Riparian vegetation six years after the Fundão dam collapse: impact on the seed bank and the growth of herbaceous and arboreal species

Zur Erlangung des akademischen Grades eines
DOKTORS DER NATURWISSENSCHAFTEN (Dr. rer. nat.)
von der KIT-Fakultät für
Bauingenieur-, Geo- und Umweltwissenschaften
des Karlsruher Instituts für Technologie (KIT)

genehmigte
DISSERTATION von

Thesis presented to obtain the title of
DOCTOR SCIENTIAE (D.SC.)
at the Graduate Program in Ecology
of Federal University of Viçosa (UFV), by

M. Sc. André Araújo da Paz

Tag der mündlichen Prüfung:
18. Dezember 2025

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Karlsruhe 2025
Viçosa 2025

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Abstract

The 2015 collapse of the Fundão iron ore tailings dam in Mariana, Brazil, constituted one of the world's largest socio-environmental disasters, burying riparian ecosystems under a massive layer of technosol. Six years post-disaster, the long-term trajectory of ecological recovery remained uncertain, particularly concerning the dual challenge of physical habitat degradation and potentially toxic elements (PTE) contamination. This thesis experimentally investigates the chronic impacts of the deposited tailings on plant recruitment, growth, and the phytoremediation potential of native species in the affected Upper Doce River Basin. The research was structured around three key environmental compartments: the initial stage of recolonization (seed bank and herbaceous plants recruitment), the short-term response of pioneer trees, and the long-term PTE accumulation in adult trees.

In the affected areas, there was a loss of biodiversity characterized by a 35% decline in regional herbaceous plant species richness (gamma diversity) compared to unaffected sites. This reduction in biodiversity is primarily attributed to habitat homogenization and poor physicochemical properties (low organic matter and nutrient content) of the affected substrate, rather than immediate toxicity from PTE. Importantly, the slurry did not prevent local plant regeneration; the abundance and local diversity of emergent seedlings (alpha diversity) remained high, indicating viable seed dispersal and persistent ruderal species capable of initiating early succession. Herbaceous native species like *Ludwigia octovalvis* and *Marsypianthes chamaedrys* demonstrated notable tolerance, successfully completing their life cycle despite growth limitations imposed by the degraded substrate.

The fast-growing pioneer tree, *Cecropia hololeuca*, exhibited remarkable resilience and improved performance on the contaminated substrate. Seedlings grown in tailings showed significantly enhanced growth, nearly doubling their height and biomass compared to controls. This success is facilitated by the species' pioneer traits and a strategic allocation of biomass to the root system, enabling it to better forage in the low-quality soil. *C. hololeuca* was identified as a differential accumulator of PTE, displaying a dual phytoremediation strategy. First, the phytostabilization, acting as an effective root accumulator for iron (Fe),

chromium (Cr), copper (Cu), manganese (Mn), and zinc (Zn), efficiently restricting their movement to aerial parts. Second, the phytoextraction, exhibiting an extremely high Translocation Factor (TF=273) for Nickel (Ni), resulting in leaf concentrations five times higher than control plants. This efficient translocation raises critical ecological concerns regarding the potential for Ni transfer into the local food web.

The analysis of growth rings from surviving adult riparian tree species (*Anadenanthera peregrina, Piptadenia gonoacantha,* and *Nectandra oppositifolia*) demonstrated the long-term impact on element cycling. The radial growth of affected trees was not significantly reduced, suggesting that the acute contamination event did not cause long-term physiological stress. Furthermore, the expected acute signal of elevated PTE concentrations immediately following the 2015 disaster was not perceived, probably due to radial transport within the diffuse-porous wood structure. Crucially, all three adult tree species qualified as effective accumulators (Bioconcentration Factor > 1 for most elements), with concentrations in the wood achieving up to 34-fold higher levels than the bioavailable fraction in the surrounding substrate (e.g., for Pb and Cr). This sequestration capacity confirms their role in the long-term stabilization of contaminants.

The findings demonstrate that while the disaster resulted in regional biodiversity loss, a suite of native pioneer and woody species possesses the resilience and sequestration mechanisms necessary for effective ecological recovery. The identified species are strong candidates for restoration programs, as their rapid growth enhances soil organic matter content and their accumulator capacity locks PTEs within stable woody tissues, thus limiting their overall mobility. Furthermore, the inclusion of two Fabaceae species is especially beneficial for ecosystem recovery as they improve soil quality through nitrogen fixation. However, the risk of trophic transfer, particularly for Ni via *C. hololeuca* leaves and non-essential metals accumulated in tree wood, necessitates careful long-term monitoring to mitigate biomagnification risks within the ecosystem. The long-term success of recovery efforts must prioritize restoring microhabitat heterogeneity and leveraging these tolerant native species for both soil improvement and phytostabilization.

Kurzfassung

Der Dammbruch des Eisenerz-Absetzbeckens von Fundão im Jahr 2015 in Mariana in Brasilien stellte eine der weltweit größten sozio-ökologischen Katastrophen dar, bei der Auenökosysteme unter einer massiven Schicht Technosol begraben wurden. Sechs Jahre nach dem Dammbruch blieb der langfristige Verlauf der ökologischen Regenerierung ungewiss, insbesondere durch die physikalische Habitatdegradation und die Kontamination mit potenziell toxischen Elementen (PTE). Diese Dissertation untersucht experimentell die langfristigen Auswirkungen der abgelagerten Bergbauabfälle auf die Keimung, das Pflanzenwachstum und das Phytosanierungspotenzial heimischer Arten im betroffenen Gebiet des oberen Doce-Flusses. Die Forschung gliederte sich in drei Hauptfelder: das Anfangsstadium der Wiederbesiedlung (Samenbank und Keimung krautiger Pflanzen), die kurzfristigen Auswirkungen auf Pionierbäume sowie die langfristige PTE-Akkumulation in adulten Bäumen späterer Sukzessionsgesellshaften.

Die Ablagerung der Bergbauabfälle reduzierte die regionale krautige Artenvielfalt (Gamma-Diversität) um 35% im Vergleich zu unbeeinträchtigten Gebieten. Dieser Verlust an Biodiversität ist primär auf die Habitat-Homogenisierung und die ungünstigen physikalisch-chemischen Eigenschaften (niedriger Gehalt an organischer Substanz und Nährstoffen) des betroffenen Substrats zurückzuführen und nicht auf eine unmittelbare PTE-Toxizität. Die Abundanz und lokale Diversität der keimenden Sämlinge (Alpha-Diversität) blieb jedoch hoch, was auf eine vitale Samenausbreitung und das Vorkommen persistenter Ruderalarten hinweist, die zur raschen Einleitung der Sukzession fähig sind. Einheimische krautige Arten wie *Ludwigia octovalvis* und *Marsypianthes chamaedrys* zeigten eine bemerkenswerte Toleranz und konnten sich weiterhin ausbreiten.

Der schnell wachsende Pionierbaum *Cecropia hololeuca* zeigte eine bemerkenswerte Resilienz und eine verbesserte Wuchsleistung auf dem kontaminierten Substrat. Auf Bergbauschlämmen gezogene Sämlinge wiesen ein signifikant gesteigertes Wachstum auf und verdoppelten nahezu ihre Höhe und Biomasse im Vergleich zu den Kontrollpflanzen. *C. hololeuca* wurde als differentieller PTE-Akkumulator identifiziert und zeigte eine zweifache

Phytosanierungsstrategie: Sie akkumuliert effektiv Eisen, Chrom, Kupfer, Mangan und Zink in ihren Wurzeln (Phytostabilisierung), während sie gleichzeitig einen sehr hohen Translokationsfaktor (TF=273) für Nickel (Ni) aufweist (Phytoextraktion). Diese effiziente Verlagerung führt zu ökologischen Bedenken aufgrund des möglichen Ni-Eintrags in die lokale Nahrungskette.

Die Analyse der Wachstumsringe überlebender adulter Auenbäume (der Arten *Anadenanthera peregrina*, *Piptadenia gonoacantha* und *Nectandra oppositifolia*) belegte den langfristigen Einfluss auf den Elementkreislauf. Das radiale Wachstum der betroffenen Bäume war nicht signifikant reduziert, was darauf hindeutet, dass die akute Kontamination keinen langfristigen physiologischen Stress verursacht. Darüber hinaus wurde der erwartete, plötzliche Anstieg erhöhter PTE-Konzentrationen unmittelbar nach der Katastrophe von 2015 nicht festgestellt, was wahrscheinlich auf den radialen Transport innerhalb der diffusporigen Holzstruktur zurückzuführen ist. Entscheidend ist, dass die adulten Individuen aller drei Baumarten als effektive Akkumulatoren (Biokonzentrationsfaktor > 1 für die meisten Elemente) eingestuft wurden, wobei die Konzentrationen im Holz bis zu 34-fach höher waren als der bioverfügbare Anteil im umgebenden Substrat (z. B. für Pb und Cr).

Zusammenfassend zeigen die Ergebnisse, dass eine Reihe heimischer Pionier- und Gehölzarten die zur wirksamen ökologischen Sanierung notwendige Resilienz und Sequestrierungsmechanismen besitzt. Die identifizierten Arten sind vielversprechende Kandidaten für die ökologische Wiederherstellung, da ihr schnelles Wachstum den Gehalt an organischer Substanz im Boden verbessert und ihre Akkumulationskapazität PTEs in stabilen Holzgeweben einschließt, wodurch deren allgemeine Mobilität eingeschränkt wird. Darüber hinaus ist die Aufnahme der beiden Fabaceae-Arten (*A. peregrina* und *P. gonoacantha*) besonders vorteilhaft für die Wiederherstellung des Ökosystems, da sie die Bodenqualität durch Stickstofffixierung verbessern. Die effiziente Translokation von Ni über die Blätter von *C. hololeuca* und die Akkumulation nicht-essenzieller Metalle im Holz erfordern jedoch ein sorgfältiges Langzeitmonitoring, um die Risiken der Biomagnifikation innerhalb des Ökosystems zu mindern. Zukünftige Arbeiten sollten auch die PTE-Konzentrationen in den Wurzeln und Blättern der Hartholzarten untersuchen, da diese Gewebe einen signifikanten Pfad für die trophische Übertragung darstellen können.

Resumo

O colapso da barragem de rejeitos de minério de ferro de Fundão, em Mariana, Brasil, em 2015, constituiu um dos maiores desastres socioambientais do mundo, soterrando ecossistemas ripários sob uma densa camada de tecnossolo. Seis anos após o desastre, a dinâmica de recuperação ecológica da área a longo prazo permaneceu incerta, particularmente em relação ao duplo desafio da degradação física do habitat e da contaminação por elementos potencialmente tóxicos (EPT). Esta tese investiga experimentalmente os impactos crônicos da deposição deste substrato sobre o recrutamento e o crescimento de plantas, considerando o potencial de fitorremediação de espécies nativas na Bacia do Alto Rio Doce. A pesquisa foi estruturada em torno de três eixos principais: o estágio inicial de recolonização (banco de sementes e recrutamento de plantas herbáceas), a resposta a curto-prazo de árvores pioneiras e a acumulação a longo prazo de EPT em árvores adultas.

Nas áreas afetadas, houve uma perda de biodiversidade, caracterizada por um declínio de 35% na riqueza regional de espécies de plantas herbáceas (diversidade gama) em comparação com áreas não afetadas. Essa redução na biodiversidade é primariamente atribuída à homogeneização do habitat e às propriedades físico-químicas desfavoráveis (baixo teor de matéria orgânica e nutrientes) do substrato afetado, em vez da toxicidade imediata por EPT. É importante ressaltar que o rejeito não impediu a regeneração ambiental; a abundância e a diversidade local de plântulas emergentes (diversidade alfa) permaneceram altas, indicando uma dispersão de sementes viável e existência de espécies ruderais capazes de continuar a sucessão. Espécies nativas herbáceas como *Ludwigia octovalvis* e *Marsypianthes chamaedrys* demonstraram notável tolerância, completando com sucesso seu ciclo de vida, apesar das limitações de crescimento impostas pelo substrato degradado. A árvore pioneira de crescimento rápido, *Cecropia hololeuca*, exibiu resiliência notável e desempenho aprimorado no substrato contaminado. As plântulas cultivadas nos substratos afetados mostraram crescimento significativamente aumentado, praticamente dobrando sua altura e biomassa em comparação com os controles. *C. hololeuca* foi identificada como uma acumuladora de EPT, exibindo uma estratégia de fitorremediação dupla. Primeiro atua como

acumuladora radicular de ferro (Fe), cromo (Cr), cobre (Cu), manganês (Mn) e zinco (Zn) (fitoestabilização), e posteriormente exibe um fator de translocação extremamente alto (TF=273) para Níquel (Ni) (fitoextração). A análise dos anéis de crescimento de espécies ripárias adultas sobreviventes (*Anadenanthera peregrina*, *Piptadenia gonoacantha* e *Nectandra oppositifolia*) demonstrou o impacto de longo prazo na ciclagem de elementos. O crescimento radial das árvores afetadas não foi significativamente reduzido, e a concentração elevada de EPT esperada após o desastre de 2015 não foi percebida, provavelmente devido ao transporte radial dentro da estrutura da madeira. Finalmente, as três espécies arbóreas adultas foram qualificadas como acumuladoras eficazes (Fator de Bioconcentração > 1 para a maioria dos elementos), com concentrações na madeira atingindo níveis até 34 vezes maiores do que a fração biodisponível no substrato circundante (e.g.: para Pb e Cr).

Em conclusão, os achados demonstram que o conjunto de espécies nativas lenhosas possui a resiliência e os mecanismos de sequestro necessários para uma recuperação ecológica eficaz. As espécies identificadas são fortes candidatas para programas de restauração, pois seu crescimento rápido melhora o teor de matéria orgânica do solo e sua capacidade de acumulação estabiliza os EPT em tecidos lenhosos, limitando sua mobilidade geral. Além disso, a inclusão das duas espécies de Fabaceae (*A. peregrina* e *P. gonoacantha*) é especialmente benéfica para a recuperação do ecossistema, pois melhoram a qualidade do solo através da fixação de nitrogênio. No entanto, a translocação eficiente de Ni para as folhas de *C. hololeuca* e o acúmulo de metais não essenciais na madeira das árvores exigem monitoramento cuidadoso de longo prazo para mitigar os riscos de biomagnificação dentro do ecossistema. Trabalhos futuros também devem investigar as concentrações de EPT nas raízes e folhas para além da madeira, pois esses tecidos podem representar uma via significativa para a transferência trófica.

Preface

This thesis represents original research conducted by me (André Araújo da Paz) during my PhD at the Karlsruhe Institute für Technology (KIT) and Universidade Federal de Viçosa (UFV). It includes 3 chapters: the chapter 1 is published in an international journal (PLoSOne). The others are finished and will be submitted right after the thesis submission. I have contributed in each part of the work, in cooperation with my advisors, co-advisors and colleagues. The work parts include funding acquisition, project administration, conceptualization, methodology, investigation, validation, data analysis, writing, visualization and presentation. The supervisors were responsible for funding acquisition, project conceptualization, and administration, mentorship and writing reviewing. All the work from this PhD was started as a Binational Doctoral Program, in partnership between members from both KIT and UFV universities, following a Doctoral agreement. The thesis was conducted without the involvement of third-party editorial assistance. The details of each chapters are as follows:

Chapter 1 has been published in PLoSOne Journal in 2025

- Paz, A.A., Shimizu-Aleixo, D., Oliveira-Wittmann, A.D., Wittmann, F., Ribeiro, C. and Campos, R.I.D., 2025. Plant recruitment six years after the Samarco's tailings-dam disaster: Impacts on species richness and plant growth. *PLoS One*, *20*(8), p.e0315990.

Chapter 2 has been completed to journal standards and will be submitted to an international indexed journal

- Paz, A.A., Shimizu-Aleixo, D., Marques, R.D., Oliveira-Wittmann, A.D., Wittmann, F. and Campos, R.I.D., 2025. Enhanced development of a pioneer tree (*Cecropia hololeuca*) on the affected substrate formed six years after the Samarco's dam break

Chapter 3 has been completed to journal standards and will be submitted to Journal Forests - Special Issue "Environmental Signals in Tree Rings"

- Paz, A.A., Shimizu-Aleixo, D., Oliveira-Wittmann, A.D., Ortega-Rodriguez D. R., Ribeiro, C., Campos, R.I.D. and Wittmann, F., 2025. Accumulation of potentially toxic elements in trees six years after the Samarco's dam collapse

The research work for this thesis was supported by the universities of UFV and KIT, with personal scholarships from Brazilian entity CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and German entity DAAD (Deutscher Akademischer Austauschdienst). In addition, the work received financial support from the project entitled *Impacts of the "Fundão" dam collapse on the aquatic biota and its habitat's structure*, with resources from FAPEMIG/RENOVA (APQ-05461-18)*. We acknowledge support by the KIT-Publication Fund of the Karlsruhe Institute of Technology (https://www.bibliothek.kit.edu/english/kit-publication-fund-services.php). * This project/product was funded by the Renova Foundation, by imposition of the Transaction and Conduct Adjustment Term – TTAC, signed for the recovery, mitigation and compensation of the socioeconomic and socio-environmental impacts of the Fundão dam collapse, in Mariana, Minas Gerais.

Acknowledgements

To all the unknown that surrounds us, and to what is yet to be discovered. To the great *Doce* River (*Watú* in the *Krenak* indigenous language) and all living beings that had their lives impacted in different ways by the crime of 2015.

To those whose love was my inspiration, and who, though no longer physically beside me, remain with me always. I dedicate this work to the memory of José Roberto Paz, Flavia Maria Carmo, Günther Wittig and Buenaventura Walter Ponce.

I'm grateful to my wife, Sara Otuki, my best friend since 2008, who accompanied me through all sorts of moments, supporting me so much to keep going. This thesis wouldn't be the same without your patience and daily love. I'm grateful to my family, my mom, who is now living by herself after my father passed away in 2021. Your love can be felt in every cell of my being, to my father- and mother-in-law, who support us in every new path of our lives with much love.

I appreciate all the support from the research groups Terra Água and Macroflora for keeping this project alive even after the passing of my former supervisor due to COVID. I'm grateful to the friends who helped in the fieldwork and data collection (Frederico Ferreira, Rafael Marques, Filipe Oliveira, Sara Otuki, Rafael Rigolon, Breno Felisberto, Caio Paz and Reginaldo Pires) and the landowners who gave us access to their properties. To all the reviewers of the first drafts of these manuscripts, they have now become Journal articles and a PhD thesis! To Diego Shimizu, my right-hand man, who was a daily support on all the plant-related work, including in my absence during the training in Germany.

Thanks to the UFV (sua linda!) and the Ecology Post-Graduation program. To my supervisor in Brazil, Dr. Ricardo Campos, who took place in the middle of the project. To the support of Dr. Ricardo Solar in this work's initial phase, and Dr. Tiago Fernandes in data visualization. To the KIT and my Doktorvater und Doktormutter, Dr. Florian and Dr. Astrid Wittmann, to show me a whole new perspective for my career. To the colleagues of the Auen-Institut, Mareike, Isabel, Christian, Ethan, Erika, Lars, Gerda, Timo and others, thank you for the support beyond the scientific matters. Also, thanks to Romy, Helmy, Niki and Seda, who became close friends and provided support during the PhD.

To the academic support and funding from Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and the Deutscher Akademischer Austauschdienst (DAAD), especially Maria Salgado, for the support.

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1. Introduction

1.1 Background and problem statement

1.1.1 Fundão dam disaster – Context and impact

A tragic disaster involving the sludge spill of an iron ore tailings dam facility occurred in November 2015 in Mariana, Minas Gerais state, Brazil, when the *Fundão* dam burst (Meira-Neto; Neri, 2017). With this, 39.2 million m³ of slurry were dumped over the *Doce* River basin, and after 16 days and 670 km, a part of this material reached the Atlantic Ocean (Sánchez et al., 2018a). The spill covered the riverbeds and a wide extension of its banks (Figure 1), with an average thickness of 50 cm, and in some places, more than 3 m high (Buch et al., 2021). This impacted an estimated area of approximately 1469 ha of vegetation (Fernandes et al., 2016a). After 110 km from the origin, 9.9 million m³ of sludge were retained in the *Risoleta Neves* Hydroelectric Power Plant (HPP), suppressing a total of 778,12 ha of vegetation just along this river stretch (Bastos, 2021). Beyond this landmark, the tailings were mainly constrained to the riverbeds but also impacted riverbanks by affecting soil quality and the life of organisms such as earthworms (Nadolny et al., 2025).

Figure 1: Areas impacted by the passage of the slurry from the Fundão dam, in Minas Gerais, Brazil. Photo credits: A) Genelício Crusoé Rocha and B) Aline Azevedo.

In the rivers, total suspended sediments reached 33,000 mg/L (Hatje et al., 2017a). Different aquatic organisms, such as fish and crustaceans, died due to the suspended solids, and the terrestrial life, whether domestic or wild, also lost access to water resources (Macêdo et al., 2024). In these areas, many people were affected, accounting for 19 deaths, three missing, and 600 homeless (Neves; Fernandes, 2016a). Furthermore, it impacted 40 cities, agricultural land

and indigenous territories, due to a reduction in water supply, electricity generation and river resources, in the two states bathed by the Doce River (Meira et al., 2016). Finally, the tailings reached the Atlantic Ocean (Figure 2) (Soares et al., 2025).

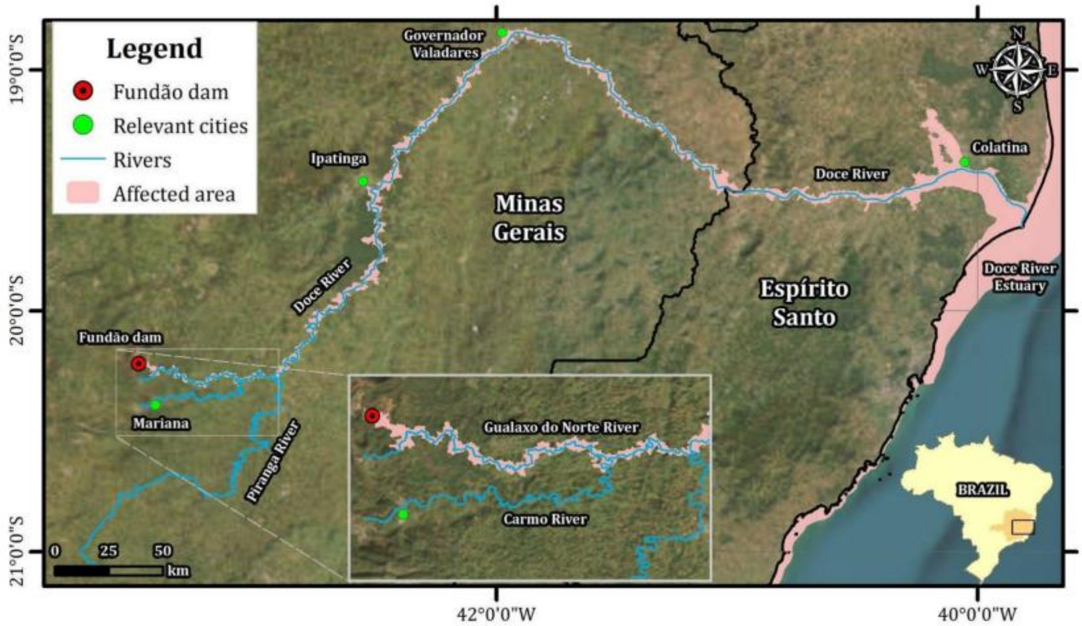

Figure 2: Map of the regions affected by the Samarco's dam disruption, from the Fundão dam to the Doce River and finally the Atlantic Ocean. Source: Soares 2025.

1.1.2 Tailings-affected substrate

The tailings substrate is classified as a Technosol (Queiroz et al., 2022a) due to its content of over 20% technogenic material (Huot et al., 2013). Throughout this work, this material will be referred to as "waste", "sludge", "slurry", or simply "substrate", accounting for its genesis and temporal alterations. *Fundão* dam's tailings consisted of two material types: one rich in sand and the other rich in clay (Norbert R. Morgenstern et al., 2016), but mainly composed of silt and fine sand (Buch et al., 2021). When dried, the sludge had a general appearance of cement, thick and dense when soaked, with irregular particle sizes (Segura et al., 2016). During the disaster, these substrates spread in different proportions along the path, mixing with the local soil (Guerra et al., 2017b).

The tailings stored at the *Fundão* Dam complex were mainly composed of goethite, hematite, quartz, and kaolinite, containing in average: Fe (57.2%), SiO_2

(14.1%), Al (1.3%) among other elements like Cd, Cr, Pb, Mn, Fe and Na (Pires et al., 2003). This mineralogy resulted in a substrate with very low cation exchange capacity (CEC) and low organic matter content (Segura et al., 2016). While an initial survey at the first impacted village found metal concentrations below the background soil, and following international guidelines (Segura et al., 2016), the presence of potentially toxic elements (PTE) was a concern. Specifically, elements like barium (Ba), arsenic (As), cadmium (Cd), strontium (Sr), iron (Fe), manganese (Mn), aluminum (Al), and lead (Pb) were shown to have high mobility from the tailings particles, presenting a clear potential for contamination. This concern was supported by bioassays demonstrating potential cytotoxicity and DNA damage, suggesting the need for environmental monitoring (Segura et al., 2016).

Contradictions regarding the contamination status persist across the upper Doce River basin. A technical report (Lactec, 2020), found anomalous values of Fe and Mn in affected soils, indicating potential environmental risk, yet concluded no contamination overall by comparing other elements to pre-disaster baselines (2012 and 2015). However, another study found higher concentrations of Fe, chromium (Cr), Pb, Mn, bismuth (Bi), and cerium (Ce) compared to unaffected areas (Silva et al., 2021a). Notably, Cr and Pb concentrations exceeded Brazilian environmental reference values (CONAMA) (Table 1). Furthermore, while high As values found in riverbank sediments (Guerra et al., 2017c) are considered non-alarming due to the region's natural geology, care is still suggested due to the possibility of biomagnification.

Table 1:Chemical characterization of soils from impacted (I) and non-impacted (NI) areas of the Gualaxo do Norte Riverbanks using Portable X-ray Fluorescence (XRF). Adapted from Silva et al 2021.

Sites	Fe (13)*	Al (960)	Cr (19)	Pb (11)	Cu (5)	Ni (5)
	mg kg^{-1}					
I	17,805 ± 46,729	30,063 ± 27,224	235.0 ± 69.5	52.7 ± 15.9	28.0 ± 6.2	6.2 ± 12.8
NI	56,654 ± 48,079	92,283 ± 14,119	48.8 ± 66.0	20.0 ± 14.0	24.2 ± 17.2	40.8 ± 33.4
VRQ	–	–	75	19.5	49	21.5
VP	–	–	75	72	60	30
VI-agr	–	–	150	180	200	70
	Zn (3)	Cl (135)	K (41)	Mn (18)	P (90)	S (80)
	mg kg^{-1}					
I	17.2 ± 9.3	595.8 ± 231.2	4306 ± 3175	1008 ± 369	563.4 ± 231.9	<LD
NI	46.8 ± 36.8	648.5 ± 137.7	9913 ± 9038	667 ± 664	366.5 ± 186.5	134.7 ± 197.0
VRQ	46.5	–	–	–	–	–
VP	300	–	–	–	–	–
VI-agr	450	–	–	–	–	–

Below the detection limit: Ag (10), As (3), Au (6.6), Ba (188.4), Cd (19), Co (5), Hf (6), Hg (6), La (166.5), Mg (7000), Mo (6), Nb (2), Pd (29), Pt (6.8), Rh (15), Sb (30), Se (3), Sn (20), Ta (4), Th (17), T1 (5), U (53), W (6).
<LD values below the detection limit, – values not defined, VRQ quality reference value (COPAM no. 166/2011), VP prevention value (CONAMA no. 420/2009), VI-agr. Amount of agricultural intervention (CONAMA no. 420/2009).
*In parentheses are the detection limits (mg kg^{-1})

A large-scale study along the sludge path (Buch et al., 2021) confirmed that concentrations of Cd (max. 1.1 mg/kg) and mercury (Hg) (max. 0.3 mg/kg) exceeded the regional reference values – COPAM, but were below the national reference values – CONAMA. While comparing three post-disaster periods (2015, 2018 and 2021) no difference was found for most of the elements (Figure 3) (Buch et al., 2024). Research showed that soil habitat quality for collembolans was significantly decreased due to high substrate avoidance, lethality, and reduced reproduction rates (Buch et al., 2021). Furthermore, the study reported high soil bioaccumulation factors in local mites, suggesting that metals are highly available for the terrestrial biota on the riparian soil (Buch et al., 2020). While the sludge flow reached the Atlantic Ocean, depositing significant quantities of Fe and Mn (Queiroz et al., 2018), the disaster led to substantial bioaccumulation in the ichthyofauna, which presented increased proportions of metals such as Cd, Cr, Cu, Fe, Mn, and Pb in their organs (Vieira et al., 2022). Finally, the toxic effects in the food chain affected human health, with high levels of Al, As, Hg, and Ni detected in blood samples from affected populations(Cavalheiro Paulelli et al., 2022). Given the conflicting findings in the literature regarding long-term contaminant risk, a further in-depth, site-specific ecotoxicological assessment is needed.

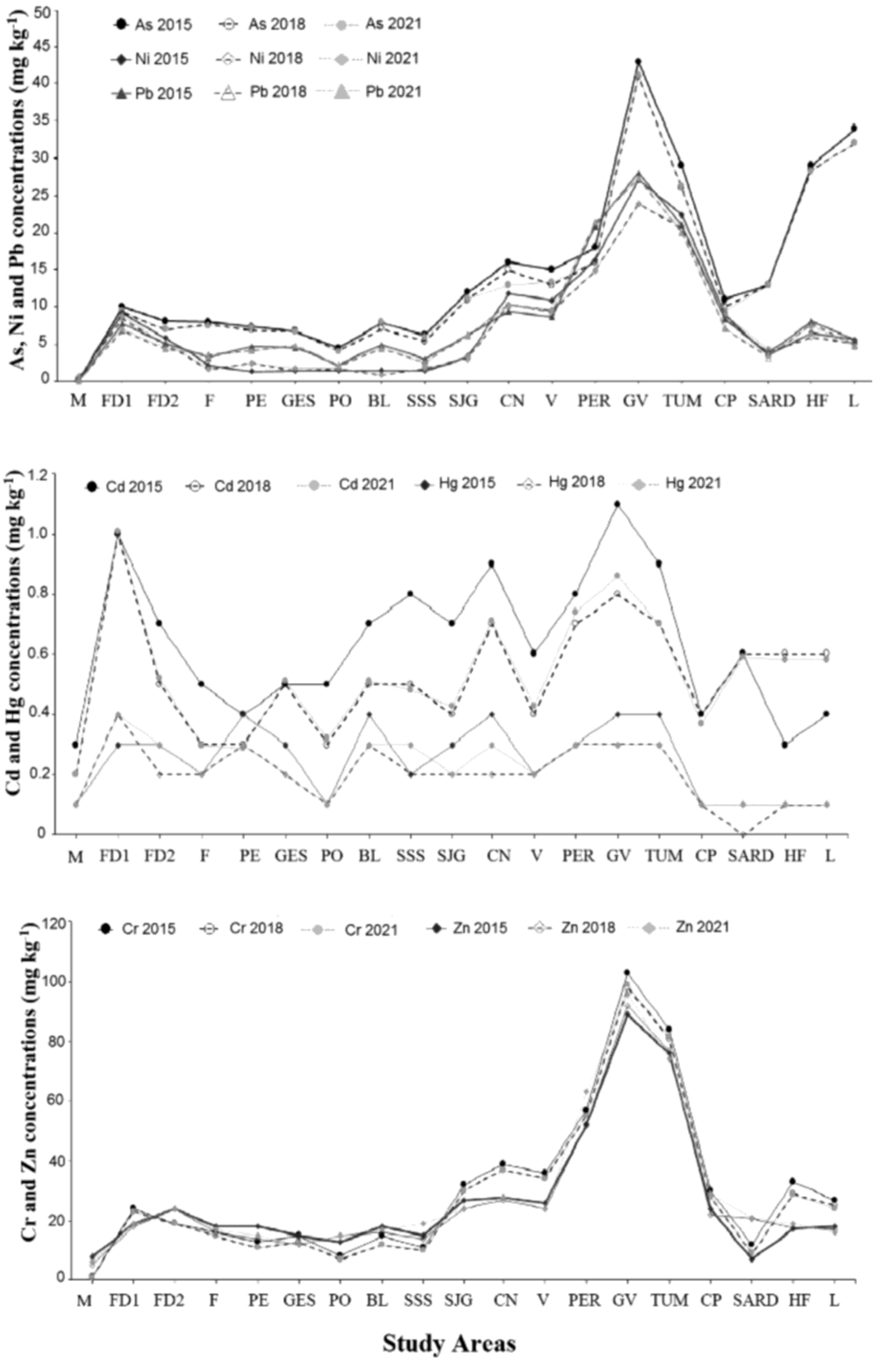

Figure 3: Concentration of trace elements in soils from areas affected by the disruption of the Fundão dam (FD), from source to sea (L-Linhares), along the 600 km along the Rio Doce and affected tributaries, with a reference area (M - Meloso). Other acronyms: FD1-fundão dam 1; FD2-fundão dam 2; F-Fonseca; PE-Pedras; GES-Gesteira; PO-Ponte do Onça; BL-Barra Longa; SSS-São sebastião do Soberbo; SJG-São José do Goiabal; CN-Córrego Novo; V-Veneza; PER-Periquito; GVGovernador Valadares; TUM-Tumiritinga; CP-Conselheiro Pena; SARD-Santo Antônio do Rio Doce; HF-Honório Fraga. (taken from Buch 2024)

Following the disaster, rehabilitation procedures were undertaken, including soil scarification, soil correction, tree planting, and seed sowing (IBAMA, 2020; SAMARCO MINERAÇÃO S.A., 2016). Propagated species included *Cajanus cajan* (L.) Huth.; *Crotalaria* spp.; *Cynodon dactylon* (L.) Pers; *Glicyne wightii* (Arnott) Verdcourt; *Neonotonia wightii* (Wight & Arn.) J.A. Lackey; and *Mimosa* sp. L. (T. Silva de Sá et al., 2023). After the remediation procedures, the landscape exhibited signs of succession, characterized by the proliferation of the opportunistic grass *Brachiaria decumbens* Stapf. and pioneer tree species over the taillings (Silva et al., 2022). This is often observed among the dead trees in the riverbanks (Figure 4).

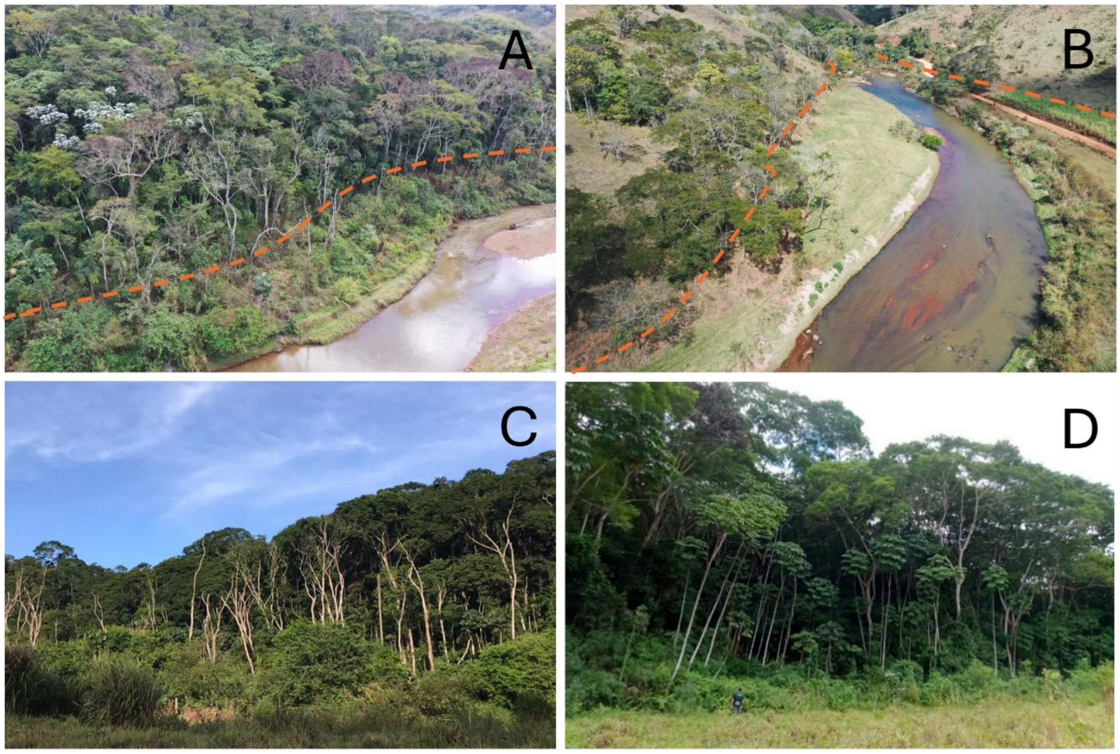

Figure 4: Post-disaster riparian vegetation in four areas affected by the passage of mining tailings. A) Secondary forest with natural succession occurring at the riverbank, with shrubs and lianas; B) floodplain revegetated with grass, and riverbed with iron-rich sediment; C) secondary forest with dead trees and D) secondary forest with pioneer Cecropia spp. trees growing near the forest edge. All images taken in 2020, except image D, taken in 2022. Dashed lines show the approximate upper limit of the tailings in each area. Drone aerial images (A and B) were taken by Dr. Ricardo Solar.

1.1.3 Physical description of the area

The study area lies within the Upper Doce River basin, a region of complex geology related to the Iron Quadrangle, and has a long history of mining for gold, diamond, iron and other minerals (Guevara et al., 2018). The geology is primarily influenced by quartzites, phyllites and itabirites (Selmi; Lagoeiro; Endo, 2009), which are associated with gneissic-granitic complexes (Figure 5) (Lobato; Ribeiro-Rodrigues; Vieira, 2001). The areas on the upper Doce River are dominated by soils classified as Cambisols and Fluvisols (IUSS Working Group WRB, 2015).

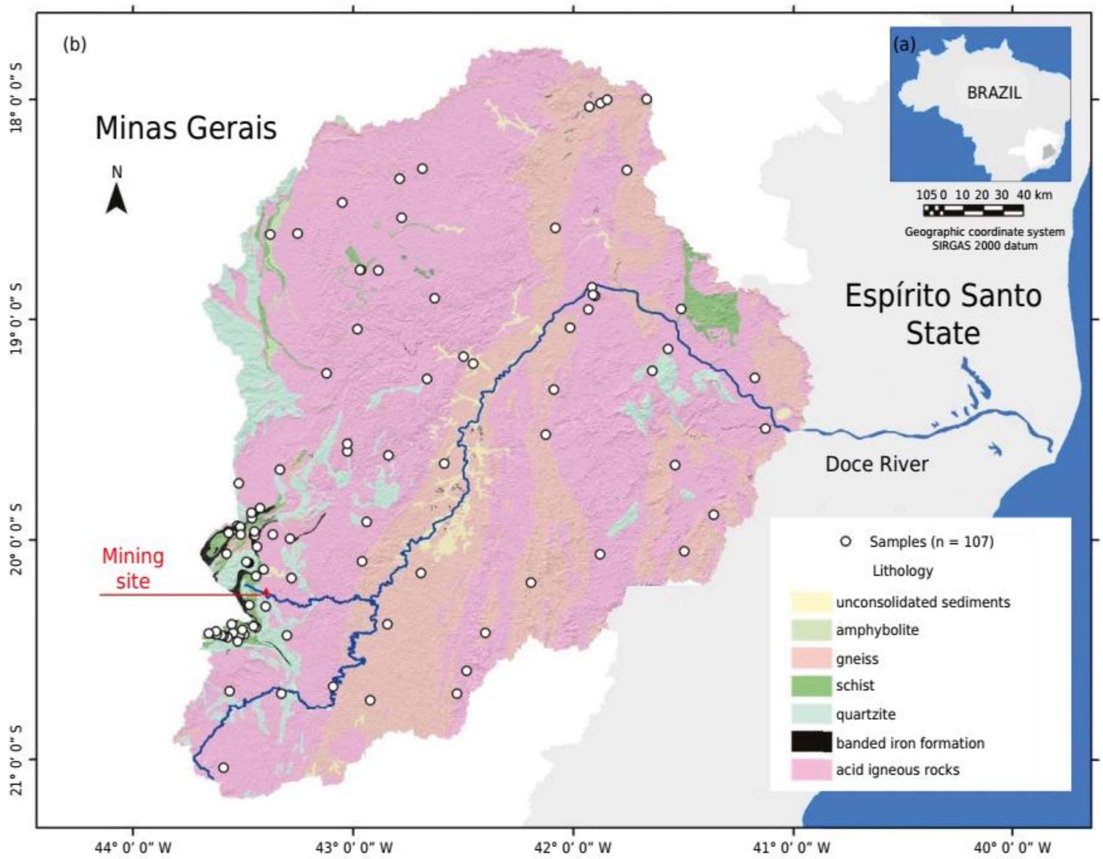

Figure 5: Location of Minas Gerais state map in Brazil (a) and lithology of the Doce River watershed (b). Small circles represents the samples from the original work: extracted from Guevara et al 2018).

The mean annual temperature of the region is 21.5°C, and the yearly rainfall is 1200 mm. The regional climate is Cwa (Köppen classification) (Alvares et al., 2013), marked by characteristic hot and rainy summers, and cold and dry winters (Figure 6). This seasonality results in annual floods (Lyra; Rigo, 2019), which are ecologically significant as they influence the transport and deposition of tailings material and subsequent successional processes. The overall land cover consists of a mosaic of native Atlantic Forest, *Eucalyptus* plantations, and pasture (T. Silva de Sá et al., 2023).

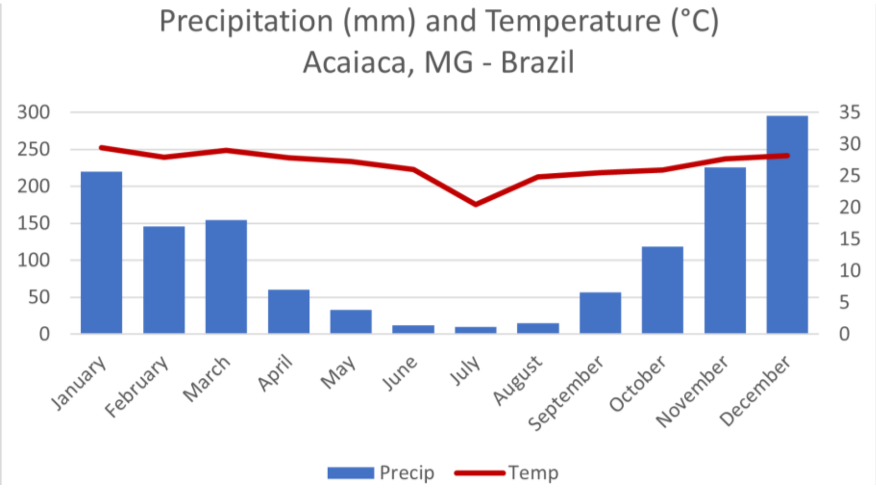

Figure 6: Monthly values of precipitation and temperature variation, between 1988 and 2016, in Acaiaca, Minas Gerais, Brazil (Upper Doce River). Data source: HidroWeb, Hydrological Information System, maintained by National Water Agency (Agência Nacional de Águas, ANA), Brazil. (https://www.snirh.gov.br/hidroweb/serieshistoricas).

1.2 Motivation of this work

The millions of cubic meters of tailings spread across the Doce River basin caused immediate mortality in riparian vegetation and buried native seed propagules, fundamentally altering the alluvial substrate. The new conditions imposed by this slurry deposition, defined by its physical constraints and chemical characteristics, shape the successional trajectory of plant development in the affected areas. After six years, the landscape still exhibits the chronic marks of mud on surviving tree trunks. Given the persistent physical and chemical constraints of the tailings and the limited, opportunistic nature of existing recovery, focused empirical research is urgently needed to assess both natural regeneration potential and long-term contaminant stabilization by native flora.

1.3 Research objectives

1.3.1 Research gaps

Previous studies investigating plant growth in the tailings spread across the Doce River basin typically compared the tailings material found on river margins with autochthonous soil from upland areas. This comparison is inherently problematic because upland soils are naturally distinct from the alluvial substrates, typical of floodplains. This thesis addresses this methodological limitation by focusing on a direct comparison between affected and unaffected floodplains within the same climatic region. Furthermore, no prior study has conducted a soil seed bank survey on a scale sufficient to account for the ecological variability across the entire region between the Fundão dam and the Risoleta Neves Power Plant. In general, existing literature has identified difficulties for plants growing in the affected substrate, primarily attributing this to nutrient deficiencies. However, few studies have focused on the native plant species that spontaneously occur in these areas (Araújo et al., 2024; Cruz et al., 2020a; Scotti et al., 2020). This includes the herbaceous plants, *Cecropia* species, and other tree species examined in this research. Finally, the dendrochronological technique has never been applied in the context of this specific environmental disaster. This technique is valuable for explaining growth patterns in plants that survived the disaster but retain chronological marks of the event, offering a unique perspective on their resilience and the impact's timing.

1.3.2 Research questions

The main general questions and the specific questions were grouped according to 1) the substrate, 2) the recruitment process and 3) the adult trees' development.

Substrate Characteristics

a) What are the differences in physicochemical composition and the concentration of potentially toxic elements between affected and unaffected substrates?

Recruitment Processes

b) What are the effects of the affected substrate deposition on seed bank richness and abundance?
c) What are the differences in growth performance and biomass accumulation of early-successional plants in the affected substrate compared to those in the unaffected soil?

d) Do any species that successfully establish in the affected substrate exhibit traits that indicate their potential for phytoremediation?

Adult Tree Development

e) How was the tree ring formation affected in surviving adult trees over the past six years?
f) What are the differences in PTE concentration within the woody tissues of affected and unaffected adult trees?

1.3.3 Expected results and contributions

This research hypothesizes that the physicochemical constraints of the affected substrate limit plant recruitment and long-term growth, but that a subset of native species demonstrates a high potential for phytostabilization of Potentially Toxic Elements.

1.3.3.1 The main contributions are:

This research encompasses a series of experiments demonstrating how plants can grow and thrive in the tailing-affected substrate, while some plants accumulate PTE in different tissues. In addition, this thesis presents:

- A list of herbaceous species capable of spontaneous germination and initial development in the affected substrate, which might be useful in future restoration projects.
- Identification of a pioneer tree species that primarily sequesters PTE in its root system, filtering the amount of elements transferred to the aerial part. This finding offers a short-term storage and containment strategy.
- Evidence that three other tree species accumulate different PTE within their wood. This represents a long-term storage of contaminants.

1.4 Thesis structure

This thesis comprises six chapters and is conceptually structured around three thematic scientific papers that collectively address the immediate and chronic ecological impacts of the tailings-affected substrate and the subsequent trajectory of natural recovery in the Doce River basin. This thematic progression allows the work to span early successional processes (recruitment) through to long-term effects (adult trees). These investigations comprise three papers (one of which is published in an international journal, and the other two are to be submitted) and culminate in the final synthesis chapter.

Fundamentals

Chapter 2 serves as the conceptual and technical guide for the entire thesis. It begins by providing a crucial overview of mining ore tailings disasters, thereby establishing the acute environmental hazard motivating this work. It reviews the physiological aspects of plant response to contaminated substrates, detailing the mechanisms of stress, tolerance, and avoidance of Potentially Toxic Elements (PTEs). It finally explores the principles and applications of phytoremediation and dendroecology, and details how the individual studies are integrated to address the overarching research objectives.

Plant recruitment six years after the Samarco's tailings-dam disaster: Impacts on species richness and plant growth

Chapter 3 presents the submitted article detailing the experimental impacts of the tailings on the herbaceous community. This research quantifies the effects on gamma-diversity (regional species richness) and plant growth in the contaminated substrate, representing the initial stage of ecological recovery.

Enhanced development of a pioneer tree (*Cecropia hololeuca*) on the affected substrate formed six years after the Samarco's dam break

Chapter 4 presents the article focusing on the fast-growing pioneer *Cecropia hololeuca* species. It details the growth impacts and the phytoaccumulation of Potentially Toxic Elements (PTE) in the roots and leaves, assessing its role in short-term element containment within the tailings-substrate.

Accumulation of potentially toxic elements in trees six years after the Samarco's dam collapse

Chapter 5 presents the article detailing the long-term effects of the disaster. This chapter features dendroecological analysis and quantifies the accumulation of PTE within the woody tissues of native adult trees, comparing affected areas to unaffected reference sites to determine long-term element storage.

Discussion and Conclusion

Chapter 6 synthesizes the cumulative effects of the three research papers. It establishes the significance of the collective findings, offers a comprehensive knowledge claim to address the research gaps, and explicitly outlines directions for future work to advance environmental remediation science in the region.

2. Fundamentals

Human-induced environmental degradation, particularly from mining activities, presents a significant challenge to global ecosystems, necessitating advanced strategies for ecological restoration. This chapter provides the foundational context for the subsequent experimental investigations. It begins by examining the nature and impacts of mining ore tailings as a primary source of environmental disturbance. Following this, it delves into the physiological adaptations of plants to contaminated soils. Finally, the chapter outlines the principles of phytoremediation and dendroecology, highlighting their roles as critical tools for understanding and addressing the complex issues of ecosystem recovery in affected areas.

2.1 Mining ore tailings

Mining activity has been a disturbance-creating phenomenon since its foundation. This important economic activity creates environmental and social impacts at every step, whether in the mine preparations, ongoing excavations and waste generation, and mine decommissioning (Neves; Fernandes, 2016b). In the first stage, such problems can be related to habitat loss for mine digging (Rudke et al., 2020) and losses of ecosystem services, such as lower water and carbon retention (Xiang et al., 2021) and depletion of groundwater (Różkowski; Różkowski; Rahmonov, 2023). During and after the extraction, some related problems are pollution of surface and groundwater, acid drainage (Adeniyi et al., 2022) and waste-related disasters.

The mining activity imposes several disturbances on nature (Azam; Li, 2010). Beyond the local impacts of mine establishment and material extraction, mining activities also disperse pollutants into the atmosphere, water, and soil. While dry or filtered tailings storage is a safer alternative (East; Fernandez, 2021), wet tailings dams remain the most widely used method. Along the milling process, the wasted rock material becomes humid tailings, generally stored layer over layer in a vast area and dammed for several years (Zheng; Xu; Xu, 2011). Moreover, various types of Tailings Storage Facilities, or dams, exist for this material. This can be done by constructing upper barriers, or crests, which are sometimes made of concrete or the tailings themselves, though the latter are significantly less durable and safe.

These impoundments can be done in three main designs, according to the position of the crest, which are called downstream, upstream and centreline methods (Kossoff et al., 2014). In the downstream design, the crest is raised downstream of the slurry, with a growing baseline supported by the ground; hence, it is safer. In the upstream design, the crests are raised inwards above the previous and over the slurry, which is more unstable. A centreline design combines the two, made after the crest's sequential upraising, with fill on the downstream side and the slurry on the upstream side (Kossoff et al., 2014). The upstream method is related to many disasters and report a higher incidence of stability issues (Franks et al., 2021). Worldwide, there are many examples of tailings disasters (Table 2) (Dong; Deng; Wang, 2020a), mainly associated with small to medium-sized dams with up to 30 m high and 5 x 10^6 m^3 total volume (Azam; Li, 2010).

Table 2:Typical accidents statistics of tailings dam, adapted from Dong et al 2020.

Year	Accident location	Consequences
2019	Vale iron mine tailings dam, Brumadinho, Minas Gerais, Brazil	260 people have died, and ten have disappeared at present
2017	Tonglvshan tailings dam, Daye, Hubei, China	Two people died; one was missing and drowning about 400 acres of fishponds
2015	Fundão iron mine tailings dam, Mariana, Minas Gerais, Brazil	19 people died, released about 60 million m^3 of tailings and destroyed 158 houses
2014	Mount Polley gold and copper mine tailings dam, Canada	Released 25 million m^3 of tailings, the ecological environment was seriously polluted
2013	Jianping Jinyuan tailings dam, Liaoning, China	12 houses were washed away
2010	Xinyi Yinyan tin mine tailings dam, Guangdong, China	At least 22 people were killed or missing
2010	Tailings dam of Fengyun Manganese Company, Hunan, China	Six people died
2010	MAL Magyar Aluminium tailings dam, Hungarian	Ten people died, and 120 were injured
2009	Karamken tailings dam, Russia	One people died, released 1 million m^3 of tailings and destroyed 11 houses
2008	Xinta Mining Company tailings dam, Shanxi, China	277 people died, and released 200,000 m^3 of tailings dam
2007	Dingyang No. 5 tailings dam, Haicheng, Liaoning, China	15 people died, two were injured, and 38 were missing
2007	Baoshan tailings dam, Xinzhou, Shanxi, China	Direct economic losses of 45 million YUAN
2006	Gold mine tailings dam, Zhen'an, Shaanxi, China	76 houses were washed away, 17 people died, and about 200,000 m^3 of tailings
2006	Miaolinggou tailings dam, Qian'an, Hebei, China	Six people died
2005	Fengguang and Chengnan tailings dam, Linyi, Shanxi, China	Nine people died
2003	CerroNegro copper mine tailings dam, Chile	Lost tailings of 50,000 tons
2001	Dechang titanium mine tailings dam, Yunnan, China	Seven people died
2000	Hongtu tin mine tailings dam, Guangxi, China	29 people died, and 56 were injured
2000	Baia Mara tailings dam, Romania	100,000 m^3 of cyanide-containing tailings into the Danube
1998	Aznalcóllar tailings dam, Spain	Washed out 46 million m^2 of farmland and lost 3.4 billion euros
1995	Omai gold mine tailings dam, Guyana	Cyanide pollution affected the lives of nearly 23,000 people in a nearby river
1994	Harmony tailings dam, South Africa	17 people died, and released 25 million tons of tailings
1994	Longjiaoshan copper mine tailings dam, Daye, Hubei, China	30 people died in the flood, and three were missing
1993	Tungsten mine tailings dam, Gannan, Jiangxi, China	Most houses in the downstream area were washed away, and 100 acres of farmland
1986	Huangmeishan iron mine tailings dam, Anhui, China	19 people died, and 95 were injured
1985	Zhuyuan Nonferrous metal mine tailings dam, Hunan, China	49 people died, direct economic losses of 13 million YUAN
1985	Stave tailings dam, Italy	268 people died, and released 200,000 m^3 of tailings
1985	Prealpi tailings dam, Italy	250 people died
1979	Church Rock Tailings Dam, New Mexico	Released 400,000 m^3 of tailings
1978	Mochikoshi tailings dam, Japan	Released 140,000 tons of tailings, the environment was polluted
1978	Corsyn Consolidated Mines gold-tailings dam, Zimbabwe	One person died, and 30000 tons of tailings were released
1976	Zletovo mine No. 4 tailings dam, Yugoslav	Released 300,000 m^3 of tailings, the ecological environment was seriously polluted
1974	Bafokeng tailings dam, South Africa	12 people died, and released more than 30 million tons of tailings
1972	Buffalo Creek tailings dam, Virginia	125 people died, released 550,000 tons of tailings, and 4,000 were homeless
1971	Phosphate mine tailings dam, Florida	Released 800,000 tons of tailings, the environment was polluted
1970	Copper mine tailings dam, Zambia	100 people died
1966	Gypsum mine tailings dam, Texas	Released 200,000 tons of tailings, the ecological environment was seriously polluted
1966	Aberfan tailings dam, Wales	144 people died, and released 150000 m^3 of tailings
1965	New El cobre tailings dam, Chile	Released 500,000 tons of tailings, the environment was polluted
1965	Old El cobre tailings dam, Chile	210 people died, and released more than 2 million tons of tailings
1962	Huogudu tailings dam, Yunnan, China	174 people died, and 92 were injured
1961	Jupille tailings dam, Belgium	11 people died, and released 300,000 tons of tailings
1933	Powell Soda Factory tailings dam, East Debel	Released hundreds of millions of cubic meters of tailings, causing severe pollution
1928	Barahana tailings dam, Chile	54 people died, and released more than 4 million tons of tailings

In Brazil, many dams have upstream-designed reservoirs, which have been decommissioned after the two most massive disasters in the last ten years in *Minas Gerais* state. The greatest of them happened in 2015 in the *Fundão* Dam, *Mariana* (Hatje et al., 2017b), which contained 56.4 x 10^6 m³ iron ore tailings in a complex of dams of up to 175 m high (Carmo et al., 2017a). The slurry was spread in the *Doce* River, affecting its riverine ecosystems and reaching the Atlantic Ocean after 660 km of disturbances (Fernandes et al., 2016b). The most recent disaster happened in 2019, affecting the *São Francisco* Basin, after the disruption of the *Feijão* stream mine dam, with 259 people killed and 11 people missing in *Brumadinho* (Almeida; Jackson Filho; Vilela, 2019).

After the disruption of a tailings dam, a massive amount of material is spread over land and rivers, altering local conditions and creating a new ecosystem (Teixeira; Fernandes, 2020). The spilled material resulting from anthropic alterations is called a technosol and has its physical and chemical characteristics changed (Lebrun et al., 2020). Therefore, heavy metals and trace elements such as Zn, Pb, Ni, Cr, Cu and Fe can be found in higher concentrations in areas affected by disasters related to iron ore extraction, compared to previous values (Quaresma et al., 2021).

Nonetheless, the impacts of mining activities can be remediated with physical, chemical or biological techniques (Rodrigues, 2013). The first can be done by material removal and relocation to a protected and secure area. The second can be made with products that may stabilize the trace elements, impeding their translocation along the soil profile or, on the opposite way, allowing their flow and facilitating their extraction (Xu et al., 2019). Finally, the most sustainable but generally longer treatment uses living beings, such as microorganisms that degrade the chemical substances or plants that may stabilize or even absorb the pollutants (Khalid et al., 2017a).

2.2 Physiological aspects of plant recruitment, establishment and growth in contaminated areas

The physical alterations concerning tailings deposition are very important for plant establishment, since they are different from the local soils. They are usually characterised by a poor porosity, low water retention, lack of organic matter and finally, a poor soil nutrition (Tordoff; Baker; Willis, 2000). Beyond that, tailings-affected areas usually have a variety of potentially toxic elements (PTE), depending on the type of ore used in the extraction process. In iron tailings such as in the Fundão dam, elements with the potential to become source of contamination were found, such as Cr, Cd, Cu, Fe, Mn, Ni, Ba, and Co (Silva 2022), Pb, Na (Pires et al., 2003), As, Sr (Segura et al., 2016), Bi, Ce, Hg (Silva et al., 2021a) and others. Among these elements are vital micronutrients for plant processes, such as Mn and Fe, which are essential for establishing electro-potentials (Mn) and facilitating electron transport (Fe) (Marschner, 2012). These micronutrients and other elements, are present in plants tissues in different concentrations and are used for an adequate plant growth (Table 3).

Table 3: Average concentration of mineral elements in plant shoot dry matter sufficient for adequate growth. Taken from Marschner 2012.

Element	Chemical symbol	μmol g^{-1} dw	mg kg^{-1}
Molybdenum	Mo	0.001	0.1
Nickel	Ni	0.001	0.1
Copper	Cu	0.1	6
Zinc	Zn	0.3	20
Manganese	Mn	1.0	50
Iron	Fe	2.0	100
Boron	B	2.0	20
Chlorine	Cl	3.0	100
Sulphur	S	30	1,000
Phosphorus	P	60	2,000
Magnesium	Mg	80	2,000
Calcium	Ca	125	5,000
Potassium	K	250	10,000
Nitrogen	N	1,000	15,000

From Epstein (1965), Epstein and Bloom (2005), Brown *et al.* (1987b).

However, beyond these nutrients, different toxic elements present in other kinds of materials are heavy metallic elements that can be non-essential (Ba, Li, Zr, and Al), less toxic (As and Sn), or highly toxic (Cd, Pb, and Hg)(Rahman et al., 2024). Each of these can be toxic in different amounts and with synergistic effects, depending on factors such as plant age, organ and species. At the same time, the upper critical level is related to the lowest tissue concentrations that lead to a reduction in plant yield and is described for a variety of plant crops (Macnicol et al., 1985). Moreover, it is not rare to find edible plants with concentrations of heavy metal/ metalloids higher than the permissible limits (Khalid et al., 2017c) (Table 4). In general, some toxicity outcomes occur by disrupting vital enzyme function, damaging or modifying biomolecules, displacing essential metal ions within biomolecular structures, and impairing antioxidant defence systems due to the creation of reactive oxygen species (ROS)(Sarwar et al., 2017).

Table 4: Concentrations of Potentially Toxic Elements (PTE) in edible plant tissues exceeding permissible safety thresholds, Based on EU and FAO/WHO Guidelines. Source: Adapted from Khalid et al. (2017).For the full reference list corresponding to the original studies cited in the last column refer to the original paper.

Heavy metals	Vegetables	Concentration in soil (mg/kg)	Concentration in edible parts (mg/kg)	*Maximum allowable limit	Fold-higher than allowable limit	References
Cd	*Lactuca sativa*	1.3	130	0.2	650	Pereira et al., 2011
	Solanum lycopersicum	11.24	13		65	Hediji et al., 2012
	Agaricus bisporus	-	10	-	50	Schlect and Siumel, 2015
	Cynosurus cristatus	0.2	9	-	45	Quezada-Hinojosa et al., 2015
	Brassica napus	1	6	-	30	Wu et al., 2015
Pb	*Daucus carota*	0.01	390	1	390	Carvalho et al., 2015
	Solanum aethiopicum	452	144	-	144	Nabulo et al., 2012
	Brassica oleracea	2.58	49	-	49	Perveen et al., 2012
	Lactuca sativa	-	28	-	28	Kang et al., 2015
	Spinacia oleracea	66.78	20	-	20	Khan et al., 2013
As	*Nicotina glauca*	14,66	92	0.15	613	Santos-Jallath et al., 2012
	Lactuca sativa	5.83	14	-	96	Caporale et al., 2014
	Oryza sativa	-	1.3	-	8	Smith et al., 2008
Zn	*Nicotina glauca*	507	1,985	50	40	Santos-Jallath et al., 2012
	Brassica juncea	190	201	-	4	Maganda et al., 2007
	Zea mays	80	148	-	3	Avci and Deveci, 2013
	Lactuca sativa	-	118	-	2.4	Bosacki and Tycistiski, 2009
	Spinacia oleracea	124	84	-	1.7	Naser et al., 2012
Ni	*Lactuca sativa*	1.11	48	0.2	238	Perveen et al., 2012
	Solanum lycopersicum	1.11	43	-	215	Perveen et al., 2012
	Portulaca oleracea	-	36	-	181	Renna et al., 2015
	Diplotaxis tenuifolia	-	35	-	175	Renna et al., 2015
	Cupressus sempervirens	11.3	7	-	35	Farahat and Linderholm, 2015
Cu	*Solanum lycopersicum*	-	202	10	20	Liu et al., 2006
	Coriandrum sativum	-	48	-	5	Gupta et al., 2013
	Zea mays	41	47	-	5	Avci and Deveci, 2013

	Agaricus bisporus	-	36	-	4	Liu et al., 2006
	Apium graveolens	46.85	11	-	1	Chao et al., 2007
Cr	*Solanum aethiopicum*	256	65	1	65	Nabulo et al., 2012
	Brassica oleracea	12.78	24	-	24	Tiwari et al., 2011
	Capsicum sp.	1.11	17	-	17	Perveen et al., 2012
	Sinapis sp.	1.11	13	-	13	Perveen et al., 2012
	Coriandrum sativum	1.11	13	-	13	Perveen et al., 2012
Mn	*Allium cepa*	573	585	500	1.17	Chiroma et al., 2014
	Lactuca sativa	619	512	-	1.02	Chiroma et al., 2014

The ROS formation is part of a plant life cycle, but is enhanced during stress conditions, where an imbalance between its production and elimination occurs (Karuppanapandan et al., 2006). Among the effects on plant metabolism are cellular component damage, DNA lesions and mutations, and further metabolic dysfunction and cell death (Karuppanapandian et al., 2011). Also, exposure to heavy metals can lead to an accumulation of H_2O_2 following lipid peroxidation, which can cause biomembrane deterioration (Karuppanapandian et al., 2011). Plants have ways to deal with these stressors, for example, by using molecules like NADPH oxidase, including situations of deficiency or excess of Cd, Cu and Ni (Hao; Wang; Chen, 2006; Quartacci; Cosi; Navari-Izzo, 2001). Beyond that, they can produce ROS-detoxifying enzymes, such as the Superoxide Dismutase (SOD) (Karuppanapandian et al., 2009) or non-enzymatic systems, like the ascorbic acid in the chloroplasts and glutathione (GSH) in many components such as chloroplasts, mitochondria, ER, vacuoles, and cytosol (Noctor; Foyer, 1998). The GSH is also important, since it is a precursor of phytochelatins (PC), which plays an important role in controlling cellular heavy metal concentrations (Pietrini et al., 2003). Finally, the phenolic compounds, including flavonoids, tannins, hydroxycinnamate esters, and lignin also have antioxidative properties, due to their high reactivity as electron donors, its ability to stabilize unpaired electrons and to chelate transition metal ions (Chen et al., 2024)2024).

In general, plants have two different strategies to deal with metals in the soil: avoidance (exclusion) or tolerance (inclusion). In the first, plants avoid intoxication by immobilizing the PTE, modifying its ionic form, exudating molecules in the roots, cell wall defence, and making symbiotic relations that guarantee protection. On the second, they tolerate it by ionic neutralization, chelation, sequestration or antioxidant defence (Nurrahma et al., 2024). Plants that absorb these PTE usually acidify the rhizosphere by exudating low-molecular-weight organic acids (LMWOAs) (Chen; Dou; Xu, 2018), enhancing the elements' migration and fluidity in the soil (Geng et al., 2020). After that, the phytosiderophores bind ions, facilitating their uptake (Coelho et al., 2020a). In the roots, the metals can be transported between the cells (apoplastic pathway), but to reach the xylem, they must pass through the cells, crossing the plasma membrane (symplastic pathway) (Yan et al., 2020). Usually, the metal transport is limited in the xylem due to the high cation exchange capacity of the negatively charged carboxyl group end in the cell wall, e.g., in hemicellulose and lignin molecules (Momoshima; Bondietti, 1990a). However, the transport of metals to the shoots is possible when the plant produces low-weight chelators that bind to metals, forming a soluble complex (Mahmood, 2010). Finally, on the leaves, the metals can be directed to different organelles where they can be used, in case of essential nutrients (e.g.: Cu, Zn, Mn, Ni and Fe), or sequestered in extracellular or sub-cellular compartments for detoxification (e.g.: Cd, Hg and Pb) (Dalvi; Bhalerao, 2013; Sarwar et al., 2017). Finally, the sequestration occurs by compartmentation of metal ions in the vacuole (Figure 7) (Rylott; Van der Ent, 2025).

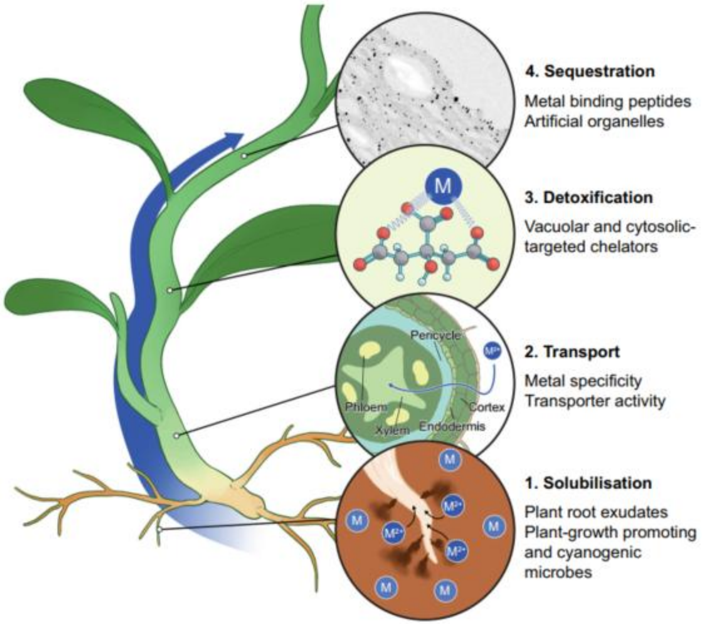

Figure 7:Pathway of metal uptake by plants. Extracted from Rylott van der Ent 2025. Harnessing hyperaccumulator plants to recover technology-critical metals: where are we at?

2.3 Restoration of degraded areas and phytoremediation

In events where a massive amount of tailings is spread in an area, destroying the natural and human environments, the consequences can be unimaginable (Fernandes et al., 2016). Nonetheless, there are different ways to actively clean or restore the impacted environment, achieving the reclamation of disturbed areas. The main approaches for such goals can be divided into physical, chemical or biological strategies (Rodrigues, 2013). The physical removal of the contaminated substrate is used when the total volume is manageable. At the same time, chemical restoration occurs when the substrate contamination has achieved a lower horizon of the soil or when the residues have spread over a certain amount of water (Xu et al., 2019). Finally, bioremediation uses microorganisms or plants that actively take the contaminants off the substrate for a more extended period but organically, leading to an ecological succession. In general, for remediation using plants (phytoremediation), short-lived herbs and temperate tree species are usually cited in the restoration literature (Boechat, 2019). Still, in tropical countries like Brazil, many herbs (Paz et al., 2024)) and arboreal species could be used for the same purpose but are neglected.

2.3.1 Phytoremediation

The use of plants for cleaning and rehabilitating an area is broadly categorized into five main approaches or mechanisms: Phytoextraction, Phytostabilisation, Phytoevaporation (of Phytovolatilization), Rhizofiltration and Rhizodegradation (Mahar et al., 2016). Typically, soils present metals and metalloids in low (200-2000 g/kg) or trace concentrations (0.1-200 g/kg), however, they may be abnormally higher in metalliferous soils (Reeves; Ent; Baker, 2017). Nonetheless, plants have evolved adaptations that allow them to survive, tolerate, or even accumulate these elements within their tissues, with an ecological outcome of reduced herbivory (Boyd, 2012). These plants can be generally classified in three types: 1) *excluders*, which limit the metal concentration in the above-ground tissues, storing them in the roots; 2) *indicators*, which uptake metals and transport them to the aerial parts, where the metal contents are similar to the soil; or 3) *accumulators*, which can store metals in a concentration significantly higher than those in the substrate, sometimes achieving up to 100 times greater accumulation than non-accumulators (Ali; Khan; Sajad, 2013). Furthermore, the concept of a hyperaccumulator is commonly applied to plants that can accumulate, per kg of aerial dry mass, thresholds such as: > 1 mg of Au and Ag, > 100 mg of Cd, Se and Ta, > 1000 mg of Cu, Co, Cr, Ni, Pb, U, As, or > 10,000 mg Mn and Zn (Mahar et al., 2016). This concept was initially defined as a guideline (Baker; Brooks, 1989) and remains flexible (Van der Ent; Mulligan, 2015).

In summary, a good extractor plant should possess the ability to grow in soils with low nutrient availability, develop a deep root system, a high growth rate, demonstrate metal resistance and preferably have an economic use for any of its parts (Pulford; Watson, 2003). To measure and compare a plant's capacity to uptake and concentrate the metals in its tissues relative to the soil, some approaches have been developed. In particular, the Bioconcentration Factor (BCF) is defined as the ratio between the metal content in the plant tissues and that in the soil. Additionally, it is essential to determine if the plant transports the absorbed metals to its shoot (e.g.: stems or leaves). This relationship is quantified by the Translocation Factor (TF), which represents the ratio of the metal concentration in the aerial part to that in the roots (Ali; Khan; Sajad, 2013).

In the initial stage of the rehabilitation of an area, herbaceous tolerant plants are essential (Araújo et al., 2020) since they spread their roots among the contaminated particles (Ferreira et al., 2019). This restricts soil particles' movement, thereby preventing erosion by wind or runoff (Ashraf; Maah; Yusoff, 2011). Foremost, these plants may absorb metals in the upper layer of the soil, allowing other plants to germinate or establish. Conversely, trees possess a longer and wider root system, reaching deeper soil layers, addressing leached underground substances that could potentially contaminate the groundwater (Mahar et al., 2016). Additionally, trees reduce the wind flow, produce litter that enhances soil organic matter, exude acids that increase nutrient flux, and finally create an upward movement of water in the soil, which helps to bring solubilized pollutants towards the surface (Atangana et al., 2014).

Most known hyperaccumulators belong to the families Brassicaceae and Phyllanthaceae; others can be found in Vochysiaceae, Rubiaceae, Melastomataceae (CHENERY; SPORNE, 1976), Asteraceae, Poaceae, Violaceae, Salicaceae, Buxaceae, Myrtaceae and Fabaceae (Reeves et al., 2018). The Fabaceae family, in particular, warrants further exploration, due to its vast number of representatives and global distribution (Safronova et al., 2011a). In the phytoremediation of tropical areas, exotic plants are often recommended or used, which can be a superficial resolution resulting from negligence regarding the ecosystemic complexity. Particularly in the tropics, various native tree species could be used in a constructed system similar to those in agroforestry (Atangana et al., 2014). This approach requires considering the distinct traits and abilities of species to manage different metals across varying concentrations and availabilities.

In this section, we present a list of tree species, currently sparsely cited in the literature, that have potential for rehabilitating metal-contaminated areas in the tropics, mainly in Brazil. First, we used the Google Scholar web search engine to survey articles or books on the "phytoremediation" subject and the "tropic" region. Second, we compiled the cited tree species and registered their information. Finally, to confirm the potential of each species, we looked up its scientific name jointly with the terms “phytoremediation” and “heavy metals” in both English and Portuguese (Table 5).

Table 5: List of tropical and Brazilian species identified as tolerant or accumulator of some heavy metals. "BR" in the Origin column refers to Brazil, among other countries of South America.

Scientific name	Common name	Origin	Family	Phytoremediation potential	Accumulation organ	Reference
Acacia angustissima	Prairie acacia	Central America	Fabaceae	Cd	Root	(Atangana et al., 2014; Pereira et al., 2012)
Acacia mangium	Black wattle, hickory wattle	Oceania (Australia)	Fabaceae	Pb, Ni, Cr	Root and stem	(Ang et al., 2010; Bongoua-Devisme et al., 2019; Rodrigues, 2013)
Acacia tortilis	Umbrella thorn	Africa	Fabaceae	Se	Root, stem, and leaves	(Atangana et al., 2014; Dhillon; Dhillon; Thind, 2008)
Albizia adianthifolia	Mepepe	Africa	Fabaceae	Co, Cu	not mentioned	(Atangana et al., 2014; Brooks et al., 1987)
Albizia amara	Bitter albizia	Africa	Fabaceae	Cr	Root	(Atangana et al., 2014; Shanker; Ravichandran; Pathmanabhan, 2005)
Albizia polycephala	Albizia	South America (BR)	Fabaceae	Mn	Shoot (not specified)	(Cruz et al., 2020b)
Anadenanthera colubrina	Vilca, Angico branco	South America (BR)	Fabaceae	Cd	Root	(Pereira et al., 2012)
Annona senegalensis	African custard-apple	Africa	Annonaceae	Co, Cu	not mentioned	(Atangana et al., 2014; Brooks et al., 1987)
Casuarina equisetifolia	Australian pine tree	Oceania (Australia)	Casuarinaceae	Pb, Zn, Cu, Cr	Stem	(Atangana et al., 2014; Ukpebor et al., 2010)
Cedrela fissilis	Argentine cedar	South and Central America (BR)	Meliaceae	Cu	Root	(Caires et al., 2011)
Cybistax antisyphilitica	Green Ipê	South and North America (BR)	Bignoniaceae	Mn	Shoot (not specified)	(Cruz et al., 2020b),
Dalbergia sissoo	North Indian rosewood, shisham	Asia (India)	Fabaceae	Se	Root, stem, and leaves	(Atangana et al., 2014; Dhillon; Dhillon; Thind, 2008)
Dendropanax cuneatus	Maria-mole; Pau de tamanco	South and Central America (BR), Asia	Araliaceae	Trace elements	stem	(Rodrigues, 2013)
Eucalyptus citriodora (sin. Corymbia citriodora)	Lemon-scented gum, spotted gum	Oceania (Australia)	Myrtaceae	Fe, Mn, Zn	Leaves	(Khattak; Jabeen, 2012; Rodrigues, 2013)
Eucalyptus grandis	Flooded gum, rose gum	Oceania (Australia)	Myrtaceae	Cu, Cd	Root	(Abbaslou; Bakhtiari, 2017; Rodrigues, 2013)

Handroanthus heptaphyllus	Pink trumpet tree, pink tab	South America (BR)	Bignoniaceae	Fe	Shoot (not specified)	(Cruz et al., 2020b)
Handroanthus impetiginosus	Pink ipê, pink lapacho, pink trumpet tree	South and North America (BR)	Bignoniaceae	Cd and Mn	Root, stem, and leaves	(Cruz et al., 2020b; Paiva et al., 2004)
Hymenaea courbaril	Courbaril, West Indian locust	South and Central America (BR), Asia	Fabaceae	Cu	Root	(Asensio et al., 2018a)
Inga edulis	Ingá, Ice cream-bean	South America (BR)	Fabaceae	Hg	Root	(Atangana et al., 2014; Marrugo-Negrete et al., 2016)
Leucaena leucocephala	White leadtree, jumbay, river tamarind	Central America	Fabaceae	Fe, Zn, Cu, Mn, Cd	Root	(Atangana et al., 2014; Bauddh; Singh; Korstad, 2017)
Melia azedarach	Cinamomo, cinnamon	Asia	Meliaceae	Cr	Root, stem, and leaves	(Atangana et al., 2014; Sakthivel; Vivekanandan, 2009)
Morus alba	Amoreira branca, blackberry	Asia	Moraceae	Pb	Leaves	(Atangana et al., 2014; Van der Ent et al., 2018)
Myroxylon peruiferum	Quina, Bálsamo-do-peru; Cabreúva	South and North America (BR)	Fabaceae	Cu, Zn e Cd	Root and shoot	(Marques et al., 2018; Rodrigues, 2013)
Nyssa sylvatica	Tupelo, black tupelo, black gum or sour gum	North America	Cornaceae	Co	Leaves	(Brooks; McCleave; Schofield, 1977; Van der Ent et al., 2018)
Peltophorum dubium	Cambuí, Canafístula	South America (BR)	Fabaceae	Fe and Mn	Shoot (not specified)	(Cruz et al., 2020b)
Phyllanthus balgooyi	Phylanthus	Oceania	Phyllanthaceae	Ni	Stem	(Mesjasz-Przybylowicz et al., 2016)
Piptadenia gonoacantha	Pau Jacaré	Brazil	Fabaceae	Cu, Zn, Pb	Root, stem, and leaves	(Horn et al., 2020a; Rodrigues, 2013)
Pycnandra acuminata	Blue sap	Oceania	Sapotaceae	Ni	Stem	(Van der Ent et al., 2018)
Salix humboldtiana	Salgueiro, Humboldt's willow	South and North America (BR)	Salicaceae	Zn, Cd, Cu	Root	(Gomes et al., 2011)
Senna macranthera	Fedegoso	Brazil	Fabaceae	Zn, Cd, Cu	Stem and leaves	(Rodrigues, 2013; Soares et al., 2001)
Syzygium cumini	Malabar plum, Java plum, Jambolão	India (naturalized in Brazil)	Myrtaceae	Se	Leaves	(Atangana et al., 2014; Dhillon; Dhillon; Thind, 2008)
Tectona grandis	Teak, Teca	Asia	Lamiaceae	Zn, Cr	Leaves	(Ajmal; Rao; Ahmad, 2011; Atangana et al., 2014)

The Fabaceae family accounted for a substantial portion of the findings (45%), with 15 species reported as occurring in Brazil. The primary beneficial attribute of this group is the ability of these plants to re-establish the soil environment through nitrogen fixation (Safronova et al., 2011). Furthermore, in addition to their metal uptake capacity, the Fabaceae are widely distributed across different biomes, presenting a high Family Importance Value (FIV) in regions such as the Doce River (França; Stehmann, 2013). Hence, legumes are well-indicated for restoring these contaminated areas.

The target metals cited, along with their respective number of literature citations (in parentheses), were: Cd (6), Co (3), Cr (3), Cu (11), Fe (4), Hg (1), Mn (6), Ni (3), Pb (4), Se (3), and Zn (6). Although Aluminum (Al) appears in relevant phytoremediation works, it was not present in this review because it is classified as a light metal and is primarily associated with acid soil toxicity, not heavy metal contamination. Since a group of the cited elements can be found in mining tailings, choosing species that can uptake a higher number of them is essential. While it is generally known that plants are often not adapted to thrive in soils contaminated with a variety of metals (Baker et al., 2012), some cited species—such as *Acacia mangium, Casuarina equisetifolia, Eucalyptus citriodora, Leucaena leucocephala, Myroxylon peruiferum, Piptadenia gonoacantha, Salix humboldtiana, Senna macranthera* —demonstrate the ability to survive and remediate soils impacted by multiple different elements.

Accumulation in the shoot (the aerial part of the plant), was cited 20 times, with specific references to the stems and leaves occurring 10 and 12 times, respectively. For environmental cleanup purposes, the translocation of metals to the upper parts is relevant because the elements can be sequestered in the bark, trunk, twigs, or leaves (Mulenga et al., 2023). Nonetheless, these stored metals may still be available to the herbivorous fauna (Destro et al., 2024) or to decomposers upon the plant’s senescence or death. Hence, it is crucial to consider the form in which these elements will be immobilized inside the plant and whether they will be stabilized. To obtain such information, studying the metal distribution within the wood of living trees can be highly valuable (McLauchlan et al., 2014).

2.3.2 Dendroecology

Trees may be studied after a disturbing event and can reveal how stressful a disturbance is for the plants, comparing growth rings previous and post-disaster, for example (Speer, 2009). The effectiveness of using trees for remediation of areas is well documented, with examples of the mechanisms of phytostabilization, phytoextraction, and phytodegradation (Khan et al., 2025). For instance, a copper mine tailings dam failure in Poland was related to a long term increase in the contents of Mn, Ni, Zn, Cr, Pb,Cu and Fe in trees of *Quercus robur* L. (Dobrzańska et al., 2021). In a field experiment of three years, ten *Quercus* species were grown in Cd and Zn-contaminated soil (Li et al., 2022). The Cd accumulation in *Q. texana* and *Q. pagoda* reached 30 and 70 mg/kg, respectively, being higher in the aboveground tissues, and higher than safety threshold values. Also, the Zn accumulation in *Q. fabri* and *Q. nigra* was up to 125 and 250 mg/kg in the leaves, higher than in most compared plants (Li et al., 2022). A study with *Salix viminalis* calculated a total Cd extraction in the order of up to 16.5 g Cd ha^{-1} $year^{-1}$, with the trees able to remove significant amounts of this pollutant and storing it in their stems (Klang-Westin; Eriksson, 2003). The Guadiamar Green Corridor (Spain) is a large-scale phytoremediation case study in which trace elements (TE) in soil were stabilized by tree planting, among them *Quercus, Celtis, Populus, Fraxinus*, etc., with positive outcomes after addition of soil amendments, tree planting and trace elements monitoring (Madejón et al., 2018). In Brazil, the pollution in urban areas was tracked using *Tipuana tipu* trees, which revealed a reduction in the levels of Cd, Cu, Pb, and Ni along three decades (Locosselli et al., 2018a). Similarly, in Zambia, the bark of three tree species was utilized to monitor the absorption of Mn, Ni, Pb, Cd, Fe, and Zn near a copper leaching plant (Mulenga et al., 2023a).

Using the dendroecology method, the pollutant concentrations inside the trees can be determined, with the support of dendrochronology techniques, which has many registered uses in the neotropics (Schöngart et al., 2017a). In this regard, a core section of the trunk is taken using a corer, which samples a wood cylinder from the tree (Algreen; Trapp, 2014). Sample preparation begins by fixing the cores in wooden mounts and meticulously polishing the surface using a sequence of progressively finer abrasives, usually ranging from 80 to 1200 grits per square inch (Schöngart et al., 2005). The growth rings are demarcated based on the species' specific wood anatomy and measured using the available device to determine radial increment rates. To ensure chronological accuracy, the resulting ring data undergoes cross-dating procedures, which requires a specific software, such as COFECHA, WinDENDRO, or TSAP (Becker; Egger; Wittmann, 2024; Izworska et al., 2022a). After that, the data is ready for comparison.

Each growth ring contains information on the radial increment achieved over one year and may exhibit varying concentrations of pollutants that were absorbed, translocated, or accumulated in the trunk during that period (Aljerf; Choukaife, 2018). This comprehensive dendroecological analysis integrates both temporal and chemical data and offers a more precise and detailed perspective on contamination history. This method leads to the development of reliable data that can inform distinct political and management decisions (Balouet et al., 2009). For monitoring an impacted area in a broader context over a prolonged period, the phytoscreening approach can be used (Bica, 2020). In this procedure, wood cores of approximately 6 cm in length are typically collected and analysed as a whole (Algreen; Trapp, 2014). While this method is useful for quickly estimating the total amount of contaminants in the tree's biomass, it does not allow for differentiation of concentration across specific years. For a more detailed, annual analysis of tree rings, a longer wood sample is required, the length of which is often determined by the size of the trunk's radius (Speer, 2009).

Chemical analysis of biological samples typically employs analytical techniques capable of scanning for various elements. Commonly used methods in metal studies include Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OS) or by Flame Atomic Absorption Spectrometer (FAAS) (Mogopodi et al., 2011). These techniques are also valuable for investigating element distribution across different plant tissues, such as roots, leaves, and seeds (Lombi; Scheckel; Kempson, 2011). However, to prepare biomass for these methods (e.g., in phytoscreening applications), the material must first be destroyed through either acid digestion or dry ashing following grinding (USEPA, 1995). A distinct and often advantageous approach for analyzing element concentrations is Energy Dispersive X-ray fluorescence (EDXRF) (Nečemer; Kump; Vogel-Mikuš, 2011). This technique is non-destructive and requires simpler sample preparation. A primary benefit is the ability to directly scan the wood core, allowing for the annual analysis of metal concentrations and clearly revealing differences in wood chemistry before and after a specific contamination event (Smith et al., 2014).

A preliminary floristic survey was conducted for the dendroecology chapter to select species for the final comparative analysis. This survey ensured that the selected species were present in both the tailings-affected and unaffected floodplains, satisfying a key methodological requirement (Table 6).

Table 6: Most common species identified by the forest guide in the region through an initial survey made in preparation for the Dendrochronological project

Scientific name	Family
Tapirira guianensis Aubl.	Anacardiaceae
Xylopia emarginata Mart.	Annonaceae
Didymopanax macrocarpum (Cham. & Schltdl.) Seem.	Araliaceae
Piptocarpha macropoda (DC.) Baker	Asteraceae
Trema micrantha (L.) Blume	Cannabaceae
Licania kunthiana Hook.f.	Chrysobalanaceae
Maprounea brasiliensis A.St.-Hil.	Euphorbiaceae
Albizia polycephala (Benth.) Killip ex Record.	Fabaceae
Anadenanthera colubrina (Vell.) Brenan	Fabaceae
Anadenanthera peregrina (L.) Speg.	Fabaceae
Apuleia leiocarpa (Vogel) J.F. Macbr.	Fabaceae
Cassia ferruginea (Schrad.) Schrad. ex DC.	Fabaceae
Copaifera langsdorffii Desf.	Fabaceae
Dalbergia nigra (Vell.) Allemão ex Benth.	Fabaceae
Inga sessilis (Vell.) Mart.	Fabaceae
Melanoxylon brauna Schott	Fabaceae
Piptadenia gonoacantha (Mart.) J.F.Macbr.	Fabaceae
Plathymenia reticulata Benth.	Fabaceae
Platypodium elegans Vog.	Fabaceae
Nectandra oppositifolia Nees	Lauraceae
Cariniana estrellensis (Raddi) Kuntze	Lecythidaceae
Byrsonima sericea DC.	Malpighiaceae
Luehea grandiflora Mart. & Zucc.	Malvaceae
Pachira glabra Pasq.	Malvaceae
Cabralea canjerana (Vell.) Mart.	Meliaceae
Sorocea bonplandii (Baill.) W.C.Burger et al.	Moraceae
Virola bicuhyba (Schott ex Spreng.) Warb.	Myristicaceae
Eugenia florida DC.	Myrtaceae
Schizocalyx cuspidatus Kainul. & B. Bremer	Rubiaceae
Hortia brasiliana Vand. ex DC.	Rutaceae
Matayba guianensis Aubl.	Sapindaceae
Siparuna guianensis Aubl.	Siparunaceae
Vochysia thyrsoidea Pohl	Vochysiaceae

3. Plant recruitment six years after the Samarco's tailings-dam disaster: Impacts on species richness and plant growth

This chapter is reproduced from the manuscript that has been published in the peer-reviewed journal ***PLoSOne***. 20(8): e0315990. https://doi.org/10.1371/journal.pone.0315990

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Abstract

One of the greatest tragedies in Brazilian mining history occurred in November 2015 in Mariana, Minas Gerais state, when a dam from the mining company Samarco was breached. Millions of mine tailings from this upstream embankment were dumped over the Doce River basin, impacting an area of approximately 1469 ha of riparian vegetation. Our objective was to experimentally investigate whether plant recruitment and establishment are impaired in areas affected by tailings six years after the deposition. To achieve this goal, in 2021 we compared soil chemical properties between affected and unaffected areas, performed a soil seed bank experiment in controlled conditions, and conducted a greenhouse growth experiment using the two most abundant plant species. Affected soils presented lower fertility and organic matter content. At the same time, the mean abundance and richness of emerging plants did not differ between soils. Still, affected areas exhibited approximately 35% lower accumulated species richness (gamma diversity) than unaffected ones. The three most abundant species in both areas represented 34% of the individuals, being *Marsypianthes chamaedrys* (Vahl) Kuntze, *Ludwigia octovalvis* (Jacq.) P.H. Raven and *Ageratum conyzoides* L. In the growth experiment, plants growing in affected soils presented reduced height and stem diameter increment (*L. octovalvis*) or allocated fewer resources to root production than aerial parts (*M. chamaedrys*), potentially in response to soil infertility and density. Even after six years, our results showed that tailings-affected areas continue to experience negative impacts on plant recruitment, highlighting its adverse effects on ecosystem functions and services.

Keywords: disturbance, revegetation, impacted areas, seed germination, Rio Doce.

3.1 Introduction

Large-scale mining generates several environmental impacts by altering the landscape before, during and after the ore extraction (Tarolli; Sofia, 2016). In addition, the establishment and operation of mining activities can cause true disasters (Dong; Deng; Wang, 2020). A tragic example occurred in November 2015 in Mariana, Minas Gerais state, southeast Brazil (Meira-Neto; Neri, 2017) when the Fundão dam (Samarco's Company), containing iron ore tailings, burst. A total of 39.2 million m^3 of slurry were released into the Doce River basin (Sánchez et al., 2018a). The disaster impacted an estimated area of approximately 1469 ha of natural vegetation (Fernandes et al., 2016), burying or carrying away the riparian plants and their diaspores in the soil.

After the passage and deposition of this large amount of tailings, the vegetation recovery where likely depended on three main factors: i) the existence of a soil seed bank (Walck et al., 2005); ii) the arrival of propagules through dispersal processes (Nazareno et al., 2021) and/or iii) restoration practices such as the addition of seeds (Campanharo et al., 2021; Doelinger, 2017). The following months after the disaster, there were some reclamation procedures such as the sowing of seeds, tree planting, and soil amendment (Fonseca, 2020; Orlandini, 2016). However, the presence of seeds alone does not guarantee the establishment of seedlings, and the recruitment and assembly of the plant community also depend on the survival of seedlings (Schupp, 1995). These stages are susceptible to environmental factors (Larson; Ebinger; Suding, 2021) and might be strongly affected by the permanence of the slurry.

The sludge passage directly affected the soil by changing its chemical and physical properties (Queiroz et al., 2022). For example, the deposited tailings differ from the predominant soil in the region by its higher sand content (Andrade et al., 2018), higher density, and lower macroporosity and clay/silt content (Silva et al., 2021a). Furthermore, in the affected banks of the Doce river tributary, Gualaxo do Norte, higher concentrations of bismuth (Bi), cerium (Ce), chromium (Cr), iron (Fe), manganese (Mn), phosphorus (P), and lead (Pb) were found compared to unaffected areas (Silva et al., 2021a). While some of these elements are essential for plants, others can be toxic in excessive amounts. For instance, the total contents of Cr (235.0 ± 69.5 mg/kg) and Pb (52.7 ± 15.9 mg/kg) were higher than the soil quality guideline values, which are 75 and 19.5 mg/kg, respectively (Silva et al., 2021a).

The changes in soil properties in the affected areas could alter the restoration process's success in the initial years after the disaster. Considering that various natural and anthropogenic factors serve as environmental filters (Bruno et al., 2016), affected soils can impact plant recruitment by reducing emergence, establishment, and growth in contaminated soils. This can occur by decreasing water absorption, gas exchange, radicle fixation, and substrate foraging (Harper, 1977). Also, plant germination and establishment can be reduced once the penetration of roots is affected by the soil compaction promoted by the tailings (Andrade et al., 2018). It has been found that soils contaminated by iron ore tailings might be responsible for lower plant biomass production (Cruz et al., 2020c), change in leaves properties (Nascimento et al., 2021) and accumulation of potentially toxic elements (Coelho et al., 2020b). It is, therefore, reasonable to expect that a smaller number of plants and fewer species will be able to germinate and grow in affected areas, with practical implications for restoration.

The present study aims to experimentally test if plant recruitment is impaired in areas affected by the passage of the tailings six years after the Samarco dam breach. We analysed soil characteristics as the primary ecological mechanism influencing plant recruitment, growth, and soil seed bank composition. More specifically, we performed experiments in controlled growth conditions to test the following hypothesis: there is a reduction in i) abundance; ii) richness of emerging seedlings, and iii) plant growth, when comparing affected to unaffected areas.

3.2 Materials and methods

3.2.1 Study area

We sampled the Upper Doce River basin in Minas Gerais in southeast Brazil (Figure 8). Semideciduous seasonal forests characterize the areas (Instituto Brasileiro de Geografia e Estatística, 2012) mainly on oxisols in an agricultural matrix of different crops and artificial cattle grazing grasslands, with a long history of degradation (Coelho, 2009). The climate according to Köppen's classification is Cwa and Cwb, humid subtropical with drought in winter and summer from hot to temperate, with annual rainfall of 1200 mm (Cupolillo; Abreu; Vianello, 2008). Map was created using software QGIS 3.34.14 (QGIS Development Team, 2024).

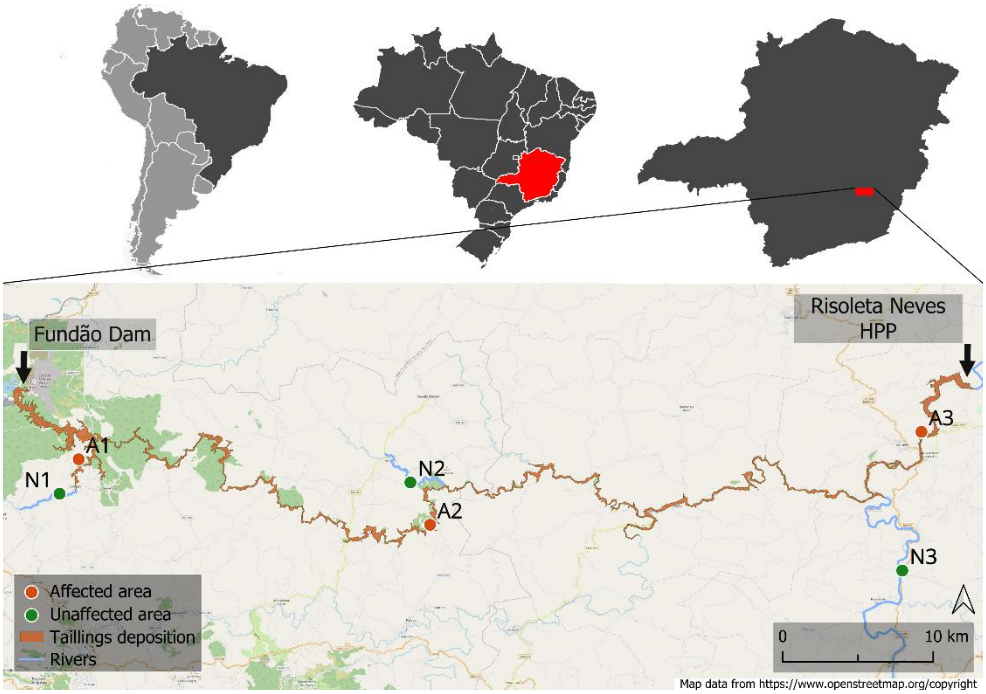

Figure 8: Location of the areas sampled in the three regions of the study. Above, from left to right: South America, Brazil, and the state of Minas Gerais. Bellow: Sampling carried out in the regions: 1) downstream of the Fundão dam, 2) downstream of Paracatu de Baixo and 3) upstream of the Risoleta Neves Hydroelectric Power Plant. The tailings deposition zone is highlighted along the sampled region (shape file "PG23_Area_Afetada_Lama" provided by Fundação RENOVA). Map created using QGIS 3.34.14. Map data from openstreetmap.org/copyright

We sampled soils in three regions between the Fundão dam and the Risoleta Neves HPP (Figure 8). The choice of sampling areas considered the different environments composing the slurry pathway, from the Fundão dam to the Risoleta Neves HPP. This limit was established since the slurry had accumulated in this reservoir and lost power after this point. Thus, we selected areas where the riparian vegetation was directly impacted, showing visible signs of the slurry on the trunks of nearby trees. The areas near Fundão dam have a history of mining activity, while the other downstream areas are primarily used for agriculture and livestock (Sánchez et al., 2018a). Using similar geomorphology and vegetation types, we compared the floodplains of affected (A) rivers and unaffected (N) nearby tributaries (Figure 9). In those areas, we sampled soils for three purposes: i) to analyze soil seed bank, ii) for chemical analysis, and iii) for plant growth experiments.

Figure 9: Riverscapes of areas in the upper Doce River basin during the dry season. Areas (A) affected by the Fundão dam disaster and (B) unaffected tributaries

3.2.2 Soil collection for seed bank experiment

We sampled soils for the seed bank experiment in three regions between the Fundão dam and the Risoleta Neves HPP (Figure 8). The affected areas and their characteristics are as follows. A1) Gualaxo do Norte River, close to Fundão Dam, with mostly forested floodplains (20º 15’ 13’’S, 43 º 25’ 16’’W); A2) Gualaxo do Norte River, near Paracatu de Baixo, with fragments of Atlantic forest mixed with pasture (20º 17’ 43’’S, 43º 11’ 51’’W); and A3) Doce River, upstream of the HPP, in a mainly farming and livestock landscape with less forest fragments (20º 14’ 10’’S, 42º 53’ 05’’W). Additionally, in the unaffected areas, we sampled the riparian zone of non-affected tributaries, with characteristics similar to those of the affected areas. N1) Gualaxo do Norte River (20º 16' 32''S, 43 º 26' 00''W); N2) Bucão stream (20º 16' 06''S, 43º 12' 36''W); and N3: Piranga River (20º 19' 28''S, 42º 53' 49''W) (Figure 8).

For the seed bank experiment, we sampled in two different seasons to perform a broader and more efficient sampling as plant phenologies vary in time (Morellato et al., 2016). We sampled during the peaks of the rainy season (Dec/2020) and the dry season (Jul/2021) in the same areas. In each studied area (Figure 8) and each season, we selected 5 points at a minimum distance of 300 m from each other, 15 points in affected areas and 15 in unaffected areas, totaling 30 samples per season. At each point, we collected the topsoil and the litterfall using a metal jig (25 × 25 cm) at 5 cm soil depth. The soil samples were kept in plastic bags at 15°C and were transferred to the laboratory. In the dry season (Jul/2021), four samples from unaffected areas were lost due to technical problems. The assessed areas were private, and the landowners were previously contacted for research approval. Since the sites are out of any conservation unit, no licence was required by Brazilian government.

3.2.3 Seedling emergence experiment in controlled growth conditions

We transferred the sampled soils to plastic trays (0.25 m × 0.5 m × 0.1 m high) and kept them in a germination room, with controlled conditions at 25 (± 2)°C, 70% humidity, with a photoperiod of 12 h. The soil samples were watered daily. To record plant recruitment from the soil seed bank, we used the seedling emergence method, adapted from (Mesgaran et al., 2007). In this way, we counted all plant individuals out of each plastic tray for six months (Figure 10). Once a month we removed all the plants from the plastic trays to count and morphotype them. We considered "emerging plants" the ones presenting a complete formation of leaves from at least three nodes. For identification purposes, at the first occurrence of each plant species, we transplanted the specimen into plastic pots filled with greenhouse soil for growing and flowering. Finally, we only considered dicotyledon plants.

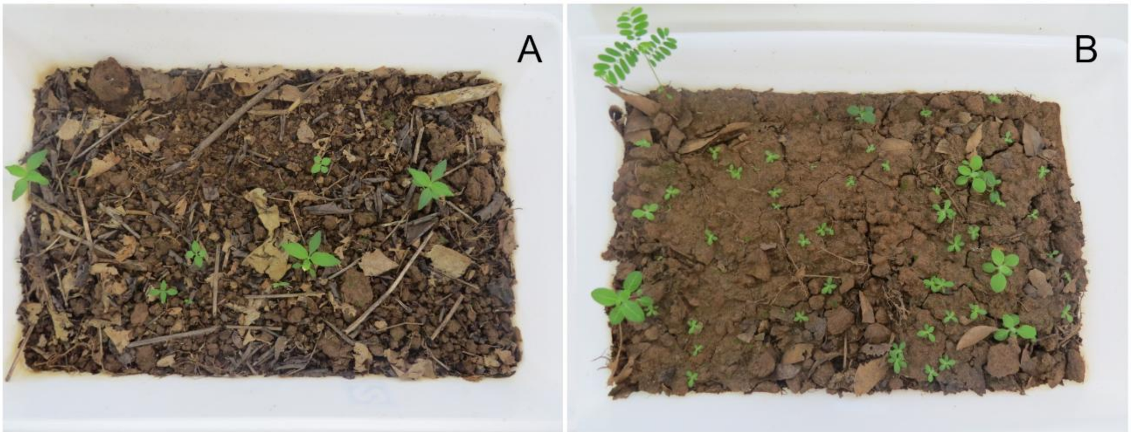

Figure 10: Substrate samples from riverine banks spread in plastic trays for seedling emergence counting. (A) Substrate from affected areas and (B) soil from unaffected tributaries

The species were identified by observing the vegetative characteristics and, when possible, reproductive characteristics of the plants, with the help of identification manuals (Brighenti, 2010; Lorenzi, 2014; Moreira; Bragrança, 2010, 2011) and local experts. Species names and authorities were standardized using the Taxonomic Name Resolution Service provided by the Missouri Botanical Garden (Piaia et al., 2019). To verify whether the species are native or not, thinking about their usage in future restoration projects, we used the Flora e Funga do Brasil website (Flora e Funga do Brasil, 2023) to classify them as native or naturalized (Richardson et al., 2000). The same nomenclature of morphotypes was used in the different seasons (e.g., Asteraceae 1 is the same morphospecies in both seasons). Some individuals could not be identified at the species level.

3.2.4 Soil collection for chemical analysis

We performed another soil collection for chemical and nutritional analysis and plant growth experiments. Those soil samples were taken from one region around the affected HPP's lake (20º 14' 10''S, 42º 53' 05''W), and in the banks of the Piranga River, the closest unaffected tributary near the reservoir (20º 19' 28''S, 42º 53' 49''W) (Figure 8, areas A3 and N3, respectively). In each of those affected and unaffected areas, we sampled three transects located at least 300m apart from each other. Within each 10 m long transect, we collected three sampling points, each consisting of three subsamples of homogenized soil. In total, we sampled nine points in each of those affected and unaffected areas.

We kept the soil samples in plastic bags at 15°C and transported them to the laboratory, where they were dried and later weighed, detorted, and sieved. The soil was divided to obtain subsamples for chemical analysis at the Soil Physics Laboratory of the Federal University of Viçosa - UFV (EMBRAPA, 1997). In the chemical analysis, we measured pH, exchangeable acidity, sum of bases, cation exchange capacity, base saturation, and organic matter content. We also measured the bioavailability of macronutrients such as phosphorus (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), and the bioavailability of potentially toxic elements such as iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), nickel (Ni), chromium (Cr), cadmium (Cd) and lead (Pb). The extractor used for Ca, Mg and Al was KCl 1mol/L, and for the other elements, it was Mehlich-1.

3.2.5 Plant growth experiment

To assess the impact of the soil change following the dam rupture on plant growth, we chose the two most common plant species from the seed bank: *Ludwigia octovalvis* (Jacq.) P.H.Raven (Onagraceae) and *Marsypianthes chamaedrys* (Vahl) Kuntze (Lamiaceae). Both species are annual herbs, *L. octovalvis* being an amphibious plant, between 0.3 to 2.5 m tall (Souza et al., 2019) and *M. chamaedrys* an aromatic plant with 0.1-1.5 m tall (Hashimoto; Ferreira, 2020). We selected twenty individuals from the seedling emergence experiment, 10 of each species. The individuals had at least three nodes with fully developed leaves and approximate height. According to their origin in the previous experiment, the individuals were planted in two types of substrates: plants originating from affected areas were transferred to soil from affected areas (A), and plants from unaffected areas were transferred to soil from unaffected areas (N). We used ten pots for each species, with five plants in each substrate. The soil used for the plant growth experiment was sampled in the same way and in the same areas as the soil used for the chemical analysis. After being taken to the laboratory, we homogenized the soil and divided it into 500 mL pots

(EMBRAPA, 1997). These plants were kept in a greenhouse for 75 d, and we measured the stem height (from the base to the apical bud) and diameter at ground level every fortnight.

At the end of the experiment, we obtained the compartmentalized dry masses of the root and shoot (leaves and stem). For this, each organ was separated, gently washed, packed in paper bags, weighed on an analytical balance and dried in an oven at 60°C to a constant weight. From these data, we calculated the ratio between the dry biomass of the root (g) and the shoot (g), representing the allocation of biomass (root:shoot ratio) (Sainju et al., 2017).

3.2.6 Statistical analysis

We used Student's t-test to compare the chemical parameters of the soils. For this test, each soil parameter was compared separately between affected and unaffected areas. We performed a Shapiro-Wilks test for the normality of the data, and when this was not achieved, we applied the non-parametrical Mann-Whitney U-test. We used the software PAST version 4.16c for this analysis.

To test if the number of individuals and species of emerging plants differ between affected and unaffected areas, we used generalized linear mixed models (GLMMs) with a Poisson distribution (counting data). For these models, we consider the soil type from affected or unaffected areas as the explanatory variable and the number of individuals (abundance) or species (richness) as the response variable. We considered the sampling regions and the sampling seasons as random effects in both models. Local abundance and richness (alpha diversity) data were calculated based on the number of individuals and species of emerging plants per tray, which served as our sampling unit. Two outliers from both treatments were removed to diminish the overdispersion for the abundance analysis. These analyses were conducted in R version 4.2.1.

We also compared the total richness (gamma diversity) of emergent plants between affected and unaffected areas using a rarefaction and extrapolation curve considering the species accumulation for all samples. Our rarefaction curves were based on the number of individuals with Hill number order ($q = 0$)

(Chao et al., 2014; Gotelli; Colwell, 2001) and were also analysed in R version 4.2.1.

To test if the plant growth is impaired in affected areas we used repeated measure Analysis of Variance (ANOVA) models in PAST version 4.16c. For this model, we used plant height and diameter as the response variables and the soil type (from affected and unaffected areas) as explanatory variables. The time after transplanting was considered as repeated measure. We also tested for changes in biomass allocation using a one-way ANOVA. For this, we used the root-to-shoot ratio as response variable and soil type (from affected and unaffected areas) as explanatory variables. These models were conducted separately for each species (*M. chamaedrys* and *L. octovalvis*). Each individual plant (10 of each species) was considered a sampling unit. We used ANOVA models to analyse the growth experiment data as it follows normal distribution.

The composition of plant species in the soil seed bank between affected and unaffected areas was compared through Non-metric Multidimensional Scaling (NMDS), made from the Bray-Curtis dissimilarity index, followed by an Analysis of Similarity (ANOSIM). We used the software PAST version 4.16c for this analysis. All analyses were conducted considering the affected and unaffected areas/soils as independent variables in a between-subjects design.

3.3 Results

3.3.1 Soil chemical analysis

The soil from affected areas showed lower fertility and organic matter content than those unaffected. In addition to the lower cation exchange capacity and sum of bases, lower levels of P and Mg were found in the affected soils. There was also lower bioavailability of the micronutrients Cu, Fe, Zn, and Ni, and the metal Cr in the affected soils when compared to the unaffected (Table 7).

Table 7: Chemical and nutritional parameters of soils used in the plant establishment and growth experiment

Parameters	A	SD	N	SD	t	p-value	
pH (H_2O)	6.10	(±0.48)	6.01	(±0.24)	0.532	0.602	
P (mg/dm³)	5.60	(±4.55)	12.27	(±2.55)	3.836	0.001	*
K (mg/dm³)	80.88	(±55.11)	108.56	(±82.13)	0.804	0.434	
Na (mg/dm³)	2.01	(±2.19)	6.46	(±7.79)	1.648	0.119	

Ca^{2+} ($cmol_c/dm^3$)	2.05	(±1.23)	2.69	(±0.74)	1.357	0.194	
Mg^{2+} ($cmol_c/dm^3$)	0.64	(±0.30)	1.43	(±0.42)	4.568	0.000	*
Al^{3+} ($cmol_c/dm^3$)	0.00	(±0.00)	0.00	(±0.00)	0.000	NA	
H+Al ($cmol_c/dm^3$)	1.47	(±0.68)	2.06	(±0.49)	2.104	0.052	
SB ($cmol_c/dm^3$)	2.88	(±1.51)	4.45	(±1.21)	2.420	0.028	*
t ($cmol_c/dm^3$)	2.88	(±1.51)	4.29	(±1.01)	2.312	0.034	*
T ($cmol_c/dm^3$)	4.35	(±1.53)	6.50	(±1.43)	3.080	0.007	*
V %	64.17	(±14.15)	67.88	(±6.04)	0.724	0.480	
m %	0.00	(±0.00)	0.00	(±0.00)	0.000	NA	
Org. Mat. (dag/kg)	1.06	(±0.52)	2.45	(±0.95)	3.871	0.001	*
P-Rem (mg/dm^3)	27.96	(±8.60)	35.59	(±4.23)	2.388	0.030	*
Cu (mg/dm^3)	3.67	(±0.98)	4.67	(±0.89)	2.262	0.038	*
Mn (mg/dm^3)	120.40	(±38.17)	130.81	(±41.19)	0.556	0.586	
Fe (mg/dm^3)	277.89	(±70.49)	494.11	(±216.53)	2.849	0.012	*
Zn (mg/dm^3)	2.82	(±0.82)	6.68	(±1.55)	6.606	0.000	*
Cr (mg/dm^3)	0.63	(±0.08)	0.81	(±0.20)	2.474	0.025	*
Ni (mg/dm^3)	1.94	(±0.40)	2.79	(±0.30)	5.033	0.000	*
Cd (mg/dm^3)	0.00	(±0.00)	0.00	(±0.00)	0.000	NA	
Pb (mg/dm^3)	2.05	(±1.42	1.09	(±0.38)	1.976	0.066	

Soils from areas affected (A) and unaffected (N) by the tailings spill from the Fundão dam. SB is the sum of bases, t is the effective Cation Exchange Capacity, and T is the potential Cation Exchange Capacity at pH 7.0. Mean values and sample standard deviation (SD), from 9 samples of each treatment are presented. * Significant difference.

3.3.2 Abundance and richness of emerging plants

In absolute values, we found 2001 individuals (1108 individuals/m²), distributed in 116 plant morphotypes and 17 families (Table 8). Also, in affected areas only, we found 1300 individuals and 83 plant morphotypes, while in unaffected areas, we found 701 individuals and 88 morphotypes.

Table 8: Species found in the soil seed bank of areas affected (A) or unaffected (N) by the passage of tailings from the Fundão dam

Family	Species	Origin[a]	Total/m²[b]		Total/m²[b]
			A	N	
Amaranthaceae	*Alternanthera* sp. 1 Forssk.	Native	0.0	0.6	0.6
	Amaranthus cf. hybridus L.	Natur.	0.5	0.0	0.5
	Amaranthus spinosus L.	Natur.	0.5	0.0	0.5
	Amaranthus sp. 1 L.	Native	13.3	1.8	15.2
Apiaceae	*Centella asiatica* (L.) Urb.	Native	4.3	1.2	5.5
Asteraceae	*Ageratum conyzoides* L.	Native	96.5	12.9	109.5
	Conyza cf. *bonariensis* (L.) Cronquist	Native	3.7	4.3	8.0

	Conyza sp. Less	Native	38.9	12.3	51.2
	Erechtites hieracifolius (L.) Raf. ex DC.	Native	4.8	0.0	4.8
	Galinsoga sp. Ruiz & Pav.	Natur.	0.0	1.2	1.2
	Gnaphalium sp. 1 L.	Native	26.7	44.9	71.6
	Mikania sp. Willd.	Native	0.5	0.0	0.5
	Pluchea sp. 1 Cass.	Native	8.0	0.0	8.0
	Pluchea sp. 2 Cass.	Native	29.9	6.2	36.0
	Sigesbeckia L.	Natur.	0.5	1.2	1.8
	Synedrella nodiflora (L.) Gaertn.	Native	2.1	3.1	5.2
	Vernonia sp.Schreb.	Native	2.1	1.8	4.0
	Youngia japonica (L.) DC.	Natur.	1.1	0.6	1.7
	Asteraceae 1	-	27.2	4.3	31.5
	Asteraceae 2	-	18.1	28.9	47.1
	Asteraceae 3	-	4.8	4.3	9.1
	Asteraceae 4	-	3.2	4.9	8.1
	Asteraceae 5	-	13.3	0.6	13.9
	Asteraceae 6	-	7.5	3.1	10.5
	Asteraceae 7	-	1.1	6.2	7.2
Boraginaceae	*Heliotropium sp.* L.	Native	0.0	0.6	0.6
Caryophillaceae	*Drymaria* sp. Willd. ex Schult.	Natur.	0.5	0.0	0.5
Euphorbiaceae	*Euphorbia hirta L.*	Native	4.3	0.6	4.9
	Ricinus communis L.	Native	2.1	0.6	2.7
Fabaceae	*Aeschynomene* L.	Native	0.5	0.6	1.1
	Crotalaria sp. L.	Native	3.2	0.0	3.2
	Senna obtusifolia (L.) H.S. Irwin & Barneby	Native	0.0	0.6	0.6
	Fabaceae 1	-	0.5	0.6	1.1
	Fabaceae 2	-	0.5	0.0	0.5
	Fabaceae 3	-	0.5	0.0	0.5
Lamiaceae	*Marsypianthes chamaedrys* (Vahl) Kuntze	Native	94.4	71.4	165.8
	Mesosphaerum suaveolens (L.) Kuntze	Native	5.9	1.8	7.7
	Lamiaceae 1	-	0.5	1.8	2.4
	Lamiaceae 2		1.1	0.0	1.1
	Lamiaceae 3	-	6.4	0.0	6.4
Lythraceae	*Cuphea* sp. P. Browne	Native	0.5	10.5	11.0
Malvaceae	*Sida* sp. L.	Native	3.7	15.4	19.1
	Malvaceae 1	-	1.1	0.6	1.7
Melastomataceae	Melastomataceae 1	-	0.0	4.3	4.3
	Melastomataceae 2	-	0.0	3.7	3.7
	Melastomataceae 3	-	18.7	6.2	24.8
	Melastomataceae 4	-	0.5	4.3	4.8
	Melastomataceae 5	-	0.0	0.6	0.6
Onagraceae	*Ludwigia* cf. *erecta* (L.) H.Hara	Native	2.1	0.6	2.7
	Ludwigia octovalvis (Jacq.) P.H.Raven	Native	72.5	30.2	102.7
Oxalidaceae	*Oxalis barrelieri* L.	Native	0.5	2.5	3.0
	Oxalis corniculata L.	Natur.	0.0	0.6	0.6
Phyllanthaceae	*Phyllanthus* cf. *amarus* Schumach. & Thonn.	Native	8.5	3.1	11.6
Plantaginaceae	*Scoparia dulcis* L.	Native	4.8	11.7	16.5

	Stemodia cf. *verticillata* (Mill.) Hassl.	Native	48.0	3.1	51.1
Rubiaceae	*Richardia* cf. *brasiliensis* Gomes	Native	1.1	9.2	10.3
Solanaceae	*Solanum* cf. *americanum* Mill.	Native	17.6	36.9	54.5
	Solanum cf. *viarum* Dunal	Native	1.1	0.0	1.1
NI	NI morphotypes	-	69.9	60.9	130.8
	Grand total				
Number of individuals			680	428	1108
Total species			82	86	116
Shared species			52	52	45%
Unique species			30	34	

[a]On the origin column, plants are classified as native or naturalized (Natur.).
[b]Seedling density is shown in the total number of emerging plants per m².

We found no difference in the mean number of individuals comparing affected and unaffected areas (X^2= 3.2148, p=0.0729). Average seedling abundance per plot (± sample standard deviation) was 35.2 (± 37.5) in the affected areas and 20.1 (± 18.5) in the unaffected areas. We found no difference in the mean species richness per sample (alpha diversity) between affected and unaffected areas (X^2= 0.544, p=0.4608). The average species richness in the affected areas was 7.7 (± 4.9), while in the unaffected areas, it was 7.3 (± 4.4).

Total plant richness, accumulated in the three regions and the two seasons (gamma diversity), was approximately 35% higher in unaffected areas than in the affected areas (Figure 11). The extrapolation curves suggest that with a sampling effort of 2000 individuals per treatment, 130 species could be expected to occur in the unaffected areas, compared to only 95 species in the affected ones.

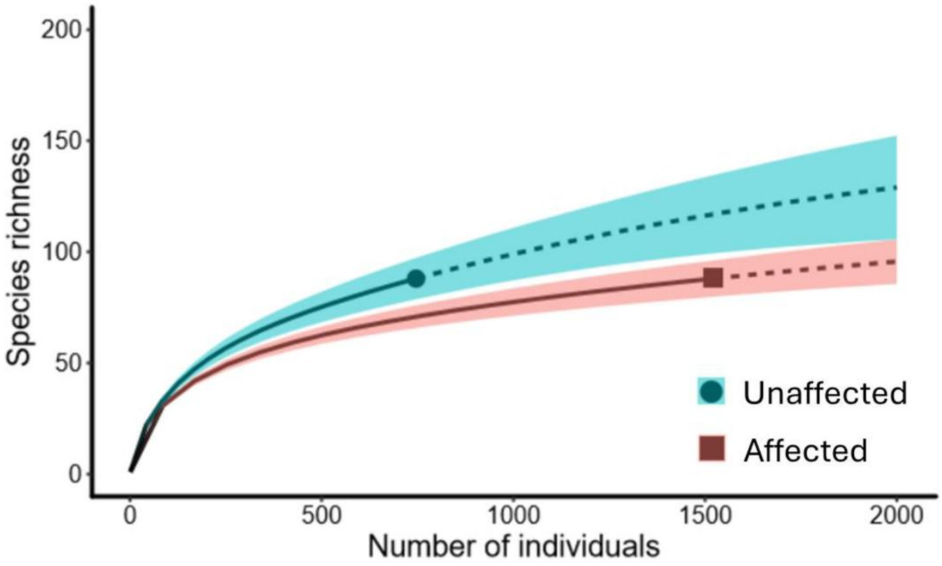

Figure 11: Species richness and abundance of emerging plants in the seed bank experiment. Sample-sized rarefaction curves (solid lines) and extrapolation (dashed lines) of the entire study. Curves from areas affected and unaffected by the passage of the tailings from the Fundão Dam, collected six years after the disaster. Species diversity based on the Hill numbers (q=0) and shaded areas represent 95% confidence intervals, which do not overlap (p<0.05).

3.3.3 Species composition

There was no statistical difference in plant species composition between affected and unaffected areas (ANOSIM, R=0.03024 and p=0.1147). Affected and unaffected areas shared 45% of all plant species (52 shared per 116 total species). The number of unique species in affected areas was 30, whereas in the unaffected areas, it was 34. The most representative family in the experiment was Asteraceae, with 20 species and 858 individuals. The most abundant species in both areas were *Marsypianthes chamaedrys* (Vahl) Kuntze, *Ludwigia octovalvis* (Jacq.) P.H. Raven and *Ageratum conyzoides* L. encompassing 34% of the total individuals per m² (377.9 from 1108). A significant part of our plant community comprises annual plants, which can accelerate nutrient cycling in affected soils, primarily through increased organic matter.

3.3.4 Plant growth experiment

The growth experiment revealed higher increment in plant height and stem diameter of *Ludwigia octovalvis* in unaffected soils, specially after 60 d (significant interaction between time and height: $F_{5,35}$= 6,558, p<0,001 and time and stem diameter: $F_{5,35}$= 5,411, p<0,001; Fig 5).There was a significative difference in plant height and diameter at the beginning of the experiment in *Marsypianthes chamaedrys*, since plants were initially bigger in the unaffected soil (p<0,001). Despite, there was no difference in the increment in height and diameter over 75 d comparing affected and unaffected soils (non significant interaction between time and height: $F_{5,40}$= 1,983, p>0,1 and time and diameter: $F_{5,40}$= 0,993, p>0,1; Figure 12). It means that the plants continued to grow with similar rate after 75 d, with no effect of soil type.

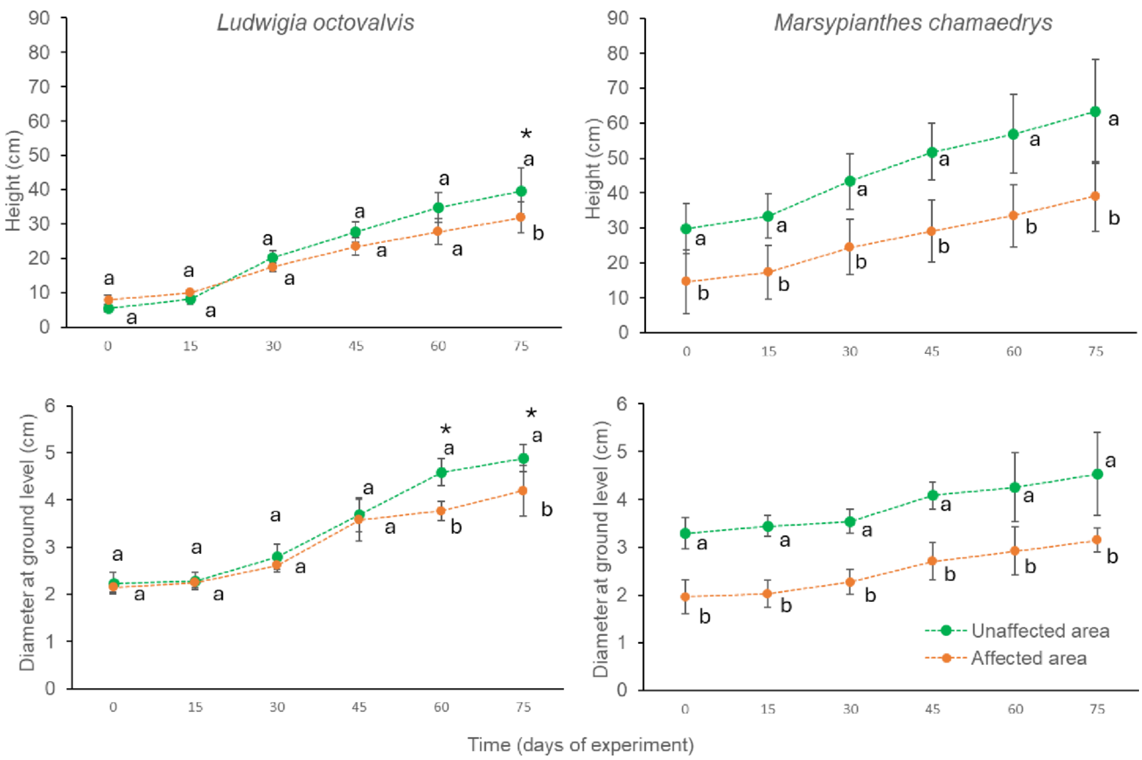

Figure 12: Variation in height and diameter of individuals from the study's two most abundant species. Vertical bars indicate the standard deviation of the mean. Different lower-case letters indicate significant differences in the parameters of the same species in time. *Significant overall difference for individuals of *L. octovalvis* plants

For *M. chamaedrys*, the root:shoot ratio (mean ± SD) in plants growing in affected soils (0.81 ± 0.18) was almost four times lower than in unaffected soils (3.52 ± 1.79) (t=4.3091 and p=0.002). This difference is mainly due to a lower production of root biomass in affected soil (1.60 ± 0.89) compared to unaffected soil (12.78 ± 6.45) (t=3.8354 and p=0.004). We found no difference in these parameters for *L. octovalvis*, between plants growing in soils affected (2.93 ± 2,57) and unaffected by the tailings (3.31 ± 1.82) (t=0.2545 and p=0.806). Plants from both species flowered during the experiment in affected and unaffected substrates (Figure 13). All plants of M. chamaedris and L. octovalvis produced flowers.

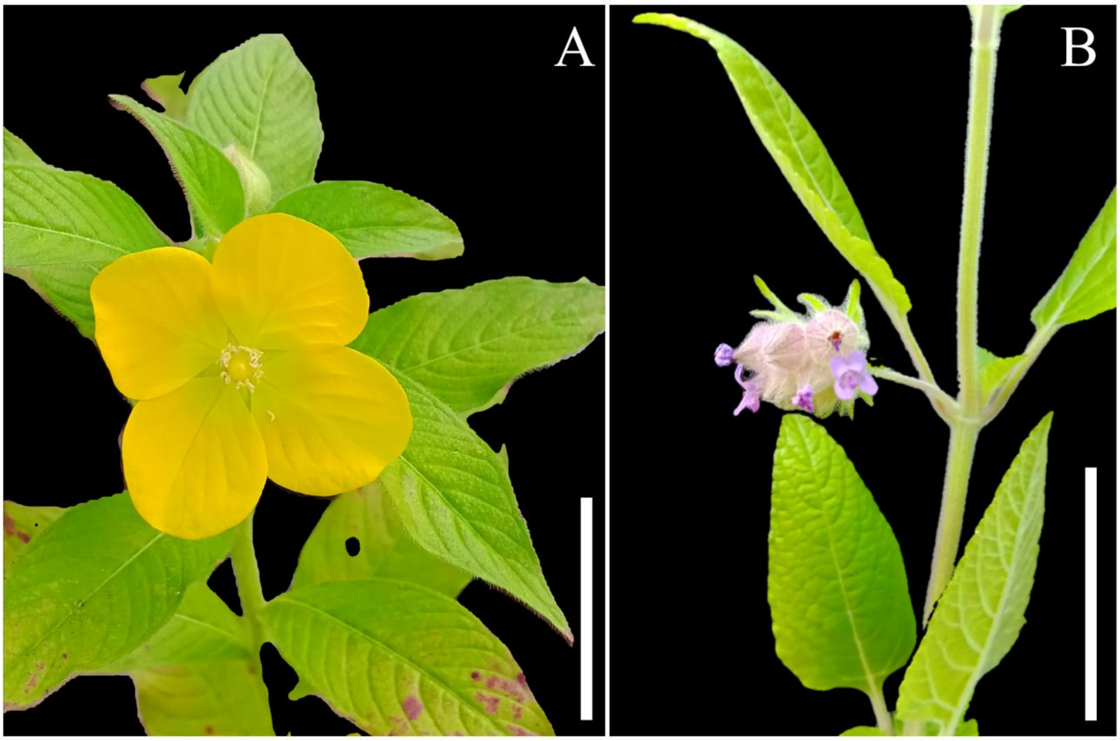

Figure 13: Growth habit and flowers from individuals of the growth experiment. (A) *Ludwigia octovalvis* and (B) *Marsypianthes chamaedrys*.

3.4 Discussion

Overall, we experimentally demonstrated that even after six years, the diversity of recruiting plants is lower in areas affected by Samarco's dam tailings. We also showed that soil affected by Samarco slurry reduced plant growth and changed root biomass allocation in two different plant species. Soil properties seem directly linked to those results as the soil from tailings-affected areas showed higher fertility and organic matter content than unaffected ones. However, despite its lower diversity, soils from affected areas demonstrated a high number of emerging plants, which shows that Samarco-slurry does not prevent plant regeneration.

3.4.1 Soil chemical analysis

In general, the soils affected by tailings showed low contents of organic matter and nutrients. Low organic matter might be stressful to colonizing plants since it reduces the availability of water and nutrients and negatively influences the soil's structure (Saldanha et al., 2016). In addition, low P and Mg bioavailability and the low sum of bases and cation exchange capacity negatively affect plant establishment in affected areas (Cruz et al., 2020c; Nascimento et al., 2021). On the other hand, the unaffected areas had higher levels of: Cu, Fe, Zn, Cr and Ni, possibly due to previous long-term anthropogenic activities, such as dairy, farming, mining, and pesticide and fertilizer use (Davila et al., 2020). However, our data showed that after six years, all tested elements are present in non-concerning amounts (Coelho et al., 2020b; Silva et al., 2021a), in both affected and unaffected areas.

3.4.2 Abundance and richness of emerging plants

The density of individuals and species richness (alpha diversity) of emerging plants did not differ locally between affected and unaffected areas. This indicates that both soils have viable seed banks, both persistent and transient. Furthermore, in our experiment, 693.3 individuals/m² emerged in the affected areas. These values are higher than the 485.4 individuals/m² reported by (Silva et al., 2021b) in the affected areas in 2019, i.e., three years after the tragedy. However, the high abundance of emerging plants in the disturbed site does not ensure plant survivorship since there is a trade-off between site attributes related to seedling recruitment and establishment (Barrett; Silander, 1992). The tailings passage completely buried all the seeds in affected areas and one explanation for the high plant recruitment in these soils is the seed arrival over the past six years.

Furthermore, there were some restoration practices after the disaster (Doelinger, 2017) such as the addition of seeds on the soil. However, it is worth noting that out of the 32 species used in the RENOVA'S seed addition, only *Crotalaria* sp. was found in our study. All the other 118 morphospecies that we found are new to this drastically disturbed environment or are recolonizing it. This suggests that numerous other plant species have been able to establish in this zone, and an alternative state has been established.

Although there was no difference in the local species richness (alpha diversity), unaffected areas accumulated nearly 35% more species than tailings-affected areas at a regional scale (130 vs 95 species for 2000 individuals respectively) (Figure 11). After the passage of the tailings, the soil became more homogenized (Davila et al., 2020), leading to a decrease in habitat heterogeneity. The affected soil's nutrient availability and organic matter content decreased (Table 7). Also, they are denser and have higher silt content and lower macroporosity, potentially affecting water infiltration and diminishing root development and microbiological activity (Silva et al., 2021a). Considering that a wider variety of microhabitats can modulate species coexistence and select species with different regenerating niches (Marques et al., 2014), the accumulation of tailings and subsequent environmental simplification might have reduced plant diversity. Hence, restoration projects in areas affected by mining activities should focus on enhancing soil structure, nutrient availability, and microhabitat variety to promote biodiversity and support species with different ecological niches.

3.4.3 Species composition

We found no statistical difference in species composition between affected and unaffected areas. This can be related to similar conditions related to historical human activities (Felippe et al., 2017). For example, many of the species found in both areas are considered weeds in agriculture (e.g., *Cyperus rotundus, Ageratum conyzoides, Conyza* sp., etc.) precisely because they have a wide dispersion, need few resources, or are good competitors (Soares et al., 2017). The two most abundant species also have favourable traits. For example, *Ludwigia octovalvis* can flower throughout the year (Souza et al., 2019), while *Marsypianthes chamaedrys* have explosive pollination and self-pollination (Amorim et al., 2022).

3.4.4 Plant growth experiment

We also found that the herbaceous plants *M. chamaedrys* and *L. octovalvis* presented different developmental responses when growing in affected and unaffected soils. While individuals of *L. octovalvis* grew less, *M. chamaedrys* produced proportionally less root biomass when growing in tailing-affected soils. Essentially, the observed traits of lower growth in the former and a poorly developed root system in the latter are responses to the unfertile and dense affected soils (Jordão et al., 2021). On the other hand, as both species grew relatively well in tailing-affected soils, those could be used in habitat restoration practices. For instance, in only three months, plants of *Kochia scoparia* (L.) Schrad. (Amaranthaceae) and *Glycine max* (Linn.) Merrill. (Fabaceae) increase soil porosity by 65% and organic matter by 21% in mining tailings areas (Cui et al., 2021).

3.5 Conclusions

Understanding the consequences of a massive tailing's deposition on plant recruitment by herbaceous species is essential to comprehend the dynamics of ecological restoration following a mining disaster. Even six years after Samarco's dam break, affected areas still experience a reduction in plant regional diversity. However, the overall abundance of emergent seedlings is high on tailings, which is a common trend for disturbed areas, but still shows that Samarco-slurry does not prevent plant regeneration. A significant part of our plant community comprises annual plants, which can accelerate nutrient cycling in affected soils, primarily through increased organic matter. In addition, plant development until reproductive age occurred in our experimental herbaceous plants on both soils, despite the challenging conditions, serving as a testament to their potential for ecological restoration. Future work may be conducted to investigate the chemical composition of plants growing on the affected soils, determining whether these plants accumulate potentially toxic elements in their tissues. Finally, our experiments show how strong the effects of a large-scale mining disaster can be on ecological restoration and, consequently, its impacts on ecosystem functions and services.

4. Enhanced development of a pioneer tree (*Cecropia hololeuca*) on the affected substrate formed six years after the Samarco's dam break

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Abstract

The 2015 collapse of the Fundão dam generated vast areas of technosol, a novel substrate whose physical and chemical properties pose a significant challenge for ecosystem regeneration. While inhibitory for many species, such disturbed landscapes can offer opportunities for stress-tolerant pioneer plants. We examined the early growth and accumulation of potentially toxic elements (PTEs) in the pioneer tree *Cecropia hololeuca*, a species that has proliferated across the affected region. We tested three hypotheses: that growth would be enhanced in tailings substrate, that tissue PTE concentrations would be higher, and that roots would act as the primary sink for these elements. In a greenhouse experiment, seedlings were cultivated for 108 days in soil collected from either areas impacted by the tailings and unaffected control sites. We measured growth bi-weekly and analysed final biomass and PTE concentrations (Fe, Mn, Ni, Zn, Cu, Cr, Cd, Pb, As). Seedlings growing in tailings substrate showed significantly faster growth in height and stem diameter, and similar leaf number. Their root and stem biomass were 140% and 167% greater, respectively, than of trees on control substrate. Leaves from plants of affected substrate contained elevated concentrations of Cu, Zn, and Ni. Overall, *C. hololeuca* accumulated high levels of several PTEs, but these were mainly restricted to the root system, with a clear decreasing concentration from roots to leaves for Fe, Cr, Cu, Mn, and Zn. Nickel was a striking exception, being strongly translocated and enriched in the leaves. Our findings indicate that *C. hololeuca* not only persists but flourishes in the tailings substrate, a response likely driven by favourable physical soil conditions combined with an efficient root sequestration strategy that mitigates the toxicity of most PTEs. We conclude that this species is a strong candidate for potential restoration initiatives, due to its rapid growth combined with PTE accumulation. However, its role in PTE cycling requires careful attention, particularly given the risk of nickel and other elements entering the food web.

Keywords: Iron ore tailings, Potentially toxic elements, phytostabilization, Biogeochemical cycling, Ecological restoration.

4.1 Introduction

Mining is an activity with high economic value, with tons of minerals being extracted daily in mines all over the world (USGS, 2025). The region of Minas Gerais in Brazil is well known for gold and iron ore extraction, among other elements, and also for two of the most lethal and dangerous environmental disasters in the country (Buch 2024). In 2015, almost ten years ago, a Tailings Storage Facility owned by Samarco company (a subsidiary of BHP Billiton and Vale SA) disrupted and spread nearly 60 million m³ of sludge in the environment (Fernandes et al., 2016a). The waste material, also called technosol (Schaefer et al., 2015), reached the tributaries of the Doce River, dispersing through the alluvial floodplain and burying a significant area of natural vegetation, pasture and agricultural lands (Meira et al., 2016). A total of 1,582 ha of riverside area (Knopff et al., 2020) and 457.6 ha of the Atlantic Forest were affected, mainly along the first 74 km of the river, where part of the sludge was trapped in the Risoleta Neves Power Plant (Omachi et al., 2018). Downstream the dam, the sludge was mainly restricted to the riverbed, as bedload and dissolved sediment that changed water turbidity (Santana et al., 2021a). It killed many species of fish and other aquatic animals (Macêdo et al., 2024), finally reaching the Atlantic Ocean, after 668 km of devastation along the Doce River (Carmo et al., 2017b). The passage of the tailings removed large part of the riparian tree vegetation (Figure 14A) and buried the emerging stratum (Fernandes et al., 2016a), causing the creation of disturbed open spaces. With the environmental reset and increased edge effect (e.g., reduced plant competition and higher availability of light) (Harper et al., 2005), the recruitment of fast-growing pioneer species, such as *C. hololeuca*, is favoured (Brando et al., 2020).

Figure 14: Riparian vegetation in two areas affected by the passage of mining tailings. A) Dead trees recorded in 2020 and B) *Cecropia* spp. trees growing in the affected area, 2022.

Beyond the opening of new disturbed areas, there was also a change in substrate and habitat quality. The physical characteristics of the riparian substrates were altered by the tailings, that spread along the first 100 km of the upper Doce river basin, with a predominant presence of silt (44.4%) and sand (26.6%) and a sandy loam texture (Cruz et al., 2020a). Furthermore, when compared to native alluvial soils, this substrate exhibits higher sand content (Andrade et al., 2018), increased density, reduced porosity, and less organic carbon (Silva et al., 2021a). Being the result of a mechanical process of rocks, its organic matter content is negligible, and the cation exchange capacity (CEC) is also low, leading to poor nutrition of individuals growing on it (Segura et al., 2016). In general, compared to unaffected areas, higher contents of some potentially toxic elements (PTE) were found in the spread substrate, like iron (Fe), chromium (Cr), lead (Pb), manganese (Mn), bismuth (Bi), cerium (Ce) (Silva et al., 2021a) and arsenic (As) (Guerra et al., 2017b). Also, some studies suggested that the PTE availability could be enhanced in the environment through acidification and other processes of reduction (Bastos, 2021).

The new substrate negatively affected plant metabolism and functioning in some tree species, as demonstrated in reduced photosynthesis, stomatal conductance, transpiration and water use efficiency in *Cassia grandis* (Matos et al., 2020). It has also been found a lower growth and biomass production, reduced tree heights and the formation of smaller leaves in the tree species *Albizia polycephala, Peltophorum dubium, Cybistax antisyphilitica, Handroanthus heptaphyllus* and *H. impetiginosus* (Cruz et al., 2020a). However, some herbaceous species demonstrated the ability to thrive in this substrate, like the invasive grass *Brachiaria decumbens* (Silva et al., 2022) and the native herbaceous *Ludwigia octovalvis* and *Marsypianthes chamaedris* (Paz et al., 2025). The resistant plants usually accumulate different proportions of PTE, like Fe and Mn, in their tissues, such as in roots and shoots of *A. polycephala, P. dubium, C. antisyphilitica, H. heptaphyllus, H. impetiginosus* (Cruz et al., 2020a); and Mn, Zn and Cu in leaves and roots of *Saccharum officinarum* and *Stylosanthes guianensis* (Coelho et al., 2020c). After five years, many individuals of the fast-growing pioneer tree genus *Cecropia* were observed growing along the affected riverbanks (Figure 14B). Cecropia trees are common in the region, with *Cecropia hololeuca* demonstrating a high natural importance (Carlos Gomes Figueiredo et al., 2022), mostly emerging from the seedbank (Moser et al., 2022). The species is known for its fast growth, high productivity in leaves and litter, and high resorption efficiency, being an important early-successional species after major disturbances (Gomes; Luizão, 2012). Moreover, it has been reported that *Cecropia* spp. are capable of accumulating high levels of Cu without showing negative effects (Asensio et al., 2018b), and accumulate higher levels of mercury (Hg) in the roots when compared to its shoots (Durango et al., 2010). In fact, the sludge reset the vegetation succession and, by consequence, reduced plant competition and increased light availability. Given this scenario, it is reasonable to expect that *C. hololeuca* recruitment would be facilitated.

Based on this, our general objective was to answer the following question: Is the initial development of *Cecropia hololeuca* enhanced in soils affected by the mining tailings, seven years after the Fundão disaster? To answer this question, we tested three hypotheses: i) plants present a greater increment in height, diameter, and number of leaves, and also greater biomass in affected substrates; ii) there is a higher accumulation of potentially toxic elements in the tissues of plants growing upon affected substrates; and iii) plants growing in affected substrates have the highest PET concentrations in the roots when compared to leaves.

4.2 Materials and methods

We sampled the substrate from the affected area close to the Risoleta Neves Hidroelectric Powerplant in October 2022. The samples were taken in three transects, spaced at least 300 m apart. Each 10m transect contained three sampling points. At each point, we collected three individual subsamples, which were homogenized to form a single composite sample per point. This resulted in a total of nine composite samples. For the control treatment, we used the same design to sample the soil from an unaffected region of the Upper Doce River basin, at the Federal University of Viçosa (UFV) campus. This is an Epieutrophic Cambisol, being moderately drained and with a sandy loam soil texture, with eutrophic conditions in the surface layer (Ferreira-Júnior et al., 2007a). The areas are 60 km apart, and according to Köppen's classification, have the climates as Cwa (hot summer) and Cwb (temperate summer), respectively, with a dry winter in both (Alvares et al., 2013; Ferreira-Júnior et al., 2007b). The yearly historical rainfall in the region is approximately 1200 mm (Lima et al., 2019), and the mean annual temperature ranges around 25 °C (Bernardino et al., 2019).

We used plants from *Cecropia hololeuca*, which belongs to one of the most common Cecropia species in the region and also in the affected area. Seeds were sampled from different trees, in natural populations of the Doce River basin in unaffected regions, collected with proper taxonomic identification by professionals of the Dendrology Department of UFV. The seeds were harvested from at least five plants, in sites at a maximum distance of 100km around the UFV campus.

Seeds were prepared according to protocols, including acid cleaning and tetrazolium germination tests (ISTA, 2015) under controlled laboratory conditions, and planted with a 12-hour photoperiod and approximately 1000 lumens of light intensity. As a trial, some seedlings were planted straight into the affected substrate, but they didn't survive, and a previous stage was added before starting the experiments. The germinated seedlings were transferred to 500 mL pots containing organic substrate and stood there for 21 days before being transplanted for the respective treatments (affected and unaffected). Thirty-one seedlings of similar age were cultivated in laboratory conditions, being 16 in substrate from affected areas and 15 in unaffected soil (experimental control). The plants were watered daily, and each fortnight the following morphological parameters were measured: height, diameter at the stem base (ground level), and number of leaves. The experiment ran between November 2022 and February 2023, totalling 108 days. Seedlings used in the experiment started with similar heights and diameters. Only one of the plants growing in the unaffected substrate died after a period of 60 days.

At the end of the experiment, five plants of each treatment were used to measure the dry biomass of leaves, stems and the root system. The roots were carefully washed with tap water to remove soil particles, avoiding contamination during chemical analysis. The plant organs were stored in paper bags, oven-dried at 60°C for 72h to achieve a constant weight and finally milled to powder (Leiterer et al., 1997). All weight measurements were taken using an analytical balance with an accuracy of 0.0001 grams. For each plant, the total shoot biomass was calculated as the sum of the dry mass of the leaves and the stem. The root:shoot ratio was determined for each plant by dividing the root dry mass by shoot dry mass (Mašková; Herben, 2018). Mean values and standard deviations for organ masses and the final ratios were calculated separately for the affected and unaffected groups. The selected plants were used for chemical analyses, of the PTE's: Fe, Mn, Ni, Zn, Pb, Cd, Cr, As and Cu. The organic matter was digested using HNO_3 and $HClO_4$ acids, and the analysis was determined using Agilent 4200 MP-AES Atomic Emission Spectrometry, following established analytical procedures (Liberato et al., 2017).

It was not possible to measure the chemical concentration of elements in all five plants from the control treatment, due to their small biomass available for analysis. Hence, only three individuals were analysed for leaves, while only two for roots, and this last organ was not considered. Therefore, our comparison between treatments is limited, and we focused on the comparison of plants of the affected substrate. For the soil chemical analysis, the extractor Mehlich-1 was used for Fe, Zn, Mn, Cu, Cd, Pb, Ni and Cr. Since the soil analysis data were initially reported in mg/L and the plant data is in mg/kg, we transformed the soil values to mg/kg to enable direct comparison. This transformation accounted for an average soil density of 1.2 mg/dm³ in unaffected areas and 2.5 mg/dm³ in affected areas, based on laboratory measurements and published literature (De Souza et al., 2016). Refer to the Appendix for the raw data (Table S1). The bioconcentration factor (BCF) and translocation factor (TF) were calculated to describe metal accumulation and distribution within the plants (Mulenga et al., 2023b). The BCF represents the plant's ability to accumulate metal from the soil and was calculated for each element as the ratio of its concentration in the root tissue (C_{root}) to its bioavailable concentration in the substrate ($C_{substrate}$) as follows: $BCF=C_{root}/C_{substrate}$. We used the bioavailable soil concentration instead of the total content, since the focus of this work is on the ability of plants to extract these elements from the soil solution, and the plant content is better correlated with the extractable fraction of the substrate (Zhang; Liu; Wang, 2010). The TF indicates the plant's efficiency in moving metals from the roots to the aerial parts (leaves and stems combined) and was calculated as the ratio of the metal concentration in the shoot tissue (C_{shoot}) to its concentration in the root tissue (Takarina; Pin, 2017), following the formula $TF=C_{shoot}/C_{root}$. All concentrations were based on dry weight.

4.2.1 Statistical analysis

We analyzed the growth responses of plant height and stem diameter to treatment over time using linear mixed-effects modelling. For each response variable, we constructed a separate model using the `lmer` function. The full model included the fixed effects of Treatment, Time, and their interaction. To control for the non-independence of repeated measurements taken from the same individual, we incorporated plant identity as a random effect. We evaluated each model through diagnostic plots to verify that it met the assumptions of homoscedasticity and normally distributed residuals.

For comparing biomass between soil treatments, we employed independent t-tests when data met normality requirements. In the case of root:shoot ratios, the normality assumption was not met, and we used the Mann-Whitney U test. For comparison between PTE concentration across different plant organs (roots, stem and leaves), we employed one-way ANOVA followed by

Tukey's tests for pairwise comparisons ($p < 0.05$). Each PTE was considered as a response variable, and the type of substrate was the explanatory variable.

As, Cd and Pb were found in concentrations below the detection limit for all samples and subsequently were not considered in the analysis. In some samples, the Ni concentration was below the detection limit (< 1 mg/kg), and they were replaced with half the detection limit to enable statistical analysis. This approach follows EPA guidelines for left-censored data (Bolks; DeWire; Harcum, 2014) and sensitivity analyses confirmed that this substitution did not affect significance patterns. One individual in the unaffected treatment died before the end of the experiment, resulting in missing data for the last three growth measurements. Consequently, this individual was excluded from the final analysis, reducing our sample size to 14.

All analyses were made using software "R version 4.4.2 (2024-10-31 ucrt)". Potential outliers were identified through visual inspection of boxplots and confirmed statistically using Grubbs' test. The normality of data distribution was verified for each treatment group using Shapiro-Wilk tests, while Levene's test confirmed the homogeneity of variances between groups.

4.3 Results

4.3.1 Plant growth

Seedlings grown in tailing-affected substrate showed a higher increment in height and stem diameter compared to unaffected controls ($p < 0.001$ for height and diameter; $p = 0.057$ for leaves). Stem diameter presented the strongest effect for treatment × time interaction ($\beta = -0.019 \pm 0.002$, $F_{(1,154.81)} = 70.41$, $p = 2.82 \times 10^{-14}$), followed by height ($\beta = -0.026 \pm 0.005$, $F_{(1,154.54)} = 25.86$, $p = 1.05 \times 10^{-6}$). At the same time, leaf number variation was marginally significant ($\beta = -0.010 \pm 0.005$, $F_{(1,164.47)} = 3.66$, $p = 0.057$), with the same trend of faster accumulation of leaves in affected substrates. In all models, the time variable was the main predictor, followed by the time and treatment interaction, with no initial differences between treatments (Figure 15).

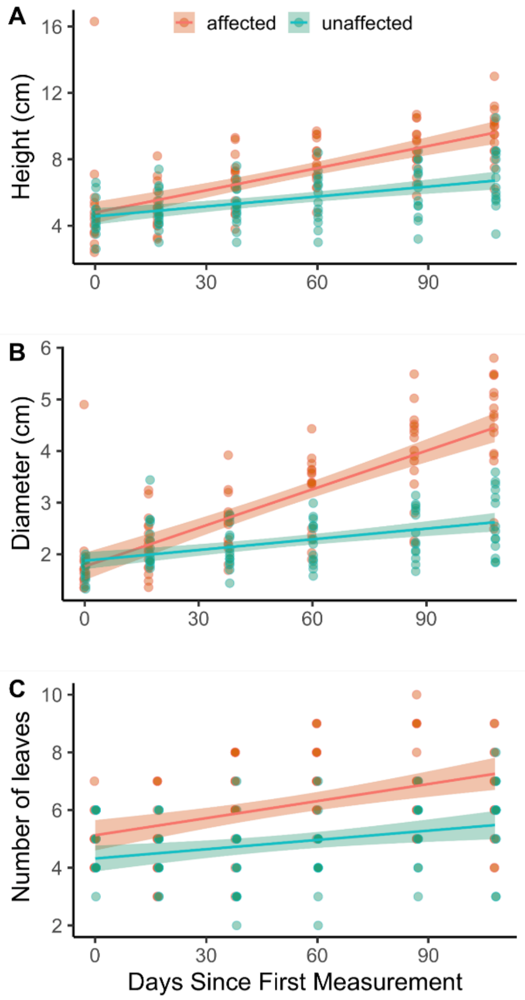

Figure 15: *Cecropia hololeuca* growth over time, in affected and unaffected substrates from the areas of the Fundão dam disaster. Points represent observed data, lines and shaded areas represent the predicted means and 95% confidence intervals from the ANOVA mixed model, respectively.

4.3.2 Plant biomass

Cecropia seedlings grown in affected substrates presented greater root ($p = 0.004$, $t_{(11)} = -3.49$) and stem biomass ($p = 0.033$, $t_{(18)} = -2.31$, Cohen's effect sizes d of 1.62 and 1.03, respectively). Leaf biomass showed a non-significant difference between affected and unaffected substrates ($p = 0.226$, $t_{(18)} = 1.25$, $d = 0.56$) (Table 9). The root:shoot ratio also exhibited a significant increase in the affected plants (median 0.732) compared to the control (0.239) ($U=10.0$, $p=0.002$).

Table 9: *Cecropia hololeuca* dry biomass (g) of leaves, stems and roots affected and unaffected substrates in the Fundão dam area.

Organ	Unaffected		Affected		Test-value	p_value
	Mean	SD	Mean	SD		
Leaves	1.43	0.58	1.06	0.74	1.25	0.226
Stem	0.09	0.06	0.24	0.2	-2.31	0.033*
Roots	0.35	0.16	0.84	0.39	-3.49	0.004*

4.3.3 PTE accumulation in leaves from plants in affected and unaffected substrates

Cu and Zn levels were 2.6- and 1.9-fold higher in affected compared to control substrates (Cu, $F_{(1,5)}$ = 24.49, p = 0.004; Zn, $F_{(1,5)}$ = 40.84, p = 0.001) while Ni concentrations only showed a marginal increase in affected substrates ($F_{(1,6)}$ = 6.00, p = 0.05). Fr, Mn and Cr did not differ between treatments (p > 0.11). For all other investigated elements, concentration was below detection limits in both treatments (Table 10).

Table 10: PTE concentration (mg/kg) in leaves of Cecropia plants grown in affected or unaffected substrates. * significant difference

Element	Unaffected		Affected		F-value	p_value	
	Mean	SD	Mean	SD			
Cu	12.04	± 6.03	31.32	± 4.38	24.49	0.004	*
Zn	13.05	± 2.51	25.45	± 2.56	40.84	0.001	*
Ni	26.61	± 45.22	136.5	± 68.13	6	0.05	*
Fe	919.16	± 859.17	428.83	± 147.33	1.73	0.236	
Mn	121.39	± 17.91	149.51	± 20.2	3.63	0.115	
Cr	476.84	± 434.7	199.35	± 51.13	2.23	0.186	

4.3.4 PTE concentration across the affected substrate, roots and leaf compartments

The Bioconcentration factor was > 1 for most of the PTE, except Ni. PTE concentrations decreased in the sequence roots> leaves> substrate in most elements (Fe, Cr, Cu, Mn and Zn) (Table 11). Fe concentration in roots was 107-fold higher than in substrates, and 28-fold higher than in leaves. For Cr, roots concentrations showed a similar trend, exceeding those in substrates by over 4,500-fold and those in leaves by 5-fold. Cu, Mn and Zn also displayed substrates-root differentiation (p < 0.001) but were not different between roots and leaves. Ni was the only element with TF >1, with a 273-fold higher leaf concentrations compared to roots ($p < 0.001$). Refer to the Appendix for the raw data (Table S2).

Table 11: Element concentrations (mg/kg) across affected substrate, roots, and leaves of *Cecropia hololeuca*. Means ± standard deviation with Tukey's post-hoc groupings

Element	Substrate	Roots	Leaves	F-value	p-value
Fe	111.16 ± 28.20^{a}	11923.73 ± 4516.25^{b}	428.83 ± 147.33^{c}	52.15	<0.001
Cr	0.25 ± 0.03^{a}	1142.43 ± 107.16^{b}	199.35 ± 51.13^{c}	620.31	<0.001
Cu	1.58 ± 0.24^{a}	39.50 ± 14.44^{b}	31.32 ± 4.38^{b}	44.84	<0.001
Mn	48.16 ± 15.27^{a}	171.50 ± 54.06^{b}	149.51 ± 20.20^{b}	30	<0.001
Zn	1.13 ± 0.33^{a}	33.68 ± 9.62^{b}	25.45 ± 2.56^{b}	83.55	<0.001
Ni	0.77 ± 0.09^{a}	0.50 ± 0.00^{a}	136.50 ± 68.13^{b}	22.2	<0.001

Means with different superscript letters (a, b, c) differ significantly (Tukey HSD, $p < 0.05$). ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, ns = not significant ($p \geq 0.05$). Outliers detected via Tukey's method.

4.4 Discussion

Our experiment showed that *Cecropia hololeuca* grew faster and accumulated more biomass on both shoots and roots when in tailing-affected substrates. These findings support field observations of many plants growing in affected areas and suggest a high tolerance to post-disaster conditions. Additionally, this species can work as a remediator, removing some metals from the substrate and storing them in its roots, while also impeding their translocation into the photosynthetic organs, which potentially could harm plant growth and metabolism. The high Ni concentrations in the leaves also indicate a certain degree of Ni-tolerance and raise concerns about possible effects on the local food chain. These results imply that *C. hololeuca* could be a valuable candidate for restoration efforts, but its impact on trophic transfer should be carefully evaluated.

Tailings exposure increased by almost two times the height, stem diameter, and leaf production in *Cecropia hololeuca* plants compared to those on unaffected soils, leading to higher dry biomass of roots and stems. The successful establishment of these individuals is typical for pioneer or early-successional trees, which have competitive advantages on sandy and nutrient-poor soils, where they typically occur in degraded areas and after major disturbances. The genus *Cecropia* is well known for recolonization after fires (Reis et al., 2018), floods, or other disturbances (Kohagura et al., 2020). For example, *C. latiloba* demonstrates a high tolerance for newly deposited sediments in alluvial zones of Amazonian floodplains (Parolin, 2002). Plants of *C. ficifolia* and *C. sciadophylla* growing in degraded soils in the Central Amazon presented a higher photosynthetic nutrient use efficiency (particularly N, P and K), especially when compared to trees of other successional stages (Santos; Gonçalves; Feldpausch, 2006). These *Cecropia* plants presented photosynthetic rates of 260 nmol g^{-1} s^{-1}, about seven times higher than *Chrysophyllum sanguinolentum*, indicating a high efficiency in using radiation to assimilate carbon. Also, plants of *C. pachystachya* under flooding stress maintained satisfactory energy production, leading to high growth rates (Batista et al., 2008). Beyond that, plants in the affected soil conditions invest a greater amount of biomass in roots (39.3% increase) compared to the aerial parts. This partitioning is typical of plants growing in disturbed areas, or under any stressors, such as lower water availability, poor nutrients, metal contamination or further alterations in soil conditions (Mašková; Herben, 2018). While *Albizia polycephala*, *Handroanthus heptaphyllus* and *H. impetiginosus* trees produced more roots in tailings, they allocated greater leaf biomass when the substrate was fertilized (Cruz et al., 2020). The results demonstrate a clear strategy of resource reallocation, suggesting that the plants are adapting to suboptimal soil conditions, such as water or nutrient limitation, by enhancing their below-ground foraging capacity.

The metal accumulation in leaves from plants grown on tailings was twice as high for Cu and Zn, and five times higher for Ni, while no difference was observed for Fe, Mn, and Cr, even considering the low number of samples from the unaffected plants (Table 10). Another study analysed the leaves of plants growing in substrates from the region of the Fundão disaster and also found a higher content of Cu in the leaves of the herbaceous species *Stylosanthes guianensis* and *Saccharum officinarum* when grown on tailings (Coelho et al., 2020). Conversely, other studies found a higher content of Zn in *Peltophorum dubium* plants growing on control soil (Araújo et al., 2024), while no Ni accumulation was reported in these studies. It is reasonable to expect a similar pattern under field conditions due to continuous absorption over the years, especially during the wet season when water and mineral uptake usually significantly increase (Gravilescu, 2021). For the other elements (i.e., Fe, Mn and

Cr), there is no difference among affected and unaffected substrates, which calls attention to the high concentrations of these elements in the soils of the Doce River Basin (Gomes et al., 2017).

Considering the individuals grown in affected substrates only, there is a higher absorption of all PTE in plant tissues (Table 11). Here, the metal concentrations in both organs analysed are significantly higher than in the substrate, with a BCF >1 for all elements. This represents a greater absorption of PTE from the substrate, which is usually favoured by the roots' exudates, like metal-chelating or reducing compounds. These exudates reduce the rhizosphere’s pH, enhancing the availability and uptake of elements like Fe and Zn (Marschner, 2012). Some heavy metals could damage plant tissues and reduce plant growth (Hao; Wang; Chen, 2006) by forming reactive oxygen species (ROS). Considering that the individuals in our study growing on the affected substrate showed no growth inhibition, it is suggested that they tolerate high concentrations of these elements, a response likely mediated by physiological defences. Such mechanisms include the formation of NADPH oxidases, to avoid stress from Cd, Cu or Ni (Hao; Wang; Chen, 2006; Quartacci; Cosi; Navari-Izzo, 2001), and Glutathione to withstand metal-induced oxidative stress by Cd, Cr and Al in different species (Karuppanapandan et al., 2006; Karuppanapandian et al., 2006; Metwally et al., 2005). Also, the metals can be stabilized by metal-binding ligands in the plant cells' cytosol, such as phytochelatins and metallothioneins (Cobbett; Goldsbrough, 2002). From there, these stable complexes can be compartmentalized in the cell vacuoles, leading to enhanced tolerance and accumulation (Krämer et al., 2000). Since our calculations utilized bioavailable fractions rather than total elemental concentrations, the resulting BCFs more accurately reflect plant-soil interactions, though they are inherently higher than values based on total concentrations. This distinction is important, as the correlation between plant uptake and soil content depends on the specific extraction method employed (Zhang; Liu; Wang, 2010). Moreover, as we utilized the Mehlich-1 extractor, these results should be compared cautiously with studies that used alternative extraction solutions.

Even though the entire plant had higher PTE concentrations than the substrate, the root system showed the highest concentration. Despite rigorous washing of the root system, any minor soil adherence would be insufficient to account for the observed values, as the elemental concentrations in the roots were markedly higher than those in the substrate (Table 3). Roots were the main sink for Fe and Cr (with BCF of 107 and 4570, respectively), whereas Ni was more likely to be translocated to leaves (TF of 273). For Fe and Cr, the very low translocation factor (TF) indicates a substantial root sequestration. The same pattern was observed in *Brachiaria decumbens* growing in affected substrates, with elevated concentrations of Fe and Cr, in both the roots and leaves (Coelho et al., 2020c). It is known that young *Cecropia hololeuca* plants can have developmental problems with Fe concentrations of 8mM in FeEDTA, which leads to the peroxidation of membrane lipids (Rodrigues-Filho et al., 2022). Although *C. hololeuca* can reduce oxidative damage by preventing elevated levels of H_2O_2, other reactive oxygen species, such as singlet oxygen and hydroxyl radicals, remain oxidizing compounds that impact its development (Rodrigues Filho et al., 2020). Cu, Mn, and Zn show moderately low TF, suggesting some translocation but a primary sink in the roots. The increased absorption of these micronutrients may be attributed to a synergistic effect, with shared plasma membrane transporters and root reductase activity, that might facilitate interactions between them (Rietra et al., 2017). However, even though the plants filter these elements into their roots, a significant part is transferred upwards. Especially for Ni, the TF is very high, demonstrating a prolific and efficient translocation to the leaves. Even though this 136 mg/kg concentration represents only 0.01% of the leaf's weight, the concentration is exceptional. The toxicity levels of Ni in sensitive and tolerant crop species range from >10 mg/kg and >50 mg/kg, respectively (Broadley et al., 2012). In *Thlaspi goesingense*, a Ni-hyperaccumulator that can absorb more than 1000-fold nickel in the leaves, this is attributed to an efficient system that pumps Ni into the vacuole of shoot cells (Krämer et al., 2000), followed by Ni complexation with organic acids like malic and citric acids (Broadley et al., 2012). Considering the higher growth in affected substrates, these elevated concentrations of PTE might not be damaging the plant, indicating a high degree of physiological tolerance that warrants further investigation of its role in impacted sites. Regarding the potential influence of Aluminum (Al), although not directly quantified, the pH levels in KCl measured in the affected area (mean 5.52) suggest that Al toxicity was likely negligible. In tropical soils, Al mobility is significantly reduced at pH levels above 5.0 (Alleoni et al., 2010). Interestingly, the affected area presented a higher pH compared to the control area (5.18), which may have created a more favorable environment for *C. hololeuca* growth by precipitating Al into non-toxic forms, thereby explaining the higher biomass observed in the impacted substrate despite the elevated Fe concentrations.

From a theoretical point of view, it is important to verify *Cecropia hololeuca*'s phytoremediation potential, contributing to phytostabilization of such substrate. Given the fast growth and high productivity, *C. hololeuca*´s removal capacity of PTE´s is substantial. However, the ecological implications of leaf PTE enrichment, especially Ni, can be harmful in the long term, considering biomagnification, mainly because *Cecropia* plants in general are visited by a variety of fauna of different organism groups, such as insects, birds, sloths, monkeys (Berg; Franco Rosselli; Davidson, 2005), and also humans (Carneiro; Conceição, 2020). From a practical point of view, it is a native plant that can be used for restoration purposes, once it can grow in soils contaminated by iron ore. It produces more roots and aerial biomass, which will become organic matter in the soils, in the form of litter for macro-, meso- and microfauna, decomposers and microorganisms (Breviglieri et al., 2019). This also enhances the soil characteristics for other plants in the successional path, such as long-lived trees like hardwoods (Campanharo et al., 2021).

4.5 Conclusion

Cecropia hololeuca can absorb a variety of PTE and detoxify the soil by means of a dual strategy. It mainly works as a root accumulator for Fe, Cr, Cu, Mn, and Zn, effectively stabilizing them in the below-ground biomass. In contrast, it behaves as an accumulator of Nickel, efficiently transporting it to its above-ground tissues in high levels. Still, as a negative fact, it can lead to PTE biomagnification, especially for Ni, intoxicating animals that interact with it, such as pollinators, dispersers and herbivores. As an implication for conservation, it can be indicated for regeneration projects due to its fast growth and tolerance to metals in the soil, with potential as a facilitator and phytoremediator, but with possible negative implications for the food chain as a whole. As seen in the field and corroborated by our results, the tailings select fast-growing pioneer species that tolerate the newly imposed conditions, even after 6 years, allowing the succession to keep tracking.

5. Accumulation of potentially toxic elements in trees six years after the Samarco's dam collapse

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Abstract

Following the collapse of the Brazilian *Fundão* tailings dam in 2015, mining tailings contaminated aquatic and terrestrial ecosystems, with potentially toxic elements (PTE) like Fe, Mn, Cd, Pb, Cu, Cr, Ni, and Hg exceeding legal limits. Our study investigated the long-term response of riparian tree species and their wood PTE accumulation capacity, testing the hypotheses: i) higher metal concentrations in growth rings post-disaster in affected trees, ii) higher concentrations in trees from affected areas, iii) higher PTE bioavailability in the substrate from affected areas, iv) higher PTE concentrations in wood compared to substrate, v) different accumulation patterns among species, and vi) reduced wood radial growth in trees from affected areas. We sampled riparian forests in affected and unaffected areas, six years post-disaster, collecting nine composite soil samples from each. We selected the tree species *Anadenanthera peregrina*, *Piptadenia gonoacantha* and *Nectandra oppositifolia* and extracted increment cores from over 30 individuals per species to analyse growth and the incorporation of PTE. We analysed the growth rings using LINTAB, TSAP, and WinDENDRO software. For PTE analysis, we selected wood segments with seven pre- and seven post-disaster annual rings and measured the concentrations of Cu, Fe, Mn, Zn, As, Cd, Pb, Cr, and Ni using ICP-MS. We also calculated Bioconcentration factors (BCF) for metal accumulation in plant tissues. PTE concentrations in the wood did not differ significantly between the pre- and post-disaster periods, reflecting a long-term elemental integration within the xylem tissues. Similarly, most elements did not differ between affected and unaffected trees, except for higher Mn in *A. peregrina* and Pb in *P. gonoacantha*. All three species qualified as PTE accumulators (BCF>1). PTE amounts in the wood were up to 34-fold higher than in the substrate (e.g., for Cr and Pb), with the highest accumulation capacity observed in *P. gonoacantha* and *N. oppositifolia*. Finally, we found that Ni, Pb, and Cr concentrations exceed FAO limits for edible parts, which highlights potential risks of biomagnification in the food chain. Radial growth was not significantly reduced in trees from affected areas. Our results highlight that the three species, due to their sequestration capacity and tolerance, are well-suited for PTE stabilization in mining-related restoration projects.

Keywords: Riparian vegetation, Growth rings, Mining tailings, Ecological restoration, potentially toxic elements

5.1 Introduction

After the collapse of the *Fundão* iron ore mining tailings dam in Minas Gerais, Brazil, in November 2015 (Meira et al. 2017), tons of sludge invaded the *Doce* River and some of its tributaries, reaching the Atlantic Ocean. The wave of waste material occupied the riverbeds and a strip of their riparian vegetation, mainly in the first 100 km upstream of the *Risoleta Neves* Hydroelectric Power Plant (SÁNCHEZ et al., 2018). The natural vegetation was suppressed in 565 ha by mechanical passage of the tailings and 212 ha by late mortality, totaling an estimated volume of 120,000 m³ of wood (LACTEC 2021). After the spill, the substrate was characterized by a low cation exchange capacity (CEC) and low organic matter, raising concerns about the high mobility of potentially toxic elements (PTE). Among the stored tailings and the spread material, there are potential source of contamination from and chromium (Cr), cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), barium (Ba), cobalt (Co)(Da Silva et al., 2022), lead (Pb), sodium (Na) (Pires et al., 2003) arsenic (As), strontium (Sr) (Segura et al., 2016), bismuth (Bi), cerium (Ce), mercury (Hg) (Silva et al., 2021a) and others.

Some of the potentially toxic elements (PTE) found in the tailings are important for plants in small concentrations, being considered micronutrients or trace elements, e.g., Cu, Mn, Zinc (Zn) and sodium (Na) (Thalassinos et al., 2023). However, there are tolerance limits and safety thresholds, which are well described for crop plants, for example, a maximum allowable limit of 10 mg/kg Cu in *Zea mays*, 50 mg/kg Zn in *Lactuca sativa*, and 500 mg/kg Mn in *Allium cepa* (Khalid et al., 2017c). The permanence of such elements in the ecosystem at higher concentrations can damage plants, causing chlorosis (Zn, Mn) and turgidity loss (Cu) (Yadav; George; Dwibedi, 2023). In general, the new conditions imposed by the tailings' passage and permanence can affect the plants' physiology, determining physiological and growth limitations and stress. For instance, Pb can decrease photosynthetic rates, reducing many plant traits like height, leaf number, dry matter, and flower and fruit production (Collin et al., 2022). It has been shown that the first visible toxic reaction of arsenic is growth inhibition, followed by a decrease in leaf biomass depending on the exposure form (BUDZYŃSKA et al., 2024).

On the other hand, some plant species demonstrate tolerance to PTE, employing strategies that range from avoidance to accumulation (Thakur et al., 2022). In accumulator species, the main mechanisms of tolerance involve root exudation to enhance PTE uptake, followed by facilitated transport to vacuoles and enhanced capacity for Reactive oxygen species (ROS) scavenging (Thalassinos et al., 2023). The accumulated PTE are subsequently stored in different plant tissues, from roots to the leaves (Coelho et al., 2020b), in the bark (Mulenga et al., 2023a) and wood (Kaivapalu et al., 2024). Moreover, element concentrations in wood seem to depend on tree species and tree age (Scharnweber et al., 2016). Wood parenchyma cells and fibers are often associated with the accumulation of specific cations due to the presence of negatively charged carboxyl groups on molecules such as hemicellulose and lignin (Momoshima; Bondietti, 1990a). These traits establish trees as important organisms for understanding the persistence of contaminants such as the sludge and their resulting ecological impacts.

Wood growth patterns typically align with environmental conditions, with precipitation and temperature being especially critical in the tropics (Schöngart et al., 2017b). A study focused on the European larch (*Larix decidua* Mill.) showed that after a strong windthrow in Slovakia, the tree growth was enhanced by the abrupt canopy opening, but this effect was influenced by tree size and only occurred several years after the event (Izworska et al., 2022b). Furthermore, growth may deviate from the expected ontogenetic trajectory, in which the growth increment naturally declines with age (Scalon et al., 2022). While natural disturbances like windthrows are documented, the long-term impact of acute, human-induced disturbances on the growth dynamics of tropical species remains poorly explored. Currently, few articles specifically describe the effect of anthropogenic disturbances on tropical trees' growth rings (Locosselli et al., 2018b; Roquette et al., 2023). Despite this knowledge gap, the dendrochronological approach is a valuable tool for evaluating tree sensitivity to disturbances (De Micco et al., 2019). Each tree ring provides an annual record of growth increment, which allows us to compare tree growth over several years before and after a disturbance event (Ortega Rodriguez et al., 2023). Besides, by analysing the chemical content of each tree ring, it is possible to determine the disturbance impacts on each element’s tree absorption, translocation, and accumulation (Aljerf; Choukaife, 2018). In Brazil, *Tipuana tipu* trees tracked a three-decade reduction in levels of Cd, Cu, Pb, and Ni in urban areas (Locosselli et al., 2018a). Similarly, in Zambia, the bark of local tree species was used to monitor the absorption of Mn, Ni, Pb, Cd, Fe, and Zn near a copper leaching plant (Mulenga et al., 2023a). A prime example of large-scale phytoremediation project using trees is the Guadiamar Green Corridor (Spain), where trace elements were stabilized by planting trees (e.g., *Quercus, Populus*) combined with soil amendments and subsequent monitoring (Madejón et al., 2018). Therefore, the dendrochronological approach may be considered ideal to determine the possible impacts of mining tailings on tropical trees following the Samarco's dam break in 2015.

In this way, using a dendroecology approach, we seek to determine the response of three highly abundant riparian tree species to PTE concentrations preceding and following Samarco's tailings dam collapse. To this end, we tested the following hypotheses: i) there is a higher concentration of metals in the tree growth rings after the disaster than pre-disaster, ii) there is a higher concentration of metals in the growth rings of trees located in affected areas than in unaffected areas, iii) the bioavailability of PTE will be higher in the substrate from affected areas, iv) the concentration of PTE will be higher in the wood tissue than in the substrate within the affected areas, v) each species will exhibit a distinct pattern of elemental accumulation in its wood tissue, and vi) a reduction in tree radial growth following the disaster will be observed exclusively in affected areas.

5.2 Materials and methods

5.2.1 Study area

We sampled the Upper *Doce* River basin in *Minas Gerais* state, in southeast Brazil (Figure 16), an area primarily characterized by Semideciduous Seasonal Forests (Instituto Brasileiro de Geografia e Estatística, 2012). This region's landscape is situated predominantly on Oxisols and comprises an agricultural matrix of various crops and artificial cattle grazing grasslands, reflecting a long history of degradation (Coelho, 2009). According to Köppen's classification, the climate is Cwa and Cwb, defined as a humid subtropical type with dry winters and hot to temperate summers, with an annual rainfall of 1200 mm (Cupolillo; Abreu; Vianello, 2008). We sampled riparian forests at two locations: areas adjacent to the affected river (*Carmo* River) near the village of *Paracatu de Baixo* (20° 17' 44" S, 43° 11' 50" W) and areas adjacent to unaffected tributaries near *Acaiaca* municipality (20° 21' 51" S, 43° 10' 28" W) (Figure 16). The sampled sites maintained a mean elevation of 4.35 m (±2.25) above the river level during the rainy season.

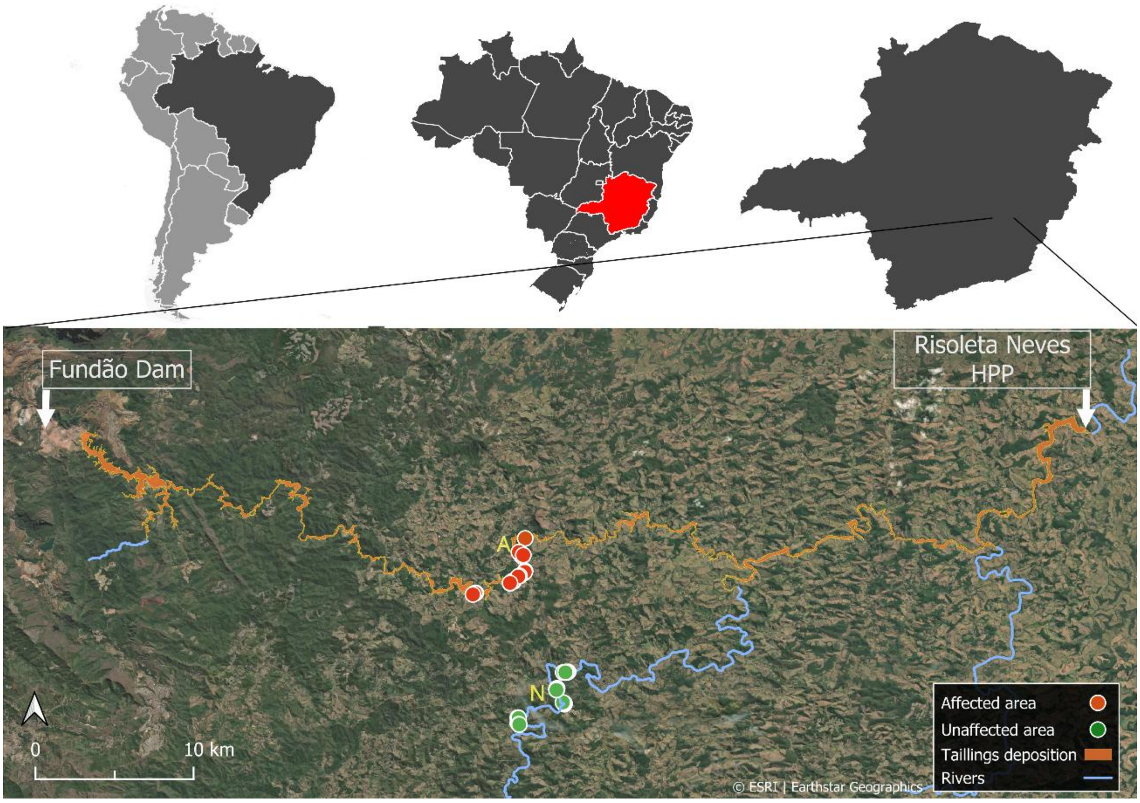

Figure 16: Tree sampling location in South America, Brazil and Minas Gerais state. Sampling was conducted in the regions between the *Fundão* dam and *Risoleta Neves* Hydroelectric Power Plant (shape file "PG23_Area_Afetada_Lama" provided by Fundação RENOVA).

5.2.2 Wood sampling

In the affected areas, we observed a mean height of 2.28 m (±1.79) for the tailings deposition mark on the sampled tree trunks, measured from the exposed trunk base. The maximum height reached 7 m (Figure 17).

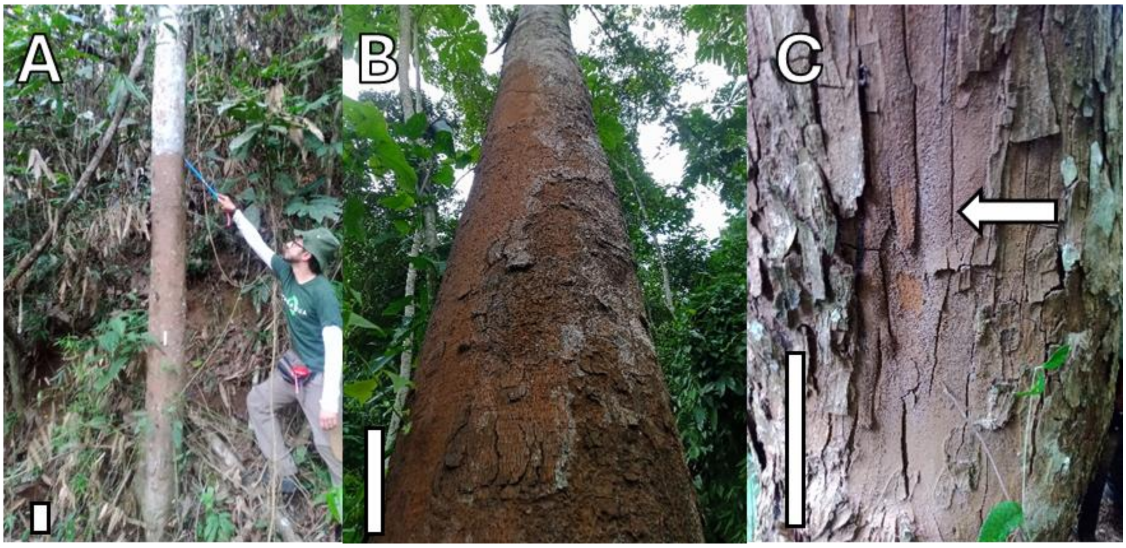

Figure 17: Marks of the tailings on different tree trunks six years after the disruption of the Fundão dam. A) Average height of the tailings marks, ca. 2m; B) Tailings reached higher levels; and C) Visible marks on trunks and bark (arrow). Scale: 20cm.

We performed an initial dendrochronological survey between March and May 2022. We established five plots parallel to the river in both affected and unaffected areas, considering the local topographic elevations. In each plot we assessed trees with a diameter at breast height (DBH) >5 cm. We cross-referenced the species lists to identify common species present in both locations. We conducted a subsequent literature review to select species with potential for PTE storage and that form visually distinguishable growth rings suitable for dendrochronological analysis (Worbes, 2002) . We selected three species for the study: *Anadenanthera peregrina* (L.) Speg. (Fabaceae), *Piptadenia gonoacantha* (Mart.) J.F.Macbr. (Fabaceae), and *Nectandra oppositifolia*. Nees & Mart. (Lauraceae). The three species are classified as pioneer species with fast radial and height growth. The genus *Anandenanthera* is known for ring formation (Mendivelso et al., 2016) and *P. gonoacantha* was used in dendrochronological studies before (Brandes et al., 2016). *N. oppositifolia* forms distinct rings with fuzzy boundaries, thick-walled latewood fibers and thin-walled earlywood fibers (Bauer; Schmitt; Oliveira, 2020) (Figure 18).

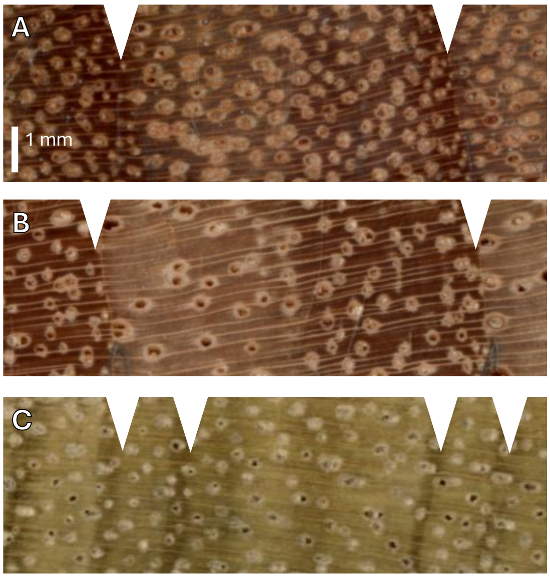

Figure 18: Wood ring anatomy for the species of the study. A) *Anadenanthera peregrina*, B) *Piptadenia gonoacantha* and C) *Nectandra oppositifolia*. Scale bar: 1mm, for all species. White triangles mark the tree ring boundary.

We recorded environmental covariates for each selected tree, including the vertical and horizontal distance to the river, terrain slope, canopy cover, geographical coordinates and the presence of tailings deposition marks on the trees or their neighbors (Table 12). In October and November 2022, we extracted increment cores (N = 206 total) from the three species: *A. peregrina* (N=62), *P. gonoacantha* (N=70) and *N. oppositifolia* (N=74). We took two samples per individual at breast height (1.3 m), using an increment borer (Friedman; Vincent; Shafroth, 2005). To account for stem eccentricity and ensure representative sampling, we extracted two increment cores in opposing directions (e.g., perpendicular to the slope or along the steepest terrain inclination) (LIN, 2022). We used all cores for radial growth comparisons and then selected a subset exhibiting the highest statistical correlation for subsequent chemical analysis, as described in the sections below. The sampled trees shared similar characteristics across species, including height and number of rings, which reduces non-environmental variance in growth comparisons.

Table 12: Characteristics of analysed trees and their surrounding from areas affected (A) or unaffected (N) by the mud passage from Samarco's Dam. Number of trees sampled differs from those used in chemical analysis, described in the text. River distance in a horizontal profile; Elevation (m) in a vertical distance to the river margins; Sludge mark is the height of the tailings on tree trunk or the surrounding trees; Number of rings counted in the dendrochronological analysis. —values are given as means and, in parentheses, the standard deviation

Parameter	*Anadenanthera peregrina*		*Piptadenia gonoacantha*		*Nectandra oppositifolia*	
	A	N	A	N	A	N
N sampled	12	19	19	16	11	26
N analysed	7	3	7	3	7	3
Diameter (cm)	39.6 (±23.3)	25.7 (±7.3)	27.5 (±9.9)	27.6 (±6)	11.6 (±6.5)	14.4 (±3.9)
Heigh (m)	14.6 (±5)	12 (±2.9)	16.3 (±4.7)	19 (±2.2)	9.1 (±3.1)	10 (±0)
River distance (m)	33.1 (±15.2)	21.7 (±13.1)	40.3 (±19.1)	21 (±8.8)	35.6 (±15.2)	14 (±6.4)
Canopy cover %	63.8 (±9.3)	76.6 (±7.3)	67.7 (±8.5)	77.7 (±2.9)	76.8 (±3.4)	80.3 (±2.9)
Elevation (m)	6.6 (±2.1)	3.7 (±0.5)	7.8 (±2.5)	3 (±0)	7.2 (±0.9)	3 (±0)
Sludge mark (m)	2.3 (±1.5)	-	1.1 (±0.8)	-	1.4 (±0.5)	-
Numbers of Rings	34.8 (±11.3)	36.8 (±9.5)	22.7 (±9.6)	19 (±10.7)	28.7 (±11.6)	30.7 (±17.1)

5.2.3 Tree growth rings identification

We used wood cores in two different work steps. First, to determine tree age and radial growth patterns (by means of increment rates), we glued the cores onto wooden supports and sanded them using a decreasing series of grain sizes, from 80 to 1,200 grits per inch² (Schöngart et al., 2005). We then differentiated the tree rings by utilizing the species' specific anatomical structure, employing a digital measuring device (LINTAB) for measurement. We performed visual and statistical cross-dating of the tree rings using the Time Series Analysis and Presentation (TSAP - Rinntech Inc.) and validated the results using the WinDENDRO tree ring analysis system (Regent Instruments Inc.) (Schöngart et al., 2005). We combined the resulting data into separate chronology-curves for the affected and unaffected areas. To check for similarity between the curves, we used the “Gleichläufigkeit correlation coefficient” (GLK), which quantifies the percentage of common signs in year-to-year radial growth change between two series (Buras; Wilmking, 2015). We selected individuals with a GLK exceeding 70% for the growth increment analysis, the second step, detailed in the Statistical Analysis section. We confirmed the sensitivity of the sampled species' anatomical rings to climatological signals, establishing their suitability for dendrochronological analysis.

5.2.4 Tree rings PTE accumulation analysis

To quantify the incorporation of potentially toxic elements (PTE) into the growth rings, we analysed a total of ten trees per species, with seven individuals from the affected areas and three from the unaffected areas. We analysed more individuals from the affected area to gain higher-resolution data on the primary study objective (the impacted zone). We isolated two distinct wood segments from each core: one represented the pre-disaster period (2001-2008) and the other represented the post-disaster period (2015-2022). This resulted in 20 samples per species for chemical analysis. We detached the samples from the support and removed the exterior material to prevent contamination. For each sample, 100 mg was weighed and ground in a ball mill. We then transferred the samples to a microwave digestion vessel and subjected them to microwave-assisted acid digestion at 200 °C under pressure in a mixture of 2 mL H_2O_2 and 8mL HNO_3 (69%) (MARS5Xpress, CEM, Matthews, USA). We used an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) system (Saint-Laurent et al., 2011) to measure the concentrations of the following elements: Cu, Fe, Mn, Zn, As, Cd, Pb, Cr and Ni. The analysis compared element concentrations across affected and unaffected trees, and pre- and post-disaster periods. The certified Standard Reference Material, SRM 1547 (Peach Leaves), provided quality control.

5.2.5 Soil sampling

We conducted soil sampling to assess substrate PTE bioavailability. We collected a total of nine composite soil samples in both the affected and unaffected areas. Sampling occurred along three transects in each area, with each transect separated by at least 300 m. Within each 10 m transect, we established three sampling points, and each point comprised three homogenized subsamples of the substrate. We preserved the material in plastic bags at 15 °C for transport to the laboratory, where we dried, weighed, ground, and sieved it.

Afterwards, we divided subsamples for chemical analysis (Embrapa, 2011). Recognizing that plant element absorption depends on soil solution concentration, rather than just on the total soil concentration (Chojnacka et al., 2005), we measured element bioavailability. We analyzed the following PTE using the Mehlich-1 extractor: Fe, Mn, Cu, Zn, Ni, Cr, Cd and Pb. Due to the specificity of this method, our results should be compared cautiously with studies employing alternative extraction solutions.

5.2.6 Statistical analysis

We designated the type of substrate, affected or unaffected by the tailings passage, as the explanatory variable for most analyses. To analyze the accumulation of PTE in the growth rings, we separately examined the concentrations of each element (mg/kg) as the response variable. The first analysis considered time (pre- and post-disaster) as the explanatory variable for trees in both affected and unaffected areas. We performed a paired t-test, or a Wilcoxon test when the normality assumption was not met. As most element concentrations did not differ significantly between these two periods, we calculated an average value for each element across the pre- and post-disaster periods. We used Mann-Whitney U-test to compare PTE concentration among trees located in affected and unaffected areas. For this model we considered tree's geographical location (affected or unaffected areas) as the explanatory variable and the PTE concentration in the wood as the response variable. We also used dplR package (v1.7.6) for the analysis of element concentration in both soil and wood.

We used Student's t-test to compare the chemical parameters between the affected substrates and unaffected soils. Each substrate parameter was separately compared between affected and unaffected areas. We performed a Shapiro-Wilks test to check the data normality, and when this assumption was not satisfied, we used the non-parametric Mann-Whitney U-test. Since the soil analysis data were initially reported in mg/L and the tree data in mg/kg, we transformed the soil values to mg/kg to enable direct comparison. This transformation accounted for an average soil density of 1,2 mg/dm³ in unaffected areas and 2,5 mg/dm³ in affected areas, based on laboratory measurements and published literature (De Souza et al., 2016).

In addition, we calculated the Bioconcentration factor (BCF) (Napoli et al., 2019) as the ratio of the element content in the wood (C_w) to its bioavailable concentration in the soil (C_s), as defined by the formula BCF= C_w/C_s. We used the bioavailable soil concentration instead of total content because plant uptake often correlates more strongly with the extractable fraction of the substrate than with total soil levels (Zhang; Liu; Wang, 2010). Consequently, the BCF reported here represents a more refined estimate of the plants' capture efficiency, though these values are inherently higher than those derived from total elemental analysis. The BCF quantifies a species' capacity to accumulate specific elements in its tissues. Plants with BCF > 1 are classified as accumulators, indicating their potential to store trace elements (Mulenga et al., 2023a) and making them suitable for phytoextraction (Napoli et al., 2019). To compare the phytoremediation potential of each species, we also calculated the average BCF using data from both affected and unaffected trees. We performed an ANOVA test followed by a Tukey's HSD test using the dplR package version (v1.7.6), retaining extreme values to capture natural variation. Finally, Arsenic (As) was excluded from this analysis as soil concentrations were below detection limits.

We compared annual increment from growth rings produced before and after 2015 (pre/post-disaster), covering the period from 2008 to 2022. Since two cores were obtained for each tree, we calculated the arithmetic mean for the annual increment. Data from a single available core were used when two were not present for a specific year. We applied filtering criteria, excluding trees with a coefficient factor lower than 70%. In *P. gonoacantha*, we entirely excluded individuals with >1 missing growth increment values (n = 5). The final number of analysed individuals in affected and unaffected areas was 10 and 17 for *A. peregrina*, 8 and 11 for *P. gonoacantha*, and 9 and 16 for *N. oppositifolia*, respectively. We fitted a Generalized Linear Mixed Model (GLMM) with a Gamma distribution and log-link function. The model incorporated the pre- and post-event periods as fixed effects and tree identification as a random intercept to account for repeated measures. Model diagnostics confirmed the absence of overdispersion, and residuals satisfied the assumptions of uniformity and homoscedasticity. We verified model assumptions via DHARMa residual diagnostics, which validated the use of the Gamma distribution with a log-link distribution. We prioritized the log-link model over the inverse-link mixed model and linear mixed model alternatives due to its superior explained variance and more precise estimates. The alternative models agreed with similar responses ($p < 0.05$). We retained extreme growth values as biologically plausible. For *N. oppositifolia*, we retained the GLMM due to its superior fit and ability to account for individual-level variability.

We analyzed wood growth and chemical parameters using R Statistical Software v.4.4.2 (R Core Team 2021). Specifically, we employed the dplR package (v1.7.6) to assess the suitability of the tree cores for the study, focusing on climatic sensibility and inter-series correlation. The growth increment analyses used lme4 (v1.1.35) and DHARMa (v0.4.6) packages.

5.3 Results

5.3.1 PTE accumulation in tree rings across time periods

The analysis of PTE concentrations in tree growth rings revealed no statistically significant difference between the rings formed before and after the 2015 tailings disaster for the majority of elements in both affected and unaffected areas (Table 13). Out of 54 comparisons performed, only two yielded significant results, both within the affected areas. The concentrations of Cu in *Piptadenia gonoacantha* increased after the disaster (t: -5.1408, p: 0.002), with mean concentrations rising from 1.36 mg/kg before 2015 to 1.95 mg/kg afterward. Concentrations of Pb in *Nectandra oppositifolia* decreased after the disaster (t: 3.303, p: 0.0163), with average concentrations of 0.43 mg/kg before and 0.21 mg/kg after the sludge deposition.

Table 13: Concentration of potentially toxic elements in the wood of *Anadenanthera peregrina*, *Piptadenia gonoacantha* and *Nectandra oppositifolia* trees considering growth rings formed before and after the passage of tailings from the Fundão dam, MG in 2015. Seven growth rings were used between the years 2001 to 2008 (before) and 2015 and 2022 (after). Different lowercase letters indicate a statistically significant difference (Mann-Whitney U-test, $p < 0.05$) between the "before" and "after" periods for the same element and species

Element	Area	Period	*A. peregrina*	*P. gonoacantha*	*N. oppositifolia*
Cr	Affected	before	0.71 ± 0.41 a	2.87 ± 2.89 a	4.11 ± 2.43 a
		after	1.88 ± 1.32 a	2.72 ± 3.16 a	3.61 ± 1.84 a
	Unaffected	before	1.26 ± 0.29 a	2.62 ± 1.50 a	5.15 ± 0.44 a
		after	1.28 ± 0.08 a	3.26 ± 1.62 a	13.84 ± 11.62 a
Mn	Affected	before	17.59 ± 4.07 a	27.94 ± 17.78 a	81.53 ± 39.57 a
		after	14.58 ± 2.90 a	19.10 ± 6.75 a	49.94 ± 29.08 a
	Unaffected	before	7.28 ± 2.30 a	16.23 ± 1.74 a	75.11 ± 36.15 a
		after	11.31 ± 3.08 a	15.45 ± 3.64 a	28.42 ± 6.11 a
Fe	Affected	before	21.18 ± 17.94 a	33.14 ± 20.16 a	79.04 ± 65.29 a
		after	30.20 ± 13.45 a	33.66 ± 27.84 a	74.63 ± 54.36 a
	Unaffected	before	18.37 ± 4.57 a	26.90 ± 23.90 a	43.61 ± 4.26 a
		after	31.82 ± 13.42 a	30.12 ± 14.60 a	119.25 ± 100.98 a
Ni	Affected	before	0.89 ± 0.77 a	0.23 ± 0.10 a	0.66 ± 0.35 a
		after	0.81 ± 0.93 a	0.28 ± 0.07 a	0.55 ± 0.28 a
	Unaffected	before	0.38 ± 0.06 a	0.46 ± 0.26 a	0.33 ± 0.04 a
		after	0.68 ± 0.19 a	0.54 ± 0.11 a	0.81 ± 0.61 a
Cu	Affected	before	1.74 ± 0.59 a	1.36 ± 0.33 a	2.16 ± 0.48 a
		after	1.32 ± 0.35 a	1.95 ± 0.14 b	2.27 ± 0.55 a
	Unaffected	before	1.48 ± 0.38 a	1.92 ± 0.94 a	1.77 ± 0.15 a
		after	1.46 ± 0.49 a	2.30 ± 0.13 a	2.64 ± 0.40 a
Zn	Affected	before	2.16 ± 3.35 a	5.27 ± 3.17 a	6.88 ± 4.21 a
		after	3.75 ± 3.10 a	5.06 ± 1.64 a	4.39 ± 3.64 a
	Unaffected	before	1.42 ± 1.89 a	3.47 ± 1.04 a	16.54 ± 6.95 a
		after	2.03 ± 1.89 a	5.23 ± 1.14 a	8.39 ± 3.43 a
As	Affected	before	0.01 ± 0.01 a	0.01 ± 0.00 a	0.00 ± 0.00 a
		after	0.01 ± 0.01 a	0.01 ± 0.00 a	0.00 ± 0.00 a
	Unaffected	before	0.02 ± 0.01 a	0.02 ± 0.02 a	0.01 ± 0.00 a
		after	0.02 ± 0.02 a	0.01 ± 0.01 a	0.01 ± 0.00 a
Cd	Affected	before	0.01 ± 0.01 a	0.01 ± 0.01 a	0.19 ± 0.14 a
		after	0.78 ± 1.65 a	0.01 ± 0.01 a	0.19 ± 0.15 a
	Unaffected	before	0.03 ± 0.01 a	0.07 ± 0.05 a	0.09 ± 0.01 a
		after	0.02 ± 0.01 a	0.02 ± 0.01 a	0.08 ± 0.01 a
Pb	Affected	before	0.50 ± 0.48 a	6.68 ± 4.49 a	0.48 ± 0.14 a
		after	0.28 ± 0.39 a	4.88 ± 3.88 a	0.21 ± 0.17 b
	Unaffected	before	1.20 ± 0.57 a	0.78 ± 0.45 a	0.16 ± 0.12 a
		after	0.71 ± 0.28 a	0.43 ± 0.02 a	0.06 ± 0.05 a

5.3.2 Tree rings PTE accumulation between areas

Comparing the average element concentrations in tree rings between affected and unaffected areas, the study found no significant difference for most elements and species (Table 14). Only two out of 27 comparisons demonstrated a significant effect. Mn content in *A. peregrina* trees was higher in affected areas (16.09 mg/kg) compared to unaffected areas (9.3 mg/kg) ($p<0.05$). Similarly, *P. gonoacantha* trees exhibited elevated Pb levels in affected areas (5.78 mg/kg) when compared to unaffected sites (0.61 mg/kg) ($p<0.05$) (Table 14).

Table 14: Contents of Cr, Mn, Fe, Ni, Cu, Zn, As, Cd and Pb, expressed in mg/kg, in the wood from three species from areas affected (A) and unaffected (N) by the Samarco's tailings disaster, Brazil. Means ± SD followed by the same lowercase letter do not differ between treatments by the Mann-Whitney U-test (p<0,05). Comparisons are made within the same species

	A. peregrina				*P. gonoacantha*				*N. oppositifolia*			
	N		A		N		A		N		A	
Cr	1.27 ± 0.2	a	1.3 ± 0.77	a	2.94 ± 1.88	a	2.8 ± 3.25	a	9.5 ± 6.9	a	3.9 ± 2.2	a
Mn	9.3 ± 3.26	a	16.09 ± 3.04	b	15.84 ± 3.29	a	23.52 ± 12.68	a	51.8 ± 25.4	a	65.7 ± 67.9	a
Fe	25.09 ± 10.49	a	25.69 ± 15.1	a	28.51 ± 22.96	a	33.4 ± 24.82	a	81.4 ± 63	a	76.8 ± 64	a
Ni	0.53 ± 0.15	a	0.85 ± 0.8	a	0.5 ± 0.22	a	0.25 ± 0.05	a	0.6 ± 0.4	a	0.6 ± 0.3	a
Cu	1.47 ± 0.36	a	1.53 ± 0.41	a	2.11 ± 0.49	a	1.65 ± 0.22	a	2.2 ± 0.2	a	2.2 ± 0.5	a
Zn	1.72 ± 1.63	a	2.95 ± 2.73	a	4.35 ± 0.51	a	5.16 ± 2.05	a	12.5 ± 6.3	a	5.6 ± 4	a
As	0.02 ± 0.02	a	0.01 ± 0.01	a	0.01 ± 0.02	a	0.01 ± 0	a	0 ± 0	a	0 ± 0	a
Cd	0.03 ± 0.01	a	0.39 ± 0.9	a	0.05 ± 0.04	a	0.01 ± 0.01	a	0.1 ± 0	a	0.2 ± 0.2	a
Pb	0.95 ± 0.51	a	0.39 ± 0.4	a	0.61 ± 0.27	a	5.78 ± 4.42	b	0.1 ± 0.1	a	0.3 ± 0.1	a

5.3.3 PTE bioavailability in the substrate

The substrate pH was significantly higher in affected areas (5.56 ± 0.17) compared to unaffected sites (4.77 ± 0.46), which likely influenced the solubility of the measured elements. Six years post-disaster (in 2022), the bioavailability of the majority of PTEs was lower in the affected substrate than in the unaffected soil (Table 15). Conversely, no significant difference was detected for Cr and Pb. However, the micronutrient Ni presented a notable exception, with substrates from affected areas exhibiting significantly higher bioavailability (0.98 mg/kg) than those from unaffected areas (0.26 mg/kg) (Table 15).

Table 15: Nutritional analysis and bioavailable concentrations of elements in substrates from areas affected (A) and unaffected (N) by the tailings spill from the *Fundão* dam. Sat. Al is the aluminium

saturation. SB is the sum of bases, and CEC is the Cation Exchange Capacity. SD is the standard deviation. * Significant difference. Average of 9 samples from each treatment

Parameter	N	SD	A	SD	t	p-value	
pH (KCL)	4.77	0.46	5.56	0.17	4.395	0.001	*
Cu (mg/kg)	1.26	0.41	0.44	0.06	-5.627	0.000	*
Fe (mg/kg)	89.72	12.98	39.97	11.95	-7.976	0.000	*
Mn (mg/kg)	70.56	6.10	57.23	10.54	-3.095	0.009	*
Zn (mg/kg)	1.69	0.53	0.43	0.08	-6.611	0.000	*
Cr (mg/kg)	0.28	0.10	0.36	0.05	2.113	0.056	
Ni (mg/kg)	0.26	0.11	0.98	0.14	11.463	0.000	*
Cd (mg/kg)	0.17	0.07	0.02	0.04	-5.416	0.000	*
Pb (mg/kg)	0.36	0.14	0.17	0.25	-1.869	0.085	

5.3.4 PTE bioconcentration in tree rings

The BCF, which compares the PTE concentration in the wood to that in the substrate, exceeded 1 for all elements across the study, indicating elemental accumulation in the plant tissue relative to the substrate (Table 16).

In affected areas, all three species accumulated higher concentrations of Cu, Zn, Pb, and Cr, achieving concentrations up to 5, 13, 34, and 10 times greater than the substrate, respectively. The highest bioconcentration was recorded for Pb in *P. gonoacantha* (BCF=34.7), which represents a wood concentration of 5.78 mg/kg against 0.17 mg/kg in the substrate. Other elements displayed species-specific accumulation patterns. For instance, *N. oppositifolia* alone stored proportionately more Fe and Mn, with concentrations up to 1.9 times greater than the soil. Meanwhile, Cd accumulation was notable in *A. peregrina* (BCF=19.2) and *N. oppositifolia* (BCF=9.4). In unaffected areas, these species also exhibited higher PTE storage in the wood compared to the soil for Cu, Zn, and Cr. Furthermore, trees in unaffected areas accumulated proportionately more Ni, with concentrations up to 2.2 times greater than the substrate.

Table 16: Bioconcentration factor between the soil and the plant in areas affected (A) or not affected (N) by the passage of Samarco's tailings. In yellow, values greater than 1, i.e., accumulation of elements in the wood

Species	Area	Cu	Fe	Mn	Zn	Cd	Pb	Cr	Ni
Anadenanthera	A	3.47	0.64	0.28	6.81	19.26	2.33	3.56	0.87
peregrina	N	1.17	0.28	0.13	1.02	0.16	2.68	4.56	2.07
Piptadenia	A	3.76	0.84	0.41	11.92	0.46	34.70	7.67	0.26
gonoacantha	N	1.67	0.32	0.22	2.58	0.27	1.70	10.59	1.96
Nectandra	A	5.03	1.92	1.15	13.01	9.43	1.92	10.59	0.62
oppositifolia	N	1.75	0.91	0.73	7.40	0.52	0.31	34.19	2.22

5.3.5 Species-Specific PTE Bioconcentration

Species exhibited varying accumulation responses when compared by their overall capacity for accumulation relative to the available PTE. *P. gonoacantha* and *N. oppositifolia* consistently showed higher BCF values for most elements. Cr storage averaged 8.5 (±8.06) for *P. gonoacantha* and 17.6 (±17.04) for *N. oppositifolia*. Fe storage averaged 0.68 (±0.57) and 1.61 (±1.43), respectively. Mn accumulation was higher in *N. oppositifolia* (1.02 ±1.00), and Pb was higher in *P. gonoacantha* (24.80 ± 26.89) (Figure 19).

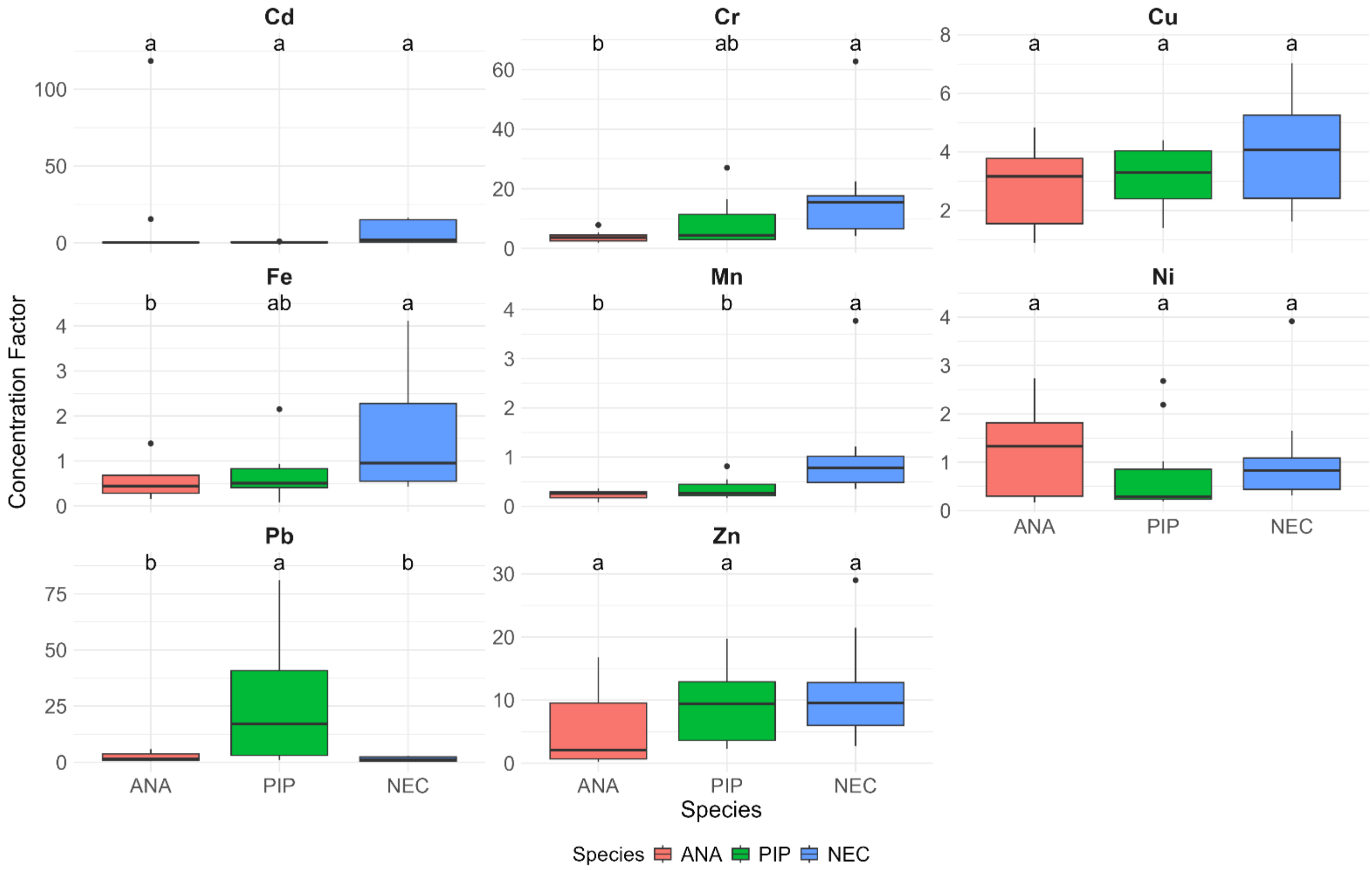

Figure 19: Interspecific Differences in Wood PTE Accumulation. Average Bioconcentration Factors (BCF) considering trees from affected and unaffected areas. Means ± SD followed by the same lowercase letter do not differ between species by Tukey's HSD test. Outliers are presented as dots. Species names in the legend: ANA – *Anadenanthera peregrina*, PIP – *Piptadenia gonoacantha*, NEC – *Nectandra oppositifolia*.

5.3.6 Tree ring growth

Ring width analysis demonstrated no significant difference in tree growth for all species when comparing the rings formed before and after the disaster in affected areas. However, the analysis of trees in unaffected areas indicated a significant reduction in growth after 2015 (Table 17, Figure 20).

Table 17: Growth increments statistics, comparing ring widths before and after 2015 in trees from areas affected and unaffected by the tailings disaster

Area	Species	X^2	df	p
Affected	*A. peregrina*	1.518	1	0.217
	P. gonoacantha	0.298	1	0.585
	N. oppositifolia	0.574	1	0.448
Unaffected	*A. peregrina*	11.347	1	0.0007
	P. gonoacantha	5.705	1	0.01691
	N. oppositifolia	346.65	1	< 2.2e-16

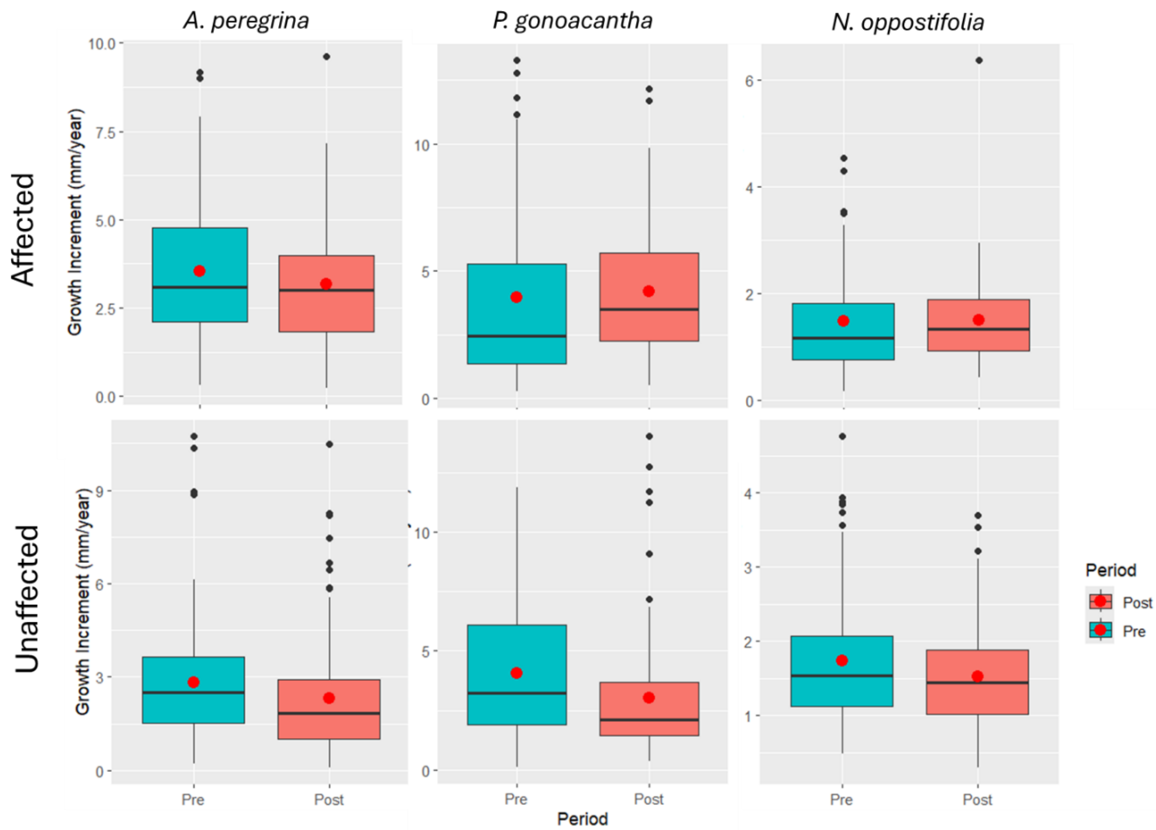

Figure 20: Growth increments before and after 2015 in trees growing in areas affected and unaffected by the tailing's disaster. Red dots represent the mean

5.4 Discussion

We found that, in general, the impact of the iron ore mining tailings on the wood chemistry or growth rings of *Anadenanthera peregrina*, *Piptadenia gonoacantha* and *Nectandra oppositifolia* trees were almost absent. The concentration of PTE in the wood did not differ between the pre- and post-disturbance periods for most elements, with only two exceptions for Cu in *P. gonoacantha* and Pb in *N.*

oppositifolia. Similarly, the mean PTE content in wood showed no difference between affected and unaffected trees, apart from elevated concentrations of Mn in *A. peregrina* and Pb in *P. gonoacantha*. Finally the radial growth of affected trees was not reduced.

Chemical analysis revealed that most element concentrations were lower in the affected soils than in unaffected areas, except for a higher value of Ni and parity for Cr and Pb. While Aluminum (Al) enrichment is common in the acidic soils of the Doce River basin (Guevara et al., 2018), it was not considered a significant phytotoxic factor in the affected areas. The tailings deposition resulted in a substrate pH of 5.56 (Table 5), a level at which Al typically precipitates into non-toxic forms (Alleoni et al., 2010). Consequently, the disaster did not appear to trigger Al-related stress, allowing our investigation to focus on the elements directly associated with the dam collapse. Nevertheless, PTE amounts were consistently higher in the trees than in the corresponding substrate, indicating absorption followed by accumulation in the xylem tissues. All three species accumulated PTEs in distinct patterns, with the highest levels of Cr, Fe, Mn, and Pb in the wood of *P. gonoacantha* and *N. oppositifolia*.

The similar PTE accumulation in the wood of the trees, comparing the same trees pre- and post-disaster, indicates that these species maintain stable elemental profiles across their growth rings. This lack of differentiation between periods reflects the high physiological resilience of these taxa and may be influenced by the internal distribution of elements within the xylem. In diffuse-porous species like those studied here (Longui et al., 2019; Stange et al., 2023), the active xylem encompasses a greater number of tree rings (Fabiani et al., 2022). This anatomical structure facilitates the lateral movement of compounds, primarily mediated by xylem ray parenchyma cells (Hristovski; Melovski, 2010), which contributes to a more uniform elemental distribution throughout the wood. Consequently, the wood chemical composition reflects a long-term integration of available elements rather than an acute record of individual events. However, these general trends are subject to element-specific physiological dynamics, and the extent of radial translocation requires further direct quantification.

While the general trend showed no temporal difference, specific deviations were observed for Cu and Pb, suggesting element- and species-specific responses (Figure 4). Consistent with our hypothesis, the Cu concentration in *P.*

gonoacantha wood was higher in the post-disaster period. This pattern is consistent with observations where divalent cation concentrations (i.e., Cu^{2+} and Fe^{2+}) naturally increase in younger tissues (Momoshima; Bondietti, 1990b). However, the measured Cu levels (max. 2.64 mg/kg in *N. oppositifolia*) are substantially lower than in other studies (23 to 183 mg/kg) and remain more than ten times below the phytotoxic thresholds for many species (Lactec, 2020; Lamb et al., 2012). These results confirm that the studied trees effectively regulate Cu translocation, maintaining xylem concentrations within a safe physiological range regardless of substrate conditions. Conversely, Pb concentrations were elevated in the older trunk segments of *N. oppositifolia*. This distribution indicates that Pb sequestration in this species occurs predominantly in the older heartwood, which is often associated with changes in the binding capacities of xylem cells as they age or as stem size increases (Scharnweber et al., 2016). Such a pattern reflects a stable, long-term accumulation strategy that appears independent of the recent tailings deposition, contributing to the overall physiological resilience observed across the studied period.

The mean PTE concentrations in wood formed between 2001 and 2022 did not differ significantly between affected and unaffected areas. This suggests that, in general, the metals present in the tailings were not extensively absorbed and translocated into the xylem tissues. However, there were two exceptions to this pattern (i.e., Mn in *A. peregrina* and Pb in *P. gonoacantha*). Trees of *A. peregrina* in affected areas showed elevated Mn concentrations (average 16.09 ± 3.04 mg/kg). While higher than unaffected trees, these concentrations remain below the general normal range for Mn in plant dry matter (20 to 500 mg/kg) (Baker; Brooks, 1989), indicating no signs of phytotoxicity or hyperaccumulation. Conversely, *P. gonoacantha* affected trees accumulated nearly ten times more Pb (5.78 ± 4.42 mg/kg) than unaffected trees (0.61 ± 0.27 mg/kg). Despite this large relative difference, the measured values are not considered environmentally concerning, as plants in severely Pb-contaminated sites can exhibit concentrations up to 50 mg/kg (Cunningham; Berti; Huang, 1995). The detection of high Pb in wood is unusual, considering that the majority of uptaken lead (often >95%) is typically retained in the roots (Ur Rahman et al., 2024). This exclusion is primarily mediated by the Casparian strip in the endodermis, which limits the element's entrance into the root vascular tissues (Kumar; Smita;

Cumbal Flores, 2017). More than the plant barriers, the absorbed concentration also depends on the soil concentration (Chojnacka et al., 2005). Consequently, this specific translocation of Pb to the wood of *P. gonoacantha* suggests a species-specific mechanism that either bypasses the typical root exclusion barrier or involves an efficient post-uptake translocation process.

The concentrations in the substrate collected in affected areas in 2022 provide important context, as they were lower than in unaffected areas for most elements, except for Ni, and remained below established regulatory thresholds (Coelho et al., 2020b; Silva et al., 2021a). These values reflect the naturally elevated background PTE levels of the Doce River basin, when compared to other regions of the state or Brazil (Guevara et al., 2018). The reduced PTE concentration in the affected substrate is likely a dual result of the iron ore processing itself, which removes these elements to produce a high-purity pulp (Segura et al., 2016), and environmental dissipation following the disaster. For example, Pb and Ni concentrations decreased substantially from values reported immediately after the disaster (e.g., 9 mg/kg Pb and 15 mg/kg Ni) (Guerra et al., 2017a) to the current levels (0.17 mg/kg Pb and 0.98 mg/kg Ni). However, cross-study comparisons must be treated cautiously due to potential differences in extraction and determination methodologies. As another example of time differences, the amount of Mn in the substrate was enhanced after the tailings deposition, but decreased in subsequent years following rainy seasons (Vasconcellos et al., 2021). This reduction over time is consistent with natural attenuation processes, such as environmental dissipation and surface runoff, which contribute to the dilution of elements like Fe, Mn, Cr, and Cu (Santana et al., 2021b). Furthermore, gradual plant absorption, as observed in this study and documented for other species in the region (i.e., *Brachiaria decumbens* and *Stylosanthes guianensis*) (Coelho et al., 2020b), works as a biological pathway for the continued reduction of bioavailable PTE in the substrate. Consequently, the current chemical state of the tailings appears to have reached a stabilized equilibrium that supports the development of the riparian forest.

The cumulative nature of the metal uptake by plants is clearly recorded in the tree rings. The element accumulation in the wood (2001–2022) was substantially higher than in the substrate sampled in 2022 (Table 16). Nearly all analyzed elements, especially Cu, Zn, Cd, Pb, Cr and Ni, showed elevated concentrations in the wood, with BCF reaching up to 34-fold higher in the plants than in the substrate (e.g., for Cr and Pb). This suggests that the trees absorbed these elements through time and stored them as they were translocated through the xylem elements. The positive charge of these cations is usually adsorbed with the carboxylic groups of the cell wall on the vase elements, which are negatively charged (Momoshima; Bondietti, 1990a). The high wood accumulation of Zn, Cd, Pb, and Cr is particularly noteworthy, given that these elements typically exhibit low vertical translocation and are sequestered in the roots by secretions, free amino acids, or proteins (Augusto et al., 2014). For instance, low-mobility ions like Pb are generally expected to accumulate predominantly in the roots (Krutul et al., 2017). Conversely, Mn, Fe, and Cd did not consistently present higher values in the wood of all species (Table 14). Despite the high bioavailable Mn concentration in the substrate (119.22 mg/kg in affected areas and 70.56 mg/kg in unaffected regions), its uptake is complex and highly influenced by factors such as soil pH, organic matter content, and redox potential (Zhang et al., 2014). It is also important to point out that the observed variations in PTE absorption and translocation are largely attributed to the specific physiological characteristics of each species, particularly differences in micronutrient requirements and toxicity tolerance (Antoniadis et al., 2017).

Comparing the three species in this work, we can see their unique accumulation profiles. *P. gonoacantha* demonstrated a high sequestration capacity, exhibiting a BCF for Pb of 34.7 (mean content of 5.7 mg/kg in affected areas) despite very low substrate bioavailability (0.17 mg/kg). This BCF significantly exceeds levels reported in common phytoremediation plants (e.g., *Ocimum sanctum*, with leaves Pb concentration of 4.59 mg/kg) (Collin 2022). Also, *N. oppositifolia* was another strong accumulator, achieving a Cr BCF up to 34.2 and being the only species to consistently accumulate Fe and Mn. Finally, *A. peregrina* accumulated six PTEs, with its highest BCF observed for Cd (19.26). While previous literature cited *P. gonoacantha* as an accumulator of Cu, Zn, and Pb, our study adds Cr and Ni to this list. Similarly, our results expanded the knowledge regarding the PTE accumulation potential of elements for *A. peregrina* (Horn et al., 2020) to include Cd, Cu, Zn, Pb, Cr, and Ni. Beyond root absorption, these elevated concentrations could also result from bark uptake following direct contact with the tailings, with subsequent translocation to the wood (Watmough;

Hutchinson, 2002). Furthermore, external sources such as atmospheric pollution, potentially from fertilizers, can introduce Cd, Pb, and Cr (Nava et al., 2011), representing an input independent of the disaster that may partially explain the high BCF values observed even in the unaffected areas.

Our findings have significant implications for ecosystem health and the related food chain. The measured concentrations of Ni, Pb, and Cr in all three species were found to exceed the reference values set by the Food and Agricultural Organization (FAO) for edible plant parts (Khalid et al., 2017b). The presence of non-essential metals, such as Cd, Pb, Cr, and As, is particularly concerning due to their lack of known function in plant metabolism (DalCorso, 2012). For instance, the sap of *A. peregrina* is a food resource for *Callithrix* monkeys (Francisco et al., 2014), and so far, the accumulated PTEs could enter the food web generating biomagnification. Furthermore, the sequestered elements will persist long-term, re-entering circulation upon tree senescence and decomposition, as advanced stages of decay facilitate the release of elements like Mn, P, Cu, Zn, and Ca (Khanina; Smirnov; Bobrovskii, 2023). It is important to note that for effective soil remediation through phytoextraction, the contaminated biomass must be harvested and removed from the site to prevent the re-release of these elements into the ecosystem. Critically, two of the species, *P. gonoacantha* and *A. peregrina*, are from Fabaceae family, known to contribute to ecosystem recovery by fixing atmospheric nitrogen (N_2). This N-fixing ability is important for the restoration of the impacted areas, as the tailings-affected substrate is typically poor in essential nutrients (Li et al., 2024). These observations highlight the need for focused studies of ecological interactions.

Finally, the disaster did not impact the trees' development, as the radial growth increment was not reduced in the years following the disaster. The expected growth releases, often associated with specific disturbances, were not observed in the post-event wood rings. It should be noted, however, that identifying such releases typically requires a 10-year interval (Nowacki; Abrams, 1997). The constant or declining radial growth observed in this riparian vegetation in our study is characteristic of trees in open-growth or gap-originated areas (Lorimer; Frelich, 1989). Alternatively, the growth patterns may be a consequence of natural ontogeny, where radial increment naturally decreases as trees age (Scalon et al., 2022). These collective growth results indicate that there was no significant physiological stress registered in the wood rings analyzed for the three species.

5.5 Conclusions

These findings establish that Anadenanthera peregrina, Piptadenia gonoacantha and Nectandra oppositifolia are effective PTE accumulators. This strong sequestration ability suggests these species play a long-term role in limiting PTE mobility within the ecosystem. Despite high PTE accumulation levels, the tailings deposited after the Samarco dam break did not result in significant long-term physiological stress, as shown by the sustained radial growth in affected areas. The consistent increment development and chemical stability of the wood across time reflect the high resilience and adaptive capacity of these riparian species to the disturbance event.

The high BCFs achieved by these species demonstrate their effective role in PTE stabilization and underscore the need for follow-up studies focusing on root and leaf element content. Since 2025 marks a decade of the dam break, further research should be conducted on tree individuals that germinated on the affected substrate post-event to assess long-term developmental responses. These results highlight the complex environmental dynamics of the Doce River region, where a long history of mining contributes to a baseline of chronic PTE availability. The shared resilience of the studied species indicates they are well-adapted to these conditions, making them collectively suitable for restoration projects throughout the basin, even in areas considered nominally unaffected.

6. Discussion and conclusion

6.1 Summary of outcomes

This thesis presents an overall image of the environmental impact, six years after the Samarco (Fundão) dam failure, revealing significant differences between the ecological scale of the impact and the specific morphophysiological responses of native species. On a broader scale, the disaster resulted in a reduction in regional plant biodiversity, with affected areas accumulating fewer species than unaffected sites. This biodiversity loss among herbaceous species is linked to habitat homogenization caused by the deposition of dense, low-nutrient tailings. However, six years post-disaster, the tailings substrate has undergone natural stabilization, and for most elements, bioavailable concentrations no longer present immediate concerns or exceed legal thresholds. Crucially, the tailings slurry did not prevent plant regeneration. Local seedling abundance and species richness (alpha diversity) remained comparable to unaffected areas, indicating active seed dispersal and viable seed banks capable of promoting an early-successional state dominated by competitive, ruderal species.

The studies on native trees highlight a surprising degree of resilience and even enhanced performance for a pioneer species under the new tailings-affected conditions. The pioneer tree *Cecropia hololeuca* showed remarkable growth, with height and biomass nearly doubling on the tailings compared to control soils, a performance attributed to its competitive advantage and a strategic increase in root biomass allocation that copes with the low-quality substrate. This contrasts with the adult riparian species analyzed using the dendrochronology technique (*A. peregrina*, *P. gonoacantha*, and *N. oppositifolia*), which showed no reduction nor enhancement in radial growth after the disaster. The wood analysis showed that the contamination impact was not visible in the tree trunks, as PTE concentrations in the wood formed after 2015 were generally similar to pre-disaster levels. This suggests that the event's contamination was punctual, and the trees effectively managed the elemental load through their active xylem.

All six species studied (two herbaceous species, one pioneer tree species and three hardwood species) demonstrate utility for the restoration of areas impacted by mining-tailings. Specifically regarding phytostabilization potential, the four woody species achieved high Bioconcentration Factors, confirming their capacity to immobilize PTE within their biomass. These species effectively absorb and sequester PTE from the contaminated substrate, primarily concentrating elements such as Fe, Cr, Cu, Mn, and Zn within woody organs like roots and trunks. However, the studies flag a critical ecological concern: Ni translocation. *C. hololeuca* exhibited an extremely high Translocation Factor, demonstrating a high concentration in its leaves. This, along with the accumulation of PTE in the wood of other species, raises concerns about trophic transfer and biomagnification in the local food web (e.g., feeding insects, birds and mammals). Therefore, while these native trees are essential candidates for long-term restoration — acting as facilitators by building organic matter and improving soil structure, their use must be carefully evaluated against the potential risks they pose to higher trophic levels via metal transfer.

6.1.1 Plant recruitment

The Samarco tailings-dam disaster reduced regional plant diversity six years post-event, although it did not inhibit local plant regeneration or overall seedling emergence. Our investigation demonstrated that areas affected by the mining tailings exhibit a 35% lower regional species accumulation compared to unaffected areas. This biodiversity loss is linked to the homogenization of the affected soil, which showed reduced nutrient availability, lower macroporosity, and greater density, simplifying microhabitat heterogeneity.

Despite this reduction in regional diversity, local seedling abundance and species richness (alpha diversity) were comparable between affected and unaffected areas, indicating viable seed banks and/or substantial seed arrival post-disaster. The high abundance of emerging plants in the disturbed site confirms that the Samarco slurry does not prevent plant regeneration.

Experimental plant growth trials on the affected soil confirmed negative impacts on development, specifically reducing overall growth in *Ludwigia octovalvis* and shifting biomass allocation toward proportionally less root development in *Marsypianthes chamaedrys*. However, the finding that both species grew relatively well suggests their high potential for use in restoration practices. The dominant recolonizing community comprises competitive, annual weed species, which can accelerate essential nutrient cycling in the affected, low-nutrient soils.

Overall, our results underscore the persistent, strong ecological footprint of a large-scale mining disaster, highlighting the critical need for restoration efforts to focus on enhancing soil structure and microhabitat diversity to effectively recover biodiversity.

6.1.2 *Cecropia* seedling growth

The pioneer species *Cecropia hololeuca* exhibits significantly enhanced growth and high tolerance to substrates from the mining-affected areas, employing a distinct, dual strategy for managing potentially toxic elements (PTE). Our experimental results show that tailings exposure nearly doubled the height, stem diameter, and leaf production of *C. hololeuca* compared to unaffected soils, resulting in greater overall dry biomass. This successful establishment is attributed to the species' pioneer traits, which confer a competitive advantage in the newly formed, nutrient-poor, sandy technosol. Plants in the affected soil demonstrated a clear resource reallocation strategy, investing a greater proportion of biomass into the roots to enhance below-ground foraging capacity.

Chemically, *C. hololeuca* acts as a differential hyper-tolerant accumulator. First, as a phytostabilizer with root sink characteristics, it acts as a highly effective root accumulator for most elements, showing very high bioconcentration factors (BCF>1) for Fe and Cr, and effectively sequestering Cu, Mn, and Zn in its below-ground tissues, thereby stabilizing the soil. Secondly, as a phytoextractor, with shoot translocation, the species demonstrates an efficient translocation of Nickel (Ni) to the leaves, reaching concentrations five times higher than control plants and exhibiting a very high Translocation Factor (TF=273).

While the enhanced growth and detoxification mechanisms make *C. hololeuca* a valuable candidate for restoration and phytostabilization, the high accumulation and translocation of Ni to aerial parts raise immediate concerns regarding its potential for trophic transfer and biomagnification in the local food chain, requiring careful ecological evaluation for long-term restoration planning.

6.1.3 PTE absorption and accumulation in trees

Dendrochronological analysis across the three diffuse-porous species (*Anadenanthera peregrina*, *Piptadenia gonoacantha*, and *Nectandra oppositifolia*) showed that concentrations in the wood formed after the disaster were similar to those formed before the event. Assuming that the contamination made the PTE bioavailable and that the trees could absorb them, this plant resilience is likely due to the limited duration of the contamination event and the capacity of diffuse-porous wood to actively transport and redistribute elements radially beyond the specific 2015 growth ring threshold. Crucially, no physiological growth stress was detected in the affected trees, suggesting the disaster did not inhibit their long-term development.

Despite the low overall impact, all three species demonstrated an ability to absorb and accumulate PTE in their xylem tissues. This resulted in Bioconcentration Factors (BCF) up to 34-fold higher than the bioavailable fraction of the surrounding substrate (as measured via Mehlich-1). Using this extractable fraction, rather than total soil content, provides a more accurate representation of the biological reality of plant uptake. This finding identifies these species as promising candidates for phytostabilization in the ore-contaminated Doce River Basin. The highest accumulation was observed for Cr, Fe, Mn, and Pb in *P. gonoacantha* and *N. oppositifolia*. However, the accumulation of PTE (Cd, Pb, Cr, Ni) raises concerns regarding trophic transfer in the local ecosystem, which calls for further ecological investigation.

6.2 Research limitations and Future research directions

It is important to acknowledge that the sample size was inherently limited for some of the analyses presented in this study. Consequently, the conclusions derived from these specific sections must be interpreted with the necessary methodological caution, serving primarily to instigate subsequent investigations rather than to establish definitive generalizations. The central value of this work lies in its ability to outline and validate multiple avenues of study within the impacted ecosystem. This research prioritized the use of the Mehlich-1 extractant to determine the readily available pool of PTE in the substrate. Consequently, comparisons with studies that utilized different extraction methods (such as total digestion or XRF) must be interpreted with caution, as extraction efficiency varies significantly between methodologies.

Given the advancing chronology since the disaster, completing a decade in November 2025, the implementation of long-term dendroecological studies is an interesting approach. Future research should explore the vegetative response (in terms of growth and xylem chemical composition) over extended temporal intervals, specifically spanning periods greater than ten years following the dam breach. This longitudinal approach will enable a more precise assessment of the chronic effects of the tailing's material, providing critical insights into the mechanisms of resilience and the successional trajectory of the flora in response to the initial disturbance. Furthermore, considering that the PTE accumulation has been detected in various tissues, future work should widen the scope of investigation to include a more comprehensive multi-organ analysis of the plant body. Research should move beyond the wood to encompass the major plant organs (roots, stems, leaves, bark, and reproductive parts) to fully quantify PTE tissue partitioning. Analyzing the whole plant will allow for a complete understanding of overall element sequestration and its impact on plant fitness and the transfer of elements in the food chain. Such comprehensive data will be fundamental for refining environmental remediation and recovery strategies within the Doce River Basin.

6.3 Main contributions/Implications for management

The findings carry several direct and indirect implications for planning and executing ecological restoration projects in mining-impacted areas.

6.3.1 Soil structure and microhabitat restoration

The reduction in regional diversity associated with the environmental disturbance caused by the tailings deposition appears to be closely related to habitat homogenization. Restoration efforts should then prioritize physically modifying the substrate to introduce heterogeneity, enhance macroporosity, and increase organic matter (OM). This can involve deep ripping, adding high-OM amendments, and creating micro-topography to modulate water and nutrient availability, thereby supporting a wider variety of plant niches.

6.3.2 Strategic use of pioneer and facilitator species

The high abundance of regenerating plants and the enhanced growth of *C. hololeuca* on tailings confirm the immediate viability of using native pioneer species. Competitive early-successional taxa, such as *C. hololeuca* and the tolerant herbaceous species identified, should be strategically introduced as "facilitator species." Their rapid growth and high biomass production will accelerate phytostabilization and increase soil OM, rapidly improving conditions for later-successional, less tolerant species.

6.3.3 Mitigation of trophic transfer risk

The high translocation of elements to the leaves of *C. hololeuca* and the accumulation of PTE in the wood of other species represent a relevant ecological consideration. Ecotoxicological studies could evaluate the transfer rates of Ni and other translocated PTE from plant tissues to local fauna (e.g., insects, birds, monkeys, bats). Long-term monitoring of PTE content in the bark, leaves, and fruits of plants used in restoration is necessary to assess potential risks of trophic transfer. Consequently, management protocols should include the periodic harvest and removal of aboveground biomass to prevent re-entry of accumulated elements into the ecosystem. This approach is particularly valuable for species like *C. hololeuca*, which demonstrates high Ni translocation to the leaves, thereby minimizing the risk of these elements moving further up the food chain.

6.3.4 Long-term monitoring

The resilience of the woody species does not negate the need for sustained monitoring. The lack of an acute chemical shift in the wood rings suggests that these trees have successfully integrated the elemental load over time. Furthermore, the current state of the Doce River basin likely reflects a baseline of chronic PTE availability to which these native species appear well-adapted. Consequently, establishing permanent dendroecological monitoring plots to track plant performance beyond the ten-year mark is required. This long-term data will allow researchers to distinguish initial physiological resilience from potential chronic, delayed effects and to fully understand the role of these trees in the long-term fate within the ecosystem.

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8. Appendix

Table S1: Raw and converted data for the affected substrate chemical analysis. Conversion based on the substrate density provided in the text.

Code	pH H_2O	pH KCl	P	K	Na	Ca	Mg	Al	H + Al	SB	t	T	V	m	ISNa	MO	P-Rem	Cu	Mn	Fe	Zn	Cr	Ni	Cd	Pb
			mg/dm³			cmolc/dm³							%			dag/kg	mg/dm³	mg/dm³							
1	6.6	5.8	6	81	0	0.46	0.12	0	0.4	0.79	0.79	1.19	66	0	0	0.27	48	1.54	78.5	267.5	1.84	0.59	1.54	0	0.43
2	5.7	5	3	23	2.3	1.48	0.52	0	2.3	2.07	2.07	4.37	47	0	0.23	1.34	23	4.82	133	349.2	3.34	0.66	2.17	0	1.3
3	6.3	5.7	5	160	0	2.06	0.86	0	1.4	3.33	3.33	4.73	70	0	0	1.88	30.3	3.68	166	302.3	3.46	0.56	1.98	0	0.86
4	5.8	5.3	4		2.3	1.95	0.53	0	2.1	2.53	2.53	4.63	55	0	0.22	1.34	21.7	4.21	143	325.6	2.86	0.55	1.88	0	1.84
5	6.2	5.5	8	27	2.3	2.83	0.8	0	1.5	3.71	3.71	5.21	71	0	0.19	1.07	23.7	4.14	175	365	3.66	0.77	2.08	0	1.29
6	7	6.1	17	45	6.33	4.72	1.12	0	0.4	5.98	5.98	6.38	94	0	0.43	0.4	33.2	3.25	122	219.4	3.62	0.71	2.71	0	1.63
7	6.1	5.4	3	137	0.29	1.96	0.67	0	1.7	2.98	2.98	4.68	64	0	0.03	1.34	27.9	4.54	115	313.3	2.93	0.67	2.07	0	2.92
8	5.9	5.3	3	133	4.32	2.09	0.82	0	2	3.27	3.27	5.27	62	0	0.36	1.21	23	3.76	87.8	179.9	2.25	0.54	1.78	0	3.22
9	5.4	-	2	41	0.29	0.86	0.33	0	1.4	1.3	1.3	2.7	48	0	0.05	0.67	20.8	3.11	64.7	178.8	1.38	0.63	1.29	0	4.97
			mg/kg			cmolc/kg							%			dag/kg	mg/kg	mg/kg							
1	6.6	5.8	2	32	0	0.18	0.05	0	0.16	0.32	0.32	0.48	66	0	0	0.27	19.2	0.62	31.4	107	0.74	0.24	0.62	0	0.17
2	5.7	5	1	9.2	0.92	0.59	0.21	0	0.92	0.83	0.83	1.75	47	0	0.23	1.34	9.2	1.93	53	139.7	1.34	0.26	0.87	0	0.52
3	6.3	5.7	2	64	0	0.82	0.34	0	0.56	1.33	1.33	1.89	70	0	0	1.88	12.12	1.47	66.3	120.9	1.38	0.22	0.79	0	0.34
4	5.8	5.3	1	0	0.92	0.78	0.21	0	0.84	1.01	1.01	1.85	55	0	0.22	1.34	8.68	1.68	57.1	130.2	1.14	0.22	0.75	0	0.74
5	6.2	5.5	3	11	0.92	1.13	0.32	0	0.6	1.48	1.48	2.08	71	0	0.19	1.07	9.48	1.66	70	146	1.46	0.31	0.83	0	0.52
6	7	6.1	7	18	2.53	1.89	0.45	0	0.16	2.39	2.39	2.55	94	0	0.43	0.4	13.28	1.3	48.8	87.76	1.45	0.28	1.08	0	0.65
7	6.1	5.4	1	55	0.12	0.78	0.27	0	0.68	1.19	1.19	1.87	64	0	0.03	1.34	11.16	1.82	45.8	125.3	1.17	0.27	0.83	0	1.17
8	5.9	5.3	1	53	1.73	0.84	0.33	0	0.8	1.31	1.31	2.11	62	0	0.36	1.21	9.2	1.5	35.1	71.96	0.9	0.22	0.71	0	1.29
9	5.4	-	1	16	0.12	0.34	0.13	0	0.56	0.52	0.52	1.08	48	0	0.05	0.67	8.32	1.24	25.9	71.52	0.55	0.25	0.52	0	1.99

Table S2: Concentration of potentially toxic elements (mg/kg dry weight) in the leaves and roots of Cecropia hololeuca individuals grown in substrates from areas affected or unaffected by Samarco's tailings disaster.

Treatment	Plant ID	Organ	Fe	Cr	Cu	Mn	Zn	Ni
Affected	1	Leaves	347.25	170.89	29.24	175.51	23.67	89.19
Affected	1	Roots	12640.82	1265.91	32.41	197.28	30.28	<1,0
Affected	2	Leaves	270.81	124.92	26.58	155.22	22.94	209.70
Affected	2	Roots	29544.99	2801.64	55.10	243.21	46.92	<1,0
Affected	3	Leaves	548.12	243.29	57.90	136.13	42.58	212.47
Affected	3	Roots	17030.76	1073.79	47.41	181.21	33.37	<1,0
Affected	4	Leaves	618.85	243.62	32.82	84.77	26.88	87.62
Affected	4	Roots	11980.97	1087.59	23.09	122.72	24.15	150.84
Affected	5	Leaves	359.10	214.01	36.65	131.18	28.31	83.50
Affected	5	Roots	6042.39	422.88	1642.52	113.10	1267.52	<1,0
Unaffected	1	Leaves	1905.70	977.48	18.68	141.23	15.76	78.83
Unaffected	1	Roots	6885.55	331.07	54.45	126.11	43.78	<1,0
Unaffected	2	Leaves	516.55	257.82	6.91	116.53	10.81	<1,0
Unaffected	2	Roots	IS	IS	IS	IS	IS	IS
Unaffected	3	Leaves	335.22	195.22	10.53	106.41	12.57	<1,0
Unaffected	3	Roots	15564.81	265.48	253.65	232.29	156.78	<1,0
Unaffected	4	Leaves	IS	IS	IS	IS	IS	IS
Unaffected	4	Roots	IS	IS	IS	IS	IS	IS
Unaffected	5	Leaves	IS	IS	IS	IS	IS	IS
Unaffected	5	Roots	IS	IS	IS	IS	IS	IS

IS (Insufficient Sample): Sample weight was below the minimum requirement for the analysis.

“…The river cannot go back...
the river needs to take the risk of entering the ocean
because only then will fear disappear,
because that’s where the river will know
it’s not about disappearing into the ocean,
but of becoming the ocean”.

(Gibran Khalil Gibran)