

# Positive interaction of *trembesi* (*Samanea saman*) and arbuscular mycorrhizae fungi in Pb stabilization of gold-mine tailing media

LULUK SETYANINGSIH<sup>1,\*</sup>, FILDZAH AISYAH RAMADHANI<sup>1</sup>, ZAINAL MUTTAQIN<sup>1</sup>,  
MAMAY MASLAHAT<sup>2</sup>

<sup>1</sup>Faculty of Forestry, Universitas Nusa Bangsa. Jl. Kh. Sholeh Iskandar KM. 4, Tanah Sareal, Bogor 16166, West Java, Indonesia. Tel.: +62-753-3189, Fax.: +62-834-0217, \*email: luluk.setya03@gmail.com

<sup>2</sup>Faculty of Mathematic and Natural Science, Universitas Nusa Bangsa. Jl. Kh. Sholeh Iskandar KM. 4, Tanah Sareal, Bogor 16166, West Java, Indonesia

Manuscript received: 18 November 2023. Revision accepted: 30 January 2024.

**Abstract.** Setyaningsih L, Ramadhani FA, Muttaqin Z, Maslahat M. 2024. Positive interaction of *trembesi* (*Samanea saman*) and arbuscular mycorrhizae fungi in Pb stabilization of gold-mine tailing media. *Biodiversitas* 25: 379-385. This experiment aimed to determine the ability of *trembesi* (*Samanea saman* Merr.) seedlings inoculated with AMF (*Glomus manihotis*) to reduce Pb from gold mine tailings media. Therefore, using plants and Arbuscular Mycorrhizae Fungi (AMF) is an alternative choice that can be considered to support a phytoremediation program to reduce Pb contamination in tailing areas. The experiment was conducted using a completely randomized design in a greenhouse with two factors, i.e., with and without AMF, and four different Pb treatments (0, 0.5, 1, and 1.5 mM). AMF-inoculated seedlings were grown on tailings media and exposed to Pb in different concentrations for 5 weeks. The results showed that the seedlings were still able to grow on the media with Pb up to 1.5 mM with a tolerance index of 91.6%. AMF induced the plant to accumulate Pb dramatically higher, even though this absorption tended to reduce plant biomass. Root tissue stored significantly higher Pb than stem and leaf tissue, with an average concentration was 526.29 mg/kg in the roots of mycorrhizal seedlings. The bioaccumulation level of Pb in mycorrhizal seedlings was significantly higher. The value of the transport factor was below 1, indicating that *S. saman* seedlings carried out the phytostabilization. The interaction of AMF with *S. saman* has a big potential to be applied in efforts to remediate Pb in tailings.

**Keywords:** Arbuscular mycorrhizal fungi, lead, phytoremediation, tailings, *trembesi*

## INTRODUCTION

One of the environmental problems that appears from gold mining activities is the large number of wastes called tailings which can potentially contain heavy metal contaminants. Soil-based tailings have very low organic matter content, dominated by sand with low clay, and higher heavy metal content, especially lead (Pb) (Hilmi et al. 2018). Revegetation on tailings land is not solely aimed at increasing land cover with plants, but plants planted in the area are also expected to be able to reduce contaminants from the tailings.

Phytoremediation is a technology that uses selected plants to clean harmful contaminants from soil and water effectively (Sarwar et al. 2017), which aligns with current sustainability principles (Grifoni et al. 2022). The implementation of phytoremediation has been developing, using the plant solely and involving other organisms in the environment, such as bacteria and fungi, known as microbe-assisted phytoremediation (Ashraf et al. 2017). Several phytoremediation mechanisms may occur to remove organic as well as inorganic contaminants, which include phytostabilization, rhizofiltration, phytoextraction, phytodegradation, and phytovolatilization (Sarwar et al. 2017). Considering that plants are the main agents as metal recipients in phytoremediation, some efforts to obtain hyperaccumulator plants are very important. The plants with the right characteristics for phytoremediation are: (i)

fast-growing, (ii) higher biomass, (iii) hyperaccumulators of heavy metals, (iv) have wide distribution, (v) translocate metals from roots to shoots, (vi) resistant to heavy metals, (vii) resistant to pathogens and pests, (viii) adapts well to prevailing climatic conditions, (ix) easy to cultivate and harvest, and (x) do not attract herbivores to avoid entry into the food chain (Sarwar et al. 2017).

*Trembesi* (*Samanea saman* Merr.) is a fast-growing and spreading tree both in tropical and sub-tropical countries (Heyne 1987). *S. saman* can grow in shallow and nutrient-poor soils, is tolerant of acid soils, and can grow in acidic to alkaline soil with little nutrient content (Staples and Elevitch 2006). Therefore, *S. saman* can be tried as a revegetation plant in media with extreme characteristics, such as tailings, while simultaneously carrying out remediation activities against contaminants often contained in tailings.

Moreover, utilizing microbes such as mycorrhizal fungus to improve plant growth is among the key successes in phytoremediation programs (Sarwar et al. 2017). Arbuscular Mycorrhizal Fungi (AMF) are obligate biotrophic microorganisms that are symbiotic mutualists with most flowering plants (Ferrol et al. 2016). AMF was reported to be able to increase plant growth under gold mine tailing, such as *Thypha angustifolia* and *Anthocephalus cadamba* (Setyaningsih et al. 2017), and increase the accumulation of heavy metals such as Cu, Pb, Zn, Fe in plant root and shoot tissues (Ferrol et al. 2016; Salim et al. 2022). The mechanism

of AMF's role in phytoremediation includes stabilizing metals in mycorrhizal roots and accumulating them in the intraradical structures of the fungus rather than in root cells (Wu et al. 2016; Ferrol et al. 2016). In many cases, AMF can stimulate plant growth because metal concentration levels decrease due to improving phosphorus nutrition. However, a decrease in the phytotoxicity of certain metals in certain plants may occur while the concentration of heavy metals in these plants is still high (Ferrol et al. 2016).

Arbuscular mycorrhizae of the *Glomus*, *Gigaspora*, and *Acaulospora* genera are reported to be found in association with plants growing on soils polluted by heavy metals (Ferrol et al. 2016; Leal et al. 2016; Salim et al. 2019). *Glomus* is a genus that is often used to help accumulate heavy metals. However, the compatibility between plant species, AMF types, plant environmental conditions, and the types of contaminants in these associations can influence the effectiveness of the phytoremediation role (Ferrol et al. 2016).

This study aimed to investigate the tolerance of *S. saman* seedlings infected with Arbuscular Mycorrhiza Fungi, *Glomus manihotis*, and their ability to remediate Pb in gold mine tailings media.

## MATERIALS AND METHODS

### Study area

The research was conducted in a greenhouse, chemical, and forestry laboratory at Universitas Nusa Bangsa, Bogor, Indonesia. The tailing material was obtained from PT ANTAM Tbk. Business Unit of Gold Mining, Pongkor, Bogor, Indonesia

### Procedures

#### Preparation of seedling media

Tailings obtained from the Pongkor gold mine with the following characteristics: dominated by sand (60%), essential Pb content 17-56 ppm, organic matter content 0.24%, pH 8, N content 0.02% and available P 6 ppm, Mg 0.73 cmol/Kg (Setyaningsih et al. 2023). Tailings were mixed with soil (25%) and compost (15%) by volume and put in polybags. Pb treatment on tailings was carried out once by adding 50 ml of  $\text{Pb}(\text{NO}_3)_2$  solution with concentrations of 0.5, 1.0, and 1.5 mM into the planting medium when the seedlings were a week old.

#### Seed preparation, mycorrhizal inoculation, and maintenance of seedlings

*Samanea saman* seeds were germinated in zeolite media for 15 days, and then the sprouts were transferred or planted in tailings media in polybags. AMF inoculant, *Glomus manihotis*, was cultivated singly from mixed inoculant (Mycofer- collected by Forest Biotech Laboratory, PPSHB IPB University) in bioassay for 3 months using sorghum as a host plant and zeolite as a carrier. AMF was inoculated 2 times, namely at the time of germination by mixing 50 g with 500 g of germination medium and when planting in tailings media in polybags by adding 10 g (equivalent to 150 spores) to each planting

hole. Seedling was watering 1-2 times a day, depending on needs.

#### Measurement of seedling biomass

After five weeks of planting, or the normal time for seedlings to transfer to the field, the plants were harvested and dried in an oven at 70°C to constant weight for approximately 36 hours. Seedling biomass was observed using analytical balance.

#### Measurement of Pb content in Samanea saman tissue

Plant material from roots, stems, and dry leaves were weighed to obtain 0.5 g for sample measurement. The sample was then crushed with the addition of  $\text{HNO}_3$  and  $\text{HClO}_4$ . Pb levels were observed with ICP-OES Agilent Technologist type 700 (Agilent Technologies 2018)

### Data analysis

The biomass and Pb content data were collected to obtain total biomass, index tolerance on the plant, factor of bioconcentration, and factor of transport, which are very important to determine plant capacity in the phytoremediation process with the formula below.

#### Total biomass of seedlings (TB) (Setyaningsih et al. 2021)

TB = Dry weight of roots + Dry weight of stem + Dry weight of leaves ..... (1)

#### Index of Tolerance (IT) (Chen et al. 2016)

IT = (Dry weight of root with Pb treatment / Dry weight of root without Pb treatment) x 100% ..... (2)

#### Factor of Bioconcentration (FB) (Yang et al. 2015)

FB = Pb concentration of plant tissue (root or shoot) / Pb concentration on media (tailing) ..... (3)

#### Factor of Transport (FT) (Yang et al. 2015)

FT = Pb concentration of plant shoot / Pb concentration of plant root ..... (4)

#### Bio-accumulation (BA) (Yang et al. 2015):

BA = Pb concentration of plant part x dry weight of plant part ..... (5)

#### Remediation effectivity (RE) (Setyaningsih et al. 2021)

RE = [(Pb concentration on media with AMF - Lead concentration on media without AMF): Pb concentration on media without AMF] X 100% .....(6)

### Experimental design

The experimental design used a completely randomized factorial design with two factors. The first factor was Pb treatment comprised of four treatments, i.e., 0, 0.5, 1, and 1.5 mM of Pb, and the second factor was AMF treatment, i.e., with and without AMF, and all units consisted of 4 replications. Data were analyzed using the software SPSS 16 and Microsoft Excel to analyze the different treatment means using ANOVA analysis followed by Duncan analysis.

## RESULTS AND DISCUSSION

### Biomass and tolerance index

Based on analysis of variance, single and interaction treatments of Pb and AMF caused significant differences ( $p < 0.05$ ) in the dry biomass variables of *S. saman* tissue, roots, stems, leaves, and plants, as well as the Tolerance Index variable. Five weeks after planting, the total dry biomass of *S. saman* seedlings grown in tailings media ranged from 7.8 to 16.3 g. The average dry biomass of mycorrhizal seedlings (+AMF: 10.4 g) was significantly ( $p < 0.05$ ) lower than that of seedlings without AMF (-AMF: 14.6 g), reaching 28.7%. Biomass in the roots (4.3 g) was higher than that in leaves (2.7). Likewise, based on biomass, the tolerance index value decreased significantly, up to 28.6%, in mycorrhizal seedlings (+AMF: 77.24 g) (Table 1).

### Pb levels in plant tissue

Based on analysis of variance, single and interaction treatments of Pb and AMF caused significant differences ( $p < 0.05$ ) in the Pb concentration of *S. saman* roots and stems. However, there was no significant Pb concentration difference ( $p < 0.05$ ) in seedling leaf. Pb levels in five-week-old *S. saman* seedlings grown on tailings media varied among the treatments in the range of 1.15-1349.9 mg/kg. Increasing Pb concentration applied to tailings media caused a significant increase of Pb levels in *S. saman* seedling tissues. The average Pb level in the roots of *S. saman* seedlings was 798.31 mg/kg, significantly higher ( $p < 0.05$ ) than in the stem, 86.65 mg/kg, and in the leaves, 2.69 mg/kg. Pb levels in the roots and stems of mycorrhizal seedlings (+AMF) were 526.29 mg/kg and 66.92 mg/kg, which significantly increased ( $P < 0.05$ ) up to 15 times in roots and 4 times in stem of seedlings as compared to those without AMF (Table 2).

### Factor of Bioconcentration (FB) and Factor of Transport (FT)

The transport of Pb from the media to the plant tissue is indicated by the value of the Factor of Bio-concentration (FB), and the transport of Pb in the aerial plant tissue is indicated by the value of the Factor of Transport (FT). The average FB value of *S. saman* seedlings exposed to lead for 5 weeks in tailings media was generally smaller than one ( $FB < 1$ ) or in the range of 0.02-2.85, with the FB value in root tissue higher than in other tissues. Mycorrhizal seedlings (+AMF) increased their FB value, but the FT values appeared inconsistent, sometimes higher or lower, in several different Pb exposure treatments in different parts of the seedling tissue. However, the FT value was less than one ( $FT < 1$ ) or ranged from 0.12-0.48, with the stem FT value being higher than the leaf FT (Figure 1).

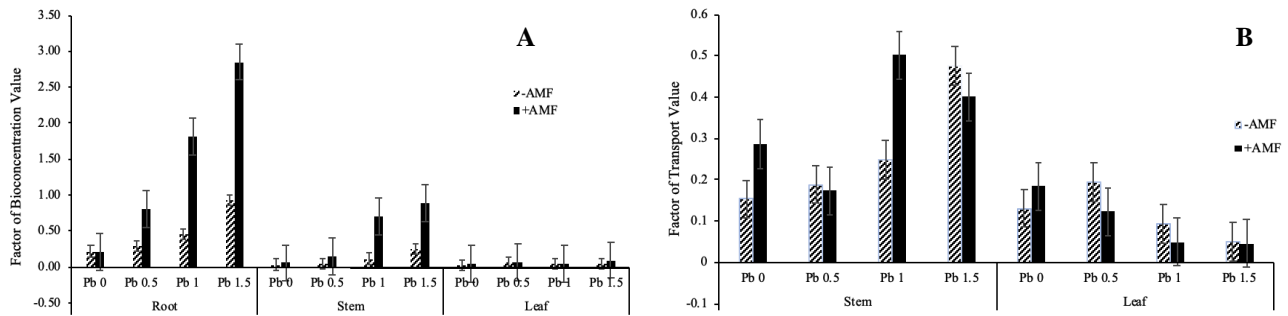
**Table 2.** Pb concentration on *Samanea saman* seedling tissue for 5 weeks exposure in tailing media

Treatment		Root	Stem	Leaf
Micorrhyza	Pb exposure	(mg/kg)		
-AMF	Pb0	25.69 e*	3.52 f*	1.19 f*
	Pb0.5	19.03 e	5.25 f	1.15 f
	Pb1	47.78 d	10.66 f	5.82 f
	Pb1.5	41.86 d	36.04 d	2.68 f
+AMF	Pb0	13.48 e	4.78 f	2.76 f
	Pb0.5	108.15 c	19.34 e	2.18 f
	Pb1	633.62 b	83.65 c	2.65 f
	Pb1.5	1349.90 a	159.93 c	3.10 f
-AMF		33.59 d	13.87 f	2.71 f
+AMF		526.29 b	66.92 d	2.67 f
Total		798.31 b	86.65 c	2.69 f

**Table 1.** Dry biomass and tolerance index of *Samanea saman* five weeks after planting with AMF application on tailing media and Pb exposure

Treatment		Dry biomass (g)				Tolerance index
AMF	Pb exposure	Root	Stem	Leaf	Plant	(%)
-AMF	Pb 0	4.6 ab*	5.6 bcd*	3.1 b*	13.2 c*	100 b*
	Pb 0.5	6.2 bc	6.7 cc	3.4 b	16.3 d	123.15 bc
	Pb 1	5.3 ab	6.4 c	3.6 b	15.2 d	115.35 b
	Pb 1.5	5.5 bc	5.2 abc	3.1 b	13.7 d	100.64 b
+AMF	Pb 0	4.1 ab	3.7 ab	2.6 ab	10.4 bc	77.24 ab
	Pb 0.5	6.2 ab	6.7 ab	3.4 b	10.9 bc	77.88 ab
	Pb 1	4.6 ab	4.8 abc	3.1 b	12.5 bc	92.17 b
	Pb 1.5	3.0 a	3.2 a	1.6 a	7.8 a	54.73 a
-AMF		5.5 b	5.7 b	3.2 b	14.6 d	108.18 b
+AMF		4.3 a	4.3 a	2.7 a	10.4 bc	77.24 ab

Note: The same letter in the same column indicates no significant difference in the error rate of 5% by the DMRT test

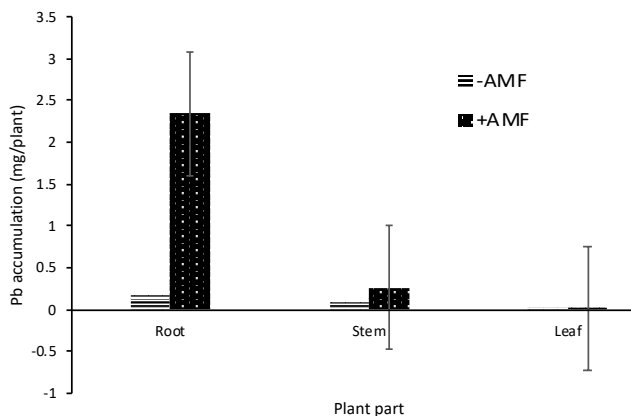


**Figure 1.** The factor of bioconcentration (FB) (Figure A) and factor of transport (FT) (Figure B) for *Samanea saman* seedlings aged five weeks in tailings media exposed to Pb with AMF application

**Table 3.** Lead Bioaccumulation on part of *Samanea saman* seedling tissue for 5 weeks with Pb exposure on tailing media

Treatment		Bioaccumulation (mg/plant)			
AMF	Pb	Root	Stem	Leaf	Total
-AMF	Pb 0	0.1182 c	0.0197 b	0.0037 a	0.1416 c
	Pb 0.5	0.1180 c	0.0352 bc	0.0039 a	0.1571 c
	Pb 1	0.2532 c	0.0682 bc	0.0210 b	0.3424 c
	Pb 1.5	0.2260 c	0.1874 c	0.0083 ab	0.4218 c
+AMF	Pb 0	0.0539 bc	0.0177 b	0.0072 ab	0.0788 bc
	Pb 0.5	0.6705 cd	0.0735 bc	0.0074 ab	0.7515 cd
	Pb 1	2.9146 d	0.4015 cd	0.0082 ab	3.3244 d
	Pb 1.5	4.0497 d	0.5118 cd	0.0050 ab	4.5664 d
Average -AMF		0.1805 c	0.0829 bc	0.0089 ab	0.2723 c
Average +AMF		2.3420 d	0.2593 c	0.0071 ab	2.6084 d

Note: The same letter in the same column indicates no significant difference in the error rate of 5% by the DMRT test



**Figure 2.** Pb accumulation in *Samanea saman* seedling tissue 5 weeks old with AMF application

**Table 4.** Pb concentration on tailing media after five weeks remediation by mycorrhizal *Samanea saman* seedling

Exposure Pb (mM)	Pb concentration in tailing media (mg/kg)		Efficiency (+AMF to -AMF) (%)
	Without AMF	AMF	
Pb 0	57.98 a*	61.06 a*	5.23
Pb 0.5	114.86 b	64.53 a	-43.82
Pb 1	171.43 c	95.08 b	-44.53
Pb 1.5	178.73 c	118.53 b	-33.68

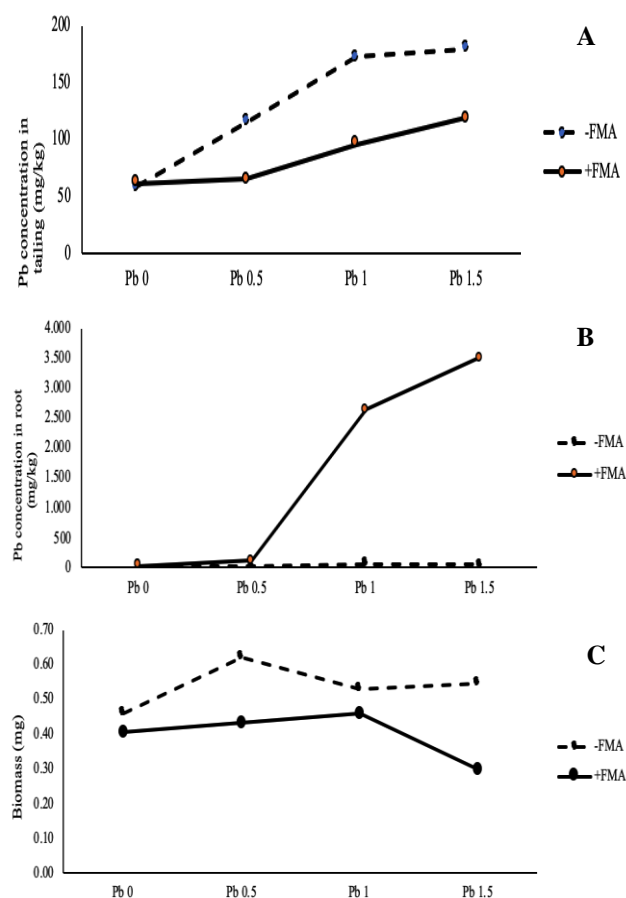
Note: The same letter indicates no significant difference in the error rate of 5% by the DMRT test

### Bioaccumulation

Based on analysis of variance, single and interaction treatments of Pb and AMF caused significant differences ( $p < 1.05$ ) in the Pb accumulation of *S. saman* roots and stems. However, there was no significant difference in the Pb accumulation in *S. saman* seedling leaves. The accumulation of Pb in the tissues of seedlings, roots, stems, or leaves of five weeks old *S. saman* grown on tailings media varied from 0.0037 mg/plant to 4.0497 mg/plant. The most considerable accumulation of Pb was in the root, while the most minor was in leaf. The total accumulation of Pb in tissues ranged from 0.14 mg/plant to 4.57 mg/plant. The accumulation of total Pb in *S. saman* seedlings infected with AMF (2.608 mg/plant) was dramatically significant ( $p < 0.05$ ) than those without AMF (0.2723 mg/plant), with an increase of up to 10 times (Table 3), particularly in root and leaf tissues of seedling (Figure 2).

### Remediation of Pb in tailings media

Based on analysis of variance, single and interaction treatments of Pb and AMF caused significant differences ( $p < 0.05$ ) in Pb concentration on tailing media. Remediation of Pb in tailings media by mycorrhizal seedlings was significantly higher ( $p < 0.05$ ) than remediation by seedlings without AMF. After 5 weeks of treatment, Pb levels in the media of the plant inoculated by AMF decreased significantly with the range of 61-118.5 mg/kg or significantly lower by 33-45%, compared to that without AMF (57.98-178 mg/kg), except on tailing without exposed Pb (Pb0) (Table 4).



**Figure 3.** Correlation of seedling biomass (A), Pb content in media (B), and root (C) with AFM and Pb exposure treatment. RF0: Pb content of seedlings without AMF; RF1: Pb content of seedlings with AMF; MF0: Pb content of tailings media without AMF; MF1: Pb content of tailings media with AMF

## Discussion

*Samanea saman* seedlings grown in the tailing media exposed to Pb up to 1.5 mM (equivalent to 450 ppm) within five weeks were alive and grew normally with upright stems and green leaves. The tolerance index of *S. saman* did not differ significantly with an increase in lead exposure, but it decreased due to the application of AMF, with the largest TI reaching 123.15%. The tolerance index value for *S. saman* seedlings was lower than that of *Acacia mangium* (TI172%) (Winata 2019) and *Anthocephalus cadamba* (TI 77%), which were exposed to Pb 450 mg/kg (Setyaningsih et al. 2018). Meanwhile, *S. saman* had higher Pb accumulation than both species (*A. mangium* and *A. cadamba*).

Plant tolerance to heavy metal exposure is closely related to its physiological adaptability, which can be recognized by the limitation of the metal absorption mechanism known as an external strategy and by detoxification of heavy metals or internal strategy (Nas and Ali 2018). Accumulation of organic acid is among the important mechanisms to bind heavy metals into a harmless form of substances for plants (Setyaningsih et al. 2021;

Collin et al. 2022). *Samanea saman* seedling was reported to be able to secrete oxalic acid and accumulate citric acid as part of their tolerance mechanism to Pb (Setyaningsih et al. 2021) and Fe toxicity (Salim et al. 2022).

The addition of Pb expose to tailings media caused a significant increase in Pb levels in *S. saman* seedling tissues. even with an increase reaching 9914% in the roots, 3246% in the stems and 12.3% in the leaves of the AMF-infected *S. saman* with exposure Pb 1.5 mM (Table 2). Several researchers have reported the same cases in other species following the principle of Pb absorption by plants, such as in *Eichhornia crassipes* (Malar et al. 2016), as well as in *Anthocephalus cadamba* (Setyaningsih et al. 2017). *S. saman* seedlings were detected to store the highest levels of Pb in the roots, which was calculated to be up to 10 times higher than the stems or leaves. Similar cases have also been reported for several other forest plants, such as *Anthocephalus cadamba*, which accumulated up to 60 ppm (Setyaningsih et al. 2017; Setyaningsih et al. 2018). The ability of plant tissues to store heavy metals is strongly influenced by the anatomical, physiological, and structural properties of metal-accumulating tissues, the physicochemical characteristics of metal ions, and the attribution of plants as excluders or accumulators (Collin et al. 2022). There is a tendency for plant organs to store heavy metals in their body with such different concentrations, i.e., roots>leaves>stems (Nas and Ali 2018; Setyaningsih et al. 2017). Plant roots can provide a higher surface for absorbing or depositing metal contaminants (Hamim et al. 2018).

The increasing Pb concentration in the tissue of *S. saman* infected with AMF is suspected due to stimulation of Pb uptake facilitated by fungal mycelia. A similar result was also reported in *A. cadamba* seedling tissue infected with AMF on compost media (Setyaningsih et al. 2017). However, Kanwal et al. (2016) observed a different result where Zn metal decreased in the tissues of AMF-inoculated wheat. The differences in AMF inoculation on absorbing heavy metals are probably due to the differences in the origin of AMF, where AMF originating from contaminated areas tends to be able to tolerate the presence of heavy metals (Zhang and Chen 2021). The metal stabilization in the roots of mycorrhizal plants and their accumulation were present in the intraradical structures of the fungus rather than in the root cells (Wu et al. 2016; Ferrol et al. 2016).

The relationship between Pb content in seedling roots and the media and the biomass of *S. saman* seedlings with and without AMF inoculation is clearly illustrated the interrelated influence (Figure 3). AMF infection significantly reduced Pb levels in tailings media up to 44% (114 mg/kg Pb without AMF and 64.52 mg/Kg with AMF), which was also indicated by an increase considerably Pb content in tissues of seedling up to 11 times (33.59 mg/kg without AMF, 526.29 mg/kg with AMF). Consequently, increasing Pb levels in *S. saman* tissue inoculated by AMF caused a negative effect, i.e., reducing the biomass of *S. saman* seedlings up to 28%. The decreased growth and increased Pb concentration in plant tissue have also been reported in other species, such as in Philippine tung exposed to 3 mM of Pb (Hamim et al. 2019).

This showed that the decrease in the growth of *S. saman* seedlings was closely related to Pb levels inside the tissues. The higher concentration of Pb in plant tissues exceeding a certain threshold would cause inhibition of plant growth. Growth inhibition of plants exposed to lead is thought to disturb photosynthetic activity as a result of the replacement of Mg elements in chloroplasts by Pb or due to the inhibition of chlorophyll synthesis, which consequently caused stunted growth (Bharwana et al. 2014; Hamim et al. 2019).

Furthermore, AMF plays a significant role in Pb remediation of tailings media using *S. saman* seedlings. There was a small decrease in the biomass of seedlings aged 2 months with an increase in Pb levels due to AMF infection, but this decrease did not affect the physical condition of the plants, so the plants still have a life and no sign of heavy metal toxicity. Therefore, this remediation role is expected to continue as the growth of the AMF seedlings progresses to the next phase.

Pb transport from the tailing medium to the *S. saman* seedling can be tracked with the BF and TF values of the seedlings. AMF infection has increased Pb seedlings' BF to more than 1. This suggests that AMF has encouraged the transfer of Pb from the media to the plant tissue. Because the TF value was still below 1 ( $TF < 1$ ), AMF infection did not change the tendency of *S. saman* seedlings to store Pb from below ground to aerial part, so Pb was stored higher in the roots compared to the stems and leaves. Thus, *S. saman* seedlings can be categorized as metal phytostabilizer. Considering the distribution of Pb in the tissue sections of the seedlings (roots, stems, and leaves), it appears that *S. saman* seedlings accumulate the most significant amount of Pb in the roots, reaching almost 90%, and in the stems reaching 8.9%. Pb accumulation by mycorrhizal seedlings reached 2.6084 mg/plant, 10 times greater than without AMF. The high capacity of plants to store contaminants in their roots is common among some species, which indicates that these plants have a phytostabilization mechanism in phytoremediation (Ferrol et al. 2016; Singh et al. 2022). Several species, such as *Anthocephalus cadamba* (Setyaningsih et al. 2017), *Brassica carinata*, *B. juncea*, and *Oryza sativa* (Tangahu et al. 2011), have been reported to accumulate the most significant amount of Pb in their roots. Phytostabilization is a phytoremediation mechanism generally carried out in plants with a strong root system (Sarwar et al. 2017).

The role of AMF in increasing the ability of *S. saman* seedlings to absorb Pb in the roots by phytostabilize mechanism is estimated to have several processes where metal may be stored in the AMF structure in plant roots (vacuoles, coils, or arbuscules) or through compounds secreted by fungi and absorbed in the cell walls, chelation of metal ions in the cytosol, compartmentation in vacuoles, activation of antioxidant defenses, stabilization with poly-P and translocation to spores (Ferrol et al. 2016). The association of *S. saman* with AMF has been shown to increase Pb remediation in gold mine tailings media which caused Pb decrease from tailings media by up to 45% for 5 weeks. This result is a good combination that can be

recommended for a phytoremediation program in the future.

In conclusion, *trembesi* (*Samanea saman* Merr.) seedlings were able to grow well on gold mine tailings exposed to high concentrations of Pb, suggesting that *S. saman* have good adaptation to the tailings. During the experiment, Pb was absorbed into the tissues, which was mostly stored in the roots up to 1349.90 mg/kg, and within 5 weeks, Pb accumulated up to 4 mg/plant without disturbing the growth of the seedlings. Application of AMF, *Glomus manihotis*, stimulated the increase of Pb uptake in root tissue, which increased Pb remediation from the tailings. The result suggests that the combination of AMF and *S. saman* has the potential in revegetation activities and remediation of heavy metals in contaminated tailings land.

## ACKNOWLEDGEMENTS

Appreciation and gratitude are conveyed to the Ministry of Education and Culture, Republic of Indonesia, Directorate General of Higher Education, Research and Technology for research funding support; to PT Antam Tbk Bussines Unit of Gold Mining Pongkor, Indonesia for the support of research materials; and to Forest Biotech Laboratory, Center for Research on Biological Resources and Biotechnology of IPB University, Bogor, Indonesia.

## REFERENCES

- Agilent Technologies, Inc. 2018. ICP-OES Agilent Technologist tipe 700- Manual Part Number 8510230100 Edition 8, June 2018. Agilent Technologies, Inc. [https://www.agilent.com/cs/library/usermanuals/public/8510230100\\_700SeriesICP\\_UserManual.pdf](https://www.agilent.com/cs/library/usermanuals/public/8510230100_700SeriesICP_UserManual.pdf).
- Ashraf MA, Hussain I, Rasheed R, Iqbal M, Riaz M, Arif MS. 2017. Advances in microbe-assisted reclamation of heavy metal contaminated soils over the last decade: A review. *J Environ Manag* 198 (1): 132-143. DOI: 10.1016/j.jenvman.2017.04.060.
- Bharwana SA, Ali S, Farooq MA, Iqbal N, Hameed A, Abbas F, Ahmad MSA. 2014. Glycine betaine-induced lead toxicity tolerance related to elevated photosynthesis, antioxidant enzymes suppressed lead uptake and oxidative stress in cotton. *Turk J Bot* 38 (2): 281-292. DOI: 10.3906/bot-1304-65.
- Chen H, Yuan X, Li T, Hu S, Ji J, Wang C. 2016. Characteristics of heavy metal transfer and their influencing factors in different soil-crop systems of the industrialization region, China. *Ecotoxicol Environ Saf* 126: 193-201. DOI: 10.1016/j.jecoenv.2015.12.042.
- Collin S, Baskar A, Geevarghese DM, Ali MNVS, Bahubali P, Choudhary R, Lvov V, Tovar GI, Senatov F, Koppala S, Swamiappan S. 2022. Bioaccumulation of lead (Pb) and its effects in plants: A review. *J Hazard Mater Lett* 3: 100064 DOI: 10.1016/j.hazl.2022.100064.
- Ferrol N, Tamayo E, Vargas P. 2016. The heavy metal paradox in arbuscular mycorrhizas: From mechanisms to biotechnological applications. *J Exp Bot* 67 (22): 6253-6265. DOI: 10.1093/jxb/erw403.
- Grifoni M, Franchi E, Fusini D, Vociante M, Barbaferi M, Pedron F, Rosellini I, Petruzzelli G. 2022. Soil remediation: Towards a resilient and adaptive approach to deal with the ever-changing environmental challenges. *Environments* 9 (2): 18. DOI: 10.3390/environments9020018.
- Hamim, Hanifatunisa, Hadisunarso, Setyaningsih L, Saprudin D. 2019. Lead (Pb) toxicity effect on physio-anatomy of bead-tree, jatropa, castor bean and Philippine-tung grown in water culture. *Biodiversitas* 20 (12): 3690-3697. DOI: 10.13057/biodiv/d201231.

- Hamim H, Miftahudin M, Setyaningsih L. 2018. Cellular and ultrastructure alteration of plant roots in response to metal stress. In: Ratnadewi D, Hamim (eds). *Plant Growth and Regulation Alterations to Sustain Unfavorable Conditions*. IntechOpen, London. DOI: 10.5772/intechopen.79110.
- Heyne K. 1987. *Tumbuhan Berguna Indonesia* (Indonesia Important Plant). Badan Penelitian dan Pengembangan Departemen Kehutanan Indonesia, Jakarta. [Indonesian]
- Hilmi M, Hamim H, Sulistyaningsih YC, Taufikurrahman T. 2018. Growth, histochemical and physiological responses of non-edible oil producing plant (*Reutealis trisperma*) to gold mine tailings. *Biodiversitas* 19 (4): 1294-1302. DOI: 10.13057/biodiv/d190416.
- Kanwal S, Bano A, Malik RN. 2016. Role of arbuscular mycorrhizal fungi in phytoremediation of heavy metals and effects on growth and biochemical activities of wheat (*Triticum aestivum* L.) plants in Zn contaminated soils. *Afr J Biotechnol* 15 (20): 872-883. DOI: 10.5897/AJB2016.15292.
- Leal PL, Varón-López M, Prado IGAO, dos Santos JV, Soares CRFS, Siqueira JO, Moreira FMdS. 2016. Enrichment of arbuscular mycorrhizal fungi in a contaminated soil after rehabilitation. *Braz J Microbiol* 47 (4): 853-862. DOI: 10.1016/j.bjm.2016.06.001.
- Malar S, Shivendra Vikram S, JC Favas P, Perumal V. 2016. Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [*Eichhornia crassipes* (Mart.)]. *Bot Stud* 55 (1): 1-11. DOI: 10.1186/s40529-014-0054-6.
- Nas FS, Ali M. 2018. The effect of lead on plants in terms of growing and biochemical parameters: A review. *MOJ Ecol Environ Sci* 3 (4): 265-268. DOI: 10.15406/mojes.2018.03.00098.
- Salim MA, Setyaningsih L, Wahyudi I, Budi SW. 2022. Growth response of three forest seedling to iron exposure. *IOP Conf Ser: Earth Environ Sci* 959 (1): 012051. DOI: 10.1088/1755-1315/959/1/012051.
- Salim MA, Budi SW, Setyaningsih L, Iskandar, Kirmi H. 2019. Diversity of arbuscular mycorrhizal fungi as affected by time consequences revegetation age in post coal mine area at PT Berau Coal Tbk, East Kalimantan Indonesia. *IOP Conf Ser: Earth Environ Sci* 394 (1): 012067. DOI: 10.1088/1755-1315/394/1/012067.
- Sarwar N, Imran M, Shaheen MR, Ishaque W, Kamran MA, Matloob A, Rehim A, Hussain S. 2017. Review phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives. *Chemosphere* 171: 710-721. DOI: 10.1016/j.chemosphere.2016.12.116.
- Singh BSM, Singh D, Dhal NK. 2022. Enhanced phytoremediation strategy for sustainable management of heavy metals and radionuclides. *Case Stud Chem Environ Eng* 5: 100176. DOI: 10.1016/j.csee.2021.100176.
- Setyaningsih L, Setiadi Y, Budi SW, Hamim, Sopandie D. 2017. Lead accumulation by jabon seedling (*Anthocephalus cadamba*) on tailing media with application of compost and arbuscular mycorrhizal fungi. *IOP Conf Ser: Earth Environ Sci* 58: 012053. DOI: 10.1088/1755-1315/58/1/012053.
- Setyaningsih L, Maslahat M, Syahrizki MA. 2021. Adaptation pattern of trembesi (*Samanea saman* Jacq. Merr.) seedling to lead exposure at nutrient culture based on the characteristics of organic acid. *IOP Conf Ser: Earth Environ Sci* 733 (1): 012033. DOI: 10.1088/1755-1315/733/1/012033.
- Setyaningsih L. 2023. Reforestation in the reclamation area of Pongkor Gold Mining. *J Sains Natural* 13 (4): 212-220. DOI: 10.31938/jsn.v13i4.649.
- Staples GW, Elevitch CR. 2006. *Samanea saman* (rain tree), ver. 2.1. In: Elevitch CR (eds). *Species Profiles for Pacific Island Agroforestry*. Permanent Agriculture Resources (PAR). Hōlualoa, Hawai'i.
- Tangahu BV, Sheikh Abdullah SR, Basri H, Idris M, Anuar N, Mukhlisin M. 2011. A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *Intl J Chem Eng* 2011: 939161. DOI: 10.1155/2011/939161.
- Winata B, Wasis B, Setiadi Y. 2019. Effect of lead on the growth and adaptability of *Acacia mangium* seedlings in gold-tailings media. *J Silviculture Tropika* 10 (2): 119-124. DOI: 10.29244/j-siltrop.10.2.119-124.
- Wu S, Zhang X, Chen B, Wu Z, Li T, Hu Y, Sun Y, Wang Y. 2016. Chromium immobilization by extraradical mycelium of arbuscular mycorrhiza contributes to plant chromium tolerance. *Environ Exp Bot* 122: 10-18. DOI: 10.1016/j.envexpbot.2015.08.006.
- Yang Y, Liang Y, Ghosh A, Song Y, Chen H, Tang M. 2015. Assessment of arbuscular mycorrhizal fungi status and heavy metal accumulation characteristics of tree species in a lead - zinc mine area: Potential application for phytoremediation. *Environ Sci Pollut Res* 22: 13179-13193. DOI: 10.1007/s11356-015-4521-8.
- Zhang C, Chen Z. 2021. Advances in study on the effects of arbuscular mycorrhizal fungi on plants in remediation of arsenic-contaminated soi. *Acta Agric Scand B Soil Plant Sci* 71 (9): 825-834. DOI: 10.1080/09064710.2021.1952300.