

Research Article

Effect of arbuscular mycorrhizal fungi on nutrients and heavy metals uptake by *Pennisetum purpureum* cv Mott in phytoremediation of gold mine tailings

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Abstract

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Mercury composite and cyanidation are gold mining methods that are frequently used. The mercury composite method produces tailings containing heavy metals that can harm living organisms. Utilization of tailings for the development of forage may be enhanced through arbuscular mycorrhizal fungi (AMF) inoculation to increase plant resistance, absorption of macro and micronutrients, and reduce levels of metal contaminants in the tailings. This study aimed to investigate the effect of arbuscular mycorrhizal fungi on nutrients and heavy metals uptake by *Pennisetum purpureum* cv Mott in the phytoremediation of gold mine tailings. Treatments consisted of four levels of AMF inoculation (0, 5, 10 and 15 g pot⁻¹) were arranged in a completely randomized design with five replications. Each pot contained 3 kg of tailings. The results showed that the best crude protein, crude fiber, crude fat, Ca, and P contents in the plant shoots was obtained by providing AMF inoculation of 15 g pot⁻¹. However, the treatment had no significant effect on dry weight, dry matter, and nitrogen-free extract. AMF significantly increased the uptake of heavy metals by the plant roots. The treatments did not significantly affect Pb uptake by plant roots and shoots and Hg uptake by plant shoots. AMF treatments significantly reduced the translocation factor (TF) value for Hg, bioconcentration factor (BCF) values for Cd and Pb, and removal efficiency (RE) values for Cd and Pb. AMF could effectively increase nutrient absorption in the plant shoots, reduce Cd, Hg, and Pb translocation in plant shoots, and reduce Cd, Hg, and Pb in the tailings.

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Introduction

Heavy metal pollution from ex-gold mines is hazardous for animals, plants, and humans (Wahl et al., 2010; Bae et al., 2011) and damages the ecosystem (Bundschuh et al., 2013). The results of a study conducted by Fashola et al. (2016) showed that the negative impact of mining is the contamination of soil and water with heavy metal poisoning. Community

mining activities are examined from an environmental point of view; they have the potential to cause severe deforestation, land degradation, and damage to forest ecosystems (Jaelani et al., 2018).

The use of *Pennisetum purpureum* cv Mott. associated with arbuscular mycorrhizal is an environmentally friendly phytoremediation solution that has the potential to reduce toxic activity due to

heavy metals, reduce heavy metals in soil, and be cost-effective (Danh et al., 2014; Wei et al., 2021), increase the growth of host plants and inhibit the translocation of heavy metals in shoots (Sharma et al., 2016; Chen et al., 2018). Boonmeerati and Sampanpanish (2021) reported that *P. purpureum* was able to increase the phytoextraction of arsenic (As) through the application of phosphate and EDTA. In addition to acting as a phytoremediation agent, *P. purpureum* is a superior grass that has a high nutrient content which is very suitable as forage for ruminants. AMF application for forage cultivation on polluted land is the right step to take (Joner et al., 2004; Ma et al., 2019; Wu et al., 2019).

Arbuscular mycorrhizal fungi can effectively interact with the roots of plants grown on polluted soil. Roots on soil polluted with heavy metals are able to interact well with arbuscular mycorrhizal fungi. Mycorrhizal symbiosis is also able to play a role in protecting roots from toxic heavy metals. The ability of mycorrhizal associations to moderate metal toxicity has been demonstrated in ericoid mycorrhizae (Tian et al., 2011), ectomycorrhizae (Colpaert et al., 2011) and arbuscular mycorrhizae (Leung et al., 2010). The native mycorrhizae that survive in a polluted environment can deposit metal oxalate in the intracellular space of the fungus or host plant so as to inhibit or limit the transport of heavy metal ions (Leyval et al., 1997). Arbuscular mycorrhizal symbiosis has a role in the phytoremediation of polluted soils (Dhalaria et al., 2020). Arbuscular mycorrhizae also reduce plant stress on polluted soils, improve soil structure, protect roots from plant

pathogens and interact with other soil microbes (Gupta and Abbott, 2021).

Numerous studies that have been conducted showed the effective role of arbuscular mycorrhizal fungi in host plants grown in soils contaminated with heavy metals. *Brachiaria mutica* inoculated with AMF is able to play a role as a phytoremediation agent in chromium contaminated (Kullu et al., 2020), and AMF is able to suppress Zn uptake in host plants in Zn polluted (Christie et al., 2004), AMF is able to reduce Al poisoning in plants in aluminium polluted (Alotaibi et al., 2021), AMF reduces Cd poisoning in plants in cadmium polluted (Liu et al., 2020), AMF inoculation on tailings can increase legume productivity (Husna et al., 2021b). The majority of previous researchers used wild plants with soil conditions polluted with one part of heavy metal. As we know that tailings are heavy metals consisting of Cd, Hg, Pb, and chromium (Putra et al., 2022). This study aimed to elucidate the effect of arbuscular mycorrhizal fungi (AMF) on nutrients and heavy metals uptake by *P. purpureum* in phytoremediation of gold mine tailings.

Materials and Methods

Research location

This study was conducted in the experimental field of Muara Bungo University, Jambi. Gold mine tailings were obtained from Rantau Pandan District, Bungo Regency, Jambi Province, with a position between 1.641450 latitudes and 101.990170 east longitude (Figure 1).



Figure 1. Location of samplings in Rantau Padan District, Bungo Regency, Jambi Province.

Treatments and experimental design

The tailings used in this study were obtained from a gold mining area in Rantau Pandan District. The physical and chemical properties of the tailings were as follows; Cd = 0.173 mg kg⁻¹, Hg = 0.635 mg kg⁻¹, Cr = 0.8 mg kg⁻¹, Fe = 1.16%, Al₂O₃ = 5.09%, water = 34.2%, pH 4.90, N = 0.141%, P = 3.14%, and K = 0.052% (Putra et al., 2022). After removing the woods, stones, and unnecessary materials, 3 kg of tailings for each treatment was mixed with vermicompost in a ratio of 6:1 and placed in a polybag pot measuring

15 × 20 cm. Four seedlings of *Pennisetum purpureum* cv Mott inoculated with arbuscular mycorrhizal fungi (AMF) were then planted in the pot containing tailings. The AMF inoculum used in this study was the *Glomus manihotis* type obtained from the Soil Science Laboratory, Faculty of Agriculture, Andalas University. Treatments tested were the amount of AMF inoculated to the tailings, i.e., 0, 5, 10, and 15 g pot⁻¹. The four treatments were arranged in a completely randomized design with five replications. Each pot received 5 g NPK basal fertilizer. The plant was grown for 2 months and kept free of weeds and

insect pathogens. The lighting conditions during the experiment were relatively normal for 12 hours, with an average temperature ranging from 27 to 30 °C.

Laboratory analysis

At the end of the second month, the branches were cut and separated from shoots and roots for nutrients and heavy metals analysis. Analysis of ash crude fiber (CF), crude protein (CP), crude fat or ether extract (EE), nitrogen-free extract (NEE), Ca, and P contents of the roots and shoots were performed at the Ruminant Nutrition Laboratory, Department of Animal Husbandry, University of Andalas. Ash, crude protein (CP), crude fiber (CF), crude fat or ether extract (EE) contents were determined according to the standard method of AOACH (2005), and NFE content was determined using the National Research Council formula (NRC, 2005). Concentrations of P and Ca were measured using Atomic Absorption Spectroscopy (AAS) through the digestion phase (Kord et al., 2010). Analysis of heavy metals (Cd, Hg and Pb) was performed at the Soil Laboratory of the Faculty of Agriculture, University of Andalas.

Calculation of removal efficiency of Cd, Hg, and Pb

The ability of AMF to reduce heavy metals (Cd, Hg, and Pb) concentrations in tailings and increase concentrations of heavy metal concentrations in roots of *P. purpureum*, i.e. the removal potential for the heavy metal concentration was calculated by the following equation (Kumar et al., 2020).

$$\text{Removal Efficiency} = \frac{C_0 - C_1}{C_0} \times 100\%$$

where:

- C₀ = initial concentrations of Cd, Hg and Pb
- C₁ = final concentration of Cd, Hg and Pb

Calculation of bioconcentration factor

The amount of heavy metal concentration absorbed by *P. purpureum* grown on the tailings is determined by the bioconcentration factor (BCF). BCF is a parameter that indicates the level of success as a phytoremediation agent; the higher the BCF value, the higher the ability to reduce heavy metals in tailings (Ndimele and Jimoh, 2011). The relationship between heavy metal concentrations absorbed by *P. purpureum* and heavy metals in tailings soil was calculated by the equation according to the following equation (Wu et al., 2011).

$$\text{BCF} = \frac{\text{Cd, Hg, and Pb in plants (ppm)}}{\text{Cd, Hg, and Pb in tailings (ppm)}}$$

Calculation of translocation factor

The TF value that determines the success rate of transferring heavy metals from roots to stems and leaves (Arnot and Gobas, 2006) was calculated to assess the potency of *P. purpureum* as a

phytoremediation agent. Plants can be called less capable of translocating heavy metals from root to shoot if the TF value < 1. Meanwhile, if TF > 1, plants are able to translocate heavy metals from roots to stems and leaves (Mellem, 2012). The TF value was calculated using a formula adopted from Patra et al. (2020) as follows,

$$\text{TCF} = \frac{\text{Cd, Hg, and Pb in shoots (ppm)}}{\text{Cd, Hg, and Pb in roots (ppm)}}$$

Data analysis

Data obtained were subjected to a one-way analysis of variance (ANOVA), followed by the test of the most significant difference (Fisher LSD) to determine the effect of AMF on nutrient content, Cd, Hg, Pb uptake, the percentage reduction in Cd, Hg, Pb concentrations in tailings, and BCF and TF values in roots and shoots of *P. purpureum*.

Results and Discussion

Effect of arbuscular mycorrhizal fungi on nutrients

Mycorrhizal fungi played an important role in increasing the nutritional residue *P. purpureum* grown on gold mine tailings. Table 1 shows that arbuscular mycorrhizal fungi inoculation up to 15 g pot⁻¹ had no significant effect (p > 0.05) on dry weight (DW), dry matter (DM), and nitrogen-free extract material (NFE). Arbuscular mycorrhizal fungi yielded significant changes in crude protein (p < 0.01), crude fiber (p < 0.01), crude fat (p < 0.05), calcium (p < 0.01), and phosphorus (p < 0.01).

Arbuscular mycorrhizal fungi inoculation significantly increased crude protein in *P. purpureum* grown on the tailings. AMF inoculation of 15 g pot⁻¹ had the highest crude protein content compared to other treatments (p < 0.01). Data presented in Table 1 show that the higher the number of AMF inoculations on *P. purpureum*, the higher the crude protein yielded. The lowest crude protein content was found in the control and 5 g AMF pot⁻¹ treatments. The high content of crude protein in the 15 g AMF pot⁻¹ treatment was due to the high nitrogen uptake in the plant roots associated with AMF. These results demonstrated that AMF plays an important role in increasing plant nitrogen by increasing the activity of nitrogen-absorbing enzymes such as glutamine synthase. The increasing nitrogen uptake by *P. purpureum* in this experiment led to an increase in crude protein and amino acid contents in the plants. The results of this study were in line with the results of research conducted by Husna et al. (2021a) that AMF significantly increased nitrogen uptake by *P. indicus* and *P. mooniana* planted in ex-gold mine areas. The high crude protein content was due to the association of *P. purpureum* roots with AMF. Arbuscular mycorrhizal fungi can secrete peptidase and protease enzymes into the soil to absorb nitrogen-containing

monomers. This is in line with Bhantana et al. (2021), who showed that arbuscular mycorrhizal fungi play an important role in increasing macronutrient uptake, especially nitrogen and phosphorus. Arbuscular mycorrhizal fungi played a role in the reduction of crude fiber in *P. purpureum*. Table 1 shows that the lowest amount of crude fiber (27.69%) was found in the 10 g AMF pot⁻¹ treatment, but it was not significantly different from the 15 g AMF pot⁻¹ treatment of 27.17%. The highest crude fiber content (30.98%) was found in the 5 g AMF pot⁻¹ treatment, but it was not significantly different from that without

AMF inoculation, with an amount of 29.99%. It is suspected that inoculation of 10 g AMF pot⁻¹ and 15 g AMF pot⁻¹ improved plant growth, such as an increase in leaf number, leaf width, and leaf area, so crude fiber content decreased (Putra et al., 2022). Table 1 shows that the higher the inoculation of arbuscular mycorrhizal fungi, the lower the crude fiber content in the plant shoots. The optimal role of arbuscular mycorrhizal fungi is the formation of crude fiber in roots. Basyal and Emery (2021) reported that arbuscular mycorrhizal fungi were effective in increasing crude fiber in roots, but not in shoots.

Table 1. Effect of arbuscular mycorrhizal fungi (AMF) on biomass and nutrient content of *P. purpureum* grown on gold mine tailings.

Treatments	Average							
	DW%	DM%	CP%	C. Fibre%	C. Fat%	NFE%	Ca%	P%
AMF (0)	95	15.20	7.31 a	29.99 a	1.36 a	50.52	0.42 a	0.40 a
AMF (5)	95	13.69	6.65 a	30.98 a	1.06 b	49.54	0.41 a	0.34 b
AMF (10)	96	14.08	8.94 b	27.69 b	1.39 a	49.47	0.30 b	0.31 b
AMF (15)	94	14.13	11.94 c	27.17 b	1.44 a	46.43	0.49 c	0.46 c
p-value	p>0.05 ^{ns}	p>0.05 ^{ns}	p<0.01 ^{**}	p<0.01 ^{**}	p<0.05 [*]	p>0.05 ^{ns}	p<0.01 ^{**}	p<0.01 ^{**}

Remarks: DW = dry weight, DM = dry matter, C.Fiber = Crude fiber, CP = Crude protein, C.fat E = crude fat, NFE = nitrogen-free extract, Ca = calcium, P = phosphorus, ** values are significantly different at p<0.01 (CP, C.Fiber, Ca, and P), * values are significantly different at p<0.05 (C.Fat/EE), ^{ns} not significantly different at p>0.05 (DW, DM, and NFE), Numbers followed by the same letters in the same column show no significant difference.

The results of this study are the same as those of research conducted by Atteya et al. (2021), that arbuscular mycorrhizal fungi could increase crude protein and decrease the amount of crude fiber of host plants. However, the results of this study differed from those of Fiorilli et al. (2009), that mycorrhizal fungi could produce glycosyltransferases (GTs) enzymes that played a role in metabolic processes, especially the formation of cellulose and hemicellulose in plants. In this study, the activity of cellulose formation was thought to be only in the roots and was seen in the shoots because of the young plant age. The increase in the number of arbuscular mycorrhizal fungi inoculation did not show a significant difference compared to that without inoculation of arbuscular mycorrhizal fungi in crude fat. It is presumed that the *P. purpureum* in this study was still young, so the changes in crude fat had not been seen.

Arbuscular mycorrhizal fungi inoculation had no significant effect on nitrogen-free extract (NFE). The NFE value in this study ranged from 46.43% to 50.52%. In this study, it was suspected that AMF did not affect NFE due to the relatively young age of the plant. This study reported significant differences in the content of Ca and P. The Ca values ranged from 0.30% to 0.49%, while P ranged from 0.31% to 0.46%. The application of 15 g AMF pot⁻¹ yielded the highest Ca and P concentrations that were not significantly different from those without FMA. This proved that arbuscular mycorrhizal fungi could increase Ca and P uptake by *P. purpureum*. The results of this study are in line with those of research conducted by Atteya et

al. (2021) on “jojoba” plants and by Zhang et al. (2019) and Rezaeian et al. (2020) on alfalfa plants. Fungal hyphae beyond the nutrient depletion area around plant roots increased the surface area available for nutrient absorption that was spread into soil pores that were too small for plant roots to enter and could access N and P elements that were inaccessible to non-plants-mycorrhizae (Morgan et al., 2005).

Phosphorus (P) is an important element in plant productivity and development (Chen et al., 2019). Phosphorus in the soil is generally immobile and unavailable to plant roots (Holford, 1997). Plant roots absorb P around the roots, but as it dominates the root area, P becomes thinner, and plant roots widen to extract more P from the Pi zone. However, the extent of plant root elongation is quite limited. AMF plays a role in root expansion so that more phosphorus can be achieved. In addition, fungal hyphae also hydrolyze organophosphate substances. Intracellular hyphae of arbuscular mycorrhizal fungi penetrate the root epidermis and cortical host cells, which then form a special structure, namely the arbuscular, to exchange nutrients with the host (Bonfante and Genre, 2010).

Effect of arbuscular mycorrhizal fungi on heavy metal uptake by plants

The inoculation of arbuscular mycorrhizal fungi did not significantly affect Cd uptake by plant roots and shoots. This made the Cd content in the tailings soil not too high. The total Cd content in the soil also determines the performance of arbuscular mycorrhizae. Arbuscular mycorrhizae can increase or

decrease Cd uptake in plant shoots and regulate Cd accumulation in plant tissues. The application of arbuscular mycorrhizae to sorghum planted on Cd-polluted soil could regulate Cd translocation in the rhizobox and concentrations in plant tissues in the right amount. Most of Cd is stored in plant roots, fungal hyphae, and mycelium, and its toxic effects on plants can be prevented (Brundrett and Tedersoo, 2020).

The inoculation of arbuscular mycorrhizal fungi did not significantly affect Hg uptake by plant shoots. This is in accordance with the statement of Wang and Greger (2004) that mercury (Hg) predominantly accumulates in plant roots and is slightly translocated to shoots. However, the difference was not significant at the roots. The higher the application of arbuscular mycorrhizal fungi, the higher the Hg uptake by the roots. The results of this study indicated that arbuscular mycorrhizal fungi are very suitable for use as phytoremediation agents for heavy metals. Forage

feed cultivated on ex-gold mines inoculated with arbuscular mycorrhizal fungi is an appropriate solution for the development of forage feeds. This is because arbuscular mycorrhizal fungi can inhibit Hg translocation to the crown. The results of this study also showed that arbuscular mycorrhizal fungi had no significant effect on Pb uptake by plant shoots but significantly ($p < 0.01$) on Pb uptake by plant roots. Table 2 shows that the higher the number of arbuscular mycorrhizal fungi inoculated, the higher the accumulation of Pb in plant roots. The symbiotic relationship between mycorrhizae and plant roots can change the chemical composition of root exudates and can alter soil pH, making it possible to increase the bioavailability of heavy metals in the soil. Kim et al. (2010) reported that *Echinochloa crusgalli* could emit citric acid and oxalic acid (natural chelating agents) and increase the translocation and bioaccumulation ability of Pb.

Table 2. Effect of arbuscular mycorrhizal fungi (AMF) on heavy metal uptake by *P. purpureum* grown on gold mine tailings.

Treatments	Cd (ppm)		Hg (ppm)		Pb (ppm)	
	Roots	Shoots	Roots	Shoots	Roots	Shoots
AMF (0)	3.58	2.24	0.91 a	0.71	3.18 a	2.28
AMF (5)	3.99	2.17	1.08 bc	0.67	4.34 bc	2.33
AMF (10)	3.78	2.14	1.18 d	0.58	4.95 b	2.60
AMF (15)	4.31	2.20	1.13 cd	0.64	4.18 c	2.65
p-value	$p > 0.05$ ^{ns}	$p > 0.05$ ^{ns}	$p < 0.01$ ^{**}	$p > 0.05$ ^{ns}	$p < 0.01$ ^{**}	$p > 0.05$ ^{ns}

Remarks: ** values are significantly different, $p < 0.01$ (Hg and Pb in roots), ^{ns} not significantly different, $p > 0.05$ (Cd in roots and shoots, Hg in shoots, and Pb in roots). Numbers followed by the same letters in the same column show no significant difference.

Plants can develop certain mechanisms to dissolve heavy metals in the soil. Plant root secretions produce metal-activating substances in the rhizosphere referred to as phytosiderophores (Lone et al., 2008). The secretion of H^+ ions by plant roots can acidify the rhizosphere and enhance metal dissolution. H^+ ions can also displace heavy metal cations that are absorbed by soil particles (Alford et al., 2010). Root exudates are also capable of lowering the pH of the rhizosphere soil by about one or two units above the bulk soil pH. The low pH value of the soil can enhance the concentration of heavy metals in the soil. Rhizosphere microorganisms such as bacteria and mycorrhizal fungi can enhance the bioavailability of heavy metals in soil (Vamerli et al., 2010; Sheoran et al., 2011).

Effect of arbuscular mycorrhizal fungi on phytoremediation

The translocation factor (TF) was counted to assess the potential of *P. purpureum* as a heavy metal accumulator plant. TF demonstrates the proficiency of plants in transferring metals from roots to stems and leaves (Arnot and Gobas, 2006). Metals accumulated by plants are mostly stored in plant roots. Table 3 shows that the treatments had no significant effect on

the TF values for Cd and Pb, but the treatments were significantly different ($p < 0.05$) on the TF for Hg. The inoculation of 15 g AMF pot^{-1} gave a lower TF value and significantly differed from that without AMF inoculation (Table 1). This indicates that arbuscular mycorrhizal fungi can effectively inhibit Hg translocation in plant shoots, so the plant shoots are suitable for livestock feed.

The inoculation of AMF had no significant effect on the BCF value for Hg, but it had a significant effect on the BCF values for Pb and Cd (Table 3). BCF value is a value that indicates the level of a plant's ability to accumulate heavy metals. The treatments with AMF inoculation gave no significantly different results with no AMF inoculation on the BCF values of Cd and Pb (Table 1). This proves that AMF plays a role in accumulating Cd and Pb in plants, especially in roots. The higher the BCF value, the better it is to be used as a phytoremediation agent. The higher the AMF application, the higher the values of BCF of Cd and Pb. This is probably due to the large accumulation of Cd and Pb in the roots.

The BCF value was obtained from the total amount accumulated in roots and shoots divided by the amount in tailings. The removal efficiency (RE) values

in the AMF treatments were not significantly different for Hg, but significantly different for Cd and Pb. Removal efficiency presents the value of the translocation efficiency of heavy metals in plants. The

higher the RE value, the better the efficiency level for heavy metal translocation. RE values in this study were 3.49%-31.85% for Cd, 9.85%-12.88% for Hg, and 41.59%-55.03% for Pb.

Table 3. Effect of arbuscular mycorrhizal fungi (AMF) on phytoremediation rate of gold mine tailing by *P. purpureum*.

Treatments	TF			BCF			RE		
	Cd	Hg	Pb	Cd	Hg	Pb	Cd	Hg	Pb
AMF (0 g pot ⁻¹)	0.626	0.782 a	0.746	2.32 a	2.77	2.02 a	3.49 a	11.62	41.59 a
AMF (5 g pot ⁻¹)	0.546	0.627 b	0.535	2.62 b	2.89	2.44 b	9.68 b	9.85	40.95 a
AMF (10 g pot ⁻¹)	0.567	0.488 b	0.541	2.78 b	2.94	3.13 c	18.15 c	12.88	47.92 b
AMF (15g pot ⁻¹)	0.516	0.565 b	0.637	3.61 c	2.99	3.29 c	31.85 d	12.88	55.03 c
p-value	p>0.05	p<0.05	p>0.05	p<0.01	p>0.05	p<0.01	p<0.01	p>0.05	p<0.01

Remarks: TF: translocation factor, BCF: bioconcentration factor, RE: removal efficiency, **values are significantly different, p< 0.01 (BCF of Cd, Pb and RE of Cd, Pb), *values are significantly different, p<0.05 (TF of Hg), ^{ns} not significantly different, p> 0.05 (TF of Cd, Pb, BCF of Hg, RE of Hg). Numbers followed by the same letters in the same column show no significant difference.

Conclusion

The arbuscular mycorrhizal fungi (AMF) of *Glomus manihotis* was effective in increasing the contents of crude protein, crude fiber, crude fat, Ca, and P in *P. purpureum* grown on gold mine tailings, as well as reducing Cd, Hg, and Pb translocations in *P. purpureum* shoots and reducing Cd, Hg, and Pb contents in gold mine tailings. The ability of AMF to reduce Cd, Hg, and Pb in the tailings indicates that AMF is suitable for enhancing the phytoremediation of soil polluted with heavy metals.

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