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Research Article
Effects of Arbuscular Mycorrhizal Fungi and Organic Material on Growth and Nutrient Uptake by *Pericopsis mooniana* in Coal Mine

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Abstract

Background and Objective: Post-coal mining land has poor soil physical characteristic and high metal toxicity and hence needs to be recovered. The recovery effort can be done through addition of organic material input, utilization of beneficial microorganism such as Arbuscular Mycorrhizal Fungi (AMF) and selection of suitable tree species. The purpose of this study was to investigate the effect of AMF and compost on the growth and uptake of nutrients and metal by *Pericopsis mooniana* in soil media of post coal mine land. **Materials and Methods:** A greenhouse experiment was carried out by using factorial Completely Randomised Design. The first factor was the AMF inoculum (Factor A): control (A_0), AMF-KG (A_1), AMF-UHO (A_2), AMF-Vale (A_3), AMF-CA (A_4), AMF-HA (A_5), AMF-BJ (A_6), AMF-Mycofer (A_7) and the second factor was an organic material (Factor B): ex-mined soil+without any organic material (B_0), soil, compost, sand (2:1:1) (B_1) and soil, compost, sand and rice husk (2:1:1:1) (B_2). The experiment was carried out for 5 months on a greenhouse scale. After 150 days the seedlings were collected to quantify growth parameters, mycorrhizal root colonization rate and number spores and nutrient uptake. **Results:** The results showed that the local AMF was able to colonize the roots of the *P. mooniana* and increase in the growth and dry weight of plants and also tend to increase the C, N, P, K, Ca and Mg accumulation. The enrichment of organic material in the coal mine media stimulated the growth and nutrient uptake. *P. mooniana* performs phytoremediation mechanisms through rhizofiltration techniques (Fe, Cd, Pb) and phytoextraction (Cr and Mn). **Conclusion:** Inoculation with AMF and the enrichment of organic materials have the potential to support post-coal mining land restoration.

Key words: *Pericopsis mooniana*, coal mine media, phytoremediation, arbuscular mycorrhizal fungi, metal toxicity, land restoration

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

One factor causing deforestation in the tropics is mining and coal mining techniques are generally done by open pit mining. This technique can change the landscape, removing soil surface and soil organic matter. In addition, mining activities produce ex-mining land with low soil pH characteristics, heavy toxic metals (Al, Fe, Mn), nutrient deficiency, soil compaction, acid mine and acid emissions[1]. The soil characteristics may be a limiting factor in plant growth in post-mining land restoration activities. Proper approach and environmentally friendly in restoration of land after coal mine needs to be done. The approach can be done through soil amendment, choice of an appropriate and utilization of beneficial soil microorganism such as mycorrhizal fungi. Soil enhancers aim for soil conditioning with organic material inputs. Organic material can improve soil structure, porosity and aeration of the soil, as an energy source for soil microbes and improve soil moisture[2]. One of the organic fertilizers used in post-mining land reclamation activities is compost[3].

Besides the addition of organic material, the use of some micro-organisms from the soil is very useful, such as arbuscular mycorrhizal fungi (AMF). It also improves soil conditions of the coal mine soils through the uptake of nutrients and water, soil structure and also improve plant resistance to environmental stress[4-8]. AMF is reported to accelerate susceptibility of vegetation and post-mined land[9-12]. In addition, based on international publications, some studies indicate that AMF inoculation effectively improves the quality, growth, dry weight and nutrient uptake on either greenhouse[13-18] or field scale[18]. In conditions of heavy metal stress, AMF involved in phytoremediation in the form of phytoextraction (transfer of metal from root to tip) and phytostabilization (metallic immobilization)[19-21]. The effectiveness of AMF, in phytoremediation, increased growth and nutrient uptake vary widely depending on AMF strains and ecotypes, plant and metallic species and ecotypes and their availability[22,23].

One type of legume tree is symbiotic with AMF and potentially developed as a type of post-mining revegetation of coal land is nedun tree, *Pericopisis mooniana*. *P. mooniana* is a type of legume of high economic value and includes the endangered species as well as the type of post nickel mining revegetation in Southeast Sulawesi[6,7]. Husna *et al.*[6] reported that indigenous AMF effectively increased the growth and Ni reduction on *P. mooniana* aged 5 months on nickel post mining media. AMF symbiosis studies (especially AMF inoculum collected from nedun trees rhizosphere) with *P. mooniana* on coal post-mining soil media have not been done. The purpose of this study was to examine the effect of AMF and organic matter enrichment on the growth and uptake of nutrients and metal by *P. mooniana* in soil media of post coal mine land.

MATERIALS AND METHODS

Time and location: This study was conducted for 5 month (June-September, 2015) in the greenhouse of Silviculture department, Faculty of Forestry IPB, Bogor. Chemical analysis of the media and absorption of nutrient and metal, were conducted in Laboratory Soils and Plants of SEAMEO BIOTROP, Bogor, Indonesia.

Growth media: The coal post-mining soil media was collected from PT. Bukit Asam, Muara Enim, South Sumatra. In addition to pure coal soil media (B0), the medium was also enriched with compost, sand (2: 1: 1) (B1) and compost, sand and charcoal husk (2: 1: 1: 1) (B2). The physical and chemical properties of each medium are presented in Table 1.

Seeds and seedlings preparation: *P. mooniana* seeds were collected from the parent tree in the village of Bali Jaya Lamedai District Kab. Kolaka, Southeast Sulawesi (04°17'57.6 "LS-121°32'59.2" BT). The collection is done by climbing the tree and then seeds of *P. mooniana* dried for 3-4 days, then dried beans extracted. Initial treatment of seed by way of scraped of the seed and soaking with giberelin 0.05 ppm for 6 h. Before being added, all the seeds were sterilized with sodium hypochlorite (5%) for 5 min. After sterilization, the seeds were washed several times with water until clean. Seeds were added to a 40×30×15 cm plastic container containing sterile zeolite media.

Inoculum and AMF inoculation: Arbuscular mycorrhizal fungi were collected from the *P. mooniana* rhizosphere in 6 places grown in Southeast Sulawesi[24]. AMF inoculum contains zeolite, spores and AMF colonized roots from trapping results by host *Pueraria javanica* seeds in greenhouse of Silviculture department, Faculty of Forestry IPB, Bogor. Five grams of AMF inoculum were inoculated on media in polybag (15 cm diameter×20 cm high). Uninoculated seeds were used as controls.

Research design: This research is based on Randomized Complete Block Design (F-RCBD) in factorial, consisting of 2 factors, AMF inoculum (A) i.e., without AMF (control, A_0), AMF was from Governor Office Arboretum (AMF-KG, A_1), AMF came

Table 1: Physical and chemical properties of growing media

Substrate	Method	B_0	B_1	B_2
pH				
H_2O (1:1)	SNI 03-6787-2002	4.00	5.40	5.80
$CaCl_2$ (1:1)		4.00	5.40	5.80
C org (%)	SNI 13-4720-1998 (Walkey and Black)	1.72	2.48	2.91
Total C (%)	SNI 13-4721-1998 (Kjeldahl)	0.08	0.09	0.20
C/N ratio		22.00	28.00	15.00
P_2O_5 availability (ppm)	SL-MU-TT-05 (Bray I/II)	3.60	61.70	346.10
CEC				
Ca (cmol kg^{-1})	SL-MU-TT-07c (Buffer extract) NH4OAc 1,0 N pH 7,0)	20.05	2.60	6.32
Mg (cmol kg^{-1})		14.22	2.09	0.74
K (cmol kg^{-1})		1.18	1.22	2.92
Na (cmol kg^{-1})		4.61	2.28	3.56
Total (cmol kg^{-1})		40.06	8.19	13.54
CEC (cmol kg^{-1})		23.70	4.90	11.81
KB (cmol kg^{-1})		100.00	100.00	100.00
Al-Hdd				
Al^{3+} (Me/100 g)	SL-MU-TT-09 (Extract KCl 1N)	0.85	0.17	0.00
H^+ (Me/100 g)		1.95	1.23	0.11
Grain distribution				
Sand (%)	SL-MU-TT-10 (Pipette)	14.90	55.00	49.30
Clay (%)		41.40	22.20	24.00
Loam (%)		43.70	22.80	26.70
Total metals				
Total Fe_2O_3 (%)	SL-MU-TT-13 (HNO_3-$HClO_4$)-AAS	5.14	2.46	1.87
Total Mn (ppm)		1290.10	544.20	651.10
Total Cd (ppm)		0.20	0.40	0.40
Total Cr (ppm)		16.10	5.20	3.90
Total Pb (ppm)		22.40	5.10	15.80

Source: SEAMEO BIOTROP SERVICES LABORATORY. B_0: Control, B_1: Soil, compost, sand-2:1:1, B_2: Soil, compost, sand, rice husk-2:1:1:1

from Unhalu Campus Environment AMF-UHO, A_2), AMF was sampled from Post Nickel Mining Land PT. Vale Indonesia Tbk Pomalaa Kolaka (AMF-Vale, A_3), AMF is derived from Lamedai Kolaka Nature Reserve (AMF-CA, A_4), AMF was derived from Tanggetada Kolaka Natural Forest (AMF-HA, A_5), AMF came from the forest of Bali Jaya (AMF-BJ, A_6), Mycofer IPB (AMF-IPB, A_7) and the second factor was an organic material (B): land mines+without any organic material (B_0), soil, compost, sand (2:1:1) (B_1) and soil, compost, sand and charcoal husk (2:1:1:1) (B_2). Each treatment repeated 5 (five) times and each replication 5 units of plants, with a total of 225 experimental plots.

Data collection and analysis on plants: A total of five replications of seedlings in the harvest for each treatment in the greenhouse of the Silviculture department of the Faculty of Forestry IPB Bogor aged 5 months after germination. The height and diameter were measured at 1 cm from the surface of the media. The number of leaves calculated based on leaf area measured at the length and width of the leaf. Root marks were measured by counting the total number of nodules at the end of the experiment:

$$\text{Seed quality index (SQI)} = \left[\frac{\text{Shoot dry weight + Dry root weight}}{\text{Height/diameter}} + \frac{\text{Dry weight of shoot}}{\text{Root dry weight}}\right]$$

Seedlings have high quality if the value of SQI $\geq$0.09 [25].

After harvest, shoots and roots were separated. They were oven-dried at 70°C for 48 h before weighting. Content of C was measured using Walkley and Black method, Total N content of plant was analyzed by using Kjeldahl and P, K, Ca, Mg with method of HNO_3-$HClO_4$. C, P, N, K, Ca and Mg uptake was calculated by multiplying the nutrient content of the plant by the dry weight of the plant. Analysis of metal content was executed using HNO_3-$HClO_4$:

$$\text{Metal transport factor (TF)} = \frac{C_{aerial}}{C_{root}}$$

where, C_{aerial} is the concentration of metal on the shoot (stems and leaves) and C_{root} is the metal concentration at the root.

AMF colonization and mycorrhizal inoculation effect (MIE): The roots of the hardwood were washed in running water.

Root cleaned in 10% KOH for 24 h, acidification with 2% HCl for 2 min and colored with tripan blue. The colonization of AMF was calculated by the equation[26,27]:

$$\text{Colonization of AMF (\%)} = \frac{\Sigma \text{ number of fields of view colonized}}{\Sigma \text{ total observed field of view}} \times 100$$

$$\text{MIE (\%)} = \frac{\text{Dry weight of morphorous plants-Dry weight of non-mycorrhizal plants}}{\text{Dried leaves of mycorrhizal plants}} \times 100$$

Data analysis: Data were statistical analyzed using two-way ANOVA. Differences between treatment means more evaluated using Duncan Multiple Range Test ($p<0.05$). All statistical analysis was conducted using SAS.

RESULTS

The ANOVA results showed that the application of AMF inoculum and organic matter significantly influences shoots Mg and Ca concentration and Ca accumulation of *P. mooniana* 5 month after transplantation. Meanwhile, the other variables are influenced by both factors, AMF inoculum and organic matter.

Plant growth: The AMF significantly increased height, length and width leaves of *P. mooniana* 5 month after transplantation (Table 2). There was no differences diameter and total leaves of *P. mooniana* between AMF and control. Total root nodules were higher of *P. mooniana* inoculated with A_1 and A_4. Organic matter enrichment significant increased height, diameter, length, width and total leaves than control seedlings. B_1 treatment increased total root nodules than B_0 and B_2.

Plant dry weight: The AMF colonization by A_1-A_4 increased shoot and total dry weight of *P. mooniana* 5 month after transplantation (Table 3). By contrast, A5-A7 did not significantly increase shoot and total dry weight of *P. mooniana*. AMF significant increased shoot root ratio of *P. mooniana*. Organic matter enrichment significant increased shoot, root and total dry weight and shoot root ratio of *P. mooniana*.

Spore, AMF colonization and mycorrhizal dependence (MD): Total spores AMF were higher in A_1 treatment and significant difference with A_0 and A_5 (Table 4). AMF colonization by A_2 treatment increased SQI of *P. mooniana*. There was no significant difference in AMF spores between organic matter and control. The highest value MD in the A_2 treatment (42%) and the lowest was in A_7. AMF structure (vesicles) in roots plants were served in Fig. 1.

Nutrient content and accumulation: Ca and Mg concentration were higher in shoots of *P. mooniana* inoculated with A_0 and B_0. There was no difference in shoot Mg concentration of *P. mooniana* between A_0B_0, A_2B_0, A_3B_0, A_5B_0 and A_7B_0 (Table 5). *P. mooniana* seedlings inoculated A_2

Table 2: Effect of treatments on *P. mooniana* seeding growth after 5 months

Treatments	Height (cm)	Diameter (mm)	Total leaves (helai)	Leave length (cm)	Leave width (cm)	Total root nodules
AMF of inoculum (A)						
A_0	15.5±0.42^b	3.08±0.07	9.7±0.78	7.6±0.47^b	4.9±0.33^b	9.9±2.90^{bc}
A_1	17.7±0.66^a	3.23±0.06	11.5±1.17	9.4±0.77^a	6.1±0.44^a	16.7±3.53^a
A_2	17.1±0.71^a	3.30±0.12	12.5±1.12	9.2±0.82^a	6.1±0.45^a	10.7±2.03^b
A_3	17.4±0.91^a	3.27±0.08	12.2±1.24	8.8±0.79^a	5.9±0.45^a	12.2±2.98^b
A_4	17.7±0.91^a	3.37±0.08	12.1±1.24	8.9±0.79^a	5.7±0.45^a	15.9±2.98^a
A_5	17.8±0.65^a	3.40±0.09	11.3±0.92	9.5±1.11^a	6.0±0.52^a	7.7±1.83^c
A_6	17.2±0.70^a	3.20±0.10	11.1±1.07	8.5±0.63^{ab}	5.7±0.42^a	11.7±2.51^b
A_7	16.9±0.72^{ab}	3.23±0.08	11.4±0.93	8.4±0.53^{ab}	5.5±0.34^{ab}	11.0±2.33^b
Organic material (B)						
B_0	15.6±0.29^b	3.08±0.03^b	8.6±0.42^b	6.2±0.20^b	4.2±0.13^b	3.3±0.48^b
B_1	18.0±0.36^a	3.31±0.06^a	13.1±0.60^a	10.4±0.33^a	6.7±0.16^a	17.8±1.05^a
B_2	17.9±0.40^a	3.39±0.06^a	12.7±0.46^a	9.8±0.22^a	6.3±0.13^a	14.7±1.16^b
Pr>F						
A	*	tn	tn	*	**	*
B	**	**	**	**	**	**
A*B	tn	tn	tn	tn	tn	tn

Average value followed by unequal letters in the same column differs significantly at the 0.05 DMRT test level. Factor AMF Inoculum (A) = A_0: Control, A_1: AMF KG, A_2: AMF UHO, A_3: AMF Vale, A_4: AMF CA, A_5: AMF HA, A_6: AMF BJ and A_7: AMF IPB. Factor organic matter (B) = B_0: Control, B_1: Soil, compost, sand-2:1:1, B_2: Soil, compost, sand, rice husk-2:1:1:1, * : Significant, ** : Very Significant, tn : Not significant

Table 3: Effect of treatments on dry weight *P. mooniana* plant after 5 months

	Dry weight (g)				
Treatments	Shoots	Root	Root nodule	Total dry weight (g)	Shoot-root ratio
Inoculum of AMF (A)					
A_0	0.62±0.049^{b}	0.24±0.008	0.040±0.010	0.91±0.057^{b}	2.2±0.169^{c}
A_1	1.17±0.043^{a}	0.28±0.010	0.047±0.009	1.51±0.048^{a}	3.4±0.165^{ab}
A_2	1.21±0.046^{a}	0.32±0.015	0.040±0.009	1.57±0.058^{a}	3.3±0.171^{ab}
A_3	1.08±0.041^{a}	0.29±0.015	0.047±0.009	1.41±0.053^{a}	3.2±0.159^{ab}
A_4	1.16±0.176^{a}	0.26±0.025	0.052±0.008	1.47±0.199^{a}	3.7±0.299^{a}
A_5	1.04±0.191^{ab}	0.29±0.028	0.051±0.007	1.39±0.218^{ab}	3.0±0.315^{ab}
A_6	1.01±0.198^{ab}	0.26±0.027	0.045±0.007	1.32±0.225^{ab}	3.3±0.343^{ab}
A_7	0.82±0.198^{ab}	0.26±0.026	0.039±0.007	1.12±0.225^{ab}	2.8±0.341^{bc}
Organic material (B)					
B_0	0.57±0.031^{b}	0.22±0.013^{b}	0.022±0.004^{b}	0.82±0.038^{b}	2.4±0.120^{b}
B_1	1.22±0.113^{a}	0.31±0.024^{a}	0.055±0.005^{a}	1.59±0.135^{a}	3.3±0.204^{a}
B_2	1.25±0.074^{a}	0.29±0.017^{a}	0.060±0.004^{a}	1.59±0.090^{a}	3.6±0.166^{a}
Pr>F					
A	*	tn	tn	*	**
B	**	**	**	**	**
A*B	tn	tn	tn	tn	tn

Average value followed by different letters in the same column differs significantly at the 0.05 DMRT test level. Factor AMF Inoculum (A) = A_0: Control, A_1: AMF KG, A_2: AMF UHO, A_3: AMF Vale, A_4: AMF CA, A_5: AMF HA, A_6: AMF BJ and A_7: AMF IPB. Factor organic matter (B) = B_0: Control, B_1: Soil, compost, sand-2:1:1, B_2: Soil, compost, sand, rice husk-2:1:1:1, * : Significant, ** : Very Significant, tn : Not significant

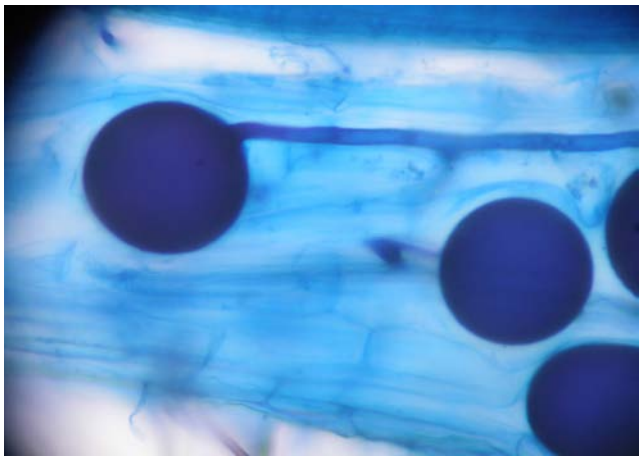

Fig. 1: AMF structure (vesicles) in roots *Pericopsis mooniana* (Thw.) plants

and enrichment material organic (B_1) were not different with these all AMF treatment (not control) and B_1 and B_2 in shoots Ca accumulation of *P. mooniana* (Table 5). AMF colonization did not increased shoots C, N, P and K level of *P. mooniana* (Table 6). Inoculation AMF-KG (A_1) increased shoot N accumulation of *P. mooniana*. There was no differences N accumulation between A_7 and control. Inoculation A_2 increased shoots Mg accumulation than A_6 and control (Table 6). Organic material enrichment increased shoots N concentration and C, N, P, K, Mg accumulation of *P. mooniana* plant. By contrast, organic material did not increased shoots C, P and K concentration of *P. mooniana* (Table 6).

Heavy metal transport factor (TF): TF of heavy metals is presented in Table 7 and it shows that TF<1 in Fe, Cd and Pb and TF>1 on Cr and Mn, except for each A_3 in Cr and A_4 in Mn.

DISCUSSION

Arbuscular Mycorrhizal Fung inoculation improves the growth of the 5 month-old *P. mooniana*. The good growth of mycotrophic plants is highly characterized by improvements in nutrient uptake and water[18,28,29] and reduction of abiotic stresses such as heavy metals[6,30] and soil properties[31] by mycorrhizal fungi. The results are consistent with some previous researchers where inoculation AMF can improve plant growth on the coal soil media among the plants of *Zea mays*[16,32], onion[33,34] and *Ropogon virginicus* and *Plantago lanceolata*[14] and *A. fruticosa*[17].

The results showed that AMF-KG (A_1) and AMF-CA (A_4) increased the number of root nodules compared to other treatments. In heavy metal stress conditions, AMF is also reported to increase root nodulation[6]. High growth and nodulation growth was also followed by increased shoot and total dry weight *P. mooniana* after 5 month in planting with AMF-KG, UHO, Vale and CA treatments. Increased plant dry weight was also reported on *Zea mays*[16], *Trifolium repens*[35], four species of trees[36] and *Medicago sativa*[13], *Paraserianthes falcataria*[18] on coal mine soil media. The effectiveness of AMF inoculum can be influenced by AMF type. Muleta and Woyessa[23] reported that the effect of fungal strains on plant

Table 4: Effect of treatment on SQI and number of AMF spores

AMF inoculum	SQI	MD	Total spore
A_0	0.124^{b}	-	0^{c}
A_1	0.163^{ab}	39.7	50^{a}
A_2	0.181^{a}	42.0	41^{ab}
A_3	0.164^{ab}	35.5	41^{ab}
A_4	0.163^{ab}	38.1	38^{ab}
A_5	0.164^{ab}	34.5	32^{b}
A_6	0.153^{ab}	31.1	43^{ab}
A_7	0.141^{ab}	18.8	47^{ab}
Organic material			
B_0	0.109^{b}		40^{a}
B_1	0.183^{a}		38^{a}
B_2	0.178^{a}		31^{a}
Pr>F			
A	tn		**
B	**		tn
A*B	tn		tn

Average value followed by different letters in the same column differs significantly at the 0.05 DMRT test level. Factor AMF Inoculum (A) = A0: Control, A_1: AMF KG, A_2: AMF UHO, A_3: AMF Vale, A_4: AMF CA, A_5: AMF HA, A_6: AMF BJ and A_7: AMF IPB. Factor organic matter (B) = B_0: Control, B_1: Soil, compost, sand-2:1:1, B_2: Soil, compost, sand, rice husk-2:1:1:1, *: Significant, **: Very Significant, tn: Not significant

growth in mining media varies by ecotype. In general, the levels of C, N, P, K at the shoots of the plant were not affected by AMF inoculum treatments, but the C, N, Mg uptake at the shoots of the plant is highest in the AMF treatment and there is a tendency for the highest accumulation of P at the inoculum AMF treatment. There is a tendency of content and accumulation of C, N, P, Ca and Mg at the highest peak of the plant on AMF treatment. Inoculation of the mixture of AMF (*Glomus mosseae* (BEG95), *G. claroideum* (BEG96) and *G, intraradices* did not affect the uptake of P *Linum usitatissimum* grown on coal mine soil-bank[37]. The influence of colonization on N, K, Ca, Mg in this study may be the largest dilution effect on the shoot as AMF mechanism for nutrient uptake efficiency on N, Ca, Mg shoot[38] and K[39].

The enrichment of coal post-mining media with organic matter can increase growth, dry weight and number of root and Seed Quality Index (SQI). The increase in plant growth is attributed to the presence of organic material on the media and also can contribute to the improvement of soil structure, facilitate root penetration, nutrient availability for growth, microbial activity[3]. Plant growth on organic matter enriched media can be linked to nutrient supply for plants. Levels of N and accumulation of C, N, P, K and Mg at the shoot were highest in the treatment of organic matter. Larney and Angers[2], the addition of organic materials can increase the production of plant biomass and soil amelioration. Juwarkar and Jambhulkar[40] mentioned that the addition of amendment and biofertilizer application can support plant

Table 5: Effect of interaction of AMF inoculum and organic material treatments on AMF colonization, Ca and Mg concentration and accumulation of Ca on shoot tissue *P. mooniana* after 5 months

	AMF	Level (%)		Accumulation (mg plant^{-1})
	colonisation	----------	----------	----------
Treatments	(%)	Ca total	Mg total	Ca
A_0				
B_0	0.0^{c}	1.08^{a}	0.87^{a}	0.46^{b}
B_1	0.0^{c}	0.68^{ab}	0.39^{b}	0.53^{b}
B_2	0.0^{c}	0.57^{b}	0.36^{b}	0.46^{b}
A_1				
B_0	17.3^{ab}	0.65^{ab}	0.59^{b}	0.37^{b}
B_1	16.4^{ab}	0.54^{b}	0.34^{b}	0.69^{ab}
B_2	23.3^{ab}	0.63^{ab}	0.44^{b}	0.69^{ab}
A_2				
B_0	21.1^{ab}	0.79^{ab}	0.62^{ab}	0.47^{b}
B_1	10.8^{b}	0.87^{ab}	0.43^{b}	1.08^{a}
B_2	24.5^{ab}	0.58^{b}	0.51^{b}	0.60^{ab}
A_3				
B_0	17.0^{ab}	0.87^{ab}	0.67^{ab}	0.36^{b}
B_1	21.2^{ab}	0.55^{b}	0.39^{b}	0.67^{ab}
B_2	15.3^{ab}	0.52^{b}	0.39^{b}	0.76^{ab}
A_4				
B_0	14.5^{ab}	0.59^{b}	0.57^{b}	0.35^{b}
B_1	10.5^{ab}	0.53^{b}	0.35^{b}	0.68^{ab}
B_2	16.4^{ab}	0.64^{ab}	0.41^{b}	0.60^{ab}
A_5				
B_0	15.8^{ab}	0.97^{ab}	0.89^{a}	0.50^{b}
B_1	26.7^{a}	0.61^{b}	0.38^{b}	0.65^{ab}
B_2	22.6^{ab}	0.39^{b}	0.38^{b}	0.65^{ab}
A_6				
B_0	9.1^{b}	0.59^{b}	0.53^{b}	0.42^{b}
B_1	10.2^{b}	0.53^{b}	0.37^{b}	0.62^{ab}
B_2	24.1^{ab}	0.53^{b}	0.36^{b}	0.60^{ab}
A_7				
B_0	12.5^{ab}	0.61^{b}	0.68^{ab}	0.60^{ab}
B_1	15.5^{ab}	0.68^{ab}	0.46^{b}	0.49^{b}
B_2	14.2^{ab}	0.97^{ab}	0.54^{b}	0.63^{ab}

Average value followed by different letters in the same column differs significantly at the 0.05 DMRT test level. Factor AMF Inoculum (A) = A_0: Control, A_1: AMF KG, A_2: AMF UHO, A_3: AMF Vale, A_4: AMF CA, A_5: AMF HA, A_6: AMF BJ and A_7: AMF IPB. Factor organic matter (B) = B_0: Control, B_1: Soil, compost, sand-2:1:1, B_2: Soil, compost, sand, rice husk-2:1:1:1

growth in coal mine soil dump. In line with the review of Mukhopadhyay and Maiti[41], high level of P and N can suppress the development of AMF.

Mechanism used by *P. mooniana* plants aged 5 months adapts to the stress condition is heavy metal rhizofiltration technique (TF<1) and phytoextraction (TF>1). In this study, TF<1 was found in Fe, Cd and Pb and TF>1 elements in Cr and Mn, except for each in AMF-Vale and AMF-CA. This indicated that the plant is able to translocate Cr and Mn to the shoot, although AMF-Vale and AMF-CA limited both elements are transported to the shoots. The ability to live in metal contamination conditions is suspected to be due to the type of heavy metal detoxification mechanism (Fe, Cd and Pb) in

Table 6: Effect of treatments on the concentration and accumulation of nutrients on *P. mooniana* plant shoots

	Level (%)				Accumulation (mg plant^{-1} dry weight)				
Treatments	C	N	P	K	C	N	P	K	Mg
Inoculum AMF (A)									
A_0	38.1	2.2	0.56	2.01	23.83^{b}	1.44^{c}	0.35	1.28	0.31^{c}
A_1	39.9	2.7	0.48	2.20	45.52^{a}	3.22^{a}	0.58	2.98	0.49^{ab}
A_2	38.9	2.5	0.48	1.58	46.79^{a}	2.86^{ab}	0.51	1.76	0.58^{a}
A_3	38.2	2.3	0.56	2.63	42.78^{a}	2.44^{ab}	0.55	2.59	0.47^{ab}
A_4	38.0	2.5	0.42	2.00	44.42^{a}	3.02^{ab}	0.50	2.45	0.48^{ab}
A_5	40.9	2.4	0.45	1.47	43.53^{a}	2.47^{ab}	0.45	1.47	0.47^{ab}
A_6	38.7	2.5	0.53	1.83	39.51^{a}	2.63^{ab}	0.57	1.99	0.41^{bc}
A_7	38.8	2.4	0.55	2.81	31.71^{ab}	2.08^{bc}	0.49	2.51	0.45^{ab}
Organic material (B)									
B_0	39.3	2.1^{b}	0.50	2.06	22.64^{b}	1.19^{b}	0.28^{b}	1.15^{b}	0.38^{b}
B_1	39.2	2.6^{a}	0.46	1.96	48.26^{a}	3.21^{a}	0.56^{a}	2.47^{a}	0.47^{a}
B_2	38.4	2.5^{a}	0.55	2.18	48.38^{a}	3.15^{a}	0.66^{a}	2.78^{a}	0.53^{a}
Pr>F									
A	tn	tn	tn	tn	*	**	tn	tn	*
B	tn	*	tn	tn	**	**	**	**	*
A*B	tn	tn	tn	tn	tn	tn	tn	tn	tn

Average value followed by different letters in the same column differs significantly at the 0.05 DMRT test level. Factor AMF Inoculum (A) = A_0: Control, A_1: AMF KG, A_2: AMF UHO, A_3: AMF Vale, A_4: AMF CA, A_5: AMF HA, A_6: AMF BJ and A_7: AMF IPB. Factor organic matter (B) = B_0: Control, B_1: Soil, compost, sand-2:1:1, B_2: Soil, compost, sand, rice husk-2:1:1:1, * : Significant, ** : Very Significant, tn : Not significant

Table 7: Heavy metal transport factor

AMF inoculum	Fe	Cd	Cr	Pb	Mn
A_0	0.058	0.39	2.2	0.54	2.81
A_1	0.061	0.38	1.1	0.50	2.04
A_2	0.042	0.36	1.0	0.50	1.06
A_3	0.038	0.34	0.6	0.42	1.41
A_4	0.060	0.29	2.1	0.48	0.89
A_5	0.061	0.31	1.1	0.45	1.98
A_6	0.071	0.28	2.5	0.49	1.91
A_7	0.067	0.33	3.2	0.55	1.58

Factor AMF Inoculum (A) = A_0: Control, A_1: AMF KG, A_2: AMF UHO, A_3: AMF Vale, A_4: AMF CA, A_5: AMF HA, A_6: AMF BJ and A_7: AMF IPB

the form of production of root exudate as metal chelating has the ability to store metal in the roots. The root exudates in question are amino acids (histidine) and carboxylic acids (citrate)[42,43]. Husna *et al.*[6] also reported that this type of hardwood performs a rhizofiltration mechanism against Ni elements. Arbuscular Mycorrhizal fungi (AMF) local improved growth and nutrient uptake of *P. mooniana* 5 month seedlings in post-coal mining media. AM fungi and organic matter enrichment should be used for reforestation of post-open cast coal mine sites. AM fungi local is potential to be developed as biological fertilizer.

CONCLUSION

Arbuscular Mycorrhizal Fungl inoculums isolated from *P. mooniana* rhizosphere enhance the growth of 5 month seedlings in post-coal mining media. This plant type performs phytoremediation mechanisms by rhizofiltration and phytoextraction. The presence of AMF limited the metal level in the roots and shoots and addition of organic matter is very vital for the quality of the plant.

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SIGNIFICANCE STATEMENT

Arbuscular Mycorrhizal Fungl is essential for post-coal mining land restoration. During the initial growth phase of the plant under environmental stress conditions, AMF can promote growth and development. AMF inoculum and *P. mooniana* interaction may be developed through physiological and structural mechanisms. On low nutrients media such as coal mine soils, addition of organic matter is very vital for the quality of the plant.

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