UNIVERSIDADE FEDERAL DE ALFENAS

PEDRO ERNESTO DOS REIS

POTENCIAL FITORREMEDIADOR DE *TALINUM PANICULATUM* NA PRESENÇA DE CÁDMIO, CHUMBO, MANGANÊS E FERRO

Alfenas/MG
2020

PEDRO ERNESTO DOS REIS

POTENCIAL FITORREMEDIADOR DE *TALINUM PANICULATUM* NA PRESENÇA DE CÁDMIO, CHUMBO, MANGANÊS E FERRO

Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Ciências Ambientais pelo Programa de Pós-graduação em Ciências Ambientais da Universidade Federal de Alfenas. Área de concentração: Tecnologias Ambientais Aplicadas.
Orientador: Prof. Dr. Plínio Rodrigues Dos Santos Filho
Coorientadora: Dra. Kamila Rezende Dázio de Souza

Alfenas/MG

2020

Dados Internacionais de Catalogação-na-Publicação (CIP)
Sistema de Bibliotecas da Universidade Federal de Alfenas
Biblioteca Central – Campus Sede

Reis, Pedro Ernesto dos
R375p Potencial fitorremediador de *Talinum paniculatum* na presença de cádmio, chumbo, manganês e ferro. / Pedro Ernesto dos Reis – Alfenas, MG, 2021.
65 f.: il. –

Orientador: Plínio Rodrigues dos Santos Filho.
Dissertação (Mestrado em Ciências Ambientais) – Universidade Federal de Alfenas, 2021.
Bibliografia.

1. Metais pesados. 2. Trocas gasosas. 3. Peroxidação lipídica. 4. Enzimas antioxidantes. 5. Massa seca. 6. Estresse oxidativo. 7. Crescimento. 8. Fitorremediação. 9. Sistema antioxidante. 10 Talinacea. I. Santos Filho, Plínio Rodrigues dos. II. Título.
CDD- 570

Ficha Catalográfica elaborada por Marlom Cesar da Silva
Bibliotecário-Documentalista CRB6/2735

PEDRO ERNESTO DOS REIS

POTENCIAL FITORREMEDIADOR DE *TALINUM PANICULATUM* NA PRESENÇA DE CÁDMIO, CHUMBO, MANGANÊS E FERRO

A Banca examinadora 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.

Aprovada em: 18 de novembro de 2020

Prof. Dr. Plinio Rodrigue dos Santos
Filho Instituição: Universidade Federal
de Alfenas

Prof. Dr. Roniel Geraldo Ávila
Instituição: Instituto Federal Goiano

Prof. Dr. Thiago Corrêa de Souza
Instituição: Universidade Federal de
Alfenas

Documento assinado eletronicamente por **Plinio Rodrigues dos Santos Filho**, **Professor do Magistério Superior**, em 18/11/2020, às 15:48, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

Documento assinado eletronicamente por **Thiago Corrêa de Souza**, **Professor do Magistério Superior**, em 18/11/2020, às 17:36, conforme horário oficial de Brasília, comfundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

Documento assinado eletronicamente por **Roniel Geraldo Avila**, **Usuário Externo**, em 18/11/2020, às 17:37, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º,do Decreto nº 8.539, de 8 de outubro de 2015.

A autenticidade deste documento pode ser conferida no site https://sei.unifal-mg.edu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador**0414280** e o código CRC **26855D6A**.

AGRADECIMENTOS

Agradeço à minha família, pilar e base que com seu esforço permitiu esta conquista, algo que para muitos é impossível ou apenas difícil de se conseguir. Agradeço por sempre me incentivarem e apoiarem nos momentos mais difíceis. O estudo é algo que ninguém pode lhe tirar, temos que praticar e nos esforçar sempre: este é o gesto mais nobre e que agradeço por ter aprendido com meus pais Sebastiana Silva dos Reis e Aristoteles José dos Reis. À minha irmã Natalia Maria dos Reis por me motivar e persistir que sempre fosse dado um passo à frente, além de todo apoio de Thais Vaz Farias e de minha tia Maria Aparecida da Silva pelas suas contribuições. À minha companheira Gabrielle Navarro que amo de maneira imensurável e que em todo momento contribuiu com seu amor e carinho em minha jornada. À tia Teresinha de Jesus dos Reis Menezes pelas orações e perseverança, ao meu primo Tarcísio Antônio dos Reis Menezes pelo auxílio na moradia e pelos momentos inesquecíveis na República RSN. À Maria Aparecida dos Santos que jaz há tempos, mas de importância tamanha por contribuir com parte da minha educação e que jamais vou esquecer. À orientação de Dr. Plínio Rodrigues dos Santos Filho e Dr. Thiago Corrêa de Souza, pela aposta e oportunidade da realização deste trabalho. À coorientação da Dra. Kamila Rezende Dázio de Souza, Phd, que se tornou uma grande amiga e pessoa importantíssima para a conclusão desta dissertação, apoiando e fazendo com que este trabalho realmente chegasse ao fim. Aos amigos que jamais iria imaginar conhecer e que vou carregar boas lembranças em meu coração: Valdir Veronezze, Letícia Braga, Gabriel Ferreira Romão, Keila Yumi Ishii, Gisele de Fátima Esteves e Joice Aparecida de Novais Portugal. Ao laboratório Biogen e sua equipe que brilha cada vez mais rumo a pesquisas de ponta e qualidade, a todos obrigado pelo carinho e atenção. Nunca podemos esquecer do nosso Deus, não importa a religião, a crença ou a cultura, mas todos devemos sempre agradecer cada dia que passa, como uma luta, uma conquista, uma glória.

Agradeço à Universidade Federal de Alfenas e ao Programa de Pós-Graduação em Ciências Ambientais.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

RESUMO

Esta presente dissertação reúne dois artigos científicos, traduzidos para a língua inglesa, que discorrem sobre o potencial fitorremediador de *Talinum paniculatum* na presença dos metais pesados cádmio (Cd), chumbo (Pb), manganês (Mn) e ferro (Fe). O objetivo do primeiro artigo é avaliar as respostas bioquímicas e ecofisiológicas dessa espécie quando exposta a altos níveis dos metais pesados supracitados. O segundo artigo tem como intuito estudar e avaliar as características fisiológicas e o potencial fitorremediador de *Talinum paniculatum*, quando exposta especificamente aos metais pesados chumbo (Pb) e Manganês (Mn). Os resultados obtidos giram em torno do potencial positivo de *Talinum paniculatum* para despoluir áreas degradadas, uma vez que a biomassa ganha permite a sobrevivência da planta, mas novos estudos devem ser realizados para confirmar a sua tolerância ao excesso de metais pesados.

Palavras-chave: Metais pesados; Trocas gasosas; Peroxidação lipídica; Enzimas antioxidantes; Massa seca; Estresse oxidativo; Crescimento; Fitorremediação; Sistema antioxidante; Talinacea.

ABSTRACT

This present dissertation gather two scientific articles, translated into English, to discuss the phytoremediation potential of *Talinum paniculatum* for excess of lead (Pb), iron (Fe), cádmium (Cd) and manganese (Mn). The goal of the first study is to evaluate the biochemical and ecophysiological responses of *Talinum paniculatum* grown under the excess of heavy metals mentioned above. The second study aims to evaluate the physiological characteristics and the potential remediation of Talinum paniculatum cuttings subjected to excess lead (Pb) and manganese (Mn). The results obtained suggest that the potential for the depollution of degraded areas, since the biomass gain allowed the plants to survive. However, further studies need to be conducted to confirm their tolerance to excess metals and under other growing conditions.

Keywords: Heavy metals; Gas Exchange; Lipid peroxidation; Antioxidant enzymes; Dry matter; Oxidative stress; Growth; Phytoremediation; Antioxidant system; Gas exchange; Talinacea.

LISTA DE SIGLAS

A_n	Taxa Fotossintética Líquida
ANOVA	Análise de Variância
Ca	Cálcio
Cd	Cádmio
C_i	Concentração Interna de CO2
CTC	Capacidade de Troca Catiônica
E	Transpiração
ERNS	Espécies Reativas de Nitrogênio
EROS	Espécies Reativas de Oxigênio
Fe	Ferro
g_s	Condutância estomática
k	Eficiência da carboxilação
K	Potássio
MDA	Malondialdeído
MF	Biomassa Fresca
Mg	Magnésio
mL	Mililitro
Mn	Manganês
MSPA	Biomassa Seca de Parte Aérea
MSR	Biomassa Seca de Raízes
PA	Parte Aérea
Pb	Chumbo
TPU	Unidade de Luz Adicional
Zn	Zinco

SUMÁRIO

1 INTRODUÇÃO

O Aumento das atividades poluidoras no campo e nas cidades, decorrentes de atividades agrícolas e industriais, contribuem para a emissão e o descarte de poluentes contendo metais pesados no meio ambiente. A incorporação destes metais na biosfera permite a sua difusão na cadeia alimentar, causando sérios danos ao meio ambiente e à saúde humana (AGUIAR *et al*., 2012; WILSON *et al*., 2008).

Substâncias minerais e alguns elementos metálicos (macro e micronutrientes), quando absorvidos pelas plantas nas concentrações corretas, atuam no metabolismo vegetal e estão envolvidos no seu crescimento e desenvolvimento. No entanto, elementos metálicos como chumbo (Pb), cádmio (Cd), Manganês (Mn), Ferro (Fe), dentre outros, quando absorvidos pelas plantas, podem causar alterações no metabolismo vegetal. (VASCONCELLOS; PAGLIUSO; SOTOMAIOR, 2012).

O processo de fitorremediação consiste na extração, retenção ou absorção de metais pesados, utilizando plantas "*in situ*", sem alterar as propriedades do solo e a atividade microbiana (RAJKUMAR *et al*., 2009; VAMERALI; BANDIERA; MOSCA, 2010). As plantas utilizadas no processo de remediação apresentam estruturas fisiológicas favoráveis à acumulação de metais pelas raízes, tecidos e membranas, além da translocação para diferentes partes da planta, permitindo a acumulação ou a incorporação de metais em folhas, flores, sementes e frutos (TAVARES; OLIVEIRA; SALGADO, 2013).

Nesse sentido, têm-se as plantas acumuladora e hiperacumuladoras, as quais absorvem elevadas concentrações dos metais, retirando-os do ambiente sem afetar completamente o metabolismo vegetal. Plantas acumuladoras produzem maior biomassa, porém acumlam menos metal, ao contrário de plantas hiperacumuladoras, suas características são alta taxa de acumulação do metal e crescimento, menor biomassa, destacando-se também a tolerância ao metal (WATANABE, 1997). Estas plantas podem ser cultivadas em ambientes contendo concentrações severas de metais pesados, uma vez que realizam fitoextração ou rizofiltração (ANDRADE *et al*., 2009). Após a extração dos metais do ambiente, as plantas podem receber destinos mais corretos, sendo descartadas ou eliminadas, visando à redução da concentração de poluentes no ambiente (CUNNINGHAM; BERTI; HUANG, 1995).

A espécie *Talinum paniculatum* (Jacq.) Gaertn., possui características desejáveis para plantas acumuladoras tais como ciclo de vida curto, rápido crescimento e acúmulo de biomassa (SOUZA *et al*., 2018). Quanto aos metais pesados, plântulas de *T. paniculatum* mostraram bons resultados para absorção e acúmulo de Pb. Por outro lado, em plantas de *T. triangulare*, uma espécie filogeneticamente próxima de T. *paniculatum*, verificou-se o potencial fitorremediador para elementos-traço como o Pb, Cd, cobre (Cu) e níquel (Ni) (KUMAR; PRASAD, 2015).

Dessa maneira, devido às características favoráveis de *T. paniculatum* para utilização como fitorremediadora, surge o interesse de conhecer o potencial fitorremediador de plantas dessa espécie, que pode ser cultivada em diferentes ambientes. Assim, o objetivo desta pesquisa será avaliar o potencial fitorremediador de plantas de *T. paniculatum* em ambientes com elevadas concentrações de Pb, Cd, Mn e Fe.

1.1 OBJETIVOS

1.1.1 Objetivo geral

Avaliar o potencial fitorremediador de plantas de *T. paniculatum* na água e no solo com elevadas concentrações de Pb, Cd, Mn e Fe.

1.1.2 Objetivos específicos

a) avaliar o processo de fitoextração com excesso de metais no solo;

b) avaliar o processo de rizofiltração com estacas de *T. paniculatum* ao excesso de metais em solução hidropônica;

c) avaliar os parâmetros biométricos de *T. paniculatum* sob diferentes concentrações de Pb, Cd, Mn e Fe;

d) avaliar parâmetros ecofisiológicos, bioquímicos associados à resposta de *T. paniculatum* ao excesso de metais.

2 DESENVOLVIMENTO

2.1 JUSTIFICATIVA

O aumento severo das atividades antropogênicas tem gerado maior número de acidentes ambientais por metais pesados. O derrame acidental de resíduos de mineração ocorreu com maior frequência em menos de uma década, começando por Aznalcollar Espanha, 1998, Ajka Hungria, 2010 e em novembro de 2015, Mariana-MG Brasil, em ambos os casos, ocorreu o rompimento da barragem de rejeitos, liberando grandes volumes de resíduos contendo metais pesados (SEGURA *et al*., 2016).

A espécie *T. paniculatum* possui infinitos benefícios para a saúde, mas também visa bons resultados para a recuperação de áreas degradadas como potencial espécie para fitoextração e rizofiltração devido à sua resistência à toxicidade por metais. Sua eficiência na remoção de Pb foi observada por (SOUZA *et al*., 2018), que cita o acúmulo de elementos-traço nos tecidos das raízes podendo acumular altas concentrações, devido aos mecanismos do sistema antioxidante enzimático e da síntese de prolina, além da maior espessura da epiderme radicular e aumento da eficiência dos fotossistemas que garantem tolerância à exposição ao metal pesado.

A fitorremediação é uma técnica de baixo custo a qual permite aplicação in situ de *T. paniculatum* em áreas contaminadas, além de esta oferecer características favoráveis, devido seu rápido cultivo, crescimento, absorção e acúmulo por metais. Essas ações minimizam altas concentrações de metais no solo ou na água, permite o controle da qualidade, para o uso posterior, evitando alta exposição à saúde humana, animal e plantas, dificultando a reinserção dos metais a cadeia trófica e a biota.

2.2 REFERENCIAL TEÓRICO

2.2.1 Metais pesados

O conceito de metal pesado está relacionado aos elementos minerais presentes na natureza, em pequenas quantidades (<0,1% em peso) e de densidade anatômica que varia entre os elementos minerais (1 a 5.0 g cm^{-3}). A partir deste conceito, os metais pesados podem ser chamados de elementos-traço, são pequenas partículas em baixas concentrações que possuem à capacidade de serem dispersos e admitidos em outros minerais. Os elementos encontrados com maior abundância na natureza são: zinco (Zn), ferro (Fe), cobre (Cu) e manganês (Mn). Os elementos Mn e Fe não são considerados contaminantes em baixas concentrações, de modo que Fe e Mn são micronutrientes para as plantas. Porém esses elementos, quando em elevadas concentrações, tornam-se perigosos na forma catiônica ou ligados a cadeias carbônicas (SEGURA, 2016). Outros elementos-traços como chumbo (Pb), cádmio (Cd), arsênio (As), mercúrio (Hg), níquel (Ni), quando absorvidos pelas plantas, podem apresentar efeitos deletérios ao metabolismo vegetal até mesmo em baixas concentrações (FADIGAS *et al*., 2002).

Os elementos-traço apresentam potencial risco de toxicidade e ecotoxicidade, para os seres vivos e a biota em geral, quando dispersados por poluentes. Embora na maioria das vezes não possuam função no metabolismo vegetal, esses elementos são absorvidos pelas plantas junto a outras substâncias e elementos minerais (COSTA; BORÉM, 2003).

A poluição por metais pesados é reflexo das atividades antrópicas, incluindo a mineração, setor industrial e beneficiamento de subprodutos como tintas, baterias, componentes elétricos e pesticidas (SOUZA; MORASSUTI; DEUS, 2018). O aumento das atividades industriais tem elevado o nível de alerta para questões a respeito de poluentes e contaminantes de alta toxicidade, como é o caso do Cd e do Hg (IORI *et al*., 2017).

Existe uma elevada quantidade de fontes antrópicas que resultam no lançamento de metais, com risco de contaminação do ambiente e acumulação ao longo da cadeia alimentar. Diante dos sérios riscos ao ambiente e à saúde humana, existem regulamentações no Brasil e no mundo para controlarem as emissões desses elementos na natureza.

No Brasil, o Ministério do Meio Ambiente dispõe da Resolução CONAMA 396/2008, que define Valores Máximos Permitidos (VMP) para substâncias químicas inorgânicas que representam risco à saúde. A Resolução CONAMA 420/2009 rege os critérios para Valores de Prevenção (VP) e Valor de Investigação (VI) para substâncias no solo, e a Resolução CONAMA 430/2011 para valores de substâncias químicas inorgânicas presentes no lançamento de efluentes. Estes valores complementam ao apresentado pela portaria nº 2.914/2011 do Ministério da Saúde, que define valores para substâncias nocivas à saúde humana (BRASIL, 2011b).

2.2.1.1 Chumbo (Pb)

O Pb é um elemento de número anatômico 82, massa atômica 207,2 g e com baixo ponto de fusão. Está presente nas rochas magmáticas e sedimentos argilosos, sendo encontrado em maiores proporções na rocha galena na forma de sulfeto de chumbo II (PbS). A oxidação do PbS ocorre lentamente formando carbonatos que são incorporados a outros elementos como Fe e Mn. A espécie catiônica mais comum desse elemento traço é Pb^{2+}, mas este pode sofrer maiores oxidações, chegando a Pb^{4+} (KABATA-PENDIAS; PENDIAS, 2000).

Atualmente o Pb é um dos principais contaminantes ambientais, sendo a sua presença notável em produtos como herbicidas, eletroeletrônicos, tintas, fumaça de automóveis e em chaminés de fábricas. O uso de Pb em grande escala nas atividades antrópicas tem acarretado sérios riscos à contaminação dos solos e da biota (RIBEIRO *et al*., 2015).

Plantas em geral não são tolerantes ao Pb, devido à sua toxicidade. No entanto, esse elemento pode ser absorvido devido ao pequeno tamanho das partículas ou por mecanismos de regulação em decorrência do desequilíbrio nutricional. O Pb é absorvido pela troca de cátions, ligado a cadeias carbônicas ou hidroxilas e a outros minerais, podendo também ser incorporado por alteração do pH do ambiente (ROMEIRO *et al*., 2007).

O excesso de Pb reduz o crescimento das plantas, influencia negativamente a fotossíntese, a nutrição mineral e leva ao estresse hídrico. A absorção de Pb ocorre primeiramente pelas raízes, envolvendo a membrana plasmática e a parede celular, onde há deposição de calose. O elemento pode se mover através dos tecidos da raiz via apoplástica e radialmente através do córtex na endoderme, subindo para o caule e partes aéreas da planta. A inibição pelo chumbo reduz o crescimento vegetal em decorrência de clorose nas folhas, redução da fotossíntese e escurecimento do sistema radicular (ROMEIRO *et al*., 2007; SOUZA *et al*., 2018).

O Pb é um elemento perigoso, venenoso e bioacumulativo e, quando ingerido por animais e pessoas, pode agravar a saúde e resultar em problemas neurológicos, além de inibir a ação de enzimas e causar dores gastrointestinais (ALMEIDA, 2009). Segundo a Agência de Proteção Ambiental (EPA), nos Estados Unidos da América (EUA), as concentrações de Pb no solo variam de 50 a 400 ppm (EPA, 2017).

O Ministério da Saúde (MS), por meio da portaria nº 2.914/2011, adverte aos perigos ocasionados à saúde humana pela exposição a metais, limitando o valor máximo permitido (VMP) em água potável para o consumo humano, para substâncias inorgânicas que apresentam riscos à saúde. O Pb possui um VMP de 0,01 mg L^{-1}, mesmo valor indicado na Resolução CONAMA 396/2008 (BRASIL, 2008).

2.2.1.2 Cádmio (Cd)

O Cd apresenta número atômico 48, massa atômica 112,4 g, baixo ponto de fusão e baixa condutividade elétrica. É um elemento-traço encontrado com abundância nas rochas magmáticas e em pequenas quantidades nas sedimentares. Encontra-se concentrado em depósitos argilosos e de xisto, onde apresenta maior afinidade ao enxofre (S) e ao (Zn). A principal forma iônica natural é Cd^{2+}, sendo que compostos de Cd são facilmente oxidados e interagem com outros compostos catiônicos como Zn^{2+}, Co^{2+}, Ni^{2+}, Fe^{2+}, Mg^{2+} e Ca^{2+} (KABATA-PENDIAS; PENDIAS, 2000).

A contaminação por poluentes contendo Cd ocorre com maior intensidade através das atividades industriais e fabricação de produtos eletrônicos. Também é possível encontrar traços de Cd em fumaça de termelétricas pela queima de carvão, a partir da fundição de chumbo, zinco e cobre (RODRIGUES *et al*., 2016).

O elemento Cd não apresenta características positivas para o desenvolvimento de plantas devido à sua ecotoxicidade, mas é facilmente absorvido e acumulado nas partes áereas de leguminosas, herbáceas e suculentas. O metal se propaga através das vias de absorção de elementos essenciais, especialmente Zn e Fe (IORI *et al*., 2017). A exposição ao Cd constitui-se um estresse aos vegetais, que apresentam alterações fisiológicas na presença desse metal (DRESLER *et al*., 2014; IORI *et al*., 2017). A fotossíntese é afetada pela ação de Cd, que se acumula inicialmente nos cloroplastos, inibindo atividades das enzimas da biossíntese da clorofila e dos complexos proteína-pigmentos. Também ocorrem perdas no fotossistema II pela oxidação de Cd^{2+} aumentando o fluxo de elétrons ao redor do fotossistema I e O_2 (YING *et al*., 2010).

A capacidade de compostos como o Cd em se acumular nas folhas, aumenta os riscos à saúde animal e humana, sendo que a presença de Cd no organismo pode causar uma grande variedade de perturbações neurológicas agudas e efeitos tóxicos crônicos (IORI *et al*., 2017).

Por conta da problemática envolvida pela contaminação de Cd no meio ambiente, o Ministério da Saúde, através da portaria 2.914/2011, regulamenta VMPs para concentrações de Cd em substâncias inorgânicas em 0,005 mg L^{-1}, mesmo valor exigido pela Resolução CONAMA 398/2008 (BRASIL, 2008) para águas subterrâneas, limitado a valores mais baixos a partir da Res. CONAMA 430/2011 (BRASIL, 2011a) que define em 0,2 mg L^{-1} para lançamento de efluentes.

2.2.1.3 Mangânes (Mn)

O Mn apresenta número atômico 25 e massa atômica 54,9 g. É o segundo elemento em maior abundância nos solos, derivado de rochas primárias, classificadas como ferromagnesianas. O Mn também está presente em outros diversos minerais em sua forma oxidada como íon Mn^{2+} ou quelada, mas também é encontrado nas formas Mn^{3+}, Mn^{4+} e Mn^{7+}. É absorvido pelos vegetais na forma Mn^{2+}, que é naturalmente mais disponível em solos ácidos, com elevado teor de matéria orgânica ou solos alagados. Por ser um micronutriente, o Mn é requerido pelas plantas em baixas concentrações, de modo que o excesso de Mn pode causar toxidade aos vegetais, resultando em redução do crescimento (COSTA; MARENCO, 2007; KABATA-PENDIAS; PENDIAS, 2000).

O Mn é um elemento que está presente em todos os organismos da terra de forma natural, mas também é liberado por intermédio das atividades antrópicas, a partir da queima de combustíveis fósseis pelo MMT (methylcyclopentadienyl manganese tricarbonyl) aditivo para gasolina, fumaça de chaminés para queima de ferro e Aço e erosão dos solos contendo Mn entre outros (ATSDR, 2017).

O excesso de Mn pode interferir na absorção e causar deficiência de outros nutrientes, tais como Ca, Mg, Cu e Zn (COSTA; MARENCO, 2007). Em espécies cultiváveis sintomas como encarquilhamento da folha, clorose marginal seguida de necrose, sendo o sistema foliar mais afetado pelo excesso de Mn do que as raízes (SHOLER *et al*., 2014).

O Mn participa do metabolismo vegetal como cofator da enzima dismutase do superóxido, constituinte do centro de evolução do oxigênio e ativador de diversas metaloenzimas (SHOLER *et al*., 2014). No entanto, por se tratar de um micronutriente, é requerido em concentrações muito baixas e pode facilmente atingir níveis tóxicos ao metabolismo vegetal. Nesse caso, está relacionado à menor ativação da Ribulose 1,5 Bifosfato Carboxilase/Oxigenase (Rubisco), redução da atividade de enzimas do metabolismo respiratório, maior produção de espécies reativas de oxigênio, alteração na estrutura da membrana celular (SANTOS *et al*., 2017; ZAMBROSI *et al*., 2016) e redução do crescimento e produção vegetal.

O principal meio de exposição ao Mn para população ocorre ingerindo vegetais cultiváveis Organização Mundial da Saúde (OMS) (VENEZUELA, OLIVEIRA, PÉREZ, 2001), mas os efeitos nocivos do excesso de Mn à saúde humana são ocasionados principalmente a altos níveis de Mn na atmosfera em ambientes industriais, onde a exposição gera problemas no sistema nervoso, movimentos lentos nas mãos e irritação nos pulmões quando ingerido grandes quantidades em pó ou fumos, acarretando em pneumonia (ATSDR, 2017).

A Portaria do MS 2.914/2011 limita o VMP para Mn presente em substâncias inorgânicas em 0,1 mg L^{-1}, enquanto a Resolução CONAMA 430/2011 (BRASIL, 2011a) cita VMP de 1,0 mg L^{-1} para Mn dissolvido para o lançamento de efluentes.

2.2.1.4 Ferro (Fe)

O elemento Ferro apresenta número atômico 26, massa atômica 55,845 g, é um dos principais constituintes da litosfera cerca de 4%, além de ser encontrado facilmente nos horizontes A, B e D do solo na forma de óxidos e hidróxidos em pequenas partículas, presente em estruturas de outros minerais, como Mn, Zn, Pb e alumínio (Al) (KABATA-PENDIAS; PENDIAS 2000). Na natureza, o ferro é encontrado em compostos ou rochas como a hematita (Fe_2O_3), sendo seu principal mineral (MEDEIROS, 2010).

O Ferro é um micronutriente essencial para o desenvolvimento das plantas, possui importante função no desempenho de metabólitos como síntese de DNA, respiração e fotossíntese (ROUT; SAHOO, 2015). Elemento necessário para a síntese de clorofilas, sendo constituinte fundamental dos citocromos, da ferredoxina e de algumas enzimas, como a catalase e as peroxidases. No solo, Fe está presente na forma Fe^{2+} e Fe^{3+} onde exerce grande afinidade para formar complexos orgânicos e quelatos, que auxiliam a absorção de nutrientes essenciais pelas raízes e sua transferência para parte aérea (JUCOSKI *et al*., 2016).

Na região Sul do Brasil o nível de Fe presente nas camadas superiores do solo é maior comparado a outras regiões, devido a predominância do horizonte B. Este elemento prevalece em níveis maiores principalmente em áreas alagadas para o cultivo de arroz. Em excesso ocasiona toxicidade e sérios danos as células, fotossíntese, clorofila e a resistência estomática (PINTO *et al*., 2016).

Já em determinadas regiões onde a concentração de Fe é menor, este implica no cultivo de grande parte das culturas como, a soja, cana de açúcar entre outras (MARIA *et al*., 2014). A aplicação de fertilizantes e adubos a fim de aumentar a capacidade de absorção de nutrientes e outros minerais essenciais, pode aumentar a capacidade de Fe disponível no solo, aumentando a quantidade de Fe presente na parta aérea da planta e desequilíbrio das atividades metabólicas, dificultando o crescimento e desenvolvimento, devendo demandar a disponibilidade de ferro no solo necessária para o cultivo de plantas (SCHMIDT; THOMINE; BUCKHOUT, 2020).

O Brasil ocupa o 7º lugar das reservaibras de minério de ferro do mundo em questão de volume, os estados de Minas-Gerais (Quadrilátero Ferrífero) e Pará lideram as maiores reservas brasileiras e concentram grandes siderúrgicas para o beneficiamento desta matéria-prima, (OMACHI, 2015). Atualmente houve queda da extração e produção de ferro no Brasil, porém os riscos que as atividades mineradoras provocam ao meio ambiente ainda são inevitáveis, como exemplo, o rompimento de barragens da Companhia Vale e Samarco no estado de Minas-Gerias nas cidades de Mariana (2015) e brumadinho (2019), provocando um grande desastre ambiental (GUERRA *et al*., 2017).

Os efeitos da toxicidade por ferro no organismo humano podem ocorrer a partir de forma orgânica ou inorgânica, pela ingestão de vegetais, carnes e remédios (GROTTO, 2010). A inalação de poluentes atmosféricos, contendo fumos de (Zn), (Mn), (S), (Pb) entre outros elementos-traço, podem causar danos (ATSDR, 2017).

A Portaria do MS 2.914/2011 limita o VMP para Fe presente em substâncias inorgânicas em 2,4 mg L^{-1}, enquanto a Resolução CONAMA 430/2011 cita VMP de 15,0 mg/L para Fe dissolvido oriundos de sistemas de disposição final de resíduos sólidos e lançamento de efluentes.

2.2.2 Fitorremediação

A fitorremediação envolve o uso de microorganismos e plantas para remover ou detoxicar poluentes do meio ambiente, a partir de diferentes técnicas e processos subdivididos em: fitoextração, rizofiltração, fitoestabilização, fitovolatização e rizodegradação. Cada técnica depende de fatores como o local e tipo de poluente a se remediar, pois efeitos adversos podem limitar ou potencializar o tratamento (SERRAT *et al*., 2014; SILVA, 2017).

Um fato importante ao avaliar a eficiência de cada espécie em absorver os metais pesados em excesso no solo é avaliar a quantidade retida em relação à matéria seca da planta. Por esta razão, as plantas hiperacumuladoras possuem baixa produção de biomassa e alta acumulação de metais, ao contrário de plantas acumuladoras, que produzem mais biomassa, mas acumulam menos metal em relação às hiperacumuladoras (GREGER, 2017).

2.2.2.1 Fitoextração

A fitoextração é um processo de remediação de metais ou sais de solos contaminados, mediante absorção pelas raízes, transporte e concentração na biomassa da parte aérea. Após a absorção do poluente retido, ocorre o armazenamento no tecido vegetal, o que facilita o descarte do material (RODRIGUES *et al*., 2016). Essa técnica pode ser aplicada na água ou no solo, porém apresenta maior viabilidade quando utilizado em solos contaminados com elevadas concentrações permitindo cultivo e controle das plantas a curto prazo além de reutilizar os metais acumulados na planta (SERRAT *et al*., 2014).

2.2.2.2 Rifiltração

A rizofiltração se concentra no processo de remediação em meios hidropônicos, onde ocorre o acumulo de contaminantes e metais nos tecidos das raízes através da adsorção, precipitação e absorção, o uso de plantas com acumulo de biomassa radicular apresentam grande viabilidade nesta técnica para remoção de metais na água e em solução hidropônica facilitando o controle do descarte e disposição final das plantas (ANDRADE *et al*., 2009).

2.2.3 Talinum paniculatum (jacq.) gaertn.

A *T. paniculatum* é uma herbácea suculenta, pertencente à família Talinaceae, grupo das Angiospermas após a sua nova taxonomia. Até o ano de 2008 era conhecida como *T. patens,* ou *T. triangulare* (KUMAR; PRASSAD, 2015). Originária de regiões dos trópicos, é considerada nativa no Brasil e ocorre nos seis domínios fitogeográficos, sendo popularmente chamada de cariru-do-mato ou beldroega. *T. paniculatum* ainda oferece vários benefícios à saúde humana, seja para alimentação ou uso medicinal (COELHO; ZAPPI, 2015; SOUZA, 2005).

Estudos demonstram que plantas pertencentes à família Talinaceae apresentam bons indicadores de características acumuladoras por metais pesados como o Pb, Cd, Cu, Ni e Zn, em seus tecidos. A absorção ocorre inicialmente pela raiz, sendo dispersados através das vias metabólicas, atingindo os demais órgãos da planta (SEKHAR *et al* 2007; SOUZA *et al*., 2018).

T. paniculatum possui rápido crescimento e desenvolvimento, rápida germinação, ocorre de 6 a 7 dias após seu cultivo, passados 15 dias, nascem às primeiras folhas, e ao longo de 50 dias, as raízes incham gerando tubérculos posteriormente com 60 dias, surgem flores e frutos (NGUYEN *et al*., 2018).

2.2.4 Normas e resoluções

Existem inúmeros estudos e pesquisas que avaliam o potencial de agentes contaminantes presente no solo, água e na atmosfera, os efeitos nocivos para os seres vivos e sua disposição no meio ambiente são limitados via resoluções e de agências internacionais, como a Agência de Proteção do Meio Ambiente dos Estados Unidos (U.S. Environmental Protection Agency – EPA), dispõe de parâmetros e técnicas para remediação de áreas degradadas, que são definidos no Brasil a partir das Resoluções CONAMA 396 CONAMA 420, CONAMA 430 e também a portaria do Ministério da Saúde de 2011, estas normas e resoluções são essenciais para limitar a dispersão e disponibilidade de contaminantes de alta toxicidade.

REFERÊNCIAS

Agency for Toxic Substances & Disease Registry – ATSDR. **Centers for Disease Control and Prevention.** 2017. Disponível em: https://www.atsdr.cdc.gov/substances/toxsubstance.asp?toxid=22. Acesso em 27 abr. 2018.

AGUIAR. C. R C., *et al*. Fitorremediação de Solos Contaminados por Petróleo Phytorremediation of soils contaminated by oil. **Revista Trópica – Ciências Agrárias e Biológicas**. Chapadinha, MA, v. 6, p. 3-9, 2012.

ALMEIDA, G. W de. **Avaliação do potencial bioindicador e fitorremediador de Salvinia auriculata Aublet na presença de cádmio e chumbo**. 2009. 72p. Dissertação (Mestrado) – Universidade Federal de Lavras, Lavras, 2009.

ANDRADE, M. *et al*. Heavy metals in soils of a lead mining and metallurgy area. I – Phytoextraction, **Revista Brasileira de Ciência do Solo**, Rio Grande do Sul, v. 33, p. 1879-1888, 2009.

BRASIL. Ministério Meio Ambiente. Conselho Nacional do Meio Ambiente. **Resolução Conama de nº 396, de 3 de abril de 2008**, p. 64-88, 2008. Disponível em: http://www2.mma.gov.br/port/conama/legiabre.cfm?codlegi=562. Acesso em: 25 out. 2020.

BRASIL. Ministério Meio Ambiente. Conselho Nacional do Meio Ambiente. **Resolução Conama de nº 420, de 28 de dezembro de 2009**. p. 81-84, 2009. Disponível em: http://www2.mma.gov.br/port/conama/legiabre.cfm?codlegi=620. Acesso em: 25 out. 2020.

BRASIL. Ministério Meio Ambiente. Conselho Nacional do Meio Ambiente. **Resolução Conama de nº 430, de 13 de maio de 2011**. p. 89, 2011a. Disponível em: http:// http://www2.mma.gov.br/port/conama/legiabre.cfm?codlegi=646. Acesso em: 25 out. 2020.

BRASIL. Ministério da Saúde. **Portaria nº 2.914, de 12 de dezembro de 2011**. p. 89, 2011b. Disponível em: https://bvsms.saude.gov.br/bvs/saudelegis/gm/2011/prt2914_12_12_2011.html Acesso em: 25 out. 2020.

COELHO, A. A. O. P.; ZAPPI, D. Portulacaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro**.** BFG. Growing knowledge: an overview of Seed Plant diversity in Brazil. **Rodriguesia**, Rio de Janeiro, v. 66, p.1085-1113, 2015.

COSTA, G. F., MARENCO, R. A. Fotossíntese, condutância estomática e potencial hídrico foliar em árvores jovens de andiroba (Carapa guianensis). **Acta Amazonica**, Manaus, v. 37, p. 229-234, 2007.

COUTINHO, H. D. E., BARBOSA, A. R., Fitorremediação: considerações gerais e características de utilização, **Silva Lusitana**, Portuguese, v. 15, p.103 - 117, 2007.

COSTA, N. M. B., BORÉM, A., **Biotecnologia e nutrição: saiba como o DNA pode enriquecer a qualidade dos alimentos**. São Paulo: Nobel, 2003.

CUNNINGHAM, S. D., BERTI, W. R., HUANG, J. W. Phytoremediation of Contaaminated Soils. **Trends in Biotechnology**, United States, v.13, p. 393-397, 1995.

DRESLER, S. *et al*. Accumulation of low-molecular-weight organic acids in roots and leaf segments of Zea mays plants treated with cadmium and copper. **Acta Physiologiae Plantarum**, Poland, v. 36, p. 1565-1575, 2014.

EPA – Agência de Proteção Ambiental dos Estados Unidos. Agency for Toxic Substances e Disease Registry CDC, 2017. **Estudos de caso em medicina ambiental**. Disponível em: https://www.atsdr.cdc.gov/csem/csem.asp?csem=34&po=8. Acesso em: 8 abr. 2018.

FADIGAS, F. S. *et al*. Solos e irrigação: concentrações naturais de metais pesados em algumas classes de solos brasileiros. **Bragantia**, Campinas, v. 61, p.151-159, 2002.

GREGER, M. Phytoremediation - Does it work? In: International conference on the biogeochemestry of trace elements, **Understanding the Geological and Medical Interface of Arsenic - As 2012**, Uppsala-Sweden**:** SLU Service, p.10-11, 2017.

GROTTO, H. Z. W. Fisiologia e metabolismo do ferro. **Revista Brasileira de Hematologia e Hemoterapia**, Brazil, v. 32, p 8-17, 2010.

GUERRA, M. B. B. *et al*. Post-catastrophe Analysis of the Fundão Tailings Dam Failure in the Doce River System, Southeast Brazil: Potentially Toxic Elements in Affected Soils. **Water, Air, and Soil Pollution**, Netherlands, v. 228, p. 1-12, 2017.

IORI, V. *et al*. Analysis of biometric, physiological, and biochemical traits to evaluate the cadmium phytoremediation ability of eucalypt plants under hydroponics. **IForest**, Viterbo, v. 10, p. 416-421, 2017.

JUCOSKI, G. *et al*. Excesso de ferro sobre o crescimento e a composição mineral em Eugenia uniflora L. **Revista Ciência Agronômica**, Ceará, v. 47, p. 720-728, 2016.

KABATA-PENDIAS, A; PENDIAS, H. **Trace elements in soils and plants**. Library of Congress Cataloging, 3 ed., p. 331, 2000.

KUMAR, A.; PRASAD, M. N. V. Lead-induced toxicity and interference in *chlorophyll fluorescence* in *Talinum triangulare* grown hydroponically. **Photosynthetica**, Czech republic, v. 53, p. 66-71, 2015.

MARIA, R. *et al*. Ferro e Manganês no perfil do solo em Saccharum Officinalis fertilizada com resíduos orgânicos. **Bioscience Journal**, Uberlândia, v. 30, p. 131-139, 2014.

MEDEIROS, M. A. Ferro. **Dictionary of Gems and Gemology**, vol. 32, n° 3, 2010.

NGUYEN T. N. L. *et al*. Use of ITS DNA barcode for identification of Jewels of Opar (*Talinum paniculatum*) collected in Thanh Hoa, Vietnam. **Vietnam Journal of Science, Technology and Engineering**, Vietnam v. 60 p. 46-49, 2018.

OMACHI, G. Y. **Estudos para o aumento da vida útil das minas de minério de ferro do Quadrilátero Ferrífero, MG**. 2015. 83f. Dissertação (Mestrado em Engenharia Mineral) - Escola de Minas, Universidade Federal de Ouro Preto, Ouro Preto, 2015.

PINTO, S. S. *et al*. Oxidative damage and photosynthetic impairment in tropical rice cultivars upon exposure to excess iron. **Scientia Agricola**, Piracicaba, v. 73, p. 217-226, 2016.

RIBEIRO, E. S. *et al*. Relações da anatomia radicular na absorção, no acúmulo e na tolerância ao chumbo em *Echinodorus grandiflorus*. **Rev. Brasileira de Engenharia Agricola e Ambiental**, Campina Grande, v. 19, p. 605-612, 2015.

RODRIGUES, A. C. D. *et al*., Mecanismos de Respostas das Plantas à Poluição por Metais Pesados: Possibilidade de Uso de Macrófitas para Remediação de Ambientes Aquáticos Contaminados. **Rev. Virtual de Química**, Niterói, RJ, v. 8, p. 262-276, 2016.

ROMEIRO, S. *et al*. Absorção de Chumbo e Potencial de Fitorremediação. **Bragantia**, Campinas, v. 66, p. 327-334, 2007.

ROUT, G. R; SAHOO, R. Role of Iron in Plant Growth and Metabolism. **Reviews in Agricultural Science**, Japan, v. 3, p. 1-24, 2015.

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

SCHMIDT, W.; THOMINE, S.; BUCKHOUT, T. J. Editorial: Iron Nutrition and Interactions in Plants. **Frontiers in Plant Science**, Suíca, v. 10, p. 1-4, 2020.

SEGURA, R. F. *et al*. Potential risks of the residue from Samarco's mine dam burst (Bento Rodrigues, Brazil). **Environmental Pollution**, United Kingdom v.218, p. 813-825, 2016.

SEKHAR, K. C. *et al*. Arsenic accumulation by Talinum cuneifolium – application for phytoremediation of arsenic-contaminated soils of Patancheru, Hyderabad, India. Arsenic in Soil and Groundwater Environment - **Biogeochemical Interactions, Health Effects and Remediation**, Netherlands, v.9, p.315-337, 2007.

SERRAT, B, M. *et al*. Metodologia para Seleção de Técnica de Fitorremediação em Áreas Contaminadas. **Revista Brasileira de Ciências Ambientais**, Brazil, n. 31, 2014.

SHOLER, A. *et al*. Biogenic manganese-calcium oxides on the cell walls of the algae chara corallina: elemental composition, atomic structure, and water-oxidation catalysis. **European Journal of Inorganic Chemistry**, Germany, n. 4, p.780–790, 2014.

SILVA, A. C. Chemical, mineralogical and physical characteristics of a material accumulated on the river margin from mud flowing from the collapse of the iron ore tailings dam in Bento Rodrigues, Minas Gerais, Brazil. **Revista Espinhaço**, Brazil, v. 5, p. 44-53, 2017.

SOUZA, A. K. R.; MORASSUTI, C. Y.; DEUS, W. B. Poluição do Ambiente por Metais Pesados e Utilização de Vegetais como Bioindicadores. **Rev. Acta Biomédica Brasiliensia**, Brazil, v. 9, p. 1-12, 2018.

SOUZA, G. *et al*. Morpho-physiological Tolerance Mechanisms of Talinum patens to Lead. **Water, Air, and Soil Pollution**, Netherlands, v. 229, p.1-9, 2018.

SOUZA V. C., 2005. **Botânica sistemática: guia ilustrado para identificação das famílias de Angiospermas da flora Brasileira, baseado em APG II**. Nova Odessa, SP. Istitutto Pantarum.

TAVARES, S. R. L.; OLIVEIRA, S. A.; SALGADO, C. M. Avaliação de Espécies Vegetais na Fitorremediação de Solos Contaminados por Metais Pesados. **HOLOS**, Portuguese, v. 5, p. 80-97, 2013.

VAMERALI, T., BANDIERA, M., MOSCA, G. Field crops for phytoremediation of metalcontaminated land. A review. **Environmental Chemistry Letters**, Germany, v. 8, p.1–17, 2010.

VENEZUELA, T. C., OLIVEIRA, A. E. X., PÉREZ, D. V. **Determinação de contaminantes metálicos (metal tóxico) num solo adubado com composto de lixo em área olerílica no município de Nova Friburgo/RJ**. 2001. 96f. Programa de Pós-graduação em Saúde Pública Centro de Saúde do Trabalhador e Ecologia Humana Escola Nacional de Saúde Pública. Rio de Janeiro, 2001.

WATANABE, M.E. Phytoremediation on the brink of commercialization. **Environmental Science & Technology**, Washington D. C., v. 31, p.182-186, 1997.

WILSON, M. A., *et al*. Geochemistry in the modern soil survey program**.** **Environmental Monitoring and Assessment**, Netherlands, v. 139, P. 151-171, 2008.

YING, R-R. *et al*. Cadmium tolerance of carbon assimilation enzymes and chloroplast in zn/cd hyperaccumulator *picris divaricata.* **Journal of Plant Physiology**, Netherlands, v.167, p. 81-87, 2010.

ZAMBROSI, F. C. B. *et al*. Anatomical and physiological bases of sugarcane tolerance to manganese toxicity. **Environmental and Experimental Botany**, United Kingdom, v. 132, p. 100-112, 2016.

APÊNDICE A – PHYTOREMEDIATION POTENTIAL OF *TALINUM PANICULATUM* FOR EXCESS LEAD, IRON, CADMIUM AND MANGANESE

Pedro Ernesto dos Reis, Kamila Rezende Dázio de Souza, Gisele de Fátima Esteves, Gabriel Ferreira Romão, Keila Yumi Ishii, Juliana Leo Poloni, Thiago Corrêa de Souza, Plinio Rodrigues dos Santos Filho

ABSTRACT

Talinum paniculatum is a herbaceous plant species common in the Northern and Southern hemispheres, with potential use in phytoremediation, since it is easily grown in poor or nutritionally deficient soils and has a short life cycle. Therefore, the objective of this study was to evaluate the biochemical and ecophysiological responses of *T. paniculatum*, grown under the excess of heavy metals Cd, Pb, Fe and Mn. Thus, plants at five months of age were used, cultivated in pots containing 500 mL of substrate, which were submitted to five concentrations of each metal: Pb (0, 0.5, 5, 25 and 50 mg L^{-1}); Cd (0, 0.2, 2, 4 and 8 mg L^{-1}); Mn (0, 1, 5, 10 and 150 mg L^{-1}); Fe (0, 15, 150, 300 and 600 mg L^{-1}). The treatments were maintained for 35 days, when the following physiological parameters were evaluated: gas exchange; pigment content; root morphology; lipid peroxidation in roots and shoots; dry biomass in roots and shoots. The parameters most affected by metals were related to root morphology, in addition to small reductions in gas exchange and increased lipid peroxidation in the leaves. Reductions in biomass were more significant in the roots than in the shoot of plants under excess metals. The use of *T. paniculatum* for phytoremediation of heavy metals has the potential for the depollution of degraded areas, since the biomass gain allowed the plants to survive. However, further studies need to be conducted to confirm their tolerance to excess metals and under other growing conditions.

Keywords: Heavy metals. Gas exchange. Lipid peroxidation. Antioxidant enzymes. Dry matter. Oxidative stress. Growth.

1 INTRODUCTION

Activities that involve the extraction and use of heavy metals for the manufacture and composition of various products contribute to environmental contamination, increasing the number of pollutants dispersed, mainly in the soil and in the water (RAJKUMAR *et al*., 2009; VASCONCELLOS; PAGLIUSO; SOTOMAIOR, 2012). Elements such as cadmium (Cd), lead (Pb), iron (Fe) and manganese (Mn) have a high degree of toxicity for living beings. These elements are discarded in the environment in different ways, as a result of both agricultural and industrial activities, domestic effluents, as well as natural and atmospheric sources. The elements Cd and Pb are present in batteries, cosmetics and electronics; Fe and Mn are associated with the use of fertilizers and mining waste (SOUZA; MORASSUTI; DEUS, 2018).

Since they are part of everyday life, there is the possibility of absorption and accumulation of these metals in plant and animal organisms, causing metabolic disturbances (ANTONIADIS *et al*., 2017; KABATA-PENDIAS; PENDIAS, 2000). The excess of metals in plants is related to the occurrence of oxidative stress, which causes cellular damage due to the greater generation of reactive oxygen (ROS) and nitrogen (RNS) species (RIBEIRO *et al*., 2015). The excess of these reactive species leads to the breakdown of cellular homeostasis, altering metabolic reactions, enzyme activities and causing cellular damage, which can culminate in plant growth reduction and, in more extreme cases, in senescence and plant death (LEÓN *et al*., 2012).

In addition to oxidative stress, other common effects of metals on plants are related to a reduction in gas exchange and chlorophyll content, an increase in respiratory rates, among others (CURVÊLO *et al*., 2013). As a result, visual symptoms such as leaf chlorosis, bending, leaf loss, root tip necrosis and plant senescence can occur (KUMAR; PRASAD; SYTAR, 2012; LI *et al*., 2013). In this context, tolerance to excess metals is related to the requirement of the antioxidant system for neutralization of reactive species, reduction in transpiratory flow, release of organic acids in order to reduce the availability of metals for absorption and complexation with organic compounds, making metals unavailable for metabolic pathways (CLEMENS, 2001).

Hyperaccumulator plants are tolerant to excess metals due to metabolic adaptations through the production of substances of the primary and secondary metabolism, existence of an efficient antioxidant system, in addition to morphological and anatomical changes that allow survival (SILVA *et al*., 2019).

Thus, hyperaccumulator plants can be used in techniques for removing metals from the environment, since they accumulate in shoots or in roots (SARWAR; IMRAN; SHAHEEN, 2017).

Phytoremediation is a technique that has great potential for removing heavy metals from soil and water, using accumulator and hyperaccumulator plants (MARQUES; CHAFIM AGUIAR; SILVA, 2011; TAVARES; OLIVEIRA; SALGADO, 2013). Once it is a low-cost technique, it can be useful for large companies in the mineral sector as a way to minimize the environmental impacts resulting from their activities (WAN; LEI; CHEN, 2016).

The species *Talinum paniculatum* (Jacq.) Gaertn. belongs to the Talinaceae group, and can be found in the Southern and Northern hemispheres, strictly in warmer regions (COELHO; ZAPPI, 2015; NGUYEN *et al*., 2018). It is easily cultivable in poor or nutritionally deficient soils and has a short life cycle, about 70 days (MACHADO; MELO; SALES, 2012; SCAFIDI; RAIMONDO, 2017). Plants of the genus Talinum (*T. triangulare*) showed tolerance to excess metals in nutrient solution (KUMAR; PRASAD, 2015), as well as seedlings (*T. paniculatum*) were tolerant to excess Pb (SOUZA *et al*., 2018), but there are still few studies involving species of this genus, which shows the novelty of this study.

Given the above, it was hypothesized that *T. paniculatum* is a species with the potential to recover degraded areas, with phytoremediation potential due to its tolerance to excess metals and characteristics of its life cycle. Therefore, the objective of this study was to evaluate the biochemical and ecophysiological responses of *T. paniculatum*, grown under the excess of heavy metals Cd, Pb, Fe and Mn, correlating to its phytoremediation potential.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL, CONDUCTION OF THE EXPERIMENT AND COLLECTIONS

The experiment was conducted in the greenhouse of Universidade Federal de Alfenas main campus. Plants containing 6 fully expanded leaves were obtained, by growing the seed bank of the research group. *T. paniculatum* plants were grown in standard soil taken from an open field area, sieved and fertilized for seedling production (soil, cattle manure and sand at a 3:1:1 ratio).

Initial plant growth, until the obtention of six fully expanded leaves, occurred in a growth room, at a temperature of ± 25 °C and white light, 12/12h photoperiod, receiving daily irrigation and weekly supplementation with 10 mL Hoagland and Arnon nutrient solution (1975). The plants were transferred to the greenhouse and underwent an acclimatization period of 15 days until the treatments were applied. The plants were again irrigated daily and had weekly application of nutrient solution. In the greenhouse, the average growing temperature was 25 ºC and relative humidity, 72%.

After the acclimatization period, treatments consisting of five doses of Pb, Cd, Mn or Fe were applied. The sources of the metals used were high-purity reagents: lead nitrate – $Pb (NO_3)_2$; cadmium nitrate – $Cd (NO_3)_2$; manganese sulfate – $MnSO_4{\cdot}H_2O$; and iron sulfate II – $FeSO_4{\cdot}7H_2O$. The concentrations used were based on the limit concentrations of CONAMA Resolution No. 420 (CONAMA, 2009), for the final disposal of residues and inorganic substances in water and soil. The concentrations used for each of the metals were: Pb (0, 0.5, 5, 25 and 50 mg L^{-1}); Cd (0, 0.2, 2, 4 and 8 mg L^{-1}); Mn (0, 1, 5, 10 and 150 mg L^{-1}); Fe (0, 15, 150, 300 and 600 mg L^{-1}). The metals were applied weekly, in the morning, together with the complete nutrient solution, which was administered directly to the substrate.

The experiment was conducted in a completely randomized design, in a 4x5 factorial scheme, consisting of four metals (Pb, Cd, Mn and Fe) and five concentrations, with four replications. Thus, the experiment contained 80 experimental plots, consisting of two plants each. The experiment was carried out for a period of 35 days after the application of the treatments and, at the end of the experimental period, gas exchange and relative chlorophyll content were analyzed. Subsequently, the plants were collected for biometric, morphological and biochemical analysis, besides metal quantification.

2.2 BIOMETRIC ANALYSIS AND ROOT MORPHOLOGY

Biometric analyses were carried out at the end of the experiment, at which the number of leaves and plant height (cm) were obtained with the aid of a caliper and ruler. The shoots were collected and stored in paper bags, for drying in an oven with forced air circulation at 65ºC, until constant weight, for the determination of shoot dry biomass (SDB). Roots were washed in trays and stored in plastic containers with 70% ethanol for root morphology using the WinRhizo Pro 2007a equipment (Régent Instr.

Inc.), coupled to a professional Epson XL 10000 scanner which, in turn, was equipped with an additional light unit (TPU). The evaluated parameters were length, root surface area and total root volume, in addition to the average diameter (SOUZA *et al*., 2012). After root analysis, they went through an oven drying process, with forced air circulation at 65ºC, until constant weight, for the determination of root dry biomass (RDB).

2.3 GAS EXCHANGE

Gas exchange was analyzed in the morning, between 8 and 11 am, with the aid of an IRGA LI 6400XT (Infra Red Gas Analyzer, LI-COR Inc., Lincoln, Nebraska, USA). All readings were performed on fully expanded leaves. A fixed flow chamber with a leaf temperature of 28 °C, a flow of 500 µmol/s of air and photosynthetically active radiation of 1,200 µmol/m^{-2} s^{-1} were used, based on the light curve previously constructed. Net photosynthetic rate (A_n), transpiration (E), stomatal conductance (g_s), internal CO_2 concentration (C_i) and carboxylation efficiency (K) were evaluated.

2.4 QUANTIFICATION OF TOTAL CHLOROPHYLL

The determination of the relative chlorophyll content was based on the readings of the SPAD 502-Plus chlorophyll meter (Konica-Minolta – Osaka, Japan). In total, eight readings were performed on the two completely expanded leaves, by replication. The chlorophyll content was then determined by the Arnon method (1949) and a calibration curve of the species was constructed, correlating the SPAD units and the readings by the Arnon biochemical method (LICHTENTHALER, LANGSDORF, LENK, 2005). The total chlorophyll content was expressed in mg g^{-1} of fresh biomass (FB).

2.5 LIPID PEROXIDATION

Lipid peroxidation was quantified in leaves and roots, according to Buege and Aust (1978). Thus, 0.2 g of leaves and 0.4 g of roots were macerated in trichloroacetic acid (0.1%). Subsequently, centrifugation was carried out at 12,000 g for 15 minutes at 4 °C, and the supernatant was collected. Aliquots of the supernatant were added: trichloroacetic acid (10%) and thiobarbituric acid (0.5%), incubated for 30 minutes in a water bath at 30 ºC. The formation of malondialdehyde (MDA) was then determined based on the absorbances of the samples at 535 and 600 nm. The MDA content was expressed as ηmol MDA mg^{-1} FB.

2.6 DETERMINATION OF HEAVY METALS

For the determination of heavy metals, dry biomass samples of four replications were used for each treatment of shoot and root, crushed in a centrifuge at 14,000 rpm. Subsequently, 0.5 g of the plant tissue was submitted to nitro-perchloric digestion. Thus, the samples were added to digestion tubes, containing nitric acid (HNO_3) and perchloric acid ($HCLO_4$) at a 3:2 ratio. Packaged in a digestion block, the tubes underwent a gradual increase in temperature until reaching 220°C. After this process, the samples were cooled, completing the volume to 13 mL. The digested samples were centrifuged at 2,000 rpm and then a 10-mL aliquot was taken to quantify metals present in the tissues, verified by readings on a flame spectrometer (Shimadzu AA-7000 Atomic Absorption, Spectrophotometer).

2.7 DATA ANALYSIS

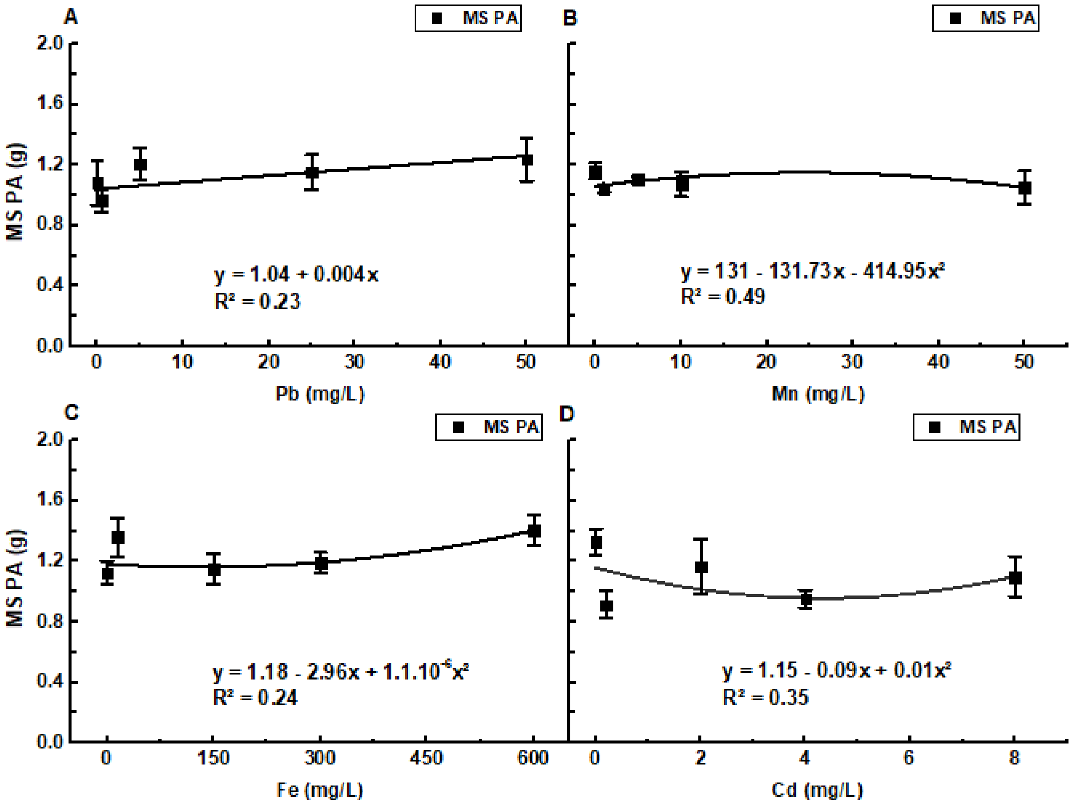
The data obtained were subjected to analysis of variance, ANOVA, linear regression, multiple linear regression, polynomial regression (2nd and 3rd degree), using the software Origin 15.0, BioEstat 5.3 and Sisvar – the choice of models was based on the significance of the coefficient regression ($P<0.05$) at 5% probability and Pearson determination coefficient for sample normality. The criterion for presenting the results was only for those that are significant.

3 RESULTS AND DISCUSSION

3.1 BIOMETRIC ANALYSIS

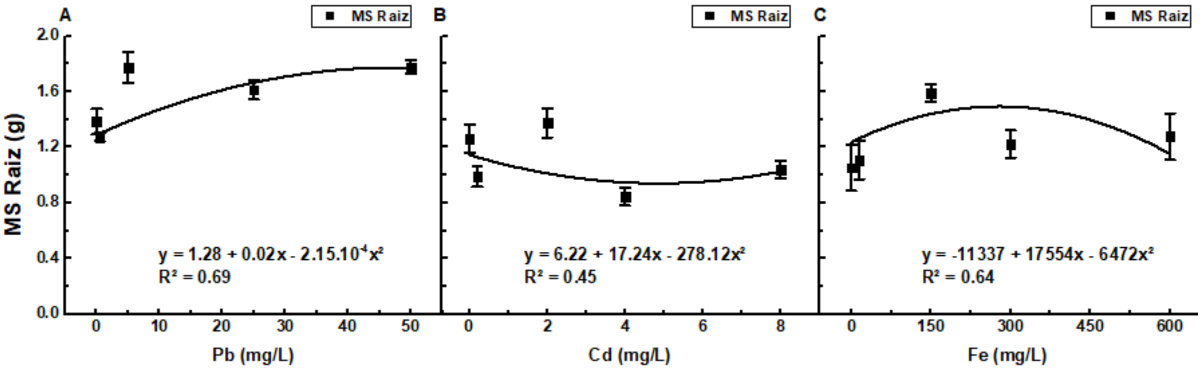
The biometric parameters of *T. paniculatum* plants, grown under the excess of different metals, suffered little variation after 35 days of treatment. Only the parameters that showed significant variations will be presented below. SDB did not demonstrate significant changes in relation to the control for all metals (Figure 1 A-D). Regarding root biomass, significant changes were observed only for Pb, Cd and Fe (Figure 2 A-C).

Figure 1 – Shoot dry biomass (SDB, A-D) of *T. paniculatum* plants, grown under excess Pb, Mn, Cd and Fe, respectively, for a period of 35 days. The bars represent the standard error of the mean (n = 4).



Source: created by the author (2020).

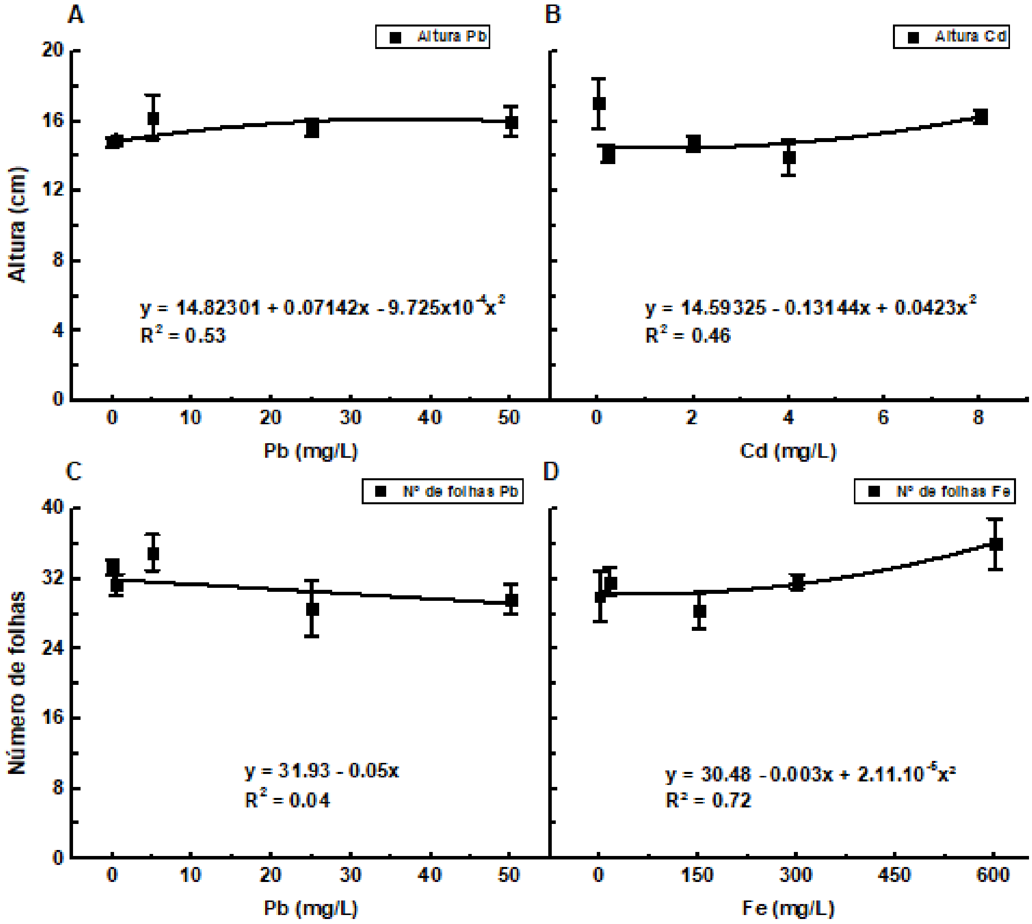
Figure 2 – Root dry biomass (RDB, A-D) of *T. paniculatum* plants, grown under excess Pb, Cd and Fe, respectively, for a period of 35 days. The bars represent the standard error of the mean (n = 4).



Source: created by the author (2020).

For height, there was a subtle increase at the highest concentrations, with a quadratic behavior in plants under Pb, Cd and Fe while, at intermediate Pb concentrations, there was an increase in height; for Cd, height was lower at intermediate concentrations (Figure 3 A, B). On the other hand, the number of leaves tended to decrease in response to the increase in Pb concentrations (Figure 3 C). Regarding Fe, there was an increase in leaf number. However, height did not differ for control plants. Mn did not lead to changes in plant height or number of leaves.

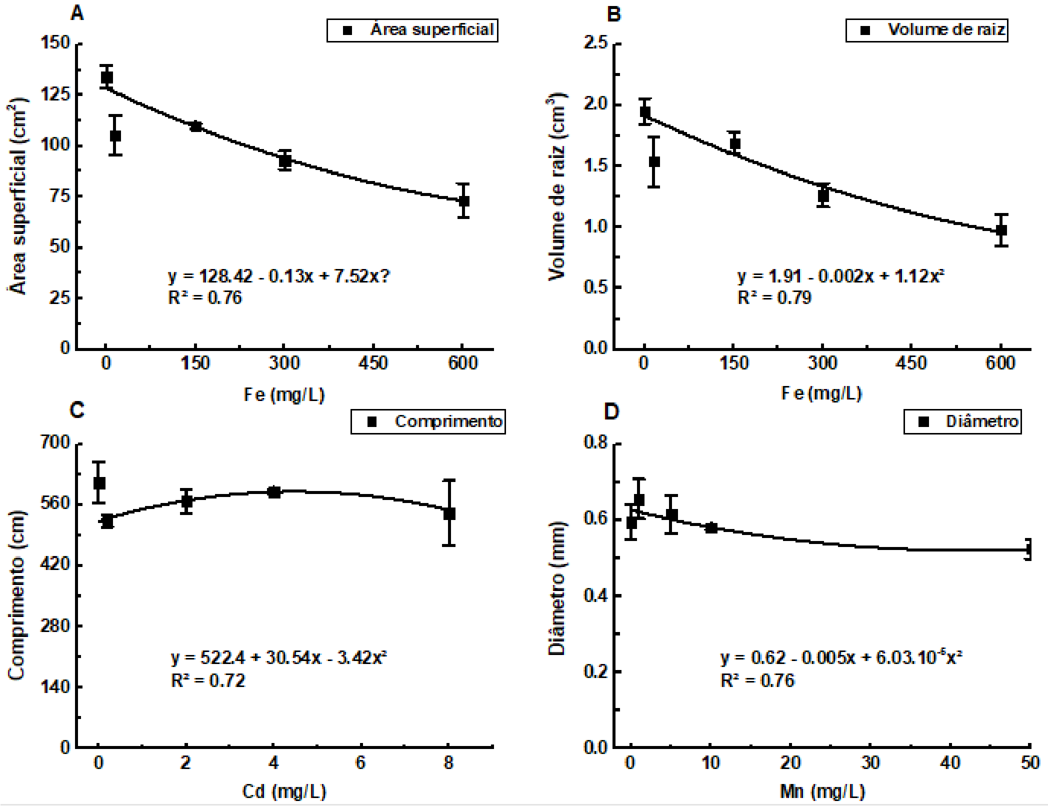
Figure 3 – Height (A, B) and leaf number (C, D) of *T. paniculatum* plants, grown under excess Pb, Cd and Fe, respectively, for a period of 35 days. The bars represent the standard error of the mean (n = 4).



Source: created by the author (2020).

Considering the root parameters, plants subjected to excess Fe had a reduction in surface area and root volume (Figure 4 A, B), while plants under excess Cd suffered a decrease in most of the analyzed parameters; only root length (Figure 4 C) remained in balance as the concentrations increased. Plants treated with Mn had only a reduction in root diameter, compared to the control (Figure 4 D).

Figure 4 – Surface area (A), root volume (B), root length (C) and mean diameter (D) of *T. paniculatum* plants, grown under excess Fe, Cd and Mn, respectively, for a period of 35 days. The bars represent the standard error of the mean (n = 4).



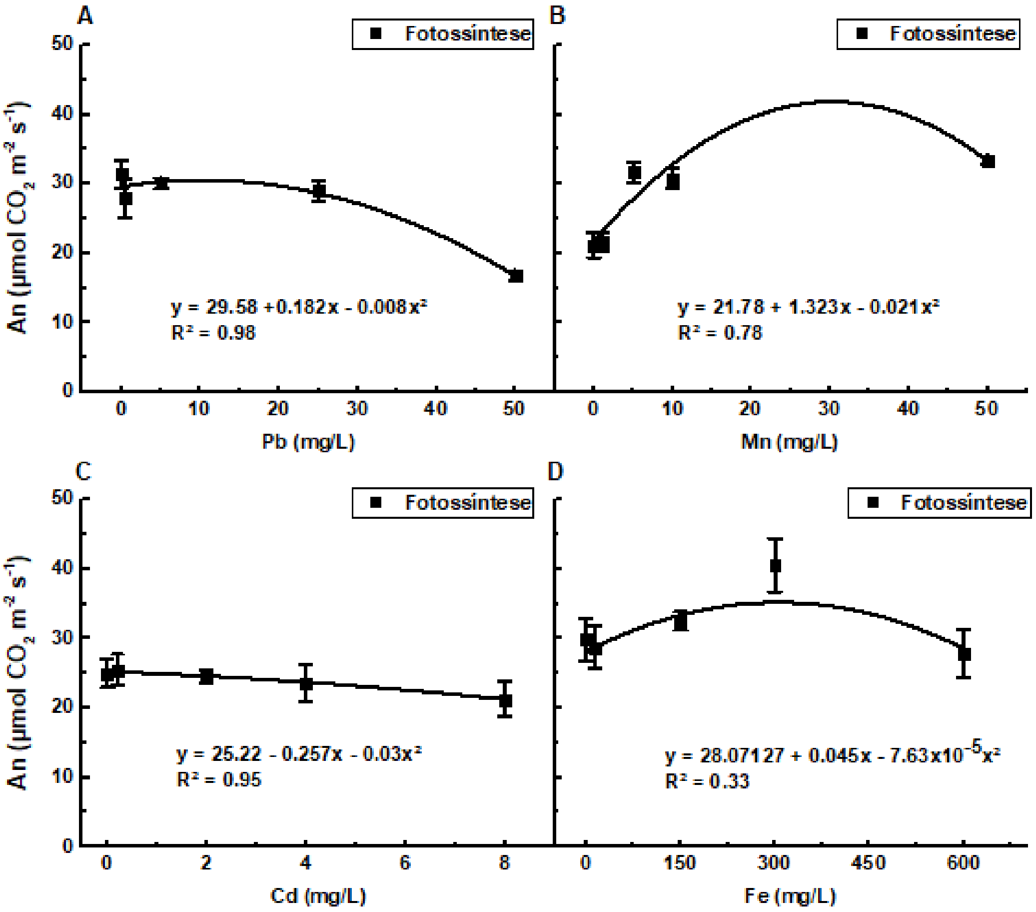
Source: created by the author (2020).

3.2 GAS EXCHANGE AND CHLOROPHYLL CONTENT

The gas exchange parameters of *T. paniculatum* plants varied according to the metal and its concentrations. Regarding the net photosynthetic rate, while plants grown under Pb and Cd showed a decrease in An (Figure 5 A, C), plants under Mn showed an increase in An (Figure 5 B) with the increase in the concentrations of this metal. In plants under excess Fe, a higher An was observed at intermediate concentrations, yielding values close to those of the control at the highest Fe concentration (Figure 5 D).

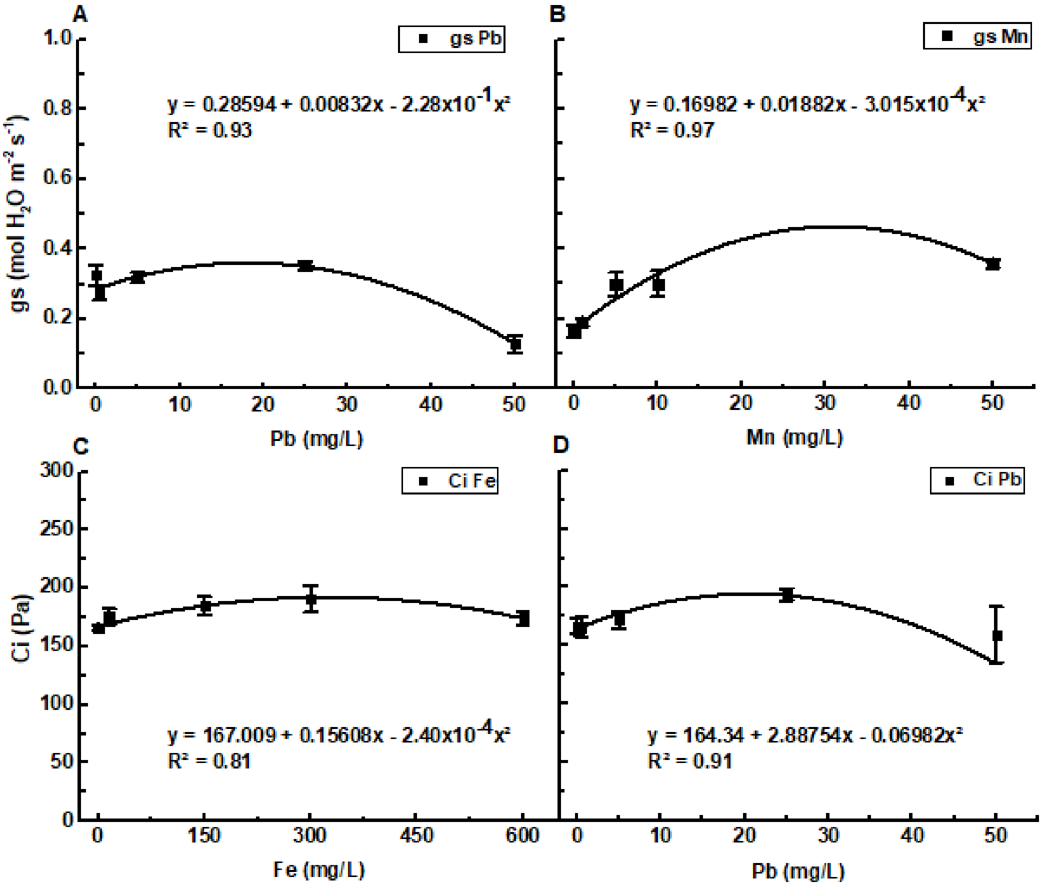
The stomatal conductance rate (gs) of plants under excess Pb showed a downward trend with the increase in the concentrations of this metal while, in plants under Mn, there was an increase in (gs) with the increase in their concentrations (Figure 6 A, B). There was an increasing tendency in relation to Ci at intermediate Fe concentrations, yielding values close to the control (Figure 6 C) and, in plants under excess Pb, there was a decrease in Ci at the highest metal concentration (Figure 6 D).

Figure 5 – Net photosynthetic rate (An, A-D) of *T. paniculatum* plants, grown under excess Pb, Mn, Cd and Fe, respectively, for a period of 35 days. The bars represent the standard error of the mean (n = 4).



Source: created by the author (2020).

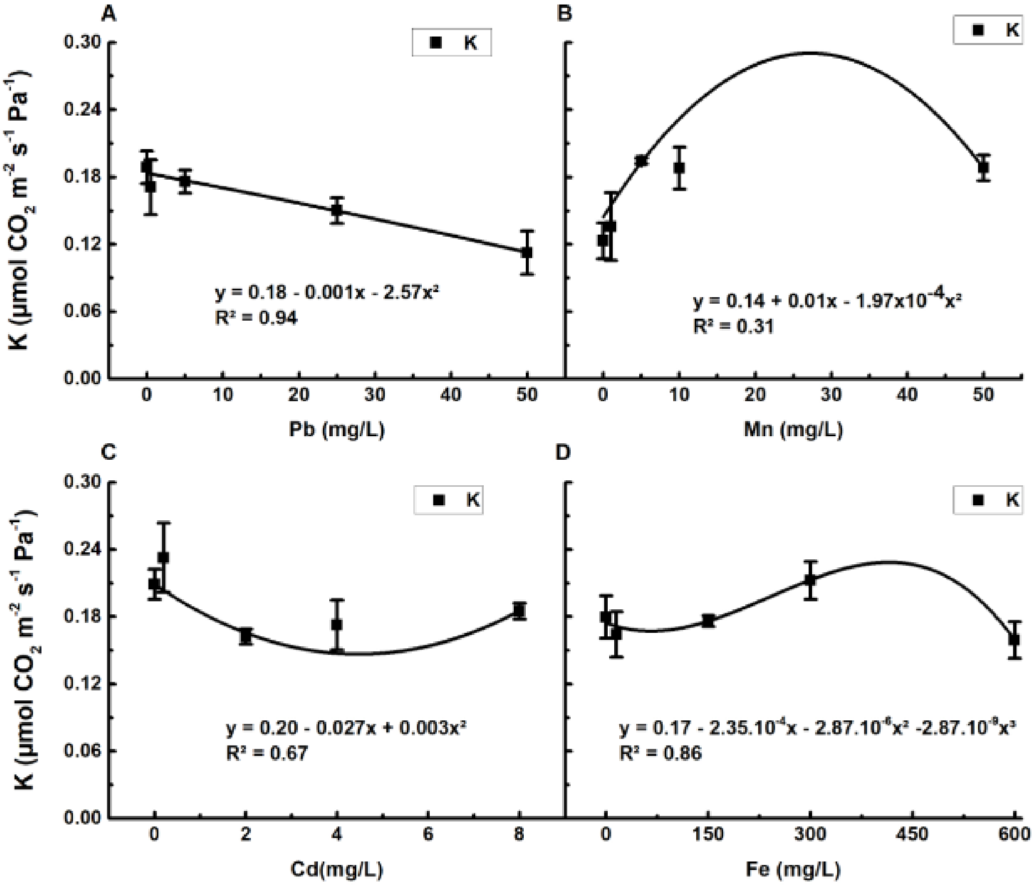
Figure 6 – Stomatal conductance (gs, A-B) of *T. paniculatum* plants, grown under excess Pb and Mn, and intercellular carbon concentration (Ci, C-D) of *T. paniculatum* plants, under excess Fe and Pb, for a period of 35 days. The bars represent the standard error of the mean (n = 4).



Source: created by the author (2020).

Regarding carboxylation efficiency (Figure 7), plants under excess Pb and Cd showed a reduction in k (Figure 7 A, C). In plants under excess Mn, there was an increasing tendency for k with increasing concentrations of this metal (Figure 6 B). Finally, it was observed that, in plants grown under excess Fe, there was a slight increase in k at intermediate metal concentrations (Figure 7 D).

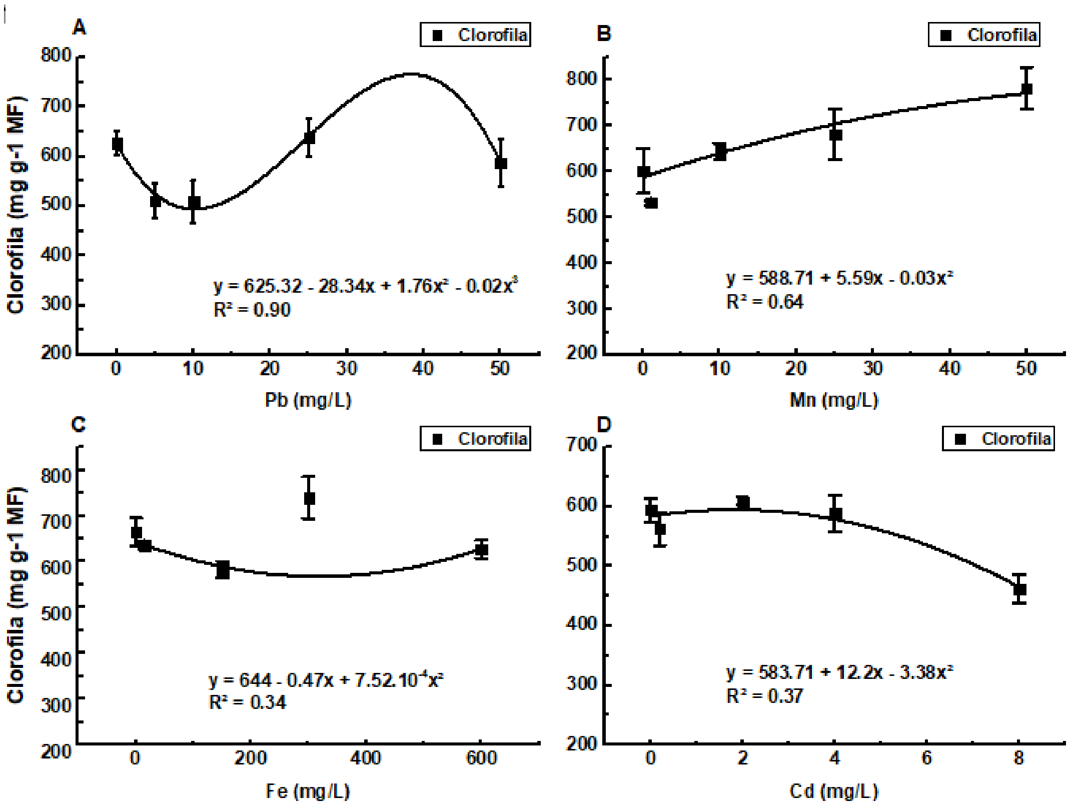
Figure 7 – Carboxylation efficiency (k, A-D) of *T. paniculatum* plants, grown under excess Pb, Mn, Cd and Fe, respectively, for a period of 35 days. The bars represent the standard error of the mean (n = 4).



Source: created by the author (2020).

The chlorophyll content increased for Fe concentrations (Figure 8 C) and effectively increased with higher concentrations of Mn (Figure 8 B). In plants under excess Pb and Cd, there was an increase in chlorophyll content at intermediate concentrations, followed by a decrease at the highest concentration of these metals (Figure 8 C, D). For Pb and Cd, there was a reduction in the chlorophyll content when compared to the control and, at the end of the experiment, yellow leaves and symptoms of leaf chlorosis were recorded, as well as loss of young leaves.

Figure 8 – Total chlorophyll concentration (A-D) of *T. paniculatum* plants, grown under excess Pb, Mn, Cd and Fe, respectively, for a period of 35 days. The bars represent the standard error of the mean (n = 4).

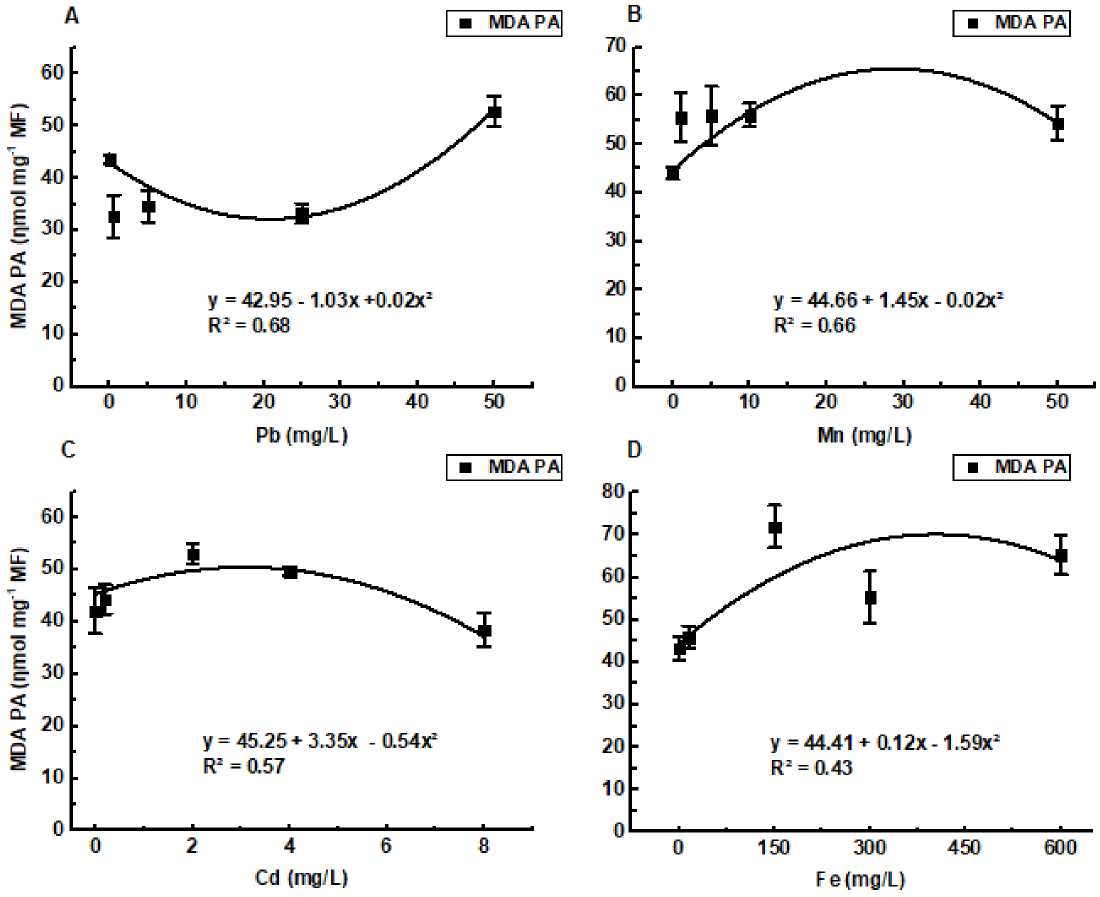


Source: created by the author (2020).

3.3 LIPID PEROXIDATION

In the shoot, *T. paniculatum* plants grown under Pb and Fe in the production of MDA showed an increase in the concentration of metals (Figure 9A, D). In plants grown under Cd, there was a tendency towards a decrease in MDA production (Figure 9 C) while, in plants under Mn, there was no significant variation in MDA content (Figure 9 B) with the increase in metal concentration.

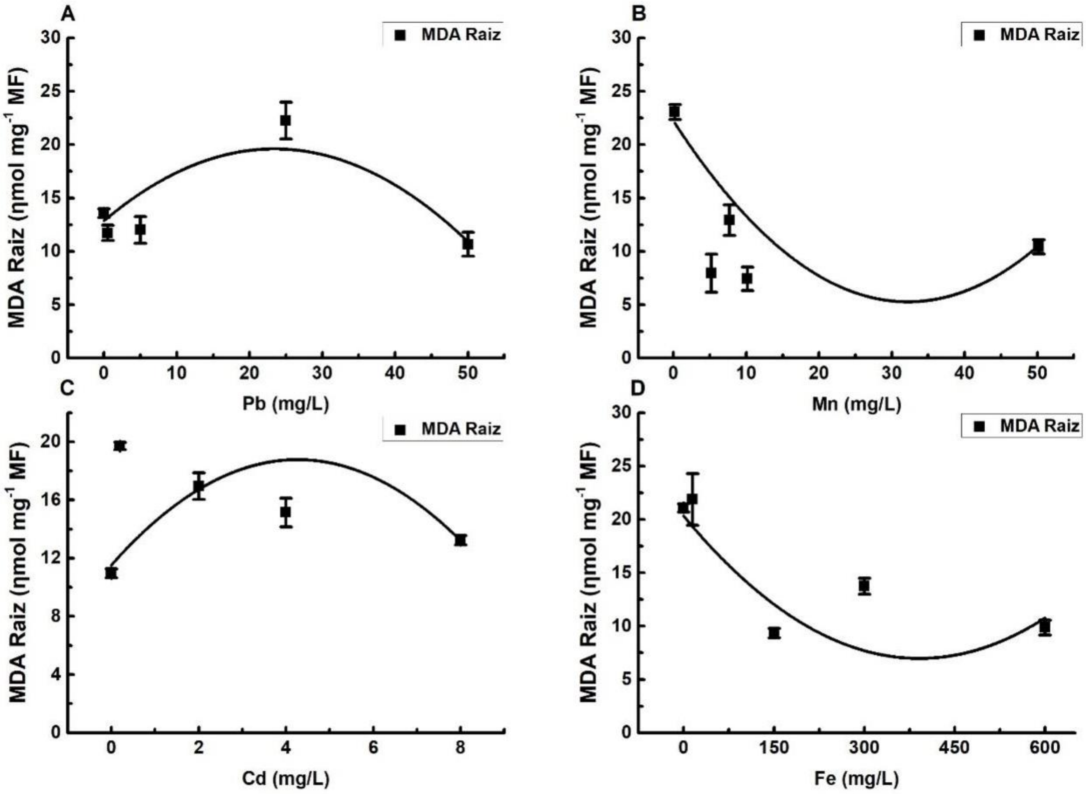
Figure 9 – Lipid peroxidation expressed by the content of malondialdehyde (MDA) in leaves (A-D) of *T. paniculatum* plants, grown under excess Pb, Mn, Cd and Fe, respectively, for a period of 35 days. The bars represent the standard error of the mean (n = 4).



Source: created by the author (2020).

In general, MDA generation was lower in the roots than in the shoot, showing lower levels of oxidative stress in the roots. In addition, plants grown on all metals showed a tendency to reduce the MDA content with the increase in metal concentrations (Figure 10 A-D).

Figure 10 – Lipid peroxidation expressed by the content of malondialdehyde (MDA) in roots (A-D) of *T. paniculatum* plants, grown under excess Pb, Mn, Cd and Fe, respectively, for a period of 35 days. The bars represent the standard error of the mean (n = 4).



Source: created by the author (2020).

As observed, *T. paniculatum* plants showed tolerance to cultivation with excess metals present in the soil. This can be demonstrated by the absence of harmful effects to the accumulation of biomass in the shoot and roots of these plants grown under Mn and Fe. On the other hand, when subjected to excess Cd, they presented only a reduction in RDB, without affecting the shoot. Plants grown under excess Pb, that is, with increased concentrations of this metal, had a greater accumulation of RDB in relation to the control plants.

Brazilian soils are rich in Mn and Fe (FADIGAS *et al*., 2002); the absorption of these micronutrients is higher in acidic soils, with a high content of organic matter, or in swampy soils (CAMARGO; SANTOS; ZONTA; 1999; PINTO *et al*., 2016). These excess metals can reduce the availability of essential nutrients and reduce plant growth in situations of remediation or even of cultivars, posing toxicity risks to plants and living beings (CARMO *et al*., 2017).

Since they are micronutrients, Mn and Fe are elements required at low concentrations to act in a favorable way to the functioning of plant metabolism and, consequently, they can easily reach toxic levels that end up implicating in plant metabolism. In the specific case of the species *T. paniculatum*, at the end of the experimental period, the plants under excess Mn presented only marginal chlorosis in the leaves, that is, mild symptoms of excess Mn. Plants under excess Mn normally show symptoms of marginal chlorosis, brown spots on their leaves, bending, cracking and leaf necrosis in more extreme cases (SANTOS *et al*., 2017).

In addition, no reductions in shoot or root dry biomass were observed in response to different Mn concentrations. The excess of Mn can be related to the following factors: lower activation of Ribulose 1,5-Bisphosphate Carboxylase/Oxygenase (Rubisco), reduced activity of enzymes in the respiratory metabolism, greater production of reactive oxygen species, changes in the structure of the cell membrane (RODRIGUES *et al*., 2016; ZAMBROSI *et al*., 2016) and reduced plant growth and production (KABATA-PENDIAS; PENDIAS, 2000). However, it was observed that the Mn concentrations used in this experiment did not cause plant responses by the excess of this metal, since there was only a small increase in net photosynthetic rate and in carboxylation efficiency, besides low levels of lipid peroxidation. Comparing the damage of the concentrations of $MnSO_4.4H_2O$, from 10, 15 and 20 mM applied in treatments with *N. tabacum*, they were much higher and difficult to contain, as well as soybean plants under concentrations of 0 to 300 µmol L^{-1} (SANTANDREA, SCHIFF, BENNICI, 1998; SANTOS *et al*., 2017).

Based on these results, there are basically two possibilities to be analyzed: the first is that *T. paniculatum* really has tolerance to excess Mn, since no oxidative stress has been detected either in shoots or in roots; the second possibility is that the applied Mn concentrations did not allow metal availability for the plant. The results of metal determination in plants are in the process of conclusion and will provide more conclusive data in this regard.

In relation to Fe, in general, the excess of this metal causes high toxicity to plants, leading to negative effects on the respiratory process and stomatal conductance, caused by the formation of reactive oxygen species ROS (JUCOSKI *et al*., 2016). Plants under excess Fe have higher generation of ROS, reduced shoots, number of leaves and root length, in addition to low dry biomass production (JUCOSKI *et al*., 2016). The greatest generation of ROS was related to photosynthetic limitation and damage to the dissipation of excess energy, as well as to the reduction in the amount of photosynthetic pigments in rice (PINTO *et al*., 2016). In the specific case of *T. paniculatum*, it was observed that the excess of Fe did not alter the biomass accumulation of these plants. Despite having caused oxidative stress in the leaves, the excess of this metal still led to an increase in An, k and chlorophyll at intermediate doses. Again, the Fe concentrations used in this experiment may not have been sufficient to cause a reduction in plant dry biomass.

Pb is currently one of the main environmental contaminants on the planet; it has brought serious risks to soil and biota contamination (KUMAR; PRASAD, 2015). In plants, Pb can be easily absorbed by tissues in the roots and dispersed to its other organs, causing disturbances in electron transfer, minimizing mechanisms of action and the ability to absorb essential nutrients linked to Pb in the form of cations (KABATA-PENDIAS; PENDIAS, 2000). The reduction in plant growth caused by Pb has been related to leaf chlorosis, reduced photosynthesis, interruption of electron flow in the electron transport chain and darkening of the root system (MARQUES *et al*., 2016; ROMEIRO *et al*., 2007; SOUZA *et al*., 2018). In *T. paniculatum* plants in particular, the presence of Pb reduced An, gs and chlorophyll content, and increased lipid peroxidation in the shoot and roots. At the end of the experimental period, the plants showed symptoms of leaf chlorosis. Despite these changes, SDB remained unchanged with the increase in Pb concentration and the roots showed an increase in biomass and a reduction in its average diameter.

Plants of the species *Talinum triangulare* showed an increase in shoot and root biomass, despite the variation in gas exchange rates at Pb concentrations of 25 mg L^{-1} in nutrient solution. However, there was a decrease in volume, diameter, root length and surface area, in addition to the generation of reactive oxygen species and cellular damage resulting from excess Pb (RAJKUMAR *et al*., 2009). Even so, the authors consider the potential of using these plants for phytoremediation due to their rooting power in the presence of metals. In addition, the low harmful effect of Pb on root growth has already been proven for *T. triangulare* (RAJKUMAR *et al*., 2009).

There are indications of *T. paniculatum* seedling tolerance to excess Pb. A study with seedlings of this species, exposed to concentrations of 50 to 500 μM $Pb(NO_3)_2$, demonstrated the absence of Pb effects on the germination index, and production of seedling fresh and dry matter. This tolerance was related to the antioxidant defense capacity to prevent cell damage caused by excess metal (SOUZA *et al*., 2018). This suggests that, in the case of *T. paniculatum* plants in this study, the antioxidant system may also be active, preventing oxidative damage from being high to the point of causing cellular damage that leads to a reduction in plant dry biomass, allowing survival for 35 days under excess Pb.

Environmental contamination by Cd is also related to anthropic action, through industrial activities and the manufacture of electronic products.

The excess of Cd in plants reduces photosynthesis, since this metal initially accumulates in chloroplasts, inhibiting the enzyme activity of chlorophyll biosynthesis and protein-pigment complexes (ALI; KHAN; SAJAD, 2013). Losses in photosystem II also occur due to Cd^{2+} oxidation and the increase in the cyclic electron transport flow around photosystem I (YING *et al*., 2010). Cd intoxication leads to an imbalance of homeostasis and damage to cell tissues, due to the input and output of chelating substances, which accumulate in the vacuole (BENAVIDES; GALLEGO; TOMARO, 2005). In addition to photosynthesis inhibition and non-assimilation of nutrients, Cd is related to the transpiration rate (SALT *et al*., 1995). As Cd is easy to accumulate in the root, it limits the transport of essential nutrients for translocation between root and shoot (CHAVES; SOUZA, 2014).

In the experiment, the presence of Cd led to a reduction in net photosynthetic rate and carboxylation efficiency at intermediate concentrations, in addition to a reduction in chlorophyll content and root growth at the highest concentrations. The highest generation of MDA was observed in shoots and roots at intermediate concentrations of Cd. At the end of the experiment, the plants showed signs of chlorosis at the highest Cd concentrations. Under low concentrations, Cd has greater plant mobility than at high concentrations, since phytochelatins allow greater Cd mobilization to the shoot, which leads to greater degradation of chlorophyll pigments (AUGUSTO *et al*., 2015). This may be related to the leaf yellowing observed at the end of the experimental period.

The decrease in chlorophyll concentration in plants under heavy metals may be related to the ability of metals to affect the biosynthesis process. In these cases, there is a competition between the absorption of metals and that of important nutrients for the synthesis of chlorophyll, such as Fe, magnesium (Mg) and zinc (Zn). In this context, due to the greater metal availability, their absorption is prioritized over nutrient absorption (CHINMAYEE *et al*., 2012; DRESLER *et al*., 2014; IORI *et al*., 2017). In addition, there is a question that the presence of metals breaks down cellular homeostasis, leading to the production of reactive oxygen species, which cause damage to cells and impair processes related to the absorption of light and the conversion of light energy into chemical energy.

Cd, which inhibits nutrient absorption, can also become an electron acceptor in photosystem II, causing changes in the activity of several enzymes that act in photosynthesis and respiration (AUGUSTO *et al*., 2015; BARCELÓ; POSCHENRIEDER, 1990). Pb is related to a lower capacity to absorb essential nutrients linked to Pb in the form of cations (KABATA-PENDIAS; PENDIAS, 2000). Excess Fe can also affect the assimilation of essential nutrients (JUCOSKI *et al*., 2016). Mn, on the other hand, can interfere with the absorption of essential nutrients, such as Calcium (Ca) and Potassium (K), and increase the concentrations of Mg and Zn (SANTOS *et al*., 2017).

Metal absorption availability depends mostly on soil characteristics such as pH, cation exchange capacity (CTC) and presence of organic matter (SOUZA; FRANÇA; FERREIRA, 2011). These factors, together with the available concentration of nutrient solution, affect the absorption of the metals under study by plants. Therefore, the application of a complete nutrient solution together with the metal may have contributed to the absorption of a greater amount of metal by the plants, causing them to suffer less from its effects, since the nutrients were also replaced. That is, a well-nourished plant may have handled excess metals better than a plant that goes through excess metals without complementary nutrition. This confirmation will be possible after determining the concentration of metals in the plants that are in the completion process.

Considering that the application of the nutrient solution may have mitigated the effects of metals on plants grown in the soil, the proposition arises that this species be analyzed in a medium with excess metals and in the absence of nutrients, to understand the physiological responses related only to metals. Other variations may be related to the concentrations of metals used and the time of plant exposure.

From the result to be obtained with metal quantification, it will be possible to analyze the potential of *T. paniculatum* in the phytoextraction of heavy metals in the soil, both in soil with and without fertilization.

4 CONCLUSION

T. paniculatum is a plant tolerant to high concentrations of Pb, Cd, Mn and Fe in the soil. For Pb and Cd, in general, despite the cellular damage caused in plants, only Cd reduces biomass accumulation in the roots. With respect to Mn and Fe, the effect of these metals on plant physiology is minimal, not altering biomass accumulation after 35 days of treatment. The analysis of metal concentration in the plants will help in the conclusion about the amount of metal absorbed by the plants and their potential use for phytoremediation purposes.

Future experiments without nutrient supplementation can be carried out to prove the effects of metals on *T. paniculatum* plants.

REFERENCES

ALI, H.; KHAN, E.; SAJAD, M. A. Phytoremediation of heavy metals–concepts and applications. **Chemosphere**, United Kingdom, v. 91, p. 869-881, 2013.

ANTONIADIS, V. *et al*. Trace elements in the soil-plant interface: Phytoavailability, translocation, and phytoremediation–A review. **Earth-Science Reviews**, Netherlands, v. 171, p. 621-645, 2017.

ATSDR – Agency for Toxic Substances and Disease Registry. **Toxicological Profile for Cadmium**. Atlanta, 2012a. Disponível em: https://www.atsdr.cdc.gov/toxprofiles/tp5.pdf. Acesso em 25 out. 2020.

ATSDR – Agency for Toxic Substances and Disease Registry. **Toxicological Profile for Manganese**. Atlanta, 2012b. Disponível em: https://www.atsdr.cdc.gov/toxprofiles/tp151.pdf. Acesso em 25 out. 2020.

ATSDR – Agency for Toxic Substances and Disease Registry. **Toxicological Profile for Lead**. Atlanta, 2020. Disponível em: https://www.atsdr.cdc.gov/toxprofiles/tp13.pdf. Acesso em 25 out. 2020.

AUGUSTO, A. *et al.* Avaliação dos efeitos tóxicos de Cd e Pb na cultura da mostarda (*Brassica juncea*), **Engenharia Sanitária e Ambiental**, Rio de Janeiro, v., 19, p. 61-68, 2015.

BARCELÓ, J.; POSCHENRIEDER, C. H. Plant water relations as affected by heavy metal stress a review. **Journal of Plant Nutrition**, London, v. 13, p. 1-37, 1990.

BELO, A. F. *et al*. Atividade fotossintética de plantas cultivadas em solo contaminado com picloram. **Planta Daninha, Viçosa**, Brazil, v. 98, p. 885-892, 2011.

BENAVIDES, M. P.; GALLEGO, S. M.; TOMARO, M. L. Cadmiun toxicity in plants. **Brazilian Journal of Plant Physiology**, Campinas, v. 17, p. 21-34, 2005.

BIEMELT, S.; KEETMAN, U.; ALBRECHT, G. Re-aeration following hypoxia or anoxia leads to activation of the antioxidative defense system in roots of wheat seedlings. **Plant Physiology**, United States, v. 116, p. 651-658, 1998.

BÓREM, A., SANTOS, F. R., ALMEIDA, M. R. **Biotecnologia de A a Z**. Metais Pesados, Viçosa, MG: UFV, 2003, cap. 6, p. 95-121.

BUEGE, J. A.; AUST, S. D. Microsomal Lipid Peroxidation. **Methods in Enzymology**, United States, v. 52, p. 302-310, 1978.

CAMARGO, F. A. O.; SANTOS, G. A.; ZONTA, E. Alterações eletroquímicas em solos inundados. **Ciência Rural**, Santa Maria, v. 29, p. 171-180, 1999.

CARMO, F. F. *et al*. Fundão tailings dam failures: the environment tragedy of the largest technological disaster of Brazilian mining in global context. **Perspectives in Ecology and Conservation**, Spain, v. 15, p. 145-151, 2017.

CHAVES, L. H.; SOUZA, R. Crescimento, distribuição e acumulação de cádmio em plantas de *Jatropha curcas*. **Rev. de Ciências Agrárias**, Brazil, v. 37, p. 286-291, 2014.

CHINMAYEE, M. D. *et al*. The Assessment of Phytoremediation Potential of Invasive Weed Amaranthus spinosus. **Appl Biochem Biotechnol**, United States, v. 167, p. 1550-1559, 2012.

CLEMENS, S. Molecular mechanisms of plant metal tolerance and homeostasis Stephan. **Planta**, Germany, v. 212, p. 475-486, 2001.

COELHO, A. A. O. P.; ZAPPI, D. Portulacaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. BFG. Growing knowledge: an overview of Seed Plant diversity in Brazil. **Rodriguesia**, Rio de Janeiro, v. 66, p.1085-1113, 2015.

CONAMA – CONSELHO NACIONAL DO MEIO AMBIENTE. **Resolução nº 420, de 28 de dezembro de 2009**. Ministério Meio Ambiente (MMA), p. 81-84, 2009. Disponível em: http://www2.mma.gov.br/port/conama/legiabre.cfm?codlegi=620. Acesso em: 25 out. 2020.

CURVÊLO, C. R. S. *et al*. Trocas gasosas e estresse oxidativo em plantas de algodoeiro supridas com silício e infectadas por *Ramularia aréola*. **Bragantia**, Campinas, v. 72, p. 346-359, 2013.

DRESLER, S. *et al*. Accumulation of low-molecular-weight organic acids in roots and leaf segments of Zea mays plants treated with cadmium and copper. Acta **Physiologiae Plantarum**, Poland, v. 36, p. 1565-1575, 2014.

FADIGAS, F. S. *et al*. Solos e irrigação: concentrações naturais de metais pesados em algumas classes de solos brasileiros. **Bragantia**, Campinas, v. 61, p.151-159, 2002.

FREIRE, M. de F. I. Metais pesados e plantas medicinais. **Revista Científica Eletrônica de Agronomia**, Brazil, v. 8, p. 1-14, 2005.

GIANNOPOLITIS, C. N.; RIES, S. K. Superoxide dismutases: I. Occurrence in higher plants. **Plant physiology**, United States, v. 59, p. 309-314, 1977.

GROTTO, H. Z. W. Fisiologia e metabolismo do ferro. **Revista Brasileira de Hematologia e Hemoterapia**, Brazil, v. 32, p 8-17, 2010.

GUERRA, M. B. B. *et al*., Post-catastrophe Analysis of the Fundão Tailings Dam Failure in the Doce River System, Southeast Brazil: Potentially Toxic Elements in Affected Soils. **Water, Air, and Soil Pollution**, Netherlands, v. 228, p. 1-12, 2017.

HAVIR, E. A.; MCHALE, N. A. Regulation of Catalase Activity in Leaves of Nicotiana sylvestris by High CO2. **Plant Physiology**, United States, v. 89, p. 952-957, 1988.

HOAGLAND, D.; ARNON, D. I. The water culture method for growing plants without soil. **California Agriculture Experiment Station**, Gainesville, v. 347, p. 1-32, 1950.

INMET – INSTITUTO NACIONAL DE METEREOLOGIA. **Instituto Nacional de Metereologia**. Disponível em: http://www.inmet.gov.br/portal/index.php?r=home/page&page=rede_estacoes_auto_graf. Acesso em: 11 out. 2019.

IORI, V. *et al*. Analysis of biometric, physiological, and biochemical traits to evaluate the cadmium phytoremediation ability of eucalypt plants under hydroponics. **IForest**, Viterbo, v. 10, p. 416-421, 2017.

JUCOSKI, G. *et al*. Excesso de ferro sobre o crescimento e a composição mineral em Eugenia uniflora L. **Revista Ciência Agronômica**, Ceará, v. 47, p. 720-728, 2016.

KABATA-PENDIAS, A; PENDIAS, H. **Trace elements in soils and plants**. Library of Congress Cataloging, 3 ed., p. 331, 2000.

KUMAR, A.; PRASAD, M. N. V. Lead-induced toxicity and interference in *chlorophyll fluorescence* in *Talinum triangulare* grown hydroponically. **Photosynthetica**, Czech republic, v. 53, p. 66-71, 2015.

KUMAR, A.; PRASAD, M. N. V.; SYTAR, O. Lead toxicity, defense strategies and associated indicative biomarkers in Talinum triangulare grown hydroponically. **Chemosphere**, United Kingdom, v. 89, p. 1056-1065, 2012.

LARCHER, W. **Ecofisiologia Vegetal**. São Paulo: RiMa Editora, 2012.

LEÓN, A. M. *et al*. Antioxidative enzymes in cultivars of pepper plants with different sensitivity to cádmium. **Plant Physiology and Biochemistry**, France, v. 40, p. 813-820, 2012.

LI, Y. *et al*. Cadmium accumulation, activities of antioxidant enzymes, and malondialdehyde (MDA) content in Pistia stratiotes L. **Environmental Science and Pollution Research**, Germany, v. 20, p. 1117-1123, 2013.

LICHTENTHALER, H. K.; LANGSDORF, G.; LENK, S. Chlorophyll fluorescence imaging of photosynthetic activity with the flash-lamp fluorescence imaging system. **Photosynthetica**, Czechia, v. 43, p. 355-369, 2005.

MACHADO, F. H. O.; MELO, J. I. M.; SALES, M. F. Flora da região de Xingó, Alagoas-Sergipe: Portulacaceae sensu lato. **Biotemas**, Brazil, v. 25, p.103-108, 2012.

MARIA, R. *et al*. Ferro e Manganês no perfil do solo em Saccharum Officinalis fertilizada com resíduos orgânicos. **Bioscience Journal**, Uberlândia, v. 30, p. 131-139, 2014.

MARQUES, É. A. *et al*. Determinação do Valor de Prevenção para Cromo em Solos do Estado de Minas Gerais. **Holos**, Brazil, v. 4, p. 142, 2016.

MARQUES, M.; CHAFIM AGUIAR C. R.; SILVA J. J. L. S. Desafios técnicos e barreiras sociais, econômicas e regulatórias na fitorremediação de solos contaminados. **Revista Brasileira de Ciência do Solo**, Brazil, v. 35, p. 1-11, 2011.

MEDEIROS, M. A. Ferro. **Dictionary of Gems and Gemology**, vol. 32, n° 3, 2010.

NAKANO, Y.; ASADA, K. Hydrogen peroxide is scavenged by ascorbato-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology, v. 22, p. 867-880, 1981.

NGUYEN T. N. L. *et al*. Use of ITS DNA barcode for identification of Jewels of Opar (*Talinum paniculatum*) collected in Thanh Hoa, Vietnam. **Vietnam Journal of Science, Technology and Engineering**, Vietnam v. 60 p. 46-49, 2018.

OMACHI, G. Y. **Estudos para o aumento da vida útil das minas de minério de ferro do Quadrilátero Ferrífero, MG**. 2015. 83 f. Dissertação (Mestrado em Engenharia Mineral) - Escola de Minas, Universidade Federal de Ouro Preto, Ouro Preto, 2015.

OREN, R. *et al*. Survey and synthesis of intra- andinterspecific variation in stomatal sensitivity to vapour pressuredeficit. **Plant Cell Environ**, United Kingdom, v. 22, p. 1515-1526, 1999.

PINTO, S. S. *et al*. Oxidative damage and photosynthetic impairment in tropical rice cultivars upon exposure to excess iron. **Scientia Agricola**, Piracicaba, v. 73, p. 217-226, 2016.

RAJKUMAR, K. *et al*. Effects of selected heavy metals (Pb, Cu, Ni, and Cd) in the aquatic medium on the restoration potential and accumulation in the stem cuttings of the terrestrial plant, *Talinum triangulare* Linn. **Ecotoxicology**, United States, v. 18, p. 952-960, 2009.

RIBEIRO, E. S. *et al*. Relações da anatomia radicular na absorção, no acúmulo e na tolerância ao chumbo em *Echinodorus grandiflorus*. **Rev. Brasileira de Engenharia Agricola e Ambiental**, Campina Grande, v. 19, p. 605-612, 2015.

RODRIGUES, A. C. D. *et al*., Mecanismos de Respostas das Plantas à Poluição por Metais Pesados: Possibilidade de Uso de Macrófitas para Remediação de Ambientes Aquáticos Contaminados. **Rev. Virtual de Química**, Niterói, RJ, v. 8, p. 262-276, 2016.

ROMEIRO, S. *et al*. Absorção de Chumbo e Potencial de Fitorremediação. **Bragantia**, Campinas, v. 66, p. 327-334, 2007.

ROUT, G. R, SAHOO, R. Role of Iron in Plant Growth and Metabolism. **Reviews in Agricultural Science**, Japan, v. 3, p. 1-24, 2015.

SALT, D. E. *et al*. Phytoremediation: A Novel Strategy for the Removal of Toxic Metals from the Environment Using Plants. **Biotechonology**, United States, v. 13, p. 468-474, 1995.

SANTANDREA, G.; SCHIFF, S.; BENNICI, A. Effects of manganese on Nicotiana species cultivated in vitro and characterization of regenerated Mn-tolerant tobacco plants. **Plant Science**, Ireland, v. 132, p. 71-82, 1998.

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

SARWAR, N.; IMRAN, M.; SHAHEEN, M, R. Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives. **Chemosphere**, United Kingdom, v. 171, p. 710-721, 2017.

SCAFIDI, F.; RAIMONDO, F. M. Principi di spontaneizzazione in Sicilia di. **Quaderni di Botanica ambientale e applicata**, Italy, v. 26, p. 23-26, 2017.

SCHMIDT, W. THOMINE, S. BUCKHOUT, T. J. Editorial: Iron Nutrition and Interactions in Plants. **Frontiers in Plant Science**, Suíça, v. 10, p. 1-4, 2020.

SHIKHOVA, N. S. Some regularities in the accumulation of lead in urban plants (by example of Vladivostok). **Contemporary Problems of Ecology**, Russian Federation, v. 5, p. 285-294, 2012.

SILVA, T. J. *et al*. Fitorremediação de Solos Contaminados com Metais: Panorama Atual e Perspectivas de uso de Espécies Florestais. **Rev. Virtual Química**, Brazil, v. 11, p. 18-34, 2019.

SOUZA, A. K. R.; MORASSUTI, C. Y.; DEUS, W. B. Poluição do Ambiente por Metais Pesados e Utilização de Vegetais como Bioindicadores. **Rev. Acta Biomédica Brasiliensia**, Brazil, v. 9, p. 1-12, 2018.

SOUZA, E. P.; FRANÇA, I.; FERREIRA, L. E. Mecanismos de tolerância a estresses por metais pesados em tolerância em plantas. **Revista Brasileira de Agrociência**, Pelotas, v.17, p. 167-173, 2011.

SOUZA, G. *et al*. Morpho-physiological Tolerance Mechanisms of Talinum patens to Lead. **Water, Air, and Soil Pollution**, Netherlands, v. 229, p.1-9, 2018.

SOUZA, T. C. *et al*. Early characterization of maize plants in selection cycles under soil flooding. **Plant Breeding,** Germany, v. 131, p. 493–501, 2012.

TAVARES, S. R. L.; OLIVEIRA, S. A.; SALGADO, C. M. Avaliação de Espécies Vegetais na Fitorremediação de Solos Contaminados por Metais Pesados. **HOLOS**, Portuguese, v. 5, p. 80-97, 2013.

VASCONCELLOS, M. C.; PAGLIUSO, D.; SOTOMAIOR, V. S. Fitorremediação: uma proposta de descontaminação do solo. **Estudos de Biologia**, Brazil, v. 34, p. 261-267, 2012.

VENEZUELA, T. C., OLIVEIRA, A. E. X. DE PÉREZ, D. V., **Determinação de contaminantes metálicos (metal tóxico) num solo adubado com composto de lixo em área olerílica no município de Nova Friburgo/RJ**, Dissertação - Programa de Pós graduação em Saúde Pública Centro de Saúde do Trabalhador e Ecologia Humana Escola Nacional de Saúde Pública. 96p.

WAN, X.; LEI, M.; CHEN, T. Cost-benefit calculation of phytoremediation technology for heavy-metal-contaminated soil. **Science of the Total Environment**, Netherlands, v. 563-564, p. 796-802, 2016.

YING, R-R. *et al*. Cadmium tolerance of carbon assimilation enzymes and chloroplast in zn/cd hyperaccumulator *picris divaricata.* **Journal of Plant Physiology**, Germany, v.167, p. 81-87, 2010.

ZAMBROSI, F. C. B. *et al*. Anatomical and physiological bases of sugarcane tolerance to manganese toxicity. **Environmental and Experimental Botany**, United Kingdom, v. 132, p. 100-112, 2016.

APÊNDICE B – POTENTIAL OF *TALINUM PANICULATUM* CUTTINGS IN LEAD AND MANGANESE RHIZOFILTRATION

Pedro Ernesto dos Reis[1], Gabriel Ferreira Romão[1], Kamila Rezende Dázio de Souza[1], Gisele de Fátima Esteves[1], Keila Yumi Ishii[1], Paulo César Magalhães[2], Plínio Rodrigues dos Santos Filho[3], Thiago Corrêa de Souza[1]*

[1]Federal University of Alfenas – UNIFAL-MG, Institute of Nature Sciences – 700, Gabriel Monteiro Street, P. O. Box 37130-001, Alfenas, MG, Brazil.
[2]Maize and Sorghum National Research Center, P. O. Box 151, 35701-970, Sete Lagoas, MG Brazil.
[3]Federal University of Alfenas – UNIFAL-MG, Institute of Biological Sciences - 700, Gabriel Monteiro Street, P. O. Box 37130-001, Alfenas, MG, Brazil.

*Corresponding author:
Thiago Corrêa de Souza
Tel.: +55-35-3701-9683
Email: thiagonepre@hotmail.com

ABSTRACT

Rhizofiltration is a technique that uses plants to remove heavy metals from water. The search for species with potential rooting and an increase in biomass for use in rhizofiltration processes becomes important, given the increased presence of metals present in contaminated water resources. Therefore, the objective of this study was to evaluate the physiological characteristics and the potential remediation of *Talinum paniculatum* cuttings subjected to excess lead (Pb) and manganese (Mn). Thus, cuttings excised from *T. paniculatum* were transferred to a nutrient solution, in which different concentrations of lead (0; 10; 50; 100; 200 mg L^{-1}) and manganese (0; 10; 50; 100; 150 mg L^{-1}) were added. The treatments were maintained for a period of 30 days, when the plant material was collected for the following analysis: biometric, root morphology, activity of antioxidant enzymes and lipid peroxidation in shoots and roots. Gas exchange, relative chlorophyll content and chlorophyll “a” fluorescence were analyzed at the beginning (C1) and at the end (C2) of the experiment. The cutting tolerance index was over 70% for Pb and Mn. The increase in dry matter and the resistance to exposure of the hydroponic solution containing Pb and Mn, showed efficiency for the analyzed parameters. While root morphology and antioxidant system activity showed tolerance to treatments, the plant response to excess Mn and Pb showed that the most related variables were gas exchange and chlorophyll fluorescence. *T. paniculatum* cuttings have the potential for use in Pb and Mn phytoextraction, mainly due to the rapid rooting and cutting growth, in addition to cutting tolerance to the excess of these metals.

Keywords: Phytoremediation. Antioxidant system. Gas exchange. Heavy metals. Talinacea.

1 INTRODUCTION

Currently, environmental contamination by heavy metals is increasingly present in agricultural soils and in water resources (SARWAR *et al*., 2017). Pollution by heavy metals occurs mostly in mineral extraction, industries, manufacture of electronic products and in their disposal. Automotive exhaust gases, coal combustion, fertilizers, insecticides and Pb-based paints, are the main ways to release the pollutant in the world. Mn has accumulated in the soil over decades, due to the presence of Fe and oxides Mn, by mineral exploration, employment in industry, and manufacturing of fertilizers (NAKBANPOTE; MEESUNGNOEN; PRASAD, 2016).

Small particles and concentrations of these metals are released into water bodies, even if pre-treatment is carried out before disposal or final disposal, but there are techniques that allow reducing the availability or extracting these metals from the water. (ANKIT, 2019). A Rhizofiltration is a technique with great poltential for removing heavy metals from water (MARQUES *et al*., 2011; TAVARES *et al*., 2013). In this technique, the environment is decontaminated through the use of accumulator or hyperaccumulator plants for the absorption, adsorption and precipitation of these metals (SARWAR *et al*., 2017). O sistema radicular das plantas trabalha como um biofiltro que reduz a mobilidade dos metais e a migração para águas subterraneas, as raízes capturam os metais contribuindo para despouluição do local contaminado, além de reduzir a biodisnibilidade dos metais na cadeia alimentar. (GALAL *et al*., 2018).

Heavy metals are mineral elements present in nature in small amounts; they have an anatomical density that varies between the mineral elements (1 to 5 g cm-3) (RASCIO; NAVARI, 2011). These elements are easily absorbed in the form of ions, salts or linked to carbon and nitrogen chains (ANDRADE *et al*., 2009). In exchange for 2 to 5 mM, manganese (Mn) has no toxicity when used in low tools; it is also a micronutrient. However, when absorbed at necessary highs, above 5mM, lead (Pb) and Mn cause serious damage to plant metabolism, including reduced growth and development (ANTONIADIS *et al*., 2017; KABATA-PENDIAS; PENDIAS 2000). However, there are plants that tolerate the presence of these metals, which gives them the ability to absorb large amounts without compromising plant metabolism. These species can be divided by their growth potential and biomass gain or through metabolism as accumulators or hyperaccumulators, and their ability to translocate metals, which are retained in their roots, aerial part or not stem (PIO, ANTONY, SANTAN, 2013).

Due to the low cost and ease of disposing of the plants through incineration, rhizofiltration is a convenient technique for large and small companies in the mineral, agricultural, livestock sector and that deposit contaminants in the water even performing a pre-treatment, in addition to minimizing the environmental impacts caused by incorrect or impractical disposal of heavy metals (WAN; LEI; CHEN, 2016).

One of the main symptoms observed in plants undergoing phytoremediation is oxidative stress resulting from the accumulation of oxygen species (ROS), generated by the breakdown of cellular homeostasis by metals (BARBOSA *et al*., 2014). The plant defense mechanism tries to block the entry of metal into cells, activating the antioxidant system and the production of enzymes and lipid peroxidation. Absorption of excess metals can inhibit nutrient uptake by plants, as well as the entry of these metals into metabolic pathways, triggering physiological changes that inhibit plant growth under excess of Pb and Mn. In general, there is a change in chlorophyll fluorescence, gas exchange and pigment content, which leads to the appearance of visual symptoms such as leaf chlorosis, leaf loss, senescence and root darkening (KUMAR; PRASAD; SYTAR, 2012; YONG *et al*., 2013). However, accumulator plants have tolerance mechanisms related to the reduction in damage caused by metals, allowing plants to survive under such conditions (MALAR *et al*., 2014).

The species *Talinum paniculatum* (Jacq.) Gaertn. or *Talinum patens* (L.) Wild belongs to the Talinaceae group, and can be found in the Southern and Northern hemispheres, strictly in warmer regions. It is easily cultivable in poor or nutritionally deficient soils and has a short life cycle, 65 days or more, depending on the care taken (MACHADO; MELO; SALES, 2012; SCAFIDI; RAIMONDO, 2017;). Souza *et al*. (2018) verified the tolerance of *T. paniculatum* seedlings to excess Pb through ecophysiological and biochemical responses. In addition, RAJKUMAR *et al*. (2009) reported the tolerance of *Talinum triangulare* cuttings - a species of the same family - to the presence of heavy metals in solution. Kumar and Prasad. (2015) also observed characteristics of tolerance to the imposition of metals in nutrient solution in *T. triangulare* plants, which shows a great propensity of plants of this genus for potential use in rhizofiltration.

In view of the growth characteristics of *T. paniculatum* and the tolerance to the excess of Pb described above, the obtention of *T. paniculatum* cuttings was hypothesized as favoring the use of this species in phytoremediation. Therefore, the objective of this study was to evaluate the physiological characteristics and their potential for rhizofiltration of *T. paniculatum* cuttings submitted to excess Pb and Mn.

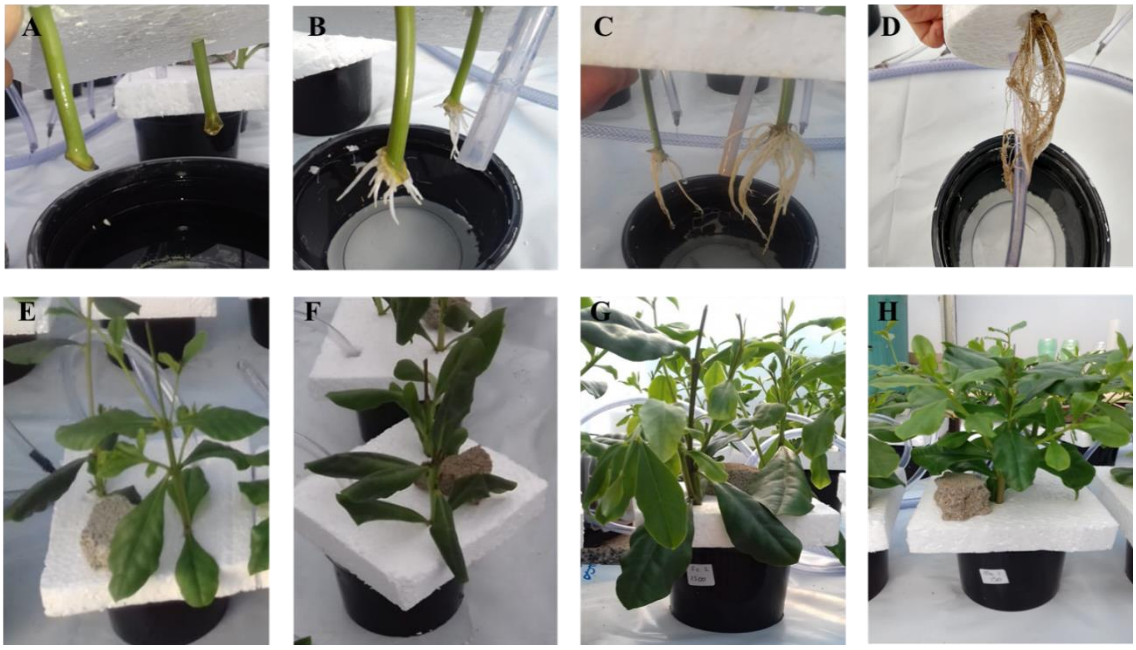
2 MATERIAL AND METHODS

2.1 OBTENTION OF PLANT MATERIAL AND CONDUCTION OF THE EXPERIMENT

The experiment was conducted in a greenhouse at Federal University of Alfenas. Seeds obtained from the seed bank of the research group were sown in pots containing substrate composed by soil and cattle manure (at the ratio 3:1) of soil and cattle manure for a period of two months.

Plants were grown for two months, when the cuttings were excised. The process from obtaining cuttings to starting treatments is detailed in Figure 1. Cuttings containing six fully expanded leaves were excised from the plants and placed in pots containing nutrient solution ¼ strength (Hoagland and Arnon 1950) (Figure 1 A, E). Five days later, when the cuttings were already rooted, the nutrient solution was changed to ½ strength (Figure 1 B, F). After ten days, the nutrient solution was changed to full strength for cutting cultivation and growth (Figure 1C, G). Finally, 20 days after the beginning of the experiment (Figure 1 D, H), rooted cuttings were selected for size uniformity and exposed to different concentrations of Pb (0; 10; 50; 100; 200 mg L^{-1}) and Mn (0; 10; 50; 100 and 150 mg L^{-1}).

Figure 1 – Period from obtaining *Talinum paniculatum* cuttings to the beginning of treatments, showing the roots (A – D) and shoots (E – H).



Source: created by the author (2020).

High purity reagents Pb $(NO_3)_2$ and $MnSO_4.H_2O$ were used as Pb and Mn sources. The concentrations used were previously defined based on Conama Resolution 430 (The National Environment Council in Brazil - CONAMA), which have allowed values for concentrations of inorganic substances in water, total Pb (0.5 mg L^{-1}) and dissolved Mn (1.0 mg L^{-1}). Metals were added to the nutrient solutions, which were changed weekly. The pH was maintained at 6 ± 0.5 and the nutrient solutions were maintained under continuous aeration.

The treatments were applied for a period of 30 days, when the plant material was collected. Biometric and ecophysiological evaluations were performed at the first day of metal exposure (C1) and at the end of the experiment (30 days of treatments, C2), while biochemical and morphological analyses, when were performed on the material collected at the end of the experiment.

2.2 VISUAL SYMPTOMS AND BIOMASS DETERMINATION

During the experimental period, visual symptoms of plant wilting, leaf chlorosis, necrosis and bending were observed. Shoot and root were dried in a forced air oven at 65 °C until constant weight for the determination of dry biomass.

2.3 DETERMINATION OF MACRO – AND MICRONUTRIENTS AND PB

For the quantification of nutrients and Pb concentrations, plants were divided in shoots and roots and were dried at 65 ^{0}C under air circulation until constant weight. Then, the samples were ground in a rotor type mill (Pulverisette 14 Classic Line, Fritsch GmbH, Germany) at 16,000 rpm. Subsequently, samples were subjected to nitro-perchloric digestion to determine the nutrient and Pb concentrations. N was determined by Sulphur digestion followed by the Kjeldahl distillation. Ca, Mg, K, Cu, Fe, Zn, Mn and Pb were determined by high sensitivity atomic emission spectrometry (MIP-AES Agilent 4200, Santa Clara, USA). P, S and B were determined by spectrofotometry (FEMTO 600S, São Paulo, Brazil). The content of macro- and micronutrients and Pb was determined according to standard laboratory protocols and methods following the recommendations of Malavolta *et al*. (1997). The limit of quantification (LOQ = 10 * standard deviation of blank/slope of calibration curve) was 0.22 mg kg^{-1} for N; 0.11 mg kg^{-1} for P; 0.32 mg kg^{-1} for K; 0.22 mg kg^{-1} for Ca; 0.11 mg kg^{-1} for Mg; 0.11 mg kg^{-1} for S; 2.03 mg kg^{-1} for Cu; 3.79 mg kg^{-1} for Fe and 4.13 mg kg^{-1} for Zn; 4.02 mg kg^{-1} for Mn and 0.01 mg kg^{-1} for Pb.

The content of macro- and micronutrients and Pb was determined by the product of dry matter x nutrient concentration in shoots and roots. The content was expressed in mg of nutrients/metals per each plant part analyzed (shoots and roots).

2.4 TRANSLOCATION FACTOR AND TOLERANCE INDEX

The translocation factor (TF) of Mn e Pb from roots to shoots were calculated by: (element content in shoot/ element content in roots) * 100. The translocation factor was expressed in percentage (Xue *et al*. 2018).

The tolerance index (IT) was determined using the formula: IT = (MS treatment/ MS control) x 100 where: treatments were considered the different concentrations of

Pb or Mn and the control was considered the concentration 0 mg L^{-1} Pb or 0 mg L^{-1} Mn (Mou *et al*. 2011).

2.5 ROOT MORPHOLOGY

The roots were properly collected, washed in running water and stored in pots containing 70% alcohol until the analyses using the Winrhizo Pro 2007 software (Regent Instruments, Sainte-Foy, QC, Canada) in order to obtain total root length, surface area and root volume.

2.6 GAS EXCHANGE AND RELATIVE CHLOROPHYLL CONTENT

Gas exchange was analyzed between 8 and 11 am, with an Infra-Red Gas Analyzer model LI 6400XT (LI-COR Inc., Lincoln, Nebraska, USA). A fixed flow chamber with a leaf temperature of 28 °C, a flow of 500 µmol/s of air and photosynthetically active radiation of 1,200 µmol/m^{-2} s^{-1} were used, based on the light curve previously plotted for the species. The rates analyzed were net photosynthesis (An), intercellular CO_2 concentration (Ci) and transpiration (E). From these parameters, water use efficiency (WUE) and instant carboxylation efficiency (k), obtained by the ratios An/E and An/Ci, respectively, were calculated.

The relative chlorophyll content was determined by the SPAD index, using a 502-Plus chlorophyll meter (Konica-Minolta – Osaka, Japan). Eight readings were performed on two completely expanded leaves, by replication.

2.7 CHLOROPHYLL “A” FLUORESCENCE

Chlorophyll “a” fluorescence was analyzed using a modulated fluorimeter (Mini-PAM – Heinz Walz, Effeltrich, Germany). After 30 minutes of adaptation in the dark, the minimum fluorescence (F_o) was measured with a sufficiently low light, avoiding photochemical reactions, and the maximum fluorescence (F_m), by applying a saturating light pulse of 7000 µm photons m^{-2} s^{-1} for 0.8 seconds. In the samples adapted in the dark, the maximum efficiency of the photosystem (PS II) was estimated by the Fv/Fm ratio. The leaves were then illuminated with actinic light at an intensity of 1500 µmol photons $m^{-2}s^{-1}$. Subsequently, constant fluorescence (Fs) was obtained and then another saturating light pulse was applied for 1s to obtain the maximum fluorescence emitted by the leaves (Fm’). The actinic light was removed and the leaves were irradiated with far-red light, for the obtention of Fo adapted to light (Fo’). Photochemical quenching was calculated as qP = (Fm’-Fs)/(Fm’-Fo’), and non-photochemical quenching was calculated as qN = (Fm-Fm’)/Fm’. Electron transport rate (ETR) = [(Fm’-Fs/Fm’) x PPFD x 0.5 x 0.84 was also evaluated (van Kooten and Snel 1990).

2.8 BIOCHEMICAL ANALYSES

The plant material (roots and leaves) was collected in liquid nitrogen and stored at -80 ºC until the analyses

2.8.1 Activity of antioxidant enzymes

The enzymes were extracted according to Biemelt *et al*. (1998). The supernatant collected at the end of the extraction process was used to quantify the activity of the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT).

SOD activity was assessed through the ability to inhibit nitrotetrazolium blue (NBT) photoreduction, as described by Giannopolitis and Reis (1977). One unit of SOD was calculated as the amount of enzyme necessary to inhibit NBT photoreduction by 50%. CAT activity was assessed by the decreasing absorbance at 240 nm (Havir and McHale 1987). The activity was expressed in μmol H_2O_2 min^{-1} mg^{-1} fresh matter (molar extinction coefficient, 36 mM^{-1} cm^{-1}). Using the methodology of Nakano and Asada (1981), APX activity was measured by monitoring the oxidation rate of ascorbate at 290 nm. The activity was expressed in μmol AsA min^{-1} mg^{-1} fresh matter (molar extinction coefficient, 2.8 mM^{-1} cm^{-1}).

2.8.2 Lipid peroxidation

Lipid peroxidation was determined according to Buege and Aust (1978)(1978 and the formation of malondialdehyde was then quantified by the following equation: [MDA] = (A535 – A600)/(ξ.b), where: ξ (extinction coefficient = 1.56 x 10^{-5} cm^{-1}); b (optical length = 1). Peroxidation will be expressed in ɳmol of malondialdehyde (MDA) g^{-1} of fresh matter.

2.9 EXPERIMENTAL DESIGN AND DATA ANALYSIS

The experiment was conducted in a completely randomized design using a factorial scheme (5 x 4), with five metal concentrations and four replications. Each replication consisted of two cuttings.

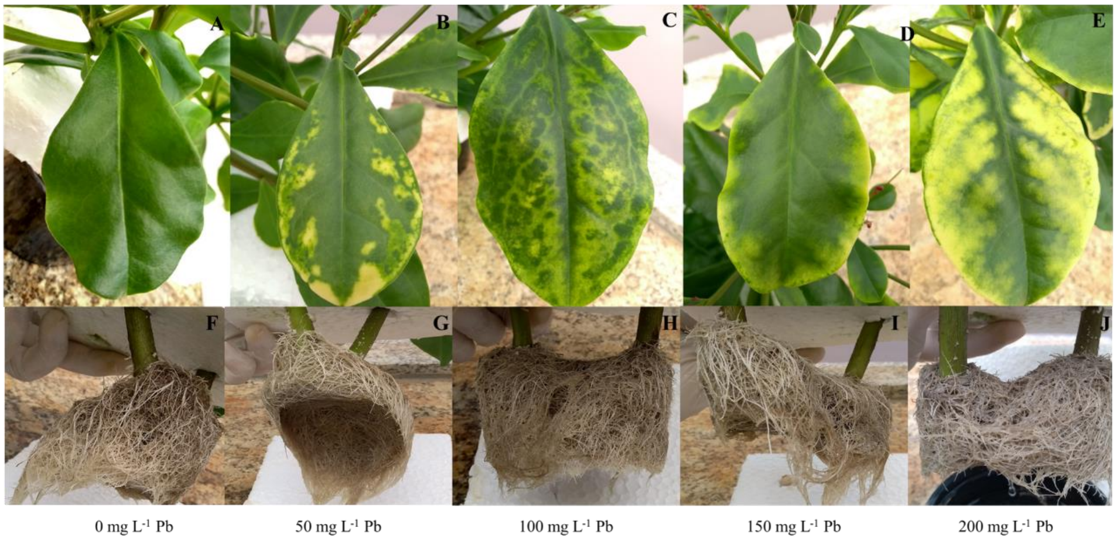
The data were tested for the assumptions and subjected to analysis of variance (ANOVA) and then multiple linear regression analyses by the Bioestat 5.3 software; when not significant, the means test was performed and they were compared using the Scott-Knott test ($p \leq 0.05$). The analyses were performed using the SISVAR statistical software.

The relationship between Pb and Mn concentrations and the variables in this study were analyzed using principal component analysis (PCA) by correlation matrix.

3 RESULTS

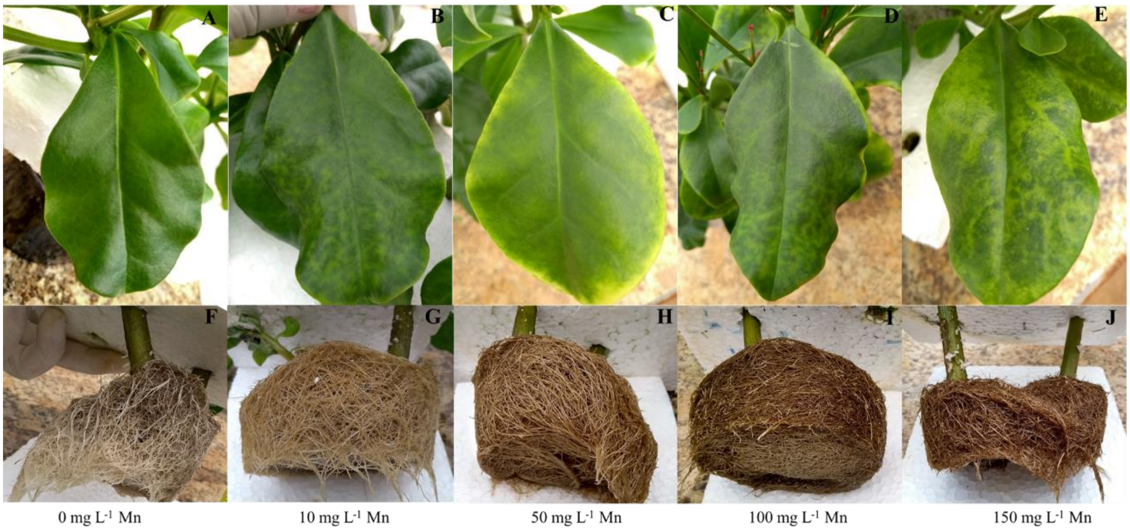
The first visual symptoms in plants were observed after 15 days of treatment imposition. In both cases, the most apparent symptom was leaf chlorosis, which did not occur in a generalized way, but only in some of the leaves of cuttings under excess Pb and Mn (Figures 2 and 3). In the case of roots, the main visual symptom observed was the darkening of the tissue under Mn excess (Figure 3).

Figure 2 – Visual symptoms of leaves (A-E) and roots (F-J) of *Talinum paniculatum* cuttings submitted to different concentrations of Pb (0, 50, 100, 150, 200 mg L^{-1}) for a period of 30 days.



Source: created by the author (2020).

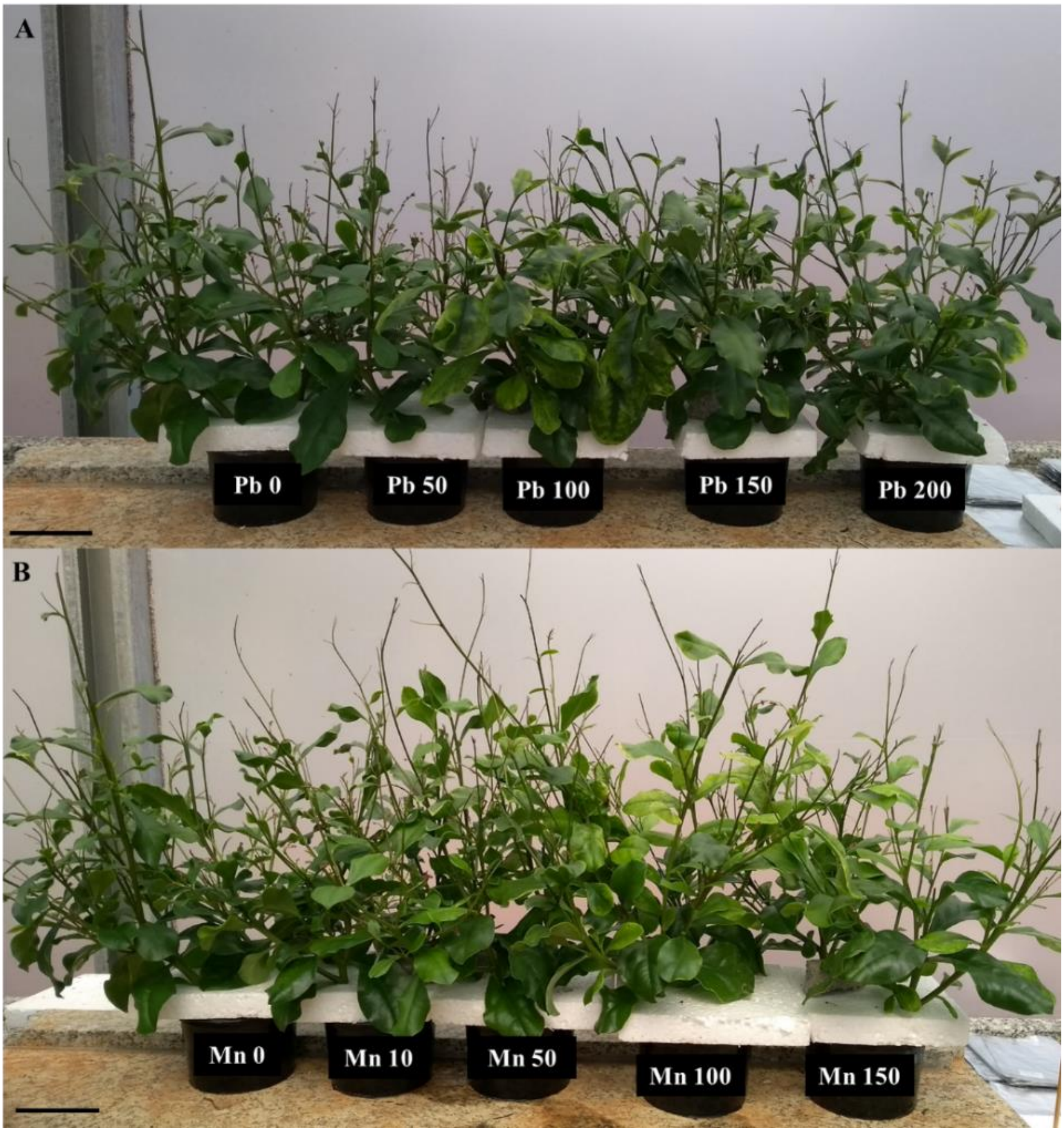
Figure 3 – Visual symptoms of leaves (A-E) and roots (F-J) of *Talinum paniculatum* cuttings submitted to different concentrations of Mn (0, 10, 50, 100, 150 mg L^{-1}) for a period of 30 days.



Source: created by the author (2020).

The general visual aspect of the plants at the end of the experiment, after 30 days of exposure to different concentrations of Pb and Mn, can be seen in Figure 4. It should be noted that the plant growth was not significantly affected by treatments when compared to the control despite the visual symptoms mentioned above.

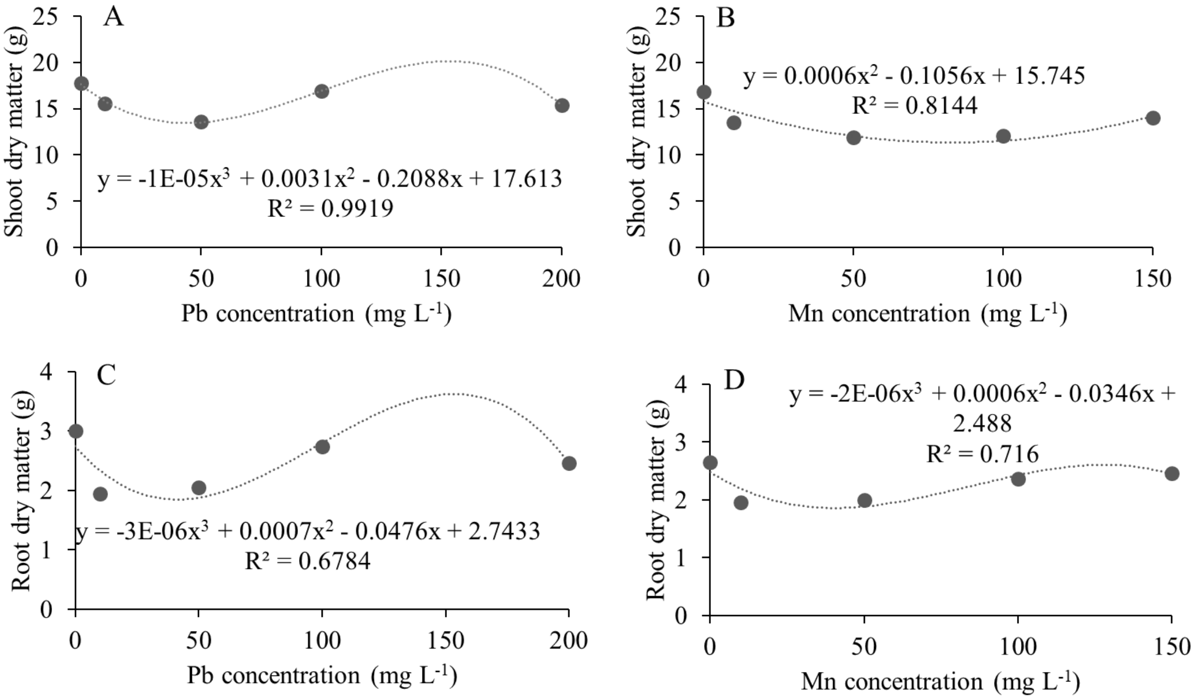
Figure 4 – Visual aspect of *Talinum paniculatum* cuttings submitted to different concentrations of Pb (0, 50, 100, 150, 200 mg L^{-1}) (A) and Mn (0, 10, 50, 100, 150 mg L^{-1}) (B) for a period of 30 days. The bar represents 10 cm.



Source: created by the author (2020).

Shoot and root dry biomass showed quadratic and cubic behavior. Shoot and root dry biomass of cuttings under Pb did not change, following a dry mass reduction trend, compared to the control (Figure 5A, C), as well as root dry biomass under Mn (Figure 5D) followed a third degree regression with a decrease in mass, followed by an increase from the concentration and again a downward trend at the highest concentration tested. Shoot dry biomass under Mn (Figure 5B) had a quadratic behavior with a decreasing hyperbole in the first treatments and a slight increase at the highest concentration. For Mn, there was a reduction in shoot and an increase in roots due to greater availability and absorption in the roots.

Figure 5 – Shoot (A, B) and root (C, D) dry biomass of rooted *Talinum paniculatum* cuttings grown under increasing concentrations of Pb and Mn, respectively, for a period of 30 days.



Source: created by the author (2020).

Under Pb and Mn excess, shoots showed higher contents of macro- and micronutrients than roots, except for Fe (Tables 1 and 2). Generally, nutrients content showed no specific dose-response to the increase in Pb concentrations (Table 1). In shoots, the most expressive variations occurred for P, K and Mg contents, once there was a decrease in P content and increase in K and Mg contents in response to the increase in Pb concentrations. In roots, Fe and Mn contents decreased in response to the increase in Pb concentrations.

Under Mn excess, the content of nutrients varied in response to the increase in Mn concentrations (Table 2). In shoots, the contents of N, P, K, Ca, Mg, S and B decreased with the increase in Mn concentrations. In roots, the contents of P, Fe and Zn increased in response to the increase in Mn concentrations in the nutrient solution.

Excess Pb and Mn influenced the concentrations of some nutrients (Tables 1 and 2). The concentrations of N, K, Ca, Mg, and B in the shoots increased with increasing Pb concentrations in the nutrient solution. In the roots, N, S, B, Fe, and Mn concentrations decreased with increasing Pb concentrations (Table 1).

With increased Mn concentrations, K increased and Ca, B, and Fe decreased in the shoots. In the roots, N, P, Fe, and Zn concentrations increased while S and B decreased with the increase in Mn (Table 2).

Table 1 – Mean concentrations of nutrients in the shoots and roots of rooted *Talinum paniculatum* cuttings grown under increasing concentrations of lead (Pb) for a period of 30 days.

	Pb concentration	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
		-------------	-------------	-------------	-------------	-------------	-------mg-------	-------------	-------------	-------------	-------------	-------------
Shoots	0	384.3 Aa	84.6 Ab	468.7 Ab	156.0 Ac	99.5 Ad	26.8 Aa	0.367 Ab	0.054 Aa	1.072 Ba	0.843 Aa	0.218 Aa
	10	399.4 Aa	86.3 Ab	647.4 Aa	245.4 Ab	113.4 Ac	21.0 Ac	0.468 Aa	0.059 Aa	0.604 Ba	0.762 Ba	0.255 Aa
	50	432.1 Aa	108.2 Aa	716.6 Aa	285.9 Aa	150.5 Aa	26.9 Aa	0.387 Ab	0.058 Aa	0.691 Ba	1.038 Aa	0.280 Aa
	100	405.0 Aa	84.9 Ab	704.4 Aa	259.1 Ab	149.2 Aa	23.8 Ab	0.469 Aa	0.062 Aa	0.800 Ba	0.962 Aa	0.266 Aa
	200	406.9 Aa	73.6 Ac	659.3 Aa	244.3 Ab	134.0 Ab	24.3 Ab	0.401 Ab	0.062 Aa	1.152 Ba	0.948 Aa	0.251 Aa
Roots	0	78.9 Ba	7.4 Bb	17.6 Ba	37.5 Ba	14.6 Ba	5.7 Bb	0.047 Ba	0.024 Bb	5.545 Ab	0.474 Bb	0.062 Ba
	10	45.1 Ba	15.2 Bb	13.2 Ba	42.2 Ba	10.7 Ba	4.5 Bb	0.028 Ba	0.017 Bb	4.799 Ab	1.205 Aa	0.048 Ba
	50	50.3 Ba	6.1 Bb	10.5 Ba	44.0 Ba	10.0 Ba	4.6 Bb	0.030 Ba	0.019 Bb	3.393 Ac	0.429 Bb	0.056 Ba
	100	74.3 Ba	23.9 Ba	17.1 Ba	67.3 Ba	16.1 Ba	8.3 Ba	0.042 Ba	0.038 Ba	8.126 Aa	0.601 Bb	0.097 Ba
	200	68.2 Ba	12.8 Bb	13.4 Ba	41.6 Ba	14.9 Ba	4.2 Bb	0.022 Ba	0.022 Bb	3.179 Ac	0.247 Bc	0.066 Ba

* Upper case letters compare the part of the plant (shoots and roots) and lower-case letters compare the concentrations in each collection time. Means followed by the same letter do not differ, according to the Scott Knott test ($p \leq 0.05$) ($n = 5$).

Source: created by the author (2020).

Table 2 – Mean concentrations of nutrients in the shoots and roots of rooted *Talinum paniculatum* cuttings grown under increasing concentrations of manganese (Mn) for a period of 30 days.

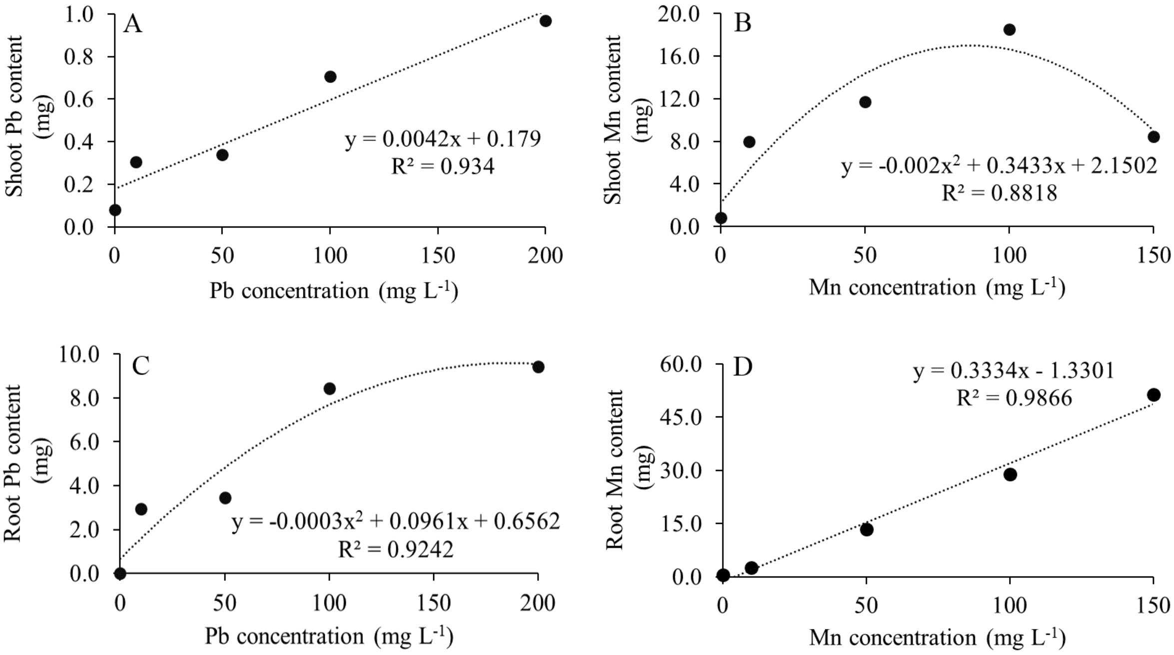
	Mn concentration	N	P	K	Ca	Mg	S	B	Cu	Fe	Zn
		mg									
Shoots	0	391.4 Aa	84.6 Aa	468.7 Aa	156.0 Aa	99.5 Ab	26.8 Aa	0.367 Aa	0.054 Ab	1.070 Ba	0.218 Aa
	10	355.3 Ab	81.8 Aa	515.4 Aa	147.0 Aa	112.8 Aa	23.3 Ab	0.349Aa	0.051 Ab	0.361 Bb	0.265 Aa
	50	294.9 Ac	73.2 Ab	488.7 Aa	133.6 Ab	112.2 Aa	24.0 Ab	0.299 Ab	0.053 Ab	0.329 Bb	0.248 Aa
	100	279.3 Ac	55.3 Ac	467.8 Aa	110.4 Ac	93.6 Ac	24.3 Ab	0.360 Aa	0.088 Aa	0.182 Bb	0.232 Aa
	150	281.4 Ac	58.0 Ac	437.6 Aa	95.0 Ad	78.7 Ad	19.0 Ac	0.207 Ac	0.057 Ab	0.262 Bb	0.260 Aa
Roots	0	78.9 Ba	7.4 Bc	17.6 Ba	37.5 Ba	14.6 Ba	5.7 Ba	0.047 Ba	0.024 Bb	5.545 Ac	0.062 Bc
	10	52.7 Ba	7.3 Bc	9.3 Ba	33.8 Ba	11.1 Ba	2.3 Ba	0.021 Ba	0.015 Bb	4.164 Ac	0.074 Bc
	50	53.6 Ba	14.1 Bb	12.3 Ba	35.9 Ba	9.8 Ba	3.2 Ba	0.019 Ba	0.048 Ba	8.108 Ab	0.110 Bb
	100	71.3 Ba	16.5 Bb	12.9 Ba	26.1 Ba	11.5 Ba	3.8 Ba	0.019 Ba	0.017 Bb	12.107 Aa	0.123 Bb
	150	82.9 Ba	21.5 Ba	17.5 Ba	31.6 Ba	15.7 Ba	4.6 Ba	0.027 Ba	0.021 Bb	12.238 Aa	0.173 Ba

* Upper case letters compare the part of the plant (shoots and roots) and lower-case letters compare the concentrations in each collection time. Means followed by the same letter do not differ, according to the Scott Knott test ($p \leq 0.05$) ($n = 5$).

Source: created by the author (2020).

Pb contents in shoots and roots increased with the increase in Pb concentrations in the solution, showing linear behavior for shoots and polynomial of second degree for roots (Figure 6 A, C). Mn content showed quadratic behavior for shoots, with an increase in the intermediary concentrations and a decrease in the higher Mn concentrations (Figure 6 B). In roots, Mn content increased linearly with the increase in Mn concentrations in the nutrient solution (Figure 6 D).

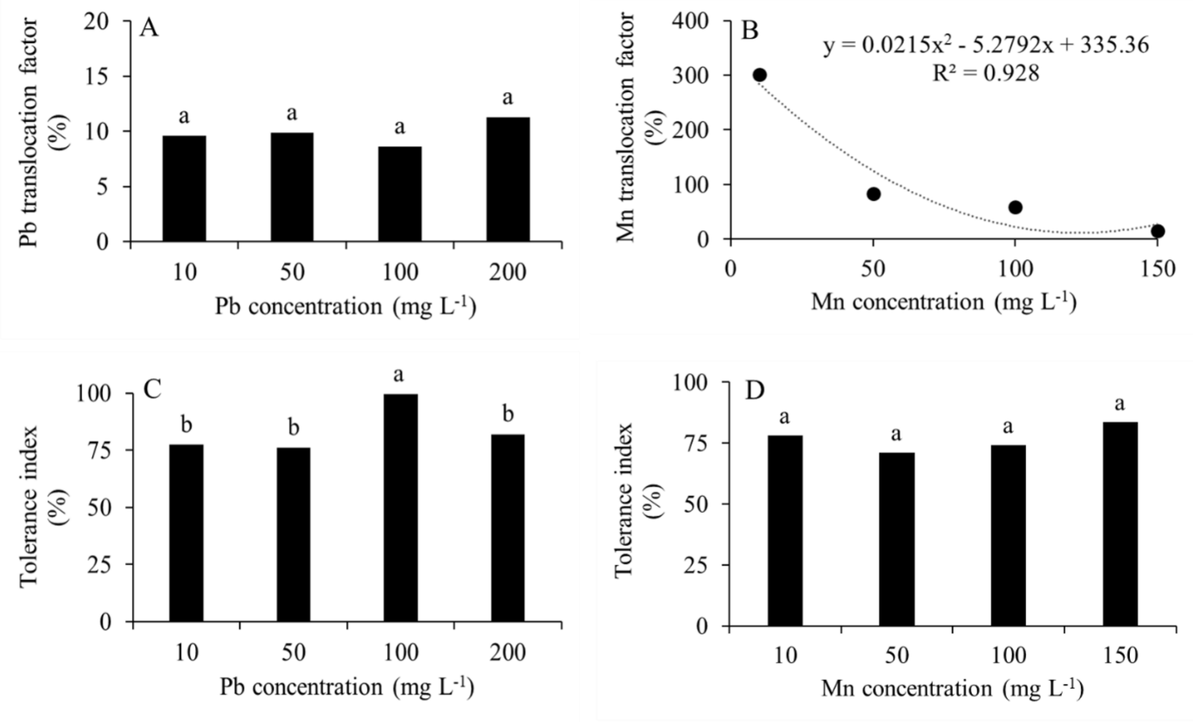
Figure 6 – Shoot and root contents of Pb and Mn of rooted *Talinum paniculatum* cuttings grown under increasing concentrations of Pb (A) and Mn (B), respectively, for a period of 30 days.



Source: created by the author (2020).

Pb translocation factor did not differ among Pb concentrations, remaining around 10% (Figure 7 A), while Mn translocation factor reduced with the increase in Mn concentrations in the solution (Figure 7 B). The cutting tolerance index for Pb and Mn was over 70%. In the case of excess Pb, a higher IT was observed at a concentration of 100 mg L^{-1} and there were no differences between the other concentrations (Figure 7 C). For Mn, the IT values were greater than 70% and there was no statistical difference between the different Mn concentrations (Figure 7 D).

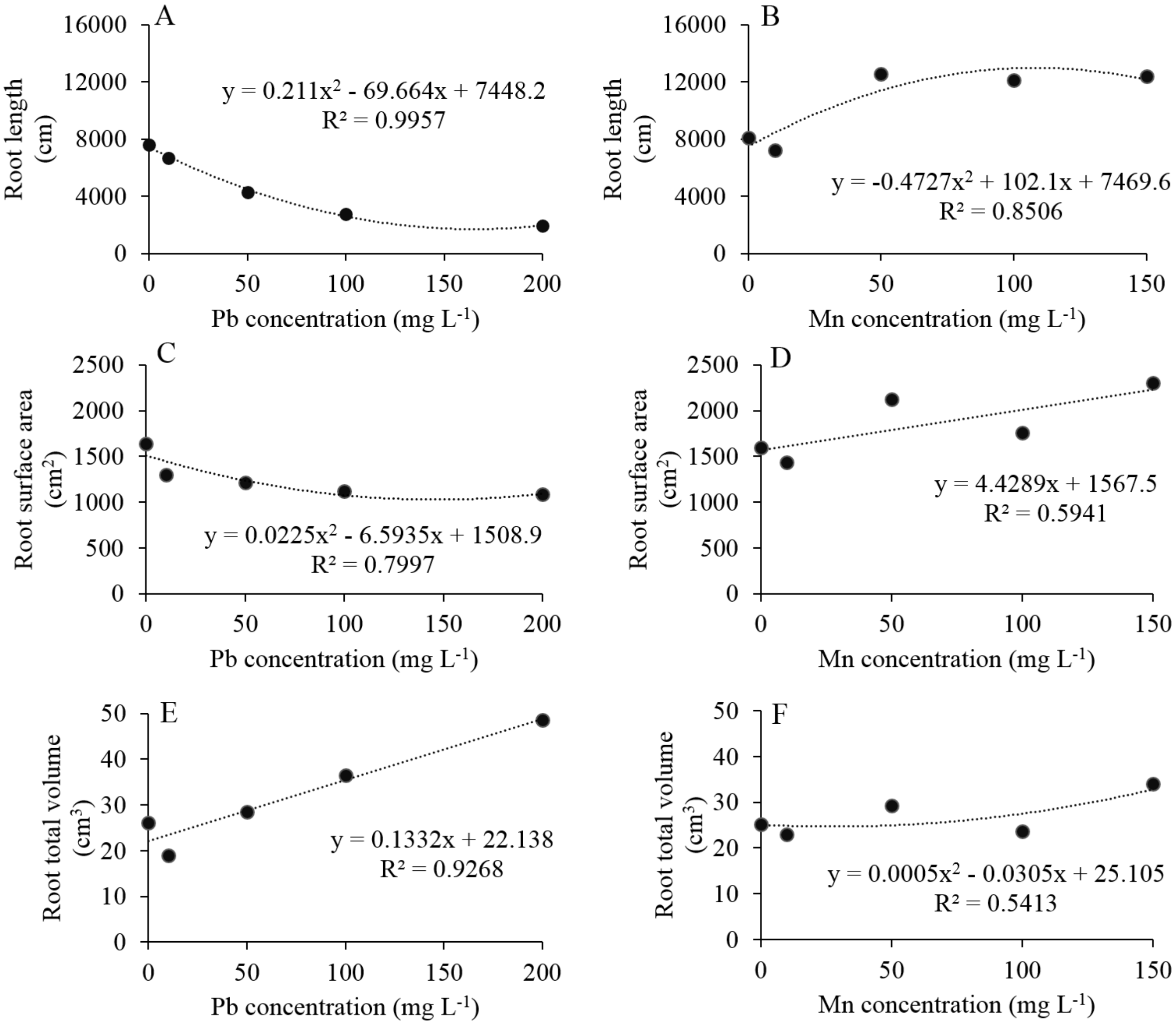
Figure 7 – Translocation factor and tolerance index of rooted *Talinum paniculatum* cuttings grown under increasing concentrations of Pb (A) and Mn (B), respectively, for a period of 30 days. Means followed by the same letter do not differ, according to the Scott Knott test ($p \leq 0.05$).



Source: created by the author (2020).

The results of root morphology are shown in Figure 8. With the increase in Pb concentrations, there was a tendency towards the reduction in root length and surface area with a second degree polynomial adjustment (Figure 8A, C), besides an increase in root volume with linear adjustment, making the roots become thicker and with a large volume (Figure 8E). In the case of increased Mn concentrations, there was an increasing tendency in length with second degree polynomial adjustment (Figure 8B), linear increase in surface area (Figure 8D) and increase in the total root volume with second degree polynomial adjustment (Figure 8F) for *T. paniculatum* plants. Cuttings that received Mn in solution in both analyzed root morphology parameters, showed an increase in the roots and decrease in the shoot.

Figure 8 – Root length (A-B), surface area (C-D) and total volume (E-F) of rooted *Talinum paniculatum* cuttings grown under increasing concentrations of Pb and Mn, respectively, for a period of 30 days.



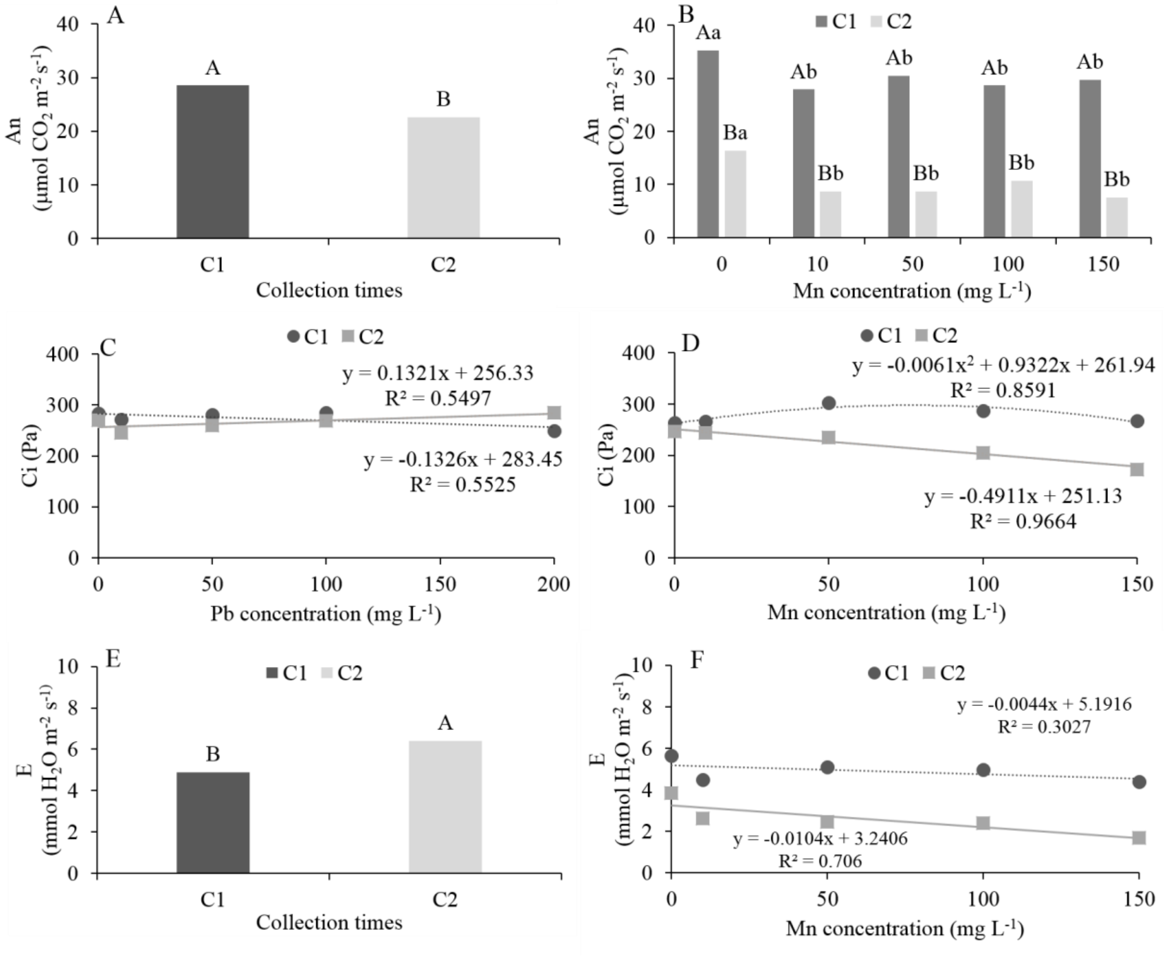
Source: created by the author (2020).

The net photosynthetic rate (An) was lower in collection 2 when compared to the collection 1 both for Pb and Mn treatments (Figure 9A, B). In addition, An was lower in cuttings of treatments with excess Mn than in the control for both collections. However, there was no variation of An in response to the different excess concentrations of Mn.

Gas exchange rates of Pb cuttings showed a reduction in the high concentrations in C1 and higher when compared to C2. Intercellular carbon concentration (Ci) of cuttings under Pb treatments showed a linear trend in both collections (Figure 9C), decreasing in collection 1 and increasing in collection 2.

In cuttings under Mn excess, there was a quadratic and linear behavior, respectively, in collections 1 and 2, both with a decreasing trend at the highest concentrations (Figure 9D). Regarding transpiration (E), it was higher in collection 2 than in collection 1 for cuttings under excess Pb (Figure 9E). Under excess Mn, E showed a linear behavior with a decreasing trend for both collections (Figure 9F).

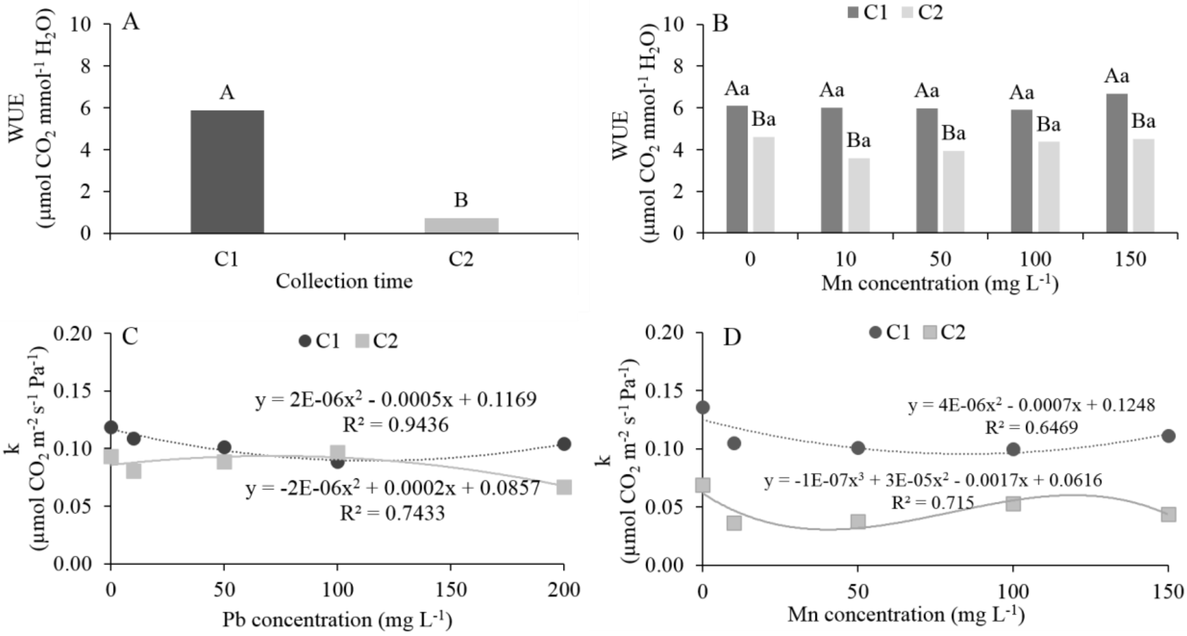
Figure 9 – Net photosynthetic rate (An, A-B), intercellular carbon concentration (Ci, C-D) and transpiration (E, E-F) of rooted *Talinum paniculatum* cuttings grown under increasing concentrations of Pb and Mn, respectively, for a period of 30 days. Means followed by the same letter do not differ, according to the Scott Knott test ($p \leq 0.05$). Upper case letters compare the collection times and lower case letters compare the concentrations in each collection time.



Source: created by the author (2020).

Water use efficiency (WUE, Figure 10 A-B) was higher in collection 1 than in collection 2 for cuttings grown under Pb and Mn. On the other hand, instant carboxylation efficiency (k) showed a second degree polynomial behavior in collection 1 for cuttings under excess Pb and Mn (Figure 10 C-D), with a lower k at intermediate concentrations. In collection 2, there was a second degree polynomial behavior in cuttings under excess Pb and a third degree polynomial behavior in those under excess Mn (Figure 9D).

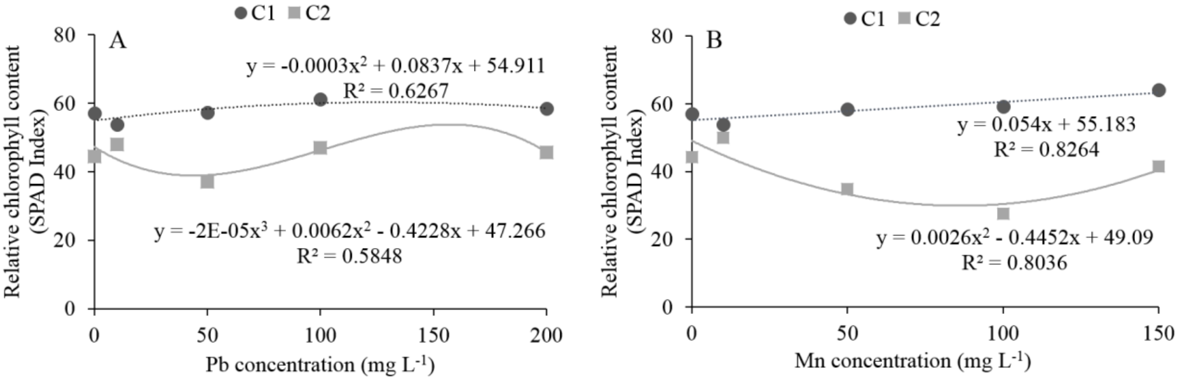
Figure 10 – Water use efficiency (WUE, A-B), and instant carboxylation efficiency (k, C-D) of rooted *Talinum paniculatum* cuttings grown under increasing concentrations of Pb and Mn, respectively, for a period of 30 days. Means followed by the same letter do not differ, according to the Scott Knott test ($p \leq 0.05$). Upper case letters compare the collection times and lower case letters compare the concentrations in each collection time.



Source: created by the author (2020).

The relative chlorophyll content (SPAD index) was higher in collection 1 than in collection 2. For cuttings grown under excess Pb, a second degree polynomial behavior was observed in collection 1 and a third degree polynomial behavior in collection 2 (Figure 11 A). In cuttings under excess Mn, there was a linear behavior with an increasing trend in collection 1 and a second degree polynomial behavior in collection 2 (Figure 11 B).

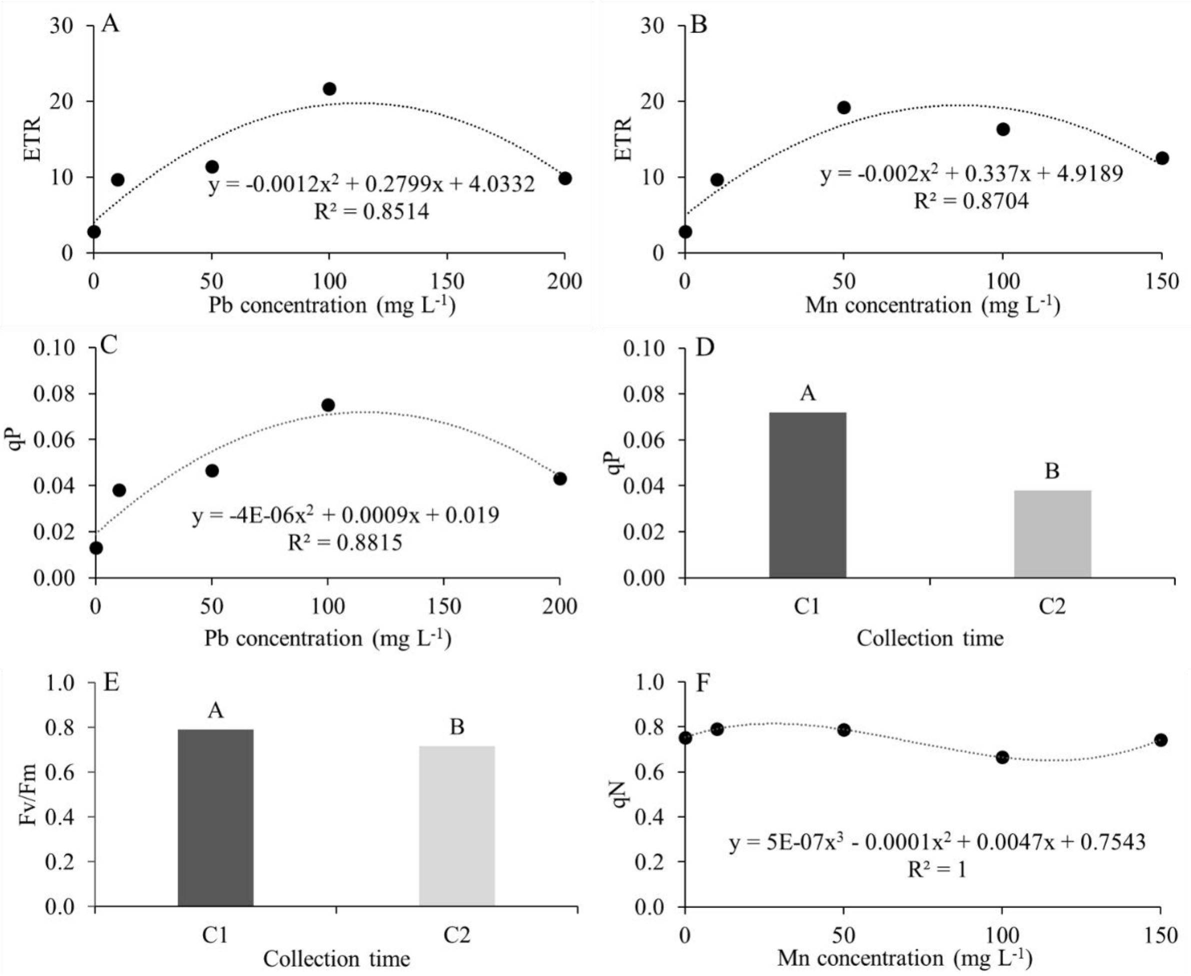
Figure 11 – Relative chlorophyll content (A, B) of rooted *Talinum paniculatum* cuttings grown under increasing concentrations of Pb and Mn, respectively, for a period of 30 days.



Source: created by the author (2020).

Regarding chlorophyll “a” fluorescence, there was only an isolated effect of the concentration of metals or collections for the analyzed variables. The electron transport rate (ETR) showed a second degree polynomial behavior for both metals, with higher values at intermediate concentrations (Figure 12 A, B). The same behavior was observed for photochemical quenching (qP) under excess Pb (Figure 12 C). Under excess Mn, qP was higher in collection 1 than in collection 2 (Figure 12 D). Under excess Pb, the maximum quantum yield of photosystem II (Fv/Fm) was higher in collection 1 than in collection 2 (Figure 12 E) and was not influenced by collections or Mn concentrations. Non-photochemical quenching showed a third-degree polynomial behavior under increasing concentrations of Mn (Figure 12 F), but was not influenced by collections or concentrations of Pb.

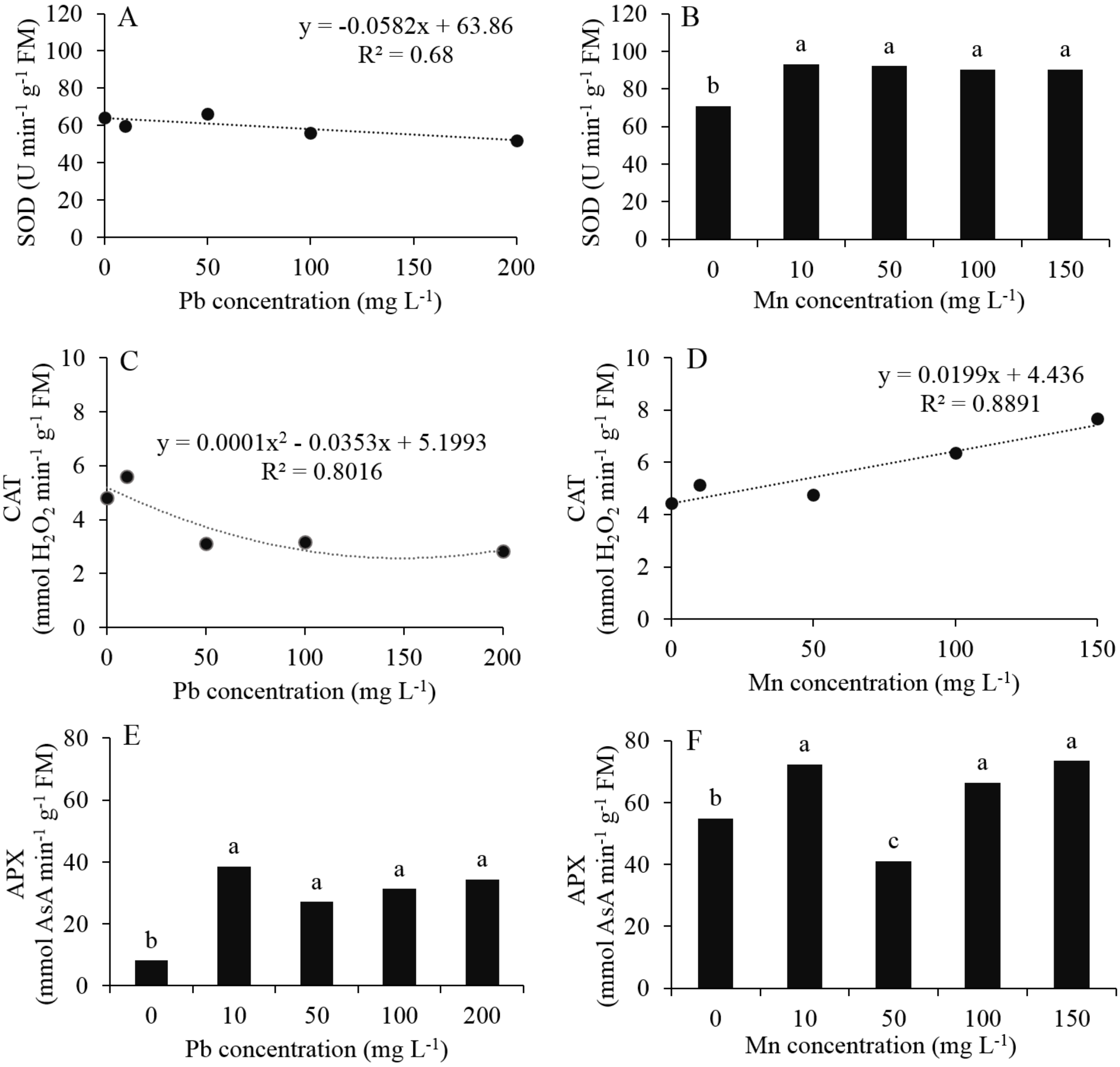
Figure 12 – Electron transport rate (ETR, A-B), photochemical quenching (qP, C-D), maximum quantum yield of photosystem II (Fv/Fm, E) and non-photochemical quenching (qN, F) of rooted *Talinum paniculatum* cuttings grown under increasing concentrations of Pb and Mn, respectively, for a period of 30 days. Means followed by the same letter do not differ, according to the Scott Knott test ($p \leq 0.05$). Upper case letters compare the collection times and lower case letters compare the concentrations in each collection time.



Source: created by the author (2020).

Considering the activity of the antioxidant system in the shoot, SOD activity presented a decreasing linear behavior under concentrations of Pb and of second degree under Mn (Figure 13A, B). CAT had a second-degree polynomial adjustment with a decreasing tendency at the highest Pb concentrations (Figure 13C) and a linear increase in activity with the increase in Mn concentrations (Figure 13D). APX activity showed a second-degree polynomial adjustment, with increased activity at the highest concentrations of Pb and Mn (Figure 13E, F).

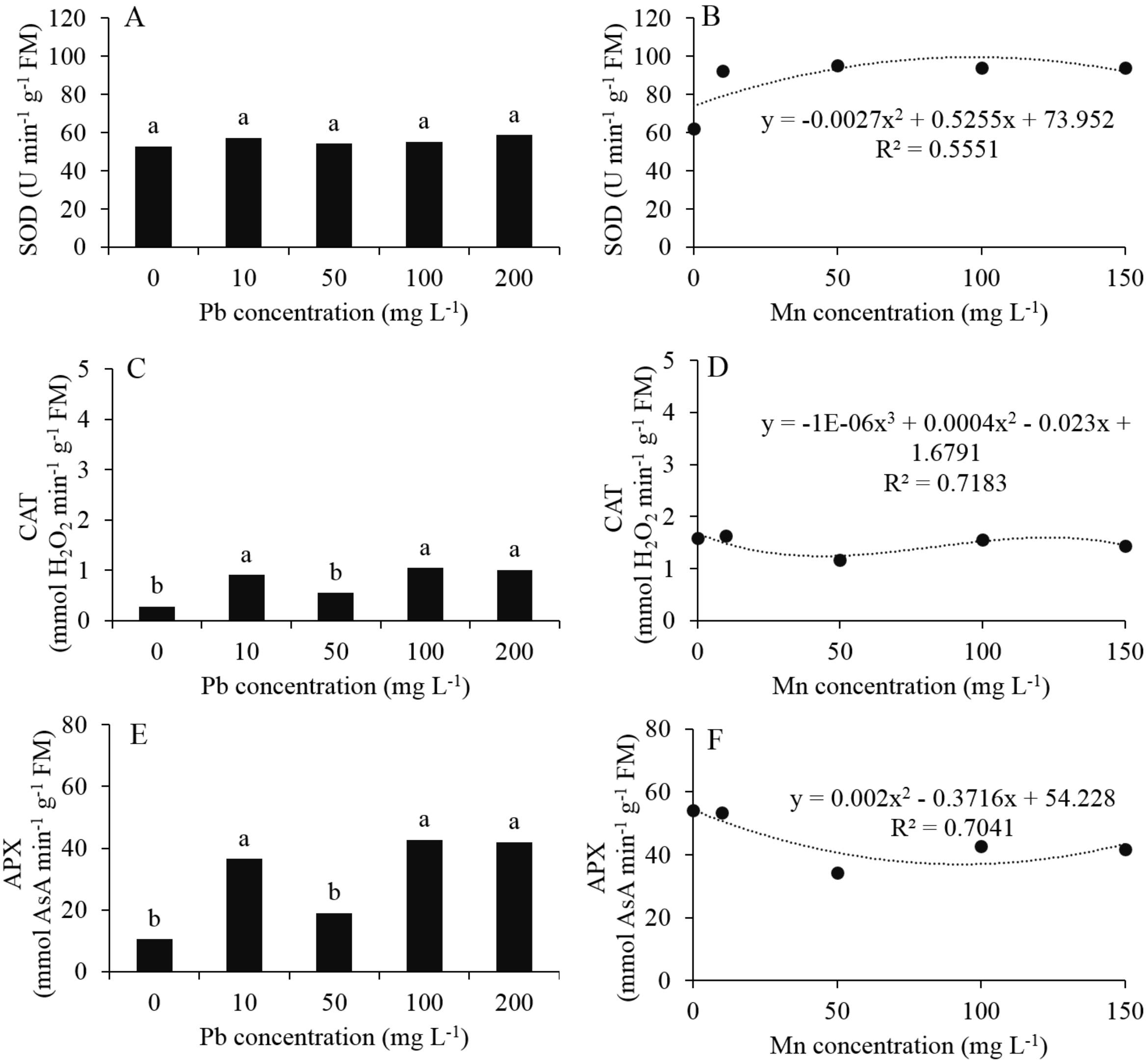
Figure 13 – Activity of antioxidant enzymes superoxide dismutase (SOD, A-B), catalase (CAT, C-D) and ascorbate peroxidase (APX, E-F) in leaves of rooted *Talinum paniculatum* cuttings grown under increasing concentrations of Pb and Mn, respectively, for a period of 30 days.



Source: created by the author (2020).

In roots, SOD activity did not vary with the increase in Pb concentrations and showed a second degree polynomial adjustment with the increase in Mn concentrations (Figure 14A, B). CAT activity had a second degree polynomial adjustment, showing an increasing tendency with the increase in Pb concentrations (Figure 14C). In the case of excess Mn, CAT activity showed a third degree polynomial tendency (Figure 14D). With regard to APX, a second degree polynomial adjustment was observed, presenting an increasing tendency in activity with an increase in Pb concentrations and a decreasing tendency in activity with increasing Mn concentrations (Figure 14E, F).

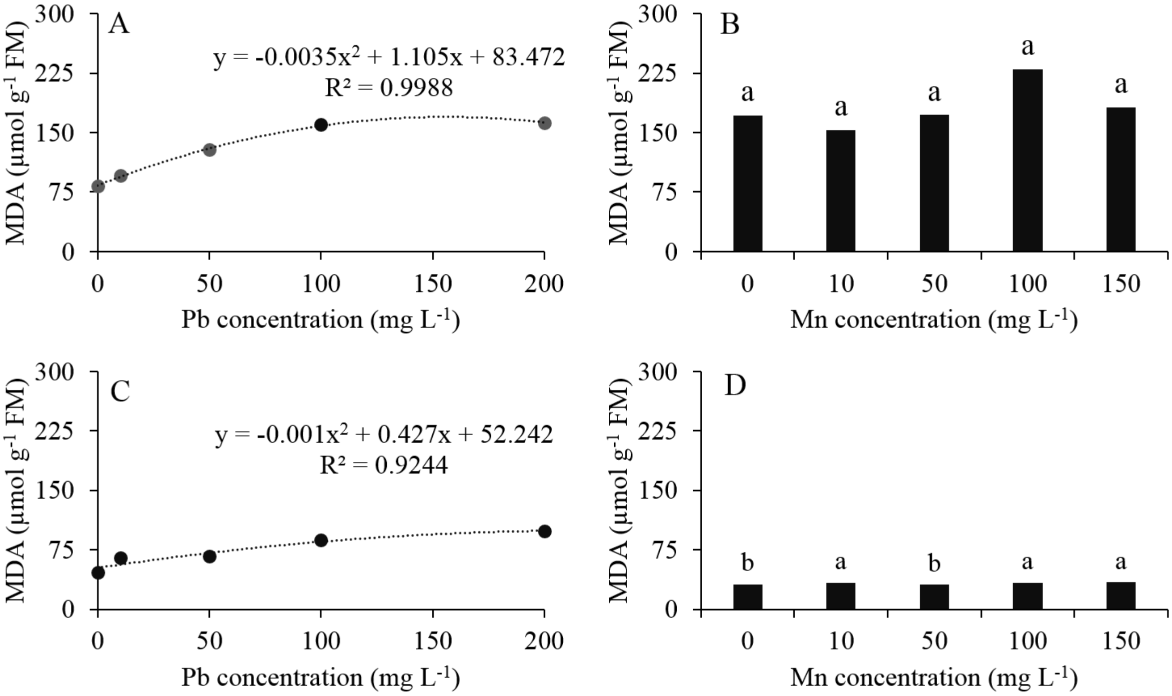
Figure 14 – Activity of antioxidant enzymes superoxide dismutase (SOD, A-B), catalase (CAT, C-D) and ascorbate peroxidase (APX, E-F) in roots of rooted *Talinum paniculatum* cuttings grown under increasing concentrations of Pb and Mn, respectively, for a period of 30 days.



Source: created by the author (2020).

Lipid peroxidation was measured by the concentration of malondialdehyde. In roots and shoot of plants under Pb, there was a second degree polynomial adjustment with an increase in lipid peroxidation in response to the increase in Pb concentrations (Figure 15A, C). On the other hand, there were no statistical differences between treatments in plant shoot due to the different concentrations of Mn (Figure 15B). In the roots, there was an increase in MDA at concentrations of 10, 100 and 150 mg L^{-1} Mn (Figure 15B).

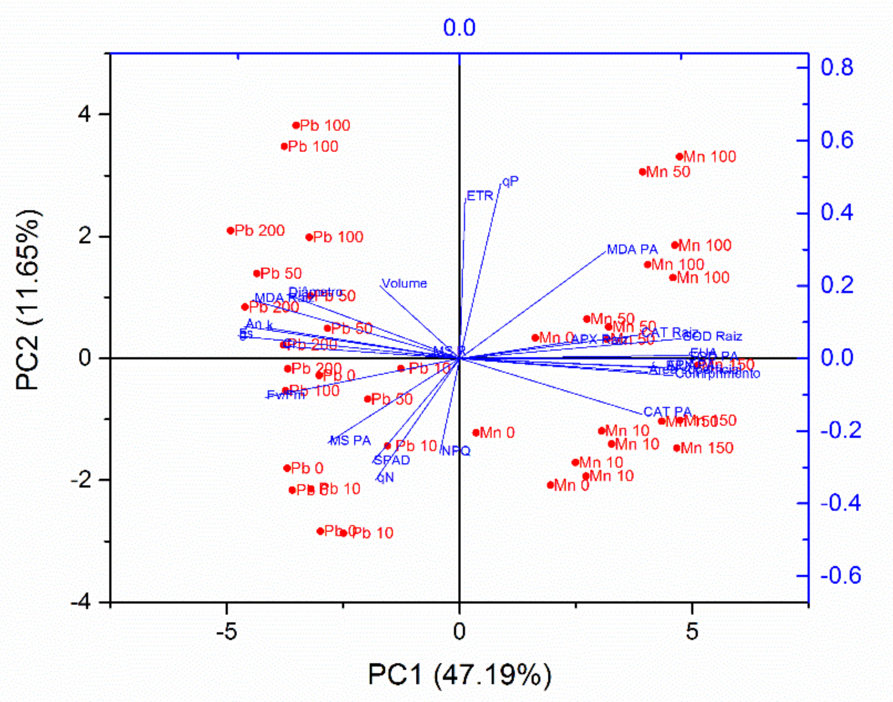
Figure 15 – Malondialdehyde (MDA) content produced in leaves (A-B) and roots (C-D) of rooted *Talinum paniculatum* cuttings grown under increasing concentrations of Pb and Mn, respectively, for a period of 30 days. Means followed by the same letter do not differ, according to the Scott Knott test ($p \leq 0.05$).



Source: created by the author (2020).

Principal component analysis showed that, although the cuttings have withstood the 30 days of treatment under different concentrations of Pb and Mn without major damage to their growth, the strategies used were different for each metal (Figure 16). The two principal components accounted for about 57% of data variation in this study and, while root morphology and antioxidant system activity were more related to the plant response to excess Mn, the most related variables under excess Pb were gas exchange and chlorophyll fluorescence.

Figure 16 – Principal component analysis relating the variables analyzed in this study (Shoot and root dry biomass, root morphology, gas exchange, chlorophyll “a” fluorescence, relative chlorophyll content, antioxidant system activity and lipid peroxidation) in *Talinum paniculatum* cuttings under increasing concentrations of Pb and Mn.



Source: created by the author (2020).

4 DISCUSSION

T. paniculatum cuttings containing six fully expanded leaves allowed the obtention of plants viable for the rhizofiltration of heavy metals. The use of nutrient solution optimized the rooting process, as well as the presence of fully expanded leaves may have contributed to the rapid cutting development. Despite the appearance of some symptoms related to leaf chlorosis and root darkening, the cuttings showed tolerance to excess metals for a period of thirty days. At the end of the experimental period, there was a 14% reduction in total plant dry biomass at the highest concentration of both metals in relation to the concentration of 0 mg L^{-1}. The species under study adopted different strategies to survive the excess of Pb and Mn, culminating in a high tolerance index, regardless of the metal concentration used. *Talinum paniculatum* has the potential for Pb and Mn rhizofiltration, which will be confirmed by the evaluation of metals in shoots and roots.

The use of cuttings in phytoremediation has already been described by Rajkumar *et al*. (2009), aiming to obtain cuttings with high rooting capacity to increase the absorption capacity of the metals present in the medium, where the rooting process was carried out in an aqueous medium and already in the presence of metals; this process was delayed by the increase in the concentration of metals and there was no formation of lateral roots. Even so, the rooted cuttings of *Talinum triangulare*, a species close to *T. paniculatum*, showed a high uptake of metals such as lead, nickel and cadmium in the medium. In this study, the use of the nutrient solution, optimized the rooting process and allowed the obtention of physiologically viable cuttings for metal absorption in a short time. Due to the large number of roots formed, *T. paniculatum* cuttings are expected to be able to absorb large amounts of the metals present in the solution. In addition, the presence of the nutrient solution allows the supply of nutrients that are essential for the maintenance of cutting growth and development.

The accumulation of dry biomass showed the same cubic behavior for shoots and roots under excess Pb, in which the highest values were found at a concentration of 100 mg L^{-1}, which culminated in a higher tolerance index for the plants at this metal concentration. On the other hand, under excess Mn, there was a decreasing tendency in relation to shoot dry biomass and increase in that of roots, which allowed the maintenance of the species tolerance index regardless of the applied Mn concentration. The trend of maintaining the tolerance index even at the highest metal concentrations can be considered as an indicative of the species tolerance to this metal (KUMAR; PRASSAD; SYTAR, 2012).

Certain plant species absorb the heavy metals present in the soil or water, as long as they are at low concentrations or available with other minerals and nutrients essential for plant development. The ability of these metals to accumulate in plant biomass allows them to be characterized by their phytoremediation potential due to the ability to survive and develop despite the presence of metals (GONG; ZHAO; WANG, 2018). *T. triangulare* plants have already been described as potential for rhizofiltration due to their ability to bioaccumulate metals both in aqueous solution (RAJKUMAR *et al*., 2009), as well as in contaminated solid medium (EZE, 2014). In these cases, there is a tendency of greater metal accumulation in the roots than in the shoot of these plants.

T. paniculatum cuttings showed tolerance in certain parameters analyzed under excess Mn. Since it is a micronutrient, Mn plays a vital role in photosynthesis and respiration (INOSTROZA-BLANCHETEAU *et al*., 2017). This is due to its role in the center of evolution of oxygen and action as an enzymatic cofactor (RODRIGUES *et al*., 2016; ZAMBROSI *et al*., 2016). However, when in excess, Mn can affect the production of essential metabolites, limit biomass growth and accumulation, reducing photosynthesis, chlorophyll and even leading to changes in the structure of chloroplasts (CARDOSO; NAVARRO; NOGUEIRA, 2003; ZAMBROSI *et al*., 2016).

In *Citrus grandis* and *Citrus sinensis* plants, Mn toxicity (0,5 mM) decreased CO_2 assimilation, stomatal conductance and transpiration rate, but had no influence on intercellular CO_2 concentration (YOU *et al*., 2017). As in this study, the photosynthetic rate, transpiration, intercellular carbon concentration, water use efficiency (WUE), instant carboxylation efficiency and SPAD index showed a reduction curve compared to the control. However, between Mn concentrations, there were no expressive variations. The drop in gas exchange rates for Mn is assigned to oxidative stress, due to the higher production of ROS, which directly affect photosystem II (SHOLER *et al*., 2014). A common response of plants to neutralize reactive oxygen species generated by excess metals is the action of the antioxidant system. Thus, there is the action of enzymes such as ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD), which neutralize excess ROS, reducing oxidative stress (SHARMA; DUBEY, 2005). Plants under excess Mn showed higher responses of antioxidant enzymes in the shoot, showing only increased activity of catalase in the roots. This better performance of the antioxidant system was sufficient to avoid oxidative stress in the shoot of these plants, but an increase in root lipid peroxidation was observed. Even so, there was a reduction in shoot biomass and a tendency to increase root biomass; the increased Mn in the plant allows greater investment of this metal in the roots, also showing that there was a certain efficiency of the antioxidant system in preventing the damage resulting from the excess of manganese in these cuttings.

Soybean plants under excess Mn in a period of 30 days showed damage to the metabolism and physiology of shoot and roots (SANTOS *et al*., 2017), at lower concentrations (2, 10, 100, 200 and 300 mM) than those used in this study. Symptoms such as marginal chlorosis in the leaves are mild symptoms of excess manganese and are related to the decrease in chlorophyll production in response to excess Mn. The appearance of these symptoms shows that the action of the antioxidant system was essential to reduce the damage caused by excess Mn in *T. paniculatum*, allowing its survival for 30 days in treatments with only mild toxicity symptoms.

The excess of Pb causes severe damage to plant physiology and biochemistry, presenting high toxicity to plants by inhibiting the activity of enzymes, reducing photosynthesis, altering water balance and mineral nutrition. Pb also changes the hormonal state, affects the structure and permeability of the cell membrane, in addition to causing visible symptoms such as reduced growth, chlorosis and darkening of the root system (SHARMA; DUBEY, 2005).

In treatments with *T. paniculatum* cuttings, it can be observed that the presence of lead with the nutrient solution reduced shoot and root dry matter; this factor is linked to the ability of this element to inhibit plant mineral nutrition (LU; ZHANG; SHAN, 2005). In *T. triangulare* plants, Pb (0, 0.25, 0.5, 0.75, 1.0, and 1.25 mM) can easily accumulate in plant tissues causing phytotoxicity and oxidative stress, resulting in a decrease in gas exchange, chlorophyll fluorescence and mechanisms of photosystem II, reducing dry matter accumulation (KUMAR; PRASAD, 2015). It is noteworthy that despite this, the metal increase did not change the accumulation in the roots, being an important mechanism to immobilize or accumulate the metals inside the plant in the short to medium term, an important factor for rhizofiltration.

At high concentrations, both Pb and Mn are harmful to the photosynthetic apparatus of plants, compromising the performance of photosystem II. Kumar and Prasad (2015) considered the fluorescence parameters of chlorophyll “a” as markers of Pb stress in *T. triangulare*. Chlorophyll fluorescence was one of the parameters that suffered the least influence from the treatments applied, indicating that, for *T. paniculatum*, these parameters are not good stress indicators for Pb and Mn.

In cuttings under excess Pb, there was no reduction in gas exchange rate or stomatal conductance compared to the control, but lipid peroxidation increased with the increase in Pb concentrations. This may be due to the exposure time of the cuttings and the use of nutrient solution, once Pb induces the production of ROS and increases lipid peroxidation and oxidative degradation of plant metabolism (ASADA, 2006). In rice cultivars with concentrations of 0,5 and 0,1 mM $Pb(NO_3)_2$ in a growth period of 5-20 days, there was a significant increase in lipid peroxidation, indicating that Pb induces oxidative stress in these plants (VERMA; DUBEY, 2003).

Pb ions induce lipid peroxidation, decrease the level of saturated fatty acids and increase the content of unsaturated fatty acids in the membrane in various plant species (KOHLI *et al*., 2020). In experiments with *Pluchea sagittalis*, the increase in the concentration of Pb (0, 0,2, 0,4, 0,6, 1 mM) led to a higher generation of H_2O_2 and an increase in lipid peroxidation, which led to a decrease in root and shoot dry matter. However, the concentration of chlorophyll and carotenoids was not affected (ROSSATO, 2010).

In plants, Pb is easily absorbed by the tissues in the roots together with essential nutrients linked to Pb in the form of cations (SILVA; SANTOS; SOUZA GUILHERME, 2015). The presence of Pb contributes to homeostasis breakdown, causing damage to cellular components or even to the DNA. In *T. paniculatum* cuttings, an increased activity of APX, CAT and SOD was detected in the roots, while APX activity increased only in the shoot. In *T. triangulare* plants under excess Pb, the essential role of the ascorbate-glutathione cycle was observed, mainly of reduced and oxidized glutathione in the protection against oxidative stress (KUMAR *et al*., 2014), which may also be related to a higher APX activity in *T. paniculatum*. In the special case of *T. triangulare* roots, there was an increase in the activity of the enzymes SOD, CAT and APX, but it was not sufficient to prevent the peroxidation of membrane lipids and the oxidation of proteins (KUMAR *et al*., 2013).

The toxic effect of Pb and its deleterious effects on plant physiology and biochemistry present several physical symptoms such as green and dark leaves, wilting of older leaves and shoot, besides darkening of low-volume, underdeveloped and brown roots, as well as marginal chlorosis (AUGUSTO *et al*., 2015). In *T. triangulare*, the excess of Pb increased root porosity, caused damage to cell membranes, in addition to rupture of the xylem, with reduced transport of water and nutrients to the shoot (KUMAR *et al*., 2013). Pb damage to roots was also observed for *T. paniculatum* cuttings in this study, characterized by a reduction in root length, surface area and volume. Despite the response of the antioxidant system, it was not sufficient to prevent damage to root tissues, resulting in a reduction in root parameters.

Despite the variation in physiological parameters, cuttings under excess Pb and Mn survived for 30 days under such conditions, showing mild symptoms of toxicity by metals. This suggests a species tolerance to these metals, which can act in rhizofiltration for both. The quantification of metals in shoot and roots will provide information on the absorption capacity and their likely of metals and their probable phytoextractor in shoot or roots.

5 CONCLUSION

Talinum paniculatum is tolerant to excess Pb and Mn, responding with an increase in the activity of the antioxidant system. The use of the nutrient solution assists in the rooting process and cutting initial development. *T. paniculatum* cuttings have potential for use in Pb and Mn rhizofiltration.

Besides the rapid cutting rooting and growth, *Talinum paniculatum* rooted cuttings absorb high amount of Pb and Mn, which is stocked mainly in roots. The tolerance mechanisms of *T. paniculatum* are different for Pb and Mn, but in both cases allow plants to survive under those conditions. Talinum paniculatum leaves open the way and interest to extrapolate the mechanisms of tolerance to heavy metals for further studies and also using other plant species under excess of these metals.

REFERENCES

ANKIT, T. J., Ecorestoration of Polluted Aquatic Ecosystems Through Rhizofiltration. **Phytomanagement of Polluted Sites**, Netherlands, v. 10, p. 179-201, 2019.

ANDRADE, M. *et al*. **Heavy metals in soils of a lead mining and metallurgy area.I – Phytoextraction**, Revista Brasileira de Ciência do Solo, Rio Grande do Sul, v. 33, p. 1879-1888, 2009.

ANTONIADIS, V. *et al*. Trace elements in the soil-plant interface: Phytoavailability, translocation, and phytoremediation–A review. **Earth-Science Reviews**, Netherlands, v. 171, p. 621-645, 2017.

ARNON, D.I. Copper enzyme in isolated chloroplast polyphenoloxid. **Plant Physiology**, United Kingdom, v. 24, p. 1-15, 1949.

ASADA. K., Production and scavenging of reactive oxygen species in chloroplasts and their functions. **Plant Physiology**, United States, v. 141, p. 391-396, 2006.

AUGUSTO, A. *et al.* Avaliação dos efeitos tóxicos de Cd e Pb na cultura da mostarda (*Brassica juncea*), **Engenharia Sanitária e Ambiental**, Rio de Janeiro, v., 19,p. 61-68, 2015.

BARBOSA *et al*., Geração e desintoxicação enzimática de espécies reativas de oxigênio em plantas. **Ciência Rural**, Brazil, v. 44, p. 453-460, 2014.

CARDOSO, E. J. B. N., NAVARRO, R. B., NOGUEIRA, M. A., Absorção e translocação de manganês por plantas de soja micorrizadas, sob doses crescentes deste nutriente. **Revista Brasileira de Ciência do Solo**, Brazil, v.21, p. 415-423, 2003.

BIEMELT, S.; KEETMAN, U.; ALBRECHT, G. Re-aeration following hypoxia or anoxia leads to activation of the antioxidative defense system in roots of wheat seedlings. **Plant Physiology**, United States, v. 116, p. 651-658, 1998.

BRASIL. Ministério Meio Ambiente. Conselho Nacional do Meio Ambiente. **Resolução Conama de nº 430, de 13 de maio de 2011**. p. 89, 2011a. Disponívelem: http://www2.mma.gov.br/port/conama/legiabre.cfm?codlegi=646. Acesso em: 25 out. 2020.

BUEGE, J. A.; AUST, S. D. Microsomal Lipid Peroxidation. **Methods in Enzymology**, United States, v. 52, p. 302-310, 1978.

EZE M. O., Evaluation of Heavy Metal Accumulation in talinum triangulare grown around Municipal solid waste dumpsites in Nigeria. **BEPLS**, v. 4, p. 92-100, 2014.

INOSTROZA-BLANCHETEAU, Claudio *et al*. Physiological and biochemical responses to manganese toxicity in ryegrass (Lolium perenne L.) genotypes. **Plant Physiology And Biochemistry**, France, v. 113, p.6-19, 2017.

GALAL, T., M. *et al*., Bioaccumulation and rhizofiltration potential of Pistia stratiotes L. for mitigating water pollution in the Egyptian wetlands. **International Journal of Phytoremediation**, United Sates, v. 20, p. 440-447, 2018.

GREGER, M. Phytoremediation - Does it work? In**:** International conference on the biogeochemestry of trace elements, Uppsala-Sweden**: SLU Service**, p.10-11, 2017.

GIANNOPOLITIS, C. N.; RIES, S. K. Superoxide dismutases: I. Occurrence in higherplants. **Plant physiology**, United States v. 59, p. 309-314, 1977.

GILL, S. S.; TUTEJA, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. **Plant physiology and biochemistry**, France, v. 48, p. 909–930, 2010.

GONG, Y., ZHAO, D. WANG, Q., An overview of field-scale studies on remediation of soil contaminated with heavy metals and metalloids: Technical progress over the last decade. **Water Research,** United Kingdom, v. 147, p. 440-460, 2018.

HAVIR, E. A; MCHALE, N. A. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. **PlantPhysiol**, United States, v. 84, p. 450-455, 1987.

KABATA-PENDIAS, A; PENDIAS, H. **Trace elements in soils and plants**. Library ofCongress Cataloging, 3 ed., p. 331, 2000.

KOHLI, S. K., *et al*. Current Scenario of Pb Toxicity in Plants: Unraveling Plethora of Physiological Responses. **Reviews of Environmental Contamination and Toxicology**, United States, v.249, p. 153-197, 2020.

KUMAR, A.; PRASAD, M. N. V.; SYTAR, O. Lead toxicity, defense strategies and associated indicative biomarkers in Talinum triangulare grown hydroponically. **Chemosphere**, United Kingdom, v. 89, p. 1056-1065, 2012.

KUMAR, A. *et al*. Elucidation of lead-induced oxidative stress in Talinum triangulare roots by analysis of antioxidant responses and DNA damageat cellular level. **Environmental Science and Pollution Research**, Germany, v. 20, p. 4551-4561, 2013.

KUMAR, A., MAJETI, N., Proteomic responses to lead-induced oxidative stress in Talinum triangulare Jacq. (Willd.) roots: Identification of key biomarkers related to glutathione metabolisms. **Environmental Science and Pollution Research**, Germany, v. 21, p. 8750-8764, 2014.

KUMAR, A.; PRASAD, M. N. V. Lead-induced toxicity and interference in *chlorophyll fluorescence* in *Talinum triangulare* grown hydroponically. **Photosynthetica**, Czechia, v. 53, p. 66-71, 2015.

LU, ZHANG, SHAN. Time effect on the fractionation of heavy metals in soils. **Geoderma**, Netherlands, v. 125, p. 225-234, 2005.

MALAR, S., *et al*., Effect of lead on phytotoxicity, growth, biochemical alterations and its role on genomic template stability in Sesbania grandiflora: A potential plant for phytoremediation. **Ecotoxicology and Environmental Safety**, Netherlands, v. 108, p. 249-257, 2014.

MACHADO, F. H. O.; MELO, J. I. M.; SALES, M. F. **Flora da região de Xingó, Alagoas-Sergipe: Portulacaceae sensu lato**. Biotemas, v. 25, p.103-108, 2012.

MARQUES, M.; CHAFIM AGUIAR C. R.; SILVA J. J. L. S. Desafios técnicos e barreiras sociais, econômicas e regulatórias na fitorremediação de solos contaminados. **Revista Brasileira de Ciência do Solo**, Brazil, v. 35, p. 1-11, 2011.

MORAES, S C., TEIXEIRA, A. M., 2014. **Guia de elaboração de planos de intervenção para o Gerenciamento de áreas contaminadas**. Instituto de Pesquisas Tecnológicas do Estado de São Paulo S.A. – IPT, P. 157-245.

NAKBANPOTE, Woranan; MEESUNGNOEN, O., PRASAD, M.N.V. **Potential of ornamental plants for phytoremediation of heavy metals and income generation**. Elsivier Inc., Russia: Ural Federal University, Bioremediation and Bioeconomy, 2016, cap. 9, p. 179-217.

NAKANO, Y.; ASADA, K. **Hydrogen peroxide is scavenged by ascorbato-specific peroxidase in spinach chloroplasts.** Plant and Cell Physiology, v.22, p. 867-880, 1981.

PIO, M. C. S., ANTONY, L. P., SANTANA, G. P., Wetlands Construídas (Terras Alagadas): Conceitos, Tipos e perspectivas para remoção de metais potencialmente tóxicos de água contaminada. **Scientia Amazonia**, Brazil, v. 2, p. 28-40, 2013.

RAJKUMAR, K. *et al*. Effects of selected heavy metals (Pb, Cu, Ni, and Cd) in the aquatic medium on the restoration potential and accumulation in the stem cuttings of the terrestrial plant, *Talinum triangulare* Linn. **Ecotoxicology**, United States, v. 18, p. 952-960, 2009.

RASCIO, N., NAVARI, F., Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? **Plant Science**, United Kingdom, v. 180, p.169-181, 2011.

ROSSATO, L. V., 2010. **Efeitos bioquímicos e fisiológicos do chumbo em plantas de quitoco (pluchea sagittalis): possível papel fitorremediador** - Dissertação - Universidade Federal de Santa Maria, Biological Sciences: Toxicological Biochemistry Post-Graduation, 83p.

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

SARWAR, N.; IMRAN, M.; SHAHEEN, M, R. Phytoremediation strategies for soilscontaminated with heavy metals: Modifications and future perspectives. **Chemosphere**, United Kingdom, v. 171, p. 710-721, 2017.

SCAFIDI, F.; RAIMONDO, F. M. Principi di spontaneizzazione in Sicilia di. **Quaderni di Botanica ambientale e applicata**, Italy, v. 26, p. 23-26, 2017.

SILVA, A.; VITTI, G.; BARROCAS, P.; SILVANA, J.; MOREIRA, J. Dietary intake and health effects of selected toxic elements. **Brazilian Journal of Plant Physiology**, Brazil, v. 17, p. 79-93, 2005.

SILVA, E., SANTOS, P., SOUZA GULHERME, M., Lead in Plants: a Brief Review of Its Effects, Mechanisms Toxicological and Remediation. **Agrarian Academy**, Brazil, v.2, p. 1-20, 2015.

SHARMA, P.; DUBEY, R. S. Pb toxicity in plants. **Brazilian Journal Plant Physiology**, Brazil, v. 17, p. 35–52, 2005.

SHOLER, A. *et al*. Biogenic manganese-calcium oxides on the cell walls of the algae chara corallina: elemental composition, atomic structure, and water-oxidation catalysis. **European Journal of Inorganic Chemistry**, Germany, n.4, p.780–790, 2014.

TAVARES, S. R. L.; OLIVEIRA, S. A.; SALGADO, C. M. Avaliação de Espécies Vegetais na Fitorremediação de Solos Contaminados por Metais Pesados. **HOLOS**, Portuguese, v. 5, p. 80-97, 2013.

VAN OOSTEN M.J., PEPE, O., DE PASCALE S. *et al*. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. – **Chemical and Biological Technologies in Agriculture**. Germany, v. 4, p. 1-12, 2017.

VERMA. S., DUBEY. R. S., Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. **Plant Science**, Ireland, v. 164, p. 645-655, 2003.

WAN, X.; LEI, M.; CHEN, T. Cost-benefit calculation of phytoremediation technology for heavy-metal-contaminated soil. **Science of the Total Environment**, Netherlands, v. 563-564, p. 796-802, 2016.

LI, Y. *et al*. Cadmium accumulation, activities of antioxidant enzymes, and malondialdehyde (MDA) content in Pistia stratiotes L. **Environmental Science and Pollution Research**, Germany, v. 20, p. 1117-1123, 2013.
YOU, X. *et al*., Long-term manganese-toxicity-induced alterations of physiology and leaf protein profiles in two Citrus species differing in manganese-tolerance. **Journal of Plant Physiology**, Germany, v. 218, p. 249-257, 2017.

ZAMBROSI, F. C. B. *et al*. Anatomical and physiological bases of sugarcane tolerance to manganese toxicity. **Environmental and Experimental Botany**, United Kingdom, v. 132, p. 100-112, 2016.

Table S1 – Concentration of nutrients and lead in shoots and roots of rooted *Talinum paniculatum* cuttings grown under increasin concentrations of Pb for a period of 30 days.

	Pb concentration	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn	Pb
		--------------------------------mg g^{-1}------------------------------						---------------------------------mg kg^{-1}---------------------------------					
Shoots	0	22.9 Bc	5.0 Ab	27.9 Ac	9.3 Bc	5.9 Ac	1.6 Bb	21.8 Ab	3.2 Ba	63.9 Ba	50.0 Ba	12.9 Ba	4.9 Ac
	10	24.0 Ac	5.1 Ab	41.4 Ab	14.6 Bb	7.9 Ab	1.3 Bb	27.7 Aa	3.8 Ba	47.1 Ba	49.2 Ba	16.3 Ba	17.8 Bc
	50	31.8 Aa	8.0 Aa	52.9 Aa	21.0 Aa	11.1 Aa	2.0 Aa	28.4 Aa	4.3 Ba	50.7 Ba	76.3 Ba	20.6 Aa	25.0 Bc
	100	25.1 Ab	5.0 Bb	41.7 Ab	15.3 Bb	8.6 Ab	1.4 Bb	27.8 Aa	3.7 Ba	47.4 Ba	56.9 Ba	15.7 Ba	43.4 Bb
	200	26.6 Ab	4.8 Ab	39.1 Ab	15.2 Ab	8.2 Ab	1.6 Ab	26.2 Aa	4.1 Ba	67.0 Ba	61.8 Ba	16.3 Ba	59.8 Ba
Roots	0	29.8 Aa	2.8 Bc	6.6 Ba	14.1 Ab	5.5 Aa	2.1 Ab	18.0 Ba	9.1 Ab	2091.0 Ab	179.1 Ac	23.3 Aa	1.8 Ad
	10	25.1 Ab	6.7 Ab	6.3 Ba	24.2 Aa	5.5 Ba	2.3 Ab	16.3 Bb	8.6 Ab	2051.1 Ab	495.4 Aa	28.2 Aa	1644.2 Ac
	50	24.5 Bb	3.5 Bc	5.1 Ba	21.5 Aa	4.5 Bb	2.3 Ab	14.5 Bb	9.3 Ab	1647.1 Ac	242.6 Ab	26.6 Aa	1876.3 Ac
	100	25.9 Ab	9.7 Aa	5.8 Ba	23.3 Aa	5.9 Ba	3.0 Aa	15.4 Bb	12.5 Aa	3143.3 Aa	173.3 Ac	31.5 Aa	2937.2 Ab
	200	26.3 Ab	5.7 Ab	5.5 Ba	15.5 Ab	5.6 Ba	1.5 Ac	8.1 Bc	9.8 Ab	1299.1 Ac	99.8 Ac	29.5 Aa	3485.4 Aa

* Means followed by the same letter do not differ, according to the Scott Knott test ($p \leq 0.05$). Upper case letters compare the part of the plant (shoots and roots) and lower-case letters compare the concentrations in each collection time.

Source: created by the author (2020).

Table S2 – Concentration of nutrients in shoots and roots of rooted *Talinum paniculatum* cuttings grown under increasing concentrations of Mn for a period of 30 days.

	Mn concentration	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
		----------------------------------mg g^{-1}					--------------------------------	---------------------------mg kg^{-1}				--------------------------
Shoots	0	22.9 Bb	5.0 Ab	28.7 Ad	9.3 Bb	5.9 Ab	1.6 Bb	21.8 Bc	3.2 Bb	63.8 Ba	48.9 Bc	12.9 Ba
	10	25.2 Ba	6.3 Aa	38.1 Ab	11.4 Ba	8.7 Aa	1.7 Ab	25.7 Ab	4.2 Bb	29.1 Bb	593.1 Bb	19.5 Ba
	50	25.9 Aa	5.8 Ba	41.2 Aa	10.8 Ba	9.1 Aa	2.0 Aa	27.2 Ab	4.4 Bb	27.8 Bb	898.4 Bb	20.8 Ba
	100	24.5 Bb	4.6 Bb	38.8 Ab	9.6 Bb	8.2 Aa	2.0 Aa	29.8 Aa	7.9 Ba	15.2 Bb	1544.6 Ba	19.2 Ba
	150	20.6 Ab	4.1 Bb	31.3 Ac	6.8 Bc	5.6 Ab	1.4 Bc	14.8 Ad	4.1 Bb	18.9 Bb	579.3 Bb	18.6 Ba
Roots	0	29.8 Ab	2.8 Bb	6.6 Ba	14.1 Ab	5.5 Aa	2.1 Aa	18.0 Aa	9.1 Ab	2091.0 Ac	179.1 Ae	23.3 Ad
	10	27.0 Ab	3.4 Bb	5.1 Ba	17.3 Aa	5.7 Ba	1.2 Bd	10.0 Bb	7.5 Ab	2005.8 Ac	1320.7 Ad	38.1 Ac
	50	26.7 Aa	7.8 Aa	6.1 Ba	17.9 Aa	5.5 Ba	1.6 Bc	8.9 Bb	23.8 Aa	4067.7 Ab	6667.2 Ac	54.7 Ab
	100	30.3 Aa	7.8 Aa	6.1 Ba	12.6 Ac	4.8 Ba	1.9 Ab	8.0 Bb	7.5 Ab	4728.4 Aa	13782.7 Ab	52.8 Ab
	150	28.7 Aa	7.5 Aa	6.1 Ba	11.0 Ad	5.4 Aa	1.6 Ac	9.5 Ab	7.4 Ab	4240.4 Aa	17799.2 Aa	61.4 Aa

* Means followed by the same letter do not differ, according to the Scott Knott test ($p \leq 0.05$). Upper case letters compare the part of the plant (shoots and roots) and lower-case letters compare the concentrations in each collection time.

Source: created by the author (2020).