1056.04 Aa	-
	Maize T+V	4.44 Ab	1.37 Ba	12.83 Ab	1.91 Cc	0.93 Cb	-	9.8 Ba	3.91 Ab	134.03 Bb	25.7 Bb	1073.54 Aa	2.97 Ba
	Millet soil	11.61 Ab	1.64 Ab	9.06 Bb	4.61 Ab	1.93 Aa	-	12.17 Ab	29.12 Ba	122.31 Bb	94.28 Ab	1041.74 Ab	-
	Millet Tailings	24.37 Aa	2.54 Aa	14.675 Ac	7.49 Aa	1.52 Ab	-	17.24 Aa	6.7 Ab	229.67 Ba	124.77 Aa	1059.42 Ab	-
	Millet T+V	7.19 Ab	1.30 Bc	7.24 Ba	4.65 Ab	1.80 Aa	-	8.96 Bc	7.23 Ab	212.48 Aa	47.7 Ac	1081.28 Aa	4.93 Ba
	Sorghum soil	14.92 Aa	1.71 Ab	16.01 Aa	2.90 Bb	1.56 Ba	-	13.36 Ab	36.38 Aa	234.49 Ab	79.71 Aa	1044.67 Ab	-
	Sorghum Tailings	16.01 Ba	2.45 Aa	13.25 Ab	4.79 Ca	0.65 Bb	-	17.24 Aa	5.35 Ab	320.02 Aa	85.79 Ba	1057.19 Ab	3.63 Aa
	Sorghum T+V	10.53 Aa	1.923 Ab	13.73 Ab	3.64 Bb	1.49 Ba	-	11.78 Ab	9.86 Ab	255.94 Ab	63.18 Aa	1086.60 Aa	4.30 Aa

* Uppercase letters compare the means between crops and lowercase letters compare the means between treatments for each crop. Means followed by the same letter do not differ by the Scott-Knott test ($p \leq 0.05$). **V= vermicompost

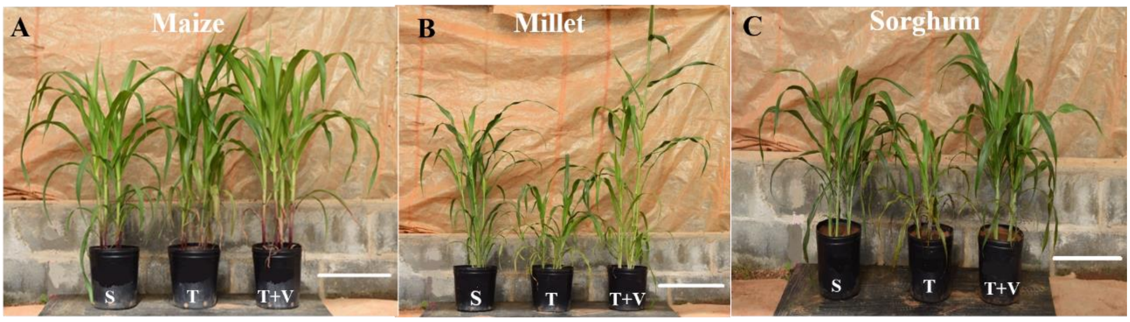

Figure S1: Plants of maize, stage V7 (A), millet, stage R5 (B) and sorghum, stage V6 (C), after 50 days of soil cultivation (S), mining tailings (T) and mining tailings + vermicompost (T+V). The white bar indicates 30 cm.

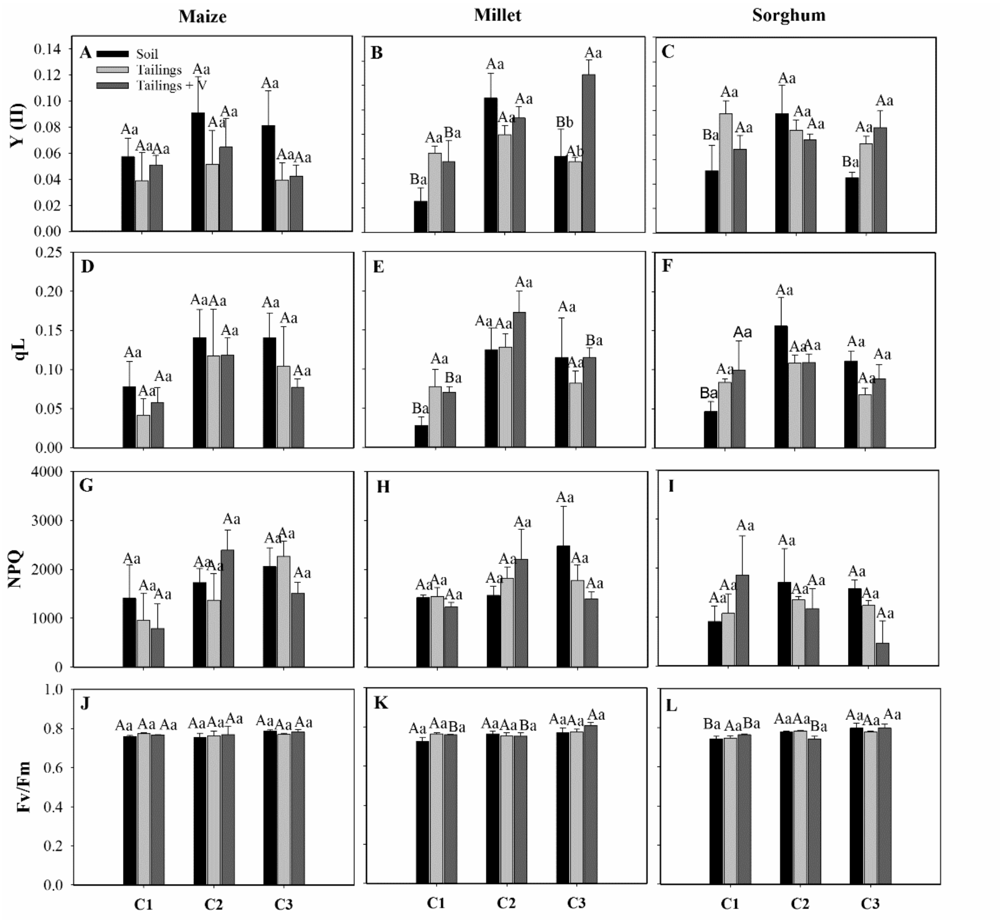

Figure S2: Effective quantum yield Y(II) (A-C); photochemical dissipation qL (D-F); non-photochemical dissipation NPQ (G-I) and maximum quantum yield of PSII (J-L) of maize, millet and sorghum plants in three harvesting seasons (C1 to C3): C1(stage V3), C2 (stage V6 for maize and V5 for millet and sorghum) and C3 (stage V7 for maize, R5 for millet and V6 for sorghum). Uppercase letters compare the means between collections and lowercase letters compare the means between treatments within each collection. Means followed by the same letter do not differ by the Scott-Knott test (p ≤ 0.05). The bars correspond to the standard error of the mean of five replications