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The role of arbuscular mycorrhizal fungi in phytoremediation of heavy metals and their effect on the growth of *Pennisetum purpureum* cv. Mott on gold mine tailings in Muara Bungo, Jambi, Indonesia

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Abstract. *Putra B, Warly L, Evitayani, Utama BP. 2021. The role of arbuscular mycorrhizal fungi in phytoremediation of heavy metals and their effect on the growth of* Pennisetum purpureum *cv. Mott on gold mine tailings in Muara Bungo, Jambi, Indonesia. Biodiversitas 23: 478-485.* The increase of heavy metal pollution in the soil results from human activities such as gold mining, which impacts human and environmental health problems. Phytoremediation is an environmentally friendly, inexpensive, and efficient solution to overcome environmental damage caused by heavy metal contamination. Inoculation of AMF in dwarf Napier grass can reduce the level of metal contaminants in soil and subsequently its absorption in plant tissue. This study investigates the effect of arbuscular mycorrhizal fungi (AMF) on dwarf Napier grass (*Pennisetum purpureum* cv. Mott) plant growth and heavy metal remediation rates. The experiment was a completely randomized design with 4 levels of AMF inoculation (0, 5, 10, and 15 g/pot) with 5 repetitions. After eight weeks of potting experiments, shoot and root biomass, plant growth, heavy metal content in potting media were assessed. The results revealed that AMF inoculation of 10 g/pot exhibited a higher growth yield of dwarf Napier grass compared to other treatments (plant height ($p<0.01$), leaf length ($P<0.01$), leaf width ($P<0.01$), stem diameter ($P<0.01$) and plant fresh weight ($P<0.01$)), but had no significant effect on the number of leaves and number of shoots ($P>0.05$). AMF (10 g/pot) significantly affected root growth of dwarf Napier grass (root length ($P<0.01$), number of roots ($P<0.05$), and root fresh weight ($P<0.01$)). The results also showed that AMF increased the uptake of Al, Co, Cr, and Fe significantly in the growing media (Al ($P<0.01$), Co ($P<0.05$), Cr ($P<0.01$), Fe ($P<0.05$) but no significant effect on Pb ($P>0.05$). This study concluded that AMF effectively increased the growth of dwarf Napier grass and reduced heavy metal contaminants on gold mines tailings.

Keywords: Arbuscular mycorrhizal fungi, dwarf Napier grass, heavy metals, tailings

INTRODUCTION

Gold mining is a very valuable mineral mining activity going on for a long time. The land used as a gold mining location in Bungo District, Jambi Province, Indonesia is very wide. Based on Bungo statistical data in 2010, gold mining locations in Bungo District, Jambi Province, include Rantau Pandan Sub-district covering an area of 1900 hectares, Pelepat Sub-district covering an area of 1800 hectares, Limbur Sub-district covering an area of 500 hectares, Tanah Sepenggal sub-district covering an area of 2000 hectares, Pelepat Ilir Sub-district covering an area of 1800 hectares, and Jujuhan Sub-district covering an area of 8000 hectares. The gold mining methods used are mercury amalgamation and cyanidation. The impact of using mercury (Hg) or cyanide is to produce sludge high in heavy metal content, called tailings (Favas et al. 2016; Oyewo et al. 2018). Mud waste from mining activities in liquid and solid form is very at risk of environmental pollution such as copper (Cu), Lead (Pb), arsenic (As), zinc (Zn), chromium (Cr), cadmium (Cd), and vanadium (V) (Krewski et al. 2007; Tchounwou et al. 2012; Fasola et al. 2016; Olobatoke and Mathuthu 2016; Singh et al. 2017; Ali et al. 2019).

Generally, tailings in gold mining areas in Bungo District are simply dumped without any further processing, resulting in environmental pollution. Previous studies have reported that there have been cases of health-related complications in residents living in areas close to mine tailings sites that were the result of contamination of agricultural soil, water sources, and air (Ngole-Jeme and Fantke 2017; Entwistle et al. 2019; Ng et al. 2019; Okereafor et al. 2020). The high levels of heavy metals found in ex-gold mining areas can be neutralized using micro-organism-based phytoremediation technology. The use of associated green plants can reduce toxic activity due to heavy metals, reduce heavy metal levels in the soil, as they are environmentally friendly, easy, and cost-effective (Danh et al. 2014; Wei et al. 2021).

Plant selection is critical to designing a phytoremediation strategy (Riaz et al. 2021). Many hyperaccumulator plants are slow growing, have low biomass, and are not widely distributed (Singh and Fulzele 2021). One of the plants that can act as phytoremediation is dwarf Napier grass (*Pennisetum purpureum* cv. Mott).

Recently, the role of dwarf Napier grass had been studied in increasing arsenic (As) phytoextraction through electronic remediation with phosphate and EDTA (Boonmeerati and Sampanpanish 2021). Apart from being a phytoremediation agent, dwarf Napier grass (*P. purpureum* cv. Mott) provides forage because this is a superior type of grass.

The use of arbuscular mycorrhizal fungi is an alternative to help increase plant growth and to inhibit the translocation of heavy metals to shoots (Sharma et al. 2016; Chen et al. 2018; Ishaq 2018), increase nutrient uptake (Riaz et al. 2021), bind toxic metal (Joner et al. 2004), immobilize heavy metals in plant roots or phytostabilization (Ambrosini et al. 2015; Wu et al. 2016) acts as a filter to block xenobiotics in its mycelium (Wu et al. 2019). Therefore, mycorrhizae act as a physical barrier and serve as an envelope for the host (Ma et al. 2019).

There have been previous studies on the success of arbuscular mycorrhizal fungi associated with various host plants in remediation of heavy metal polluted environments have been carried out. For example, in sunflower plants (Ashofie and Prasetya 2019), *Hordeum vulgare* L. (Khan et al. 2020), *Zea mays* L. (Zhuo et al. 2020), *Trifolium repens* (Xiao et al. 2020), *Medicago sativa* L. (Zhang et al. 2019). The majority of previous studies used weeds as hosts for the remediation of polluted land. There is little information about the use of forage plants as phytoremediation. The arbuscular mycorrhizal fungi can bind heavy metals so that they do not pollute in plant shoots and have the potential to be used to develop healthy forage.

The aim of this study was to investigate the effect of arbuscular mycorrhizal fungi (AMF) on dwarf Napier grass (*P. purpureum* cv. Mott) plant growth and heavy metal remediation.

MATERIALS AND METHODS

Study area

This research was conducted in Rantau Pandan Sub-district, Bungo District, Jambi Province, Indonesia, located between -1.641450 S and 101.990170 E at an altitude of 97 meters above sea level. The map of the study site is presented in Figure 1.

Procedures

Preparation of mine tailings and Pennisetum purpureum

Mining tailings used in the study were collected from the gold mining area of Rantau Pandan Sub-district, Bungo District, Jambi Province, Indonesia, from a depth of 0–30 cm. Pieces of wood, stones, plants, and unwanted material have been removed. For each treatment, tailings soil was taken as much as 3 kg/pot. The initial physical and chemical characteristics of mine tailings were analyzed in the Padang Industrial Standardization and Research Institute laboratory. The results of the analysis are shown in Table 1. The tailings soil medium was mixed with vermicompost (6 : 1). The plant used in this study was dwarf Napier grass (*P. purpureum* cv. Mott). Experimental plants were grown by cuttings method and selected plants with the same age, size, and fresh weight.

Preparation of arbuscular mycorrhizal fungi inoculum

The inoculum of arbuscular mycorrhizal fungi i.e., *Glomus manihotis,* was obtained from the Laboratory of Soil Science, Faculty of Agriculture, Andalas University, Indonesia. AMF was inoculated on the roots of dwarf Napier grass seedlings planted in 15 × 20 cm polybags containing sterile gold tailings soil media. The inoculated seedlings were maintained, watered, and observed for 2 months.

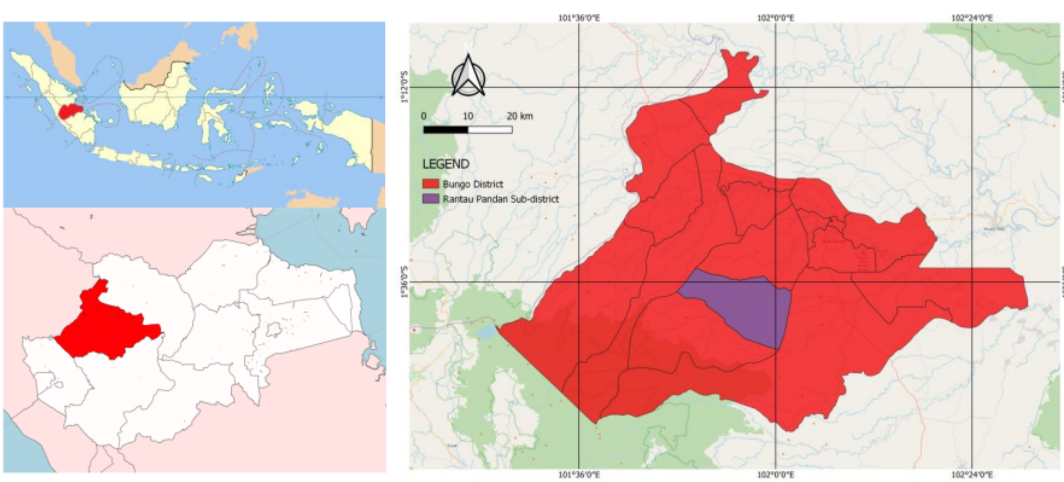

Figure 1. Location of samplings in Rantau Pandan Sub-district, Bungo District, Jambi Province, Indonesia

Table 1. Basic physical and chemical properties of mine tailings

Parameters	Total content	Measuring method
CD	0.173 mg/kg	ASTM C1301-95 (2001)
Hg	0.635 mg/kg	ASTM C1301-95 (2001)
Cr	10.8 mg/kg	ASTM C1301-95 (2001)
Co	6.23 mg/kg	ASTM C1301-95 (2001)
Fe	1.16%	ASTM C1301-95 (2001)
Al2O3	5.09 %	SNI 2049:2015
Water content	34.2%	SNI 7763:2018
pH	4.90	SNI 7763:2018
N	0.141 %	SNI 7763:2018
P	3.14%	SNI 7763:2018
K	0.052%	SNI 7763:2018

Experimental design

The experiment was based on a completely randomized design (CRD), with 4 treatments consisting of 4 levels of arbuscular mycorrhizal fungi (AMF) (0, 5, 10, and 15 g/pot). Each treatment consisted of 5 replications, each consisting 4 plant seeds with a total of 80 plant seeds.

Research location

Experimental garden located at Mura Bungo University Jambi where dwarf Napier grass was planted; arbuscular mycorrhizal spores was collected from Soil Laboratory, Faculty of Agriculture, Andalas University; heavy metal analysis of soil before treatment was performed in Padang Industrial Standardization Research and Standardization Laboratory and heavy metal analysis (Cd, Hg, Pb, Cr, Co, Fe, Al, Sn) of soil after research was done in Laboratory of PT. Saraswanti Indo Genetech Bogor.

Research flow

This study was initiated by analyzing the heavy metal content of ex-gold mine soil, and identifying the spores of arbuscular mycorrhizal fungus. Planted dwarf Napier grass (*P. purpureum* cv. Mott) was inoculated with arbuscular mycorrhizal fungi (AMF) for each treatment (0, 5, 10, and 15 g/pot). Each treatment was given NPK fertilizer as much as 5 g/pot. Plants were maintained for 2 months to observe the growth of dwarf Napier grass. The environmental conditions of the study area were normal lighting (12 hours) and temperature conditions (27 to 30°C). At the end of 2^{nd} month after planting, harvesting and separation between plants and roots were carried out to measure the fresh weight of plants and roots. The soil of each treatment was tested for heavy metal content by the method of Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) to record the success rate of phytoremediation.

Data analysis

The research data were analyzed for variance based on a randomized design with four treatments and five replications. Duncan's multiple tests were used further to determine differences between treatments.

RESULTS AND DISCUSSION

Plant growth and shoot biomass

The various arbuscular mycorrhizal fungi treatments (0, 5, 10, 15 g pot^{-1}) on growth parameters (leaf length, number of leaves, plant height, number of shoots, and fresh weight) is presented in Table 2. It was observed from the results that inoculation of arbuscular mycorrhizal fungi had a positive effect on the growth and biomass of dwarf Napier grass plants on tailings soil.

Effect of treatment on leaf length, leaf width and fresh weight

Arbuscular mycorrhizal fungi had a significant positive effect on leaf length and fresh wight parameters of dwarf Napier grass (*P. purpureum* cv. Mott). The highest leaf length and fresh plant weight were found in the treatment with arbuscular mycorrhizal fungi inoculation of 10 g/pot. Inoculation of arbuscular mycorrhizal fungi increased the uptake of nutrients for dwarf Napier grass. Wu et al. (2011) studied ex-gold mine lands where arbuscular mycorrhizal fungi were inoculated on vetiver grass. The results showed that the uptake of N and P was significantly higher in the leaves of vetiver grass after mycorrhizal treatment (N: 13.6 mg/kg; P: 1.26 mg/kg) compared to controls. The present study results are consistent with the results of Mallick et al. (2018), who reported that arbuscular mycorrhizal fungi are able to increase total dry weight, leaf area, and plant growth in heavy metal contaminated soil. Arbuscular mycorrhizal fungi are also able to increase nitrogen uptake in host plants. This is thought to be the main factor determining leaf length on dwarf Napier grass plants. Arbuscular mycorrhizal fungi can optimally increase N uptake in plants at the level of 10 g/pot. Wu et al. (2021) observed that arbuscular mycorrhizal fungi are able to increase N uptake in maize plants.

Effect of treatment on number of leaves and shoots

Table 2 shows that treatments were not significantly ($P>0.05$) different in the number of leaves and shoots of dwarf Napier grass (*P. purpureum* cv. Mott). The effect of arbuscular mycorrhizal fungi was not observed for the parameters of number of leaves and shoots. This is presumably because the root system of dwarf Napier grass is not too broad due to the young planting age, so that the infection with arbuscular mycorrhizal fungi is not optimal. In contrast, Basyal and Emery (2021) reported that arbuscular mycorrhizal fungi could increase the number of tillers of *Panicum virgatum* by 32% compared to no arbuscular mycorrhizal fungi inoculation. In this study inoculation of arbuscular mycorrhizal fungi only affected the growth of stem, plant height, leaf width, rod diameter, and fresh weight. However, it was not seen in shoots of dwarf Napier grass. The light intensity in all treatments was relatively the same, which was the reason there was no difference in the number of shoots. Raffo et al. (2020) observed that light intensity affected the growth of *Rosmarinus officinalis* L. Optimal light conditions can trigger the formation of new shoots on plants dwarf Napier grass. The light conditions were similar for all treatments, showing no significant difference in number of leaves and shoots of dwarf Napier grass.

Table 2. Effect of arbuscular mycorrhizal fungi on leaf length, number of leaves, plant height, and number of shoots, leaf width, rod diameter, and fresh weight of dwarf Napier grass on gold mining tailings

Treatments	Average						
	Leaf length	**Number of leaves**	**Plant height**	**Number of shoots**	**Leaf width**	**Stem diameter (mm)**	**Fresh weight (g)**
AMF (0 g/pot)	49.47^{ac}	27.60	62.80^{ab}	5.25	20.40^{a}	14.20^{a}	57.46^{a}
AMF (5 g/pot)	47.56^{a}	30.80	59.50^{a}	5.80	22.20^{ab}	13.40^{a}	61.55^{a}
AMF (10 g/pot)	56.50^{b}	31.00	69.75^{b}	5.40	26.40^{bc}	18.60^{b}	79.08^{b}
AMF (15g/pot)	53.20^{bc}	29.40	65.80^{ab}	6.69	26.60^{c}	18.30^{b}	63.70^{a}
P- value	P<0.01	P>0.05	P<0.01	P>0.05	P<0.01	P<0.01	P<0.01

Note: Different letter notations in the table show significantly different values with p-values less than 0.01 (leaf length, plant height, leaf width, stem diameter, and fresh weight). Without letter notations in the table show non significantly different values with p-value more than 0.05 (number of leaves and number of shoots).

Effect of treatment on plant height and stem diameter

It is evident from table 2 that treatments had a very significant (P<0.01) difference in plant height and stem diameter of dwarf Napier grass (*P. purpureum* cv. Mott). The optimum plant height and stem diameter were found to be significantly different from without arbuscular mycorrhizal fungi inoculation of 10 g/pot treatment. The 10 g/pot inoculation treatment was also significantly different from 5 g/pot. Highest number of arbuscular mycorrhizal fungi inoculum showed high growth of dwarf Napier grass). This proved that arbuscular mycorrhizal fungi played an important role in increasing the height of dwarf Napier grass on ex-gold mines (tailings). Arbuscular mycorrhizal fungi can repair and increase plant growth which is related to its role in phosphorus absorption. It was observed that arbuscular mycorrhizal fungi were able to increase nutrient uptake, resulting in increased plant height and stem diameter as compared to without arbuscular mycorrhizal fungi inoculation. Arbuscular mycorrhizal fungi have extensive hyphae that can increase water and mineral uptake in plants under abiotic stress environments (Begum et al. 2019). Adeyemi et al. (2021) observed that arbuscular mycorrhizal fungi significantly increased soybean leaf production in the vegetative period. A similar finding was also reported by Tisarum et al. (2020), who noticed that rice plants inoculated with arbuscular mycorrhizal fungi produced a higher number of leaves than without inoculated plants. Arbuscular mycorrhizal fungi can also absorb organic phosphate and convert it into inorganic P that can be absorbed by plants with the help of phosphatase enzymes which are also produced by arbuscular mycorrhizal fungi and plant cells. This fungus activates phosphatase enzymes on the root surface, causing inorganic phosphate to be liberated from organic phosphate near the cell surface and then absorbed through nutrient uptake mechanisms and affect leaf growth.

The results of this study are in line with the results of Husna et al. (2021), who studied the role of arbuscular mycorrhizal fungi on the growth of *Kalappia celebica* on tailings soil. The results showed that arbuscular mycorrhizal fungi significantly affected plant height growth of *Kalappia celebica* on tailings soil.

Effect of treatment on root length

Based on Table 3, it was observed that treatments had a very significant (P<0.01) difference in root length of dwarf Napier grass. The highest root length was noted in treatment with arbuscular mycorrhizal fungi inoculation of 10 g/pot, while the lowest root length was found in without arbuscular mycorrhizal fungi inoculated plants. Arbuscular mycorrhizal fungi inoculation of 10 g/pot was significantly different from other treatments. Arbuscular mycorrhizal fungi may play a role in assisting roots in uptake of nutrients in the soil. The role of arbuscular mycorrhizal fungi on root length was seen in this study. The effectiveness of arbuscular mycorrhizal fungi with host plants determines plant root growth. On ex-gold mines (tailings) it can cause damage to plant roots. The presence of arbuscular mycorrhizal fungi inoculation can restore root damage caused by nematode factors or heavy metal poisoning. This is in line with the research by Vallejos-Torres et al. (2021), showing that coffee plants infected with roots by nematodes could be recovered by inoculation of arbuscular mycorrhizal fungi.

Table 3. Effect of arbuscular mycorrhizal fungi on root length, number of roots, and root weight

Treatments	Average		
	Root length (cm)	**Number of roots (cm)**	**Root fresh weight (cm)**
AMF (0 g/pot)	30.70^{a}	33.77^{a}	24.43^{a}
AMF (5 g/pot)	32.00^{ab}	43.75^{ab}	31.40^{a}
AMF (10 g/pot)	58.10^{c}	49.89^{b}	52.39^{b}
AMF (15g/pot)	42.40^{b}	40.25^{ab}	30.97^{a}
P- value	P<0.01	P<0.05	P<0.01

Note: Different letter notations in the table show significantly different values with p-values less than 0.01 for root length and root fresh weight and p-value less than 0.05 for number of roots.

Table 4. Effect of arbuscular mycorrhizal fungi on the reduction of heavy metal content in gold mine soil tailings

Treatments	Average				
	Al (mg/kg)	Co (mg/kg)	Cr (mcg/100g)	Fe (mg/100g)	Pb (mg/kg)
AMF (0 g/pot)	4730.35[a]	2.52[a]	481.10[a]	835.99[a]	11.92
AMF (5 g/pot)	5287.94[a]	1.91[b]	403.05[b]	719.38[b]	10.22
AMF (10 g/pot)	3211.65[b]	1.95[b]	361.59[b]	686.47[b]	10.29
AMF (15g/pot)	4471.60[a]	2.16[ab]	406.89[b]	760.83[ab]	10.71
P- value	P<0.01	P<0.05	P<0.01	P<0.05	P>0.05

Note: The table's different letter notations show significantly different values with p-values less than 0.01 (Al and Cr) and p-values less than 0.05 (C0 and Fe). Without letter notations in the table show non significantly different value with p-value more than 0.05 (Pb).

Table 5. Effect of arbuscular mycorrhizal fungi on heavy metal content in the roots of dwarf Napier grass

Treatments	Average			
	Al (mg/kg)	Co (mg/kg)	Cr (mcg/100g)	Fe (mg/100g)
AMF (0 g/pot)	243.31[a]	0.18[ac]	17.85	71.60
AMF (5 g/pot)	239.23[a]	0.17[a]	20.54	80.97
AMF (10 g/pot)	446.76[b]	0.28[b]	17.72	54.32
AMF (15g/pot)	534.36[b]	0.24[bc]	20.81	97.30
P- value	P<0.01	P<0.01	P>0.05	P>0.05

Note: Different letter notations in the table show significantly different values with p-values less than 0.01 (Al and Co). Without letter notations in the table show non significantly different value with p-value more than 0.05 (Cr and Fe)

Effect of treatment on number of roots

Table 3 shows that treatments had significantly (P<0.05) different on the number of roots of dwarf Napier grass (*P. purpureum* cv. Mott). Arbuscular mycorrhizal fungi inoculation as much as 10 g/pot showed the highest yield and was significantly different from those without arbuscular mycorrhizal fungi. The formation of root tissue may be strongly influenced by the presence of arbuscular mycorrhizal fungi. Turan (2021) observed that arbuscular mycorrhizal fungi could increase plant root weight, increase antioxidants and improve soil enzymes. Lubis et al. (2021) also reported that the uptake of N, P, and K are influenced by arbuscular mycorrhizal fungi. Püschel et al. (2020) noted that arbuscular mycorrhizal fungi significantly increased the number of roots and plant root area.

Effect of treatment on fresh weight of roots

Based on Table 3, it was observed that the treatments with arbuscular mycorrhizal inoculation were found very significant (P<0.01) on the fresh weight of dwarf Napier grass roots (*P. purpureum* cv. Mott). The highest fresh weight of root was found in the treatment of arbuscular mycorrhizal fungi inoculation of 10 g/pot, while the lowest was recorded in the treatment without arbuscular mycorrhizal fungi inoculation.

Fresh root weight was influenced by the total number of roots, root length, and root density. It was assumed that in this study, the role of arbuscular mycorrhizae greatly affects the number of roots, root length, and root density. Plant roots may experience an increase in length due to the low availability of nutrients around the main root, thus triggering the roots to reach other locations. The role of arbuscular mycorrhizal fungi made the roots of dwarf Napier grass optimal in nutrient absorption around the main roots. Turan (2021) reported that arbuscular mycorrhizal fungi significantly increased the root weight of host plants.

The arbuscular mycorrhizal fungi also make reaching nutrients easier for the roots. Chen et al. (2018) observed that plant roots could trigger root hair growth in the presence of competition with other plants.

Effect of treatment on aluminum content

Treatment with arbuscular mycorrhizal fungi inoculation of 10 g/pot showed the lowest aluminum content and was very significantly different (P<0.01) compared to treatment without inoculation (Table 4). However, Table 5 shows that highest aluminum content in roots was found in the treatment of arbuscular mycorrhizal fungi inoculation of 15 g/pot, but not significantly different from that of arbuscular mycorrhizal fungi inoculation of 10 g/pot. This shows that 10 g/pot arbuscular mycorrhizal fungi inoculation was able to reduce the levels of aluminum in the soil of ex-gold mines. The ability of arbuscular mycorrhizal fungi to reduce aluminum levels in the soil is a solution to utilize ex-gold mines, which are generally high in aluminum content. Besides being able to be used as phytoremediation, arbuscular mycorrhizal fungi can also increase plant productivity on land contaminated with heavy metals.

The optimal role of arbuscular mycorrhizal fungi in 10 g/pot inoculation treatment was thought to be due to the high production of glomalin related soil protein (GRSP) so that it could absorb Al from the experimental soil. Wang et al. (2019) reported that arbuscular mycorrhizal fungi could produce glomalin related soil protein (GRSP) through mycelium and spore walls. Glomalin related soil protein plays a role in absorbing aluminum (Al^{3+}) (Seguel et al. 2013), and reducing the bioavailability of heavy metals (Siani et al. 2017). Aguilera et al. (2018) reported that arbuscular mycorrhizal fungi have the potential to reduce Al phytotoxicity through the role of glomalin.

Effect of treatment on cobalt content

Table 4 shows that treatment without arbuscular mycorrhizal fungi showed the highest cobalt content, which was significantly different from arbuscular mycorrhizal fungi inoculation treatment. The lowest cobalt content of the treated soil was found in 10 g/pot of inoculated arbuscular mycorrhizal fungi. The lowest cobalt content in the treated soil indicates the translocation of heavy metals to the roots (Table 5). The results of this study differ from those of Crossay et al. (2021), who reported that arbuscular mycorrhizal fungi have no significant effect on cobalt translocation in roots and shoots of sorghum plants. The effect of arbuscular mycorrhizal fungi in reducing cobalt levels in the treated soil indicated that it was translocated to roots, shoots or structures of arbuscular mycorrhizal fungi. The accumulation of metallic elements by arbuscular mycorrhizae is carried out by intraradical and extraradical mycelium (Wu et al. 2019). Roychoudhury and Chakraborty (2021) stated that cobalt from the soil accumulates in the root system and root nodules in legumes and added that higher Co accumulation in plant roots indicates that less than less cobalt was transferred to shoots. In addition, the high accumulation of Co in plant roots indicated that less cobalt was translocated to shoots. Conversa et al. (2019) noted that inoculation of arbuscular mycorrhizal fungi could translocate cobalt to plant shoots.

Effect of treatment on chromium content

Table 4 shows that arbuscular mycorrhizal fungi had a very significant effect on the chromium content of the treated soil. Further test results showed that arbuscular mycorrhizal fungi inoculation showed a very significant ($P<0.01$) difference in chromium content to the treatment without arbuscular mycorrhizal fungi inoculation. The highest (481.10 mcg/100 g) chromium content of the experimental soil was found in the treatment without arbuscular mycorrhizal fungi, while the lowest (361.59 mcg/100 g) chromium content was found in the inoculation treatment of arbuscular mycorrhizal fungi at 10 g/pot. These results indicated that the inoculation of arbuscular mycorrhizal fungi was able to reduce the chromium content in the soil. There was no significant difference in the process of translocation to the root (Table 5). These results are in accordance with the results of Hu et al. (2021) which reported that the concentration of Cr in water decreased significantly with the addition of arbuscular mycorrhizal fungi

The reduction in heavy metal content, especially chromium, was due to the combined effect of plants, microorganisms, and arbuscular mycorrhizal fungi. Previous studies have shown that the removal and immobilization of heavy metals depend on several factors such as pH value, redox state, plant species, and microbial activity. Heavy metal removal and immobilization depended on pH value, redox state, the composition of influent water, dominant plant species, and microbial activity (Vymazal et al. 2010). Chen et al. (2018a) stated that arbuscular mycorrhizal fungi are important microorganisms for increasing heavy metal remediation through accumulation of heavy metals in fungal structures, such as arbuscular intraradical mycelium, and vesicles. Hu et al. (2021) reported that the reduction of chromium levels in growing media after inoculation of arbuscular mycorrhizal fungi through the role of extraradical mycelium. Kullu et al. (2020) observed that the extraradical mycelium of arbuscular mycorrhizal fungi extends plant roots, which can move beyond the root depletion zone and absorb heavy metals from the environment. The concentration of chromium in the roots of *Brachiaria mutica* plants inoculated with arbuscular mycorrhizal fungi was significantly higher than without inoculation, but the concentration of chromium was lower in shoots of plants inoculated with arbuscular mycorrhizal fungi.

Effect of treatment on iron (Fe) content

The test results showed that the inoculation of arbuscular mycorrhizal fungi showed significantly ($P<0.05$) different iron content results to the treatment without arbuscular mycorrhizal fungi inoculation. The lowest iron content was found in the treatment of 10 g/pot arbuscular mycorrhizal fungi inoculation, while the highest was found in the treatment without arbuscular mycorrhizal inoculation (Table 4). The translocation of Fe from the treated soil to the roots was not significant (Table 5). This indicates the translocation of Fe to dwarf Napier grass shoots.

Arbuscular mycorrhizal fungi can absorb iron in the experimental soil. Mishra et al. (2016) reported that arbuscular mycorrhizal fungi could significantly increase Fe uptake in *Pennisetum glaucum* and *Sorghum bicolor* plants grown on soil contaminated with iron (Fe). The high absorption of Fe in this study may be due to be the role of siderophores produced by arbuscular mycorrhizal fungi that act as Fe solvents. Haselwandter (2008) explained that most fungi synthesize siderophores as chelating agents, forming soluble complexes with Fe^{3+} with very high stability constants, thereby dissolving iron Fe. Manoharan et al. (2021) stated that siderophores are secondary metabolites produced by arbuscular mycorrhizal fungi that are important for accessing host cellular components for growth and development. Siderophores produced by arbuscular mycorrhizal fungi have roles such as extracellular iron acquisition, intracellular iron storage, conidia iron acquisition, and storage during infection.

Effect of treatment on Pb content

Based on table 4, it was observed that arbuscular mycorrhizal fungi had no significant ($P>0.05$) effect on Pb content of the treated soil. In this study, there was no effect of arbuscular mycorrhizal fungi on reducing Pb levels in the treated soil. It is assumed that Pb is heavy metal with low mobility properties. The role of arbuscular mycorrhizal fungi does not appear significant in the Pb remediation process. The soil's chemical composition and absorption properties can affect the mobility and bioavailability of metals. Phytoextraction efficiency is influenced by bioavailability or bioavailability of heavy metals in the soil. The low bioavailability in the soil is a limiting factor for the success of phytoextraction of heavy metal contamination specifically Pb. Generally, only a small amount of soil metal is bioavailable for absorption by plants. Strong heavy metal binding to soil particles or precipitation causes most of the heavy metals in the soil to be insoluble and therefore unavailable for uptake by plants (Sheoran et al. 2011). The bioavailability of heavy metals or metalloids in soil can be categorized into three parts: fast bioavailability such as (Cd, Zn, Cu, Se, As, and Ni), moderate bioavailability such as (Co, Fe, and Mn), and low bioavailability such as Pb, Cr, U (Prasad 2003). Kim et al. (2015) also reported that Pb is a heavy metal with low mobility. Vermicompost triggered the invisible effect of arbuscular mycorrhizal fungi on Pb uptake. Chu et al. (2017) observed that the addition of vermicompost increases the accumulation of Zn and Pb in the soil because vermicompost contains a heterogeneous substance that can simultaneously mobilize and stabilize Pb.

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