

Can *Pongamia pinnata* be an effective phytoremediation tool for tailings in the Copperbelt of Zambia?

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Abstract

Mining in Zambia has left a legacy of degraded land that is unsuitable for most forms of agriculture, and contaminated land surrounds numerous mining towns in the Copperbelt. As mining activities decline, communities are left heavily impacted by the negative environmental and social conditions surrounding them. The challenge is to understand and promote an effective revegetation approach that is economically productive, providing environmental benefits, employment, and diversification. Pongamia pinnata (L.) Pierre, a tree member of the Fabaceae family, has generated interest as a potentially sustainable biofuel feedstock. It produces seeds with high oil content and Pongamia reforestation systems can potentially provide a perennial climate change resilient, drought tolerant, carbon negative alternative to annual oilseed crops for the provision of oil and biomass products capable of providing fuel and food protein products. Additionally, Pongamia can be cultivated in degraded and/or marginal land and, given its phytoremediation potential, it is an ideal candidate for the regeneration of the Copperbelt area. Our study aimed to gain a broader understanding of the potential of P. Pinnata as a phytoremediation tool in the Copperbelt. We have carried out bioassays to assess the toxicity of copper (Cu) in the tailings and measured copper concentrations in both the tailings and the organic amendment around the trees, and in Pongamia roots and leaves growing in a large-scale field trial. Results show high survival rates (98.95%) at relatively high levels of Cu in the tailings (2,997.42 mg.kg⁻¹ on average), with Pongamia trees exhibiting normal development 4 months after being transplanted. Our results suggest that elite Pongamia trees have the potential to be a viable option as a phytoremediation tool in the Copperbelt of Zambia.

Keywords: *Pongamia, phytoremediation, copper, soil*

1 Introduction

Economic growth in Zambia during the past few decades has been largely underpinned by the mining sector. However, it has left a legacy of degraded land that is unsuitable for most forms of agriculture, and contaminated land surrounds numerous mining towns in the Copperbelt (Mwaanga et al. 2019). With declines in mining activities, communities are left jobless and heavily impacted by the negative environmental and social conditions surrounding them. Recently, there have been efforts to reduce environmental health risks through localised interventions while at the same time supporting local income generation (The World Bank 2022). Mining activities have also both directly and indirectly led to extensive deforestation around mining towns (Mwitwa et al. 2012). All of these, combined with efforts to increase access to clean energy by 2030, provide a great opportunity for regeneration strategies combining environmental, social, and economic benefits.

Pongamia pinnata (L.) Pierre (Pongamia) is a tree with a history of use in India and neighbouring countries as a source of traditional medicines, green manure, wood, animal fodder, fuel, biopesticide, and fish poison (Islam et al. 2021). More recently, interest in Pongamia focused on its potential as a biofuel source as its seeds contain around 40% oil (Biswas et al. 2011). Pongamia can be cultivated in marginal land, as it is able to tolerate a broad range of climatic and edaphic conditions. Risk assessment reports done in Zambia concluded that there is no evidence of Pongamia exhibiting invasive behaviour (Warr 2013). Its extensive

network of lateral roots is known for controlling erosion and, due to its large tap roots, it is resistant to drought and can potentially reduce competition for water with other crops as it is able to remove water from deeper soil layers (Kesari et al. 2013). It is also a legume so it can fix nitrogen symbiotically, therefore reducing the need for synthetic nitrogen fertiliser (Gresshoff et al. 2015). *Pongamia* can also contribute to climate change mitigation through CO₂ sequestration. The sequestration potential of *Pongamia* during the 10 to 15 years of its growth has been found to be many times that of several other tree species (Prasad 2021). Additionally, a growing body of evidence supports the potential of *Pongamia* as an effective phytoremediation tool, as it can selectively uptake and remove heavy metals (copper, chromium and nickel) from polluted soils (Prasad 2019; Shirbhate & Malode 2004). However, gaps in knowledge on the levels of tolerance to different contaminants, interactions with symbionts, impact of contaminants on tree survival and yields, and the mechanisms underpinning these, still remain especially in a field context where very little evidence exists (Degani et al. 2022).

Mining tailings are associated with multiple environmental challenges, and if not managed properly, they can result in severe environmental pollution. Low pH conditions in tailings can increase copper (and other heavy metals) mobility in the soil, which can result in contamination of water sources and potentially endanger human health and ecosystems (Gitari et al. 2018). Copper concentration in tailings varies considerably around the world and studies suggest values from 455 to 9,979 mg.kg⁻¹ (Loredo Portales et al. 2015). Edaphic factors play a vital role in plant growth, especially in degraded land where interactions between plants and mine soils are context dependent. Therefore, given its resilience to harsh edaphic conditions, testing the potential of *Pongamia* to regenerate mining tailings using a large-scale field trial is vital to inform effective regeneration strategies.

The challenge is to understand and promote an effective revegetation approach using *Pongamia* that combines environmental, social, and economic benefits. The use of crops with high bioenergy potential for the phytoremediation of contaminated soils can potentially provide these. Additionally, increasing scientific evidence backing the potential of *Pongamia* as a phytoremediation tool may increase consensus across multiple stakeholders, leading to further investments and government support required to transform the lives of communities in the Copperbelt. Our study aims to assess the edaphic conditions and levels of phytotoxicity in an experimental tailing rehabilitation in order to gain a broader understanding of the potential of *Pongamia* as a phytoremediation tool in the Copperbelt of Zambia.

2 Methodology

2.1 Study site

The experimental field is in Chingola, Zambia, on a Konkola Copper Mine (KCM) Plc mine facility. The facility is a floor-washed tailings dam. The tailings dam received crushed and processed materials from the mine beneficiation facility, following acid leaching, to liberate copper and cobalt from the ores. Materials accumulated at the 165 ha site for over a decade, prior to floor washing, which involved washing tailings with a high-pressure hose to a collection point, where they are then pumped as a sludge for reprocessing. This process did not remove all the tailings and a layer extending variably some 1 to 4 meters in depths remains at the site. Entirely dominating the town of Chingola, KCM covers an area of 10,445 ha. This comprises an open pit (1,100 ha), tailing storage facility (1,300 ha) and also a large unexploited area of land (approx. 6,000 ha). KCM has followed standard practice for mining companies in Zambia. They initially obtain a large exploration licence. Once exploration is completed and a resource is defined, they relinquish much of the superficies of the original exploration licence and convert this to a mining licence.

The trial is laid out in a 4 ha field (set up on the abandoned Tailings Dam 2) with a total of 2,007 elite variety trees (625 trees.ha⁻¹), which were transplanted between December 2016 and January 2017, when they were ~1 year old, to copper tailings with a layer of organic amendment consisting of organic matter, sawdust, and chicken manure mixed in with the tailings, and applied to a 1 m² area around the trees. There are 36 different

elite *Pongamia* varieties randomly spaced across the field. Figure 1 shows the field site before planting (a) and two years after planting with elite *Pongamia* trees (b).



Figure 1 (a) Abandoned Chingola Tailings Storage facility before planting; (b) Chingola Tailings Storage facility two years after planting with elite *Pongamia pinnata*

2.2 Experimental design

2.2.1 Bio-toxicity assay of tailings

Seeds of oilseed rape (*Brassica napus*), rye grass (*Lolium perenne*), and onion (*Allium cepa*) were used in a bio-toxicity assay where garden soil and tailings from the experimental field were mixed with increasing % of garden soil to tailings (0%, 1%, 5%, 10%, 50%, and 100%). Three seeds per species were planted in pots containing the different soil/tailing medium and these were replicated three times in a 3 × 6 × 3 factorial design. The mixed growing medium was analysed for (i) organic carbon (Walkley–Black method) and (ii) pH (KCL). Seedlings were assessed for (i) % germination after 17 days, (ii) shoot length, (iii) root length, (iv) dry shoot weight, and (v) dry root weight.

2.2.2 Early tree performance

In April 2017, all *Pongamia* trees were visually assessed for (i) % survival rate, (ii) presence of fully formed flowers, (iii) presence of both young and mature pods, (iv) presence of insect damage, and (v) whether trees were stunted (height <0.5 m or stem diameter <0.1 m).

2.2.3 Soil and plant analyses

Soil and plant samples were taken in April 2021. The experimental plot was divided into three blocks: (i) default tailings, which consist of non-aggregated sandy material; (ii) termite mound, which consists of a raised area with a large termite mound; and (iii) accumulating area where water and lime tend to accumulate. Soil samples were taken from the 1 m² soil pits around the trees at 3 depths (0–300 mm, 300–600 mm, 600–1,000 mm) × 3 replicates per block. Tailings between the trees were sampled at 1 depth (0–300 mm) × 3 replicates per block. Soil samples were analysed for copper concentration (Cu). Root and leaf samples were collected and analysed for copper (Cu), Zinc (Zn), Iron (Fe), and Manganese (Mn). Three replicates per block were collected for root and leaf analyses.

2.3 Statistical analyses

For the seed germination rates analyses, there were no significant differences to the common species responses to the different mixtures of garden soil and tailings in the medium, therefore all species were grouped together for analyses. One-way analysis of variance (ANOVA) was used to test the effect of the

different percentages of garden soil in the medium on the proportion of germinated seeds. Species were also grouped together, and two-way ANOVA (day and treatment) was used to test the effect of the different percentages of garden soil to tailings, on different days, in the medium, on shoot and root length, and dry shoot and root weight. Linear mixed models were used to test the effect of tailings and soil pit on Cu concentration in the soil. Linear mixed models were also used to detect differences in metal concentration (Cu, Fe, Zn, and Mn) in roots and leaf. Tukey’s post hoc test was used for pairwise comparisons. Block was included in all models as a random effect. All models were visually checked for homoscedasticity and normality of residuals and were fitted in R version 3.5.2 (R Core Team 2020).

3 Results

3.1 Bio-toxicity assay

3.1.1 Soil parameters

Results for the soil parameters measured in the different treatments (increased % of garden soil to tailings) are shown in Table 1. Both % of organic carbon and soil pH increased with increases in the % of soil garden in the medium.

Table 1 Summary of soil parameters (organic carbon, pH, and EC) measured on medium containing increasing percentage of garden soil to tailings

% of garden soil in the medium						
	0	1	5	10	50	100
Organic carbon (%)	0.1	0.1	0.4	0.16	0.54	1.02
Soil pH	4.4	4.9	5.4	5.6	5.8	5.9

3.1.2 Seed germination rates

There were no significant differences in seed germination rates among the different soil treatments on day 17 after sowing ($p = 0.68$). All treatments had a mean of >80% germination for all species grouped together (Figure 2).

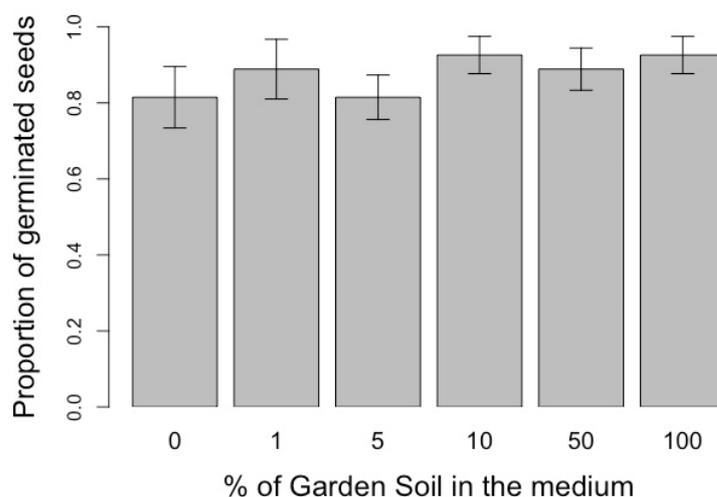
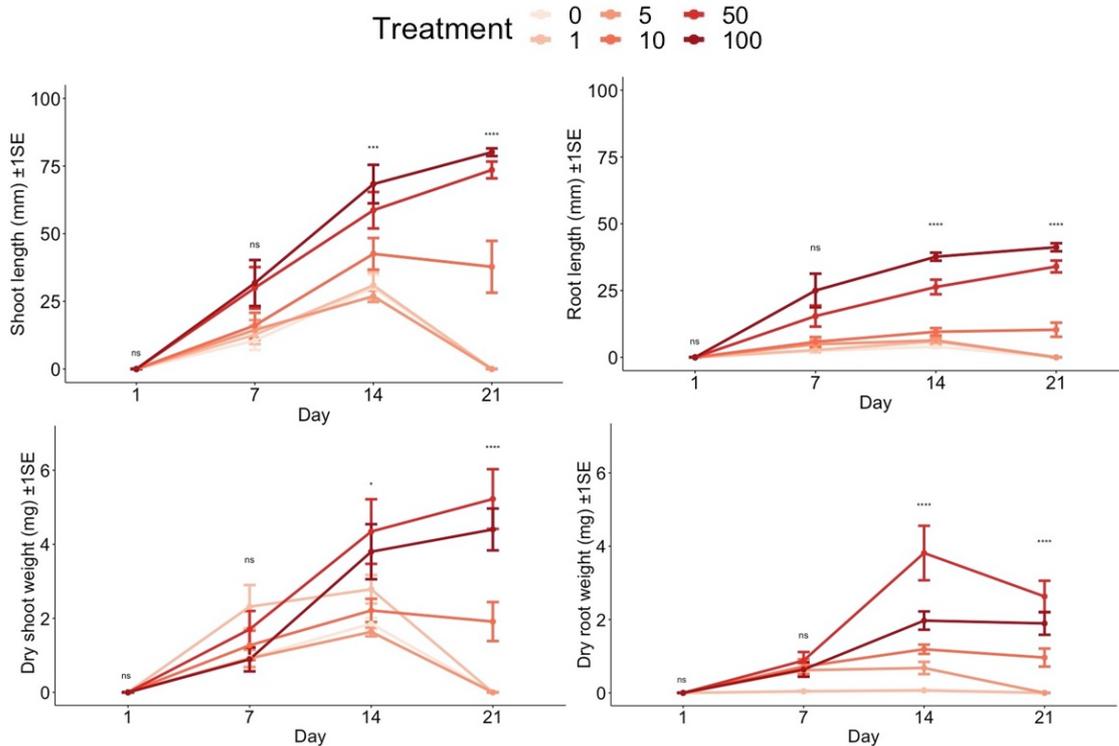


Figure 2 Mean proportion of germinated seeds (± 1 SE) 17 days after sowing for all species (onion, oilseed rape and rye grass) in soil medium with increasing % of garden soil to tailings

3.1.3 Plant parameters

All seedlings in the medium containing no garden soil (0%), 1% and 5% died by day 21. Shoot length of the germinated seedlings in the 0%, 1%, and 5% medium increased until day 14 but were significantly lower than the other treatments. Shoot length of seedlings in the 10% medium increased until day 14, with no further increases by day 21. Shoot length in the 50% and 100% medium increased until day 21 and was significantly higher than in the other treatments. On day 21, shoot length of seedlings in the medium containing 100% garden soil was on average 6.56 mm longer than in the 50% medium and 42.35 mm longer than in the 10% medium (Figure 3a). Root length in the 0%, 1%, 5% and 10% medium increased until day 14 but were significantly lower than in the 50% and 100% medium. No further increases were recorded on the 10% medium. In the 50% and 100% medium, root length increased until day 21, with roots in the 100% medium being 7.22 mm higher than in the 50% medium and 30.89 mm higher than in the 10% treatment (Figure 3b). Dry shoot weight increased until day 14 on all treatments, with seedlings in the 10% medium having no further increases between day 14 and day 21. There were no significant differences between 50% and 100% on day 21. Dry root weight in the 50% medium weighed 0.82 mg more than the 100% medium and 3.31 mg more than in the 10% medium (Figure 3c). Dry root weight in the 0%, 1% and 5% medium increased only to 0.08 mg, 0.06 mg, and 0.68 mg respectively by day 14. By day 21, dry root weight in the 50% treatment was 0.73 mg higher than in the 100% medium, but this difference was not significant, and 1.67 mg higher than in the 10% medium (Figure 3d).



ns=non-significant, *** = p<0.001, **** = p<0.0001

Figure 3 (a) Mean shoot length; (b) Root length; (c) Dry shoot weight; (d) Dry root weight of seedlings planted in 0%, 1%, 5%, 10%, 50%, and 100% garden soil to tailings medium over 21 days

3.2 Early tree performance

From the 2007 trees assessed, 1,986 1.5-year-old trees were alive 4 months after being transplanted to the tailings. Out of the 1,986 live trees, 1,677 contained new shoots, 93 had fully formed flowers, 11 had both young and mature pods present, 3 presented insect damage and 208 were considered stunted (Table 2).

Table 2 Results from tree visual assessment 4 months after being transplanted to the field

	Number of trees	Survival rate (%)	New shoots (%)	Flowering (%)	Bearing pods (%)	Insect damage (%)	Stunted (%)
Alive	1,986	98.95	84.45	4.68	0.55	0.15	10.47
Dead	21	–	–	–	–	–	–

3.3 Soil and plant analyses

3.3.1 *Cu concentration in the soil*

Copper concentration was relatively high in both the tailings and the soil pit. The *p* value for the difference between tailing and soil pits across all depths was marginal, $\chi^2(1) = 3.72$, $p = 0.053$, with the mean Cu concentration in the tailings being 974.45 mg.kg⁻¹ higher than in the pits and the Cu concentration in the tailings being significantly higher than in the deep layer (60–100 cm) in the pits ($p = 0.02$) by 1,412.6 mg.kg⁻¹ (Figure 4).

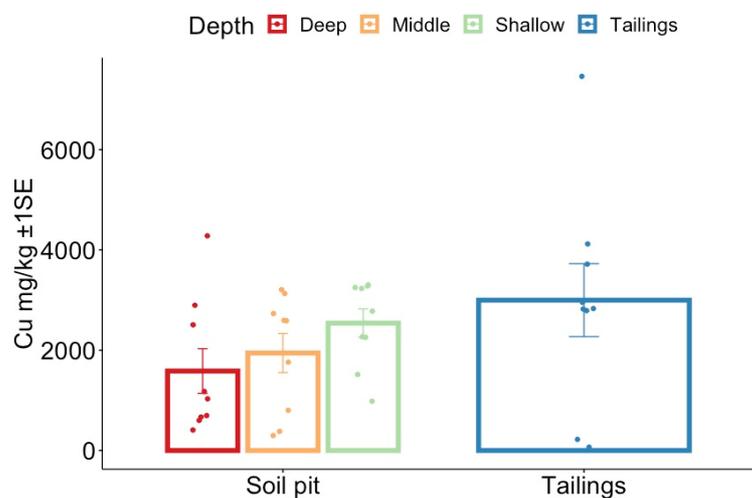


Figure 4 Cu concentration (mg.kg⁻¹) in soil pits with organic amendment at different depths (shallow = 0–30 cm, medium = 30–50 cm and deep = 60–100 cm) and tailings (0–30 cm)

3.3.2 *Cu, Zn, Fe, and Mn concentration in plant parts (root and leaf)*

There were no differences in Cu concentration between leaf and root, $\chi^2(1) = 0.31$, $p = 0.57$, with the mean Cu concentration in leaves being 303.77 mg.kg⁻¹ (± 40.04 SE), and in roots, 264.37 mg.kg⁻¹ (± 77.5 SE) (Figure 5a). There were no differences in Zn concentration between leaf and root, $\chi^2(1) = 0.52$, $p = 0.46$, with the mean Zn concentration in leaves being 28.65 mg.kg⁻¹ (± 1.94 SE), and in roots, 35.09 mg.kg⁻¹ (± 9.52 SE) (Figure 5b). There were no differences in Fe concentration between leaf and root, $\chi^2(1) = 0.38$, $p = 0.53$, with the mean Fe concentration in leaves being 114.98 mg.kg⁻¹ (± 14.1 SE), and in roots, 136.98 mg.kg⁻¹ (± 35.25 SE) (Figure 5c). Mn concentration was higher in leaves, $\chi^2(1) = 13.07$, $p < 0.001$, with the mean Mn concentration in leaves being 130.55 mg.kg⁻¹ (± 23.55 SE), and in roots, 43.03 mg.kg⁻¹ (± 11.03 SE) (Figure 5d).

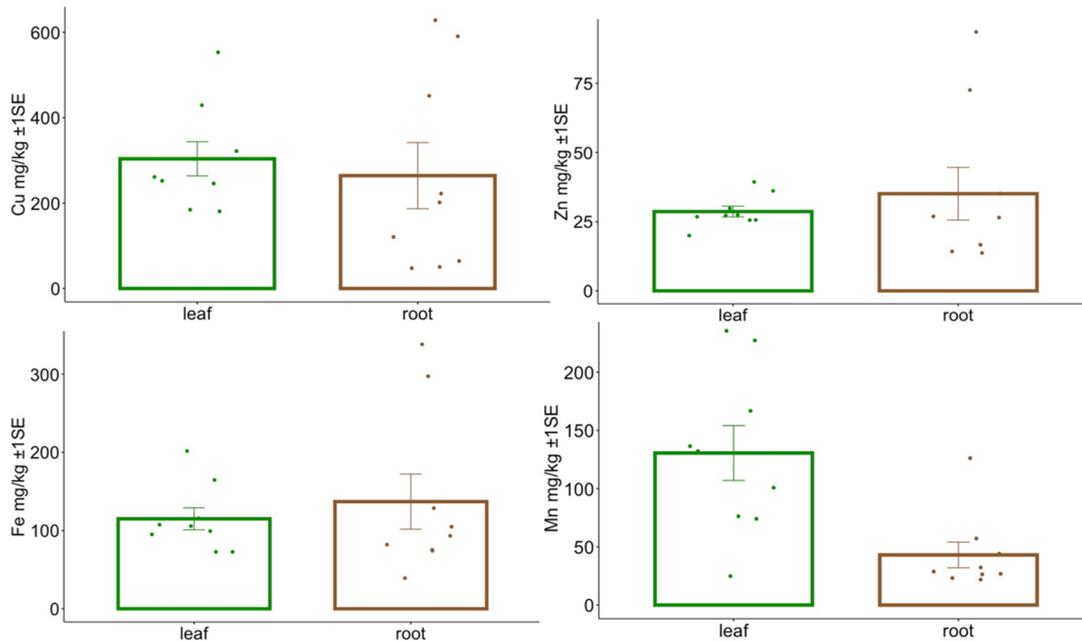


Figure 5 (a) Cu concentration (mg.kg^{-1}); (b) Zn concentration (mg.kg^{-1}); (c) Fe concentration (mg.kg^{-1}); (d) Mn concentration (mg.kg^{-1}) in roots and leaves of elite *Pongamia pinnata* in copper tailings

4 Discussion

4.1 Bio-toxicity assay

Results from the soil parameters assessed after the tailings and garden soil mixing process show that the protocol was effective and that the toxicity results are proportionate. Although germination was not affected by tailings at any level, with high levels of germination across all treatments, the tailings were shown to eventually be highly toxic to plant growth. Contrasting levels of Cu tolerance have been shown for the species used here (oilseed rape, rye grass, and onion) (De Conti et al. 2020; Geremias et al. 2010; Ivanova et al. 2010). In our experiment, all seedlings in treatments with 0%, 1%, and 5% garden soil were dead by day 21. Although plants in treatments with 10% garden soil survived, root and shoot length, as well as dry weight, were significantly inhibited in comparison with treatments with a higher proportion of garden soil, suggesting that despite high survival rates, high proportion of tailings in the medium had a significant impact on plant development. Interestingly, on day 21, there were no significant differences between 50% and 100% proportion of garden soil to tailings on any of the parameters measured here, suggesting that 50% mixture is sufficient, at least during the early growth stages. However, the long-term impact on the plants remains unknown and would need to be tested. The lethal effect of undiluted and weakly diluted tailings shows that, for most plants, persistence in substrates with Cu concentration of $\sim 3,000 \text{ mg.kg}^{-1}$ is unlikely without specific adaptations or tolerances.

4.2 Early tree performance

One-year-old elite *Pongamia pinnata* trees transplanted to copper mining tailings with a layer of organic amendment had survival rates of 98.95%, with a high proportion of trees (84.45%) displaying new shoots. Although flower and pod production were low, 4.68% and 0.55% respectively, this is expected as full flowering events are not expected until the trees reach 4 to 5 years, with precocious flowering in 2-year-old trees being observed in 6% of trees in one trial in Australia (Murphy et al. 2012). Although 10.47% of trees were considered stunted due to a drop in pH at the nursery stage, it is possible that the stunting might have occurred as a result of the conditions before they were transplanted to the field. Since these trees affected by the lowered pH were not tagged before transplanting, it is not possible to disentangle the potential effect of copper in the tailings and the lowered pH on the stunted trees. However, high survival rates at high copper

concentration in both the soil pit and tailings, with 2,022.97 (\pm 223.97 SE) mean Cu concentration in the soil pits and 2,997.42 (\pm 727.30 SE) in the tailings, suggest high levels of tolerance to Cu in the soil in elite *P. pinnata* trees. This is in line with previous studies that have demonstrated that *P. pinnata* is able to tolerate varying levels of Cu (Kumar et al. 2009; Shirbhate & Malode 2004; Tulod et al. 2012). However, with the exception of Tulod et al. (2012), which also included a commercial arbuscular mycorrhiza inoculum, all other studies were carried out in vitro or using young seedlings with much lower Cu concentrations. Therefore, to our knowledge, the current study is the first to demonstrate *P. pinnata* tolerance to Cu in soils, in older trees, in a large-scale field trial.

4.3 Soil and plant analyses

Cu concentration was 974.45 mg.kg⁻¹ higher in the tailings when compared to the mean Cu concentration across all depths in the soil pits. However, Cu concentration in the soil pits was still high with a mean across all depths of 2,022.97 mg.kg⁻¹, which suggests a high level of Cu leaching from the tailings to the soil pits indicating that the Cu in the soil is quite mobile. Visual observations of shallow roots (not included here), indicate that the roots have not extended beyond the soil pits into the tailings. Given that the levels of Cu in the pits are still high, it suggests that factors other than Cu levels are preventing the roots from expanding beyond the pit. High salinity levels in the tailings or water and nutrient supply could potentially be preventing the roots from expanding into the tailings, but more tests need to be done before we can come to a more definitive conclusion. There were no significant differences in Cu concentration between the leaves and the roots, indicating that *Pongamia* can potentially act as a hyperaccumulator. However, translocation and bioaccumulation factors have not been calculated. There were also no differences in the amount of Fe and Zn in the leaves and roots, with Mn being significantly higher in the roots.

5 Conclusion

To our knowledge, our study is the first to test the potential of *Pongamia pinnata* to tolerate high levels of Cu in a field trial. Our results show that despite high levels of germination rates, the lethal effect of undiluted and weakly diluted tailings shows that, for most plants, growth in substrates with these levels of Cu concentration is unlikely without specific adaptations or tolerances. Despite high levels of Cu leaching from the tailings to the soil pits, 1-year-old *Pongamia* trees transplanted to the soil pits had a 98.95% survival rate and exhibited normal development 4 months after being transplanted. However, more tests are necessary to establish the potential impact of the tailings on flowering, pod formation, and yield. The fact that shallow roots have not expanded beyond the soil pits despite high levels of Cu indicate that factors other than Cu concentration are inhibiting root growth into the tailings, but further analyses are necessary to establish what these are. However, our results suggest that elite *Pongamia* trees have the potential to be a viable option as a phytoremediation tool in the Copperbelt of Zambia.

Acknowledgement

This work was supported by the UKRI Global Challenges Research Fund grant, 'The development of networks and science to transform degraded land into a productive economic asset using *Pongamia pinnata* as a tool to engage stakeholders and remediate wasteland' and the Global Research and Innovation Programme grant, 'UK-India-Zambia Collaboration on *Millettia* (formerly *Pongamia*) *pinnata* as a tool to remediate contaminated land in the tropics'.

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