Phytoremediation of Mercury Contaminated Soils by *Swietenia macrophylla* Inoculated with Arbuscular Mycorrhizal Fungi (*Acaulospora* sp.)

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Abstract. The high mercury (Hg) content in tailings from artisanal gold mining areas requires efforts to reduce or overcome the effects of contaminants through remediation. A widely used bioremediation technique in developing countries is phytoremediation because it is considered efficient and can be applied in the long term. Phytoremediation can run more effectively with Arbuscular Mycorrhizal Fungi (AMF) support. This study aimed to analyze the ability of *S. macrophylla* inoculated with AMF in Hg phytoremediation. The experimental design used in this study was a split-plot design. The treatment tested was AMF inoculation, which consisted of six levels: Without AMF, *Acaulospora* sp.1, *Acaulospora* sp.2, *Acaulospora* sp.3, *Acaulospora* sp.4, and *Acaulospora* sp.5. The planting medium used was tailings from artisanal gold mining areas which contained 42.61 ppm Hg. *S. macrophylla* leaves inoculated with *Acaulospora* sp. 4 have the ability to absorb Hg greater than plants inoculated with other types of AMF. The leaves of plants inoculated with AMF type *Acaulospora* sp. 4 absorbed 28.65% more Hg than the control treatment. *S. macrophylla* plants inoculated with *Acaulospora* sp. 4 produced the highest percentage of AMF colonization with plants inoculated with other types of AMF.

1 Introduction

The enormous environmental impact of gold mining activities occurs due to the chemical processes used to extract gold [1]. Gold mining results in the accumulation of heavy metals from the processing of gold ore and the disposal of tailings and waste around the mining area [2]. Gold ore from mining is processed by crushing and screening. This processing leaves waste rock in the form of tailings containing heavy metals, especially Hg, Pb, and As, which can be released into the environment [3]. One of the environmental pollutions caused by gold mining and processing activities occurred in Bunut Village, Way Ratai District, Pesawaran Regency, Lampung Province. This is because in this place there are smallholder gold mining processing activities without any effort to reduce pollutants.

The high content of Hg in tailings from gold mine processing requires an effort to reduce or overcome the effects of contaminants through remediation. Remediation is necessary because heavy metals can accumulate in sediments and soil for a long time [4]. One of the remediation techniques is bioremediation which is an effort to remove contaminants using natural biological activity. The bioremediation technique that is widely used in developing countries is phytoremediation because it is considered efficient and can be applied in the long term [5]. Phytoremediation is an effort to extract contaminants using plants.

The type of plant is important to consider in carrying out phytoremediation. Plant species for remediation should be local plants because they adapt better to polluted environments than exotic plants [6]. Plants in phytoremediation should be hyperaccumulator plants that can translocate very high concentrations of contaminants to plant tissues without causing abnormal growth (stunting and poisoning) [7]. Based on the above considerations, this study used *S. macrophylla* plants. This plant is a pioneer plant that is able to grow on marginal land and support the natural succession process, so it is widely used in rehabilitation activities.

One of the functions of pioneer plants is to stabilize the macro and micro ecosystem conditions around the former mining area [8]. *S. macrophylla* plants used to rehabilitate the former nickel mine area in Sorowako, East Luwu Regency have a survival percentage of 95% at the age of 12 months [9]. The

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selection of plant species in this study was also based on the ability of the two plants to grow in Hg-contaminated media. *S. macrophylla* plants can adapt to Hg-polluted tailings growing media [10]. *S. macrophylla* plants were found around the gold mine processing site in Bunut Village, Way Ratai District, Pesawaran Regency, Lampung Province. In addition, the types of plants chosen are also not plants that produce fruit or plants used for animal feed. This is to avoid Hg entering the body of living things.

Phytoremediation can run more effectively with Arbuscular Mycorrhizal Fungi (FMA) support. This means that FMA can support the phytoremediation acceleration process [11]. Acceleration is a term used for increasing speed [12]. Mycorrhiza is able to increase the growth ability of plants growing on marginal land, such as land polluted with heavy metals [13]. The presence of FMA benefits host plants that grow in soil conditions that lack nutrients and contain heavy metals. This research aims to analyze the ability of *Swietenia macrophylla* inoculated with FMA in phytoremediating Hg.

2 Method

2.1 Research Area

Tailings were collected from artisanal gold mining area, Bunut Village, Way Ratai District, Pesawaran Regency, Lampung Province. Seeding and inoculation of AMF on seedlings were carried out in the greenhouse of the Faculty of Agriculture, University of Lampung. Measurement of seedling biomass and observation of AMF colonization were carried out at the Plant Science Laboratory, Faculty of Agriculture, University of Lampung. Hg content testing on leaves was conducted at the Iskandar Zulkarnain Tanjung Bintang BRIN Science Area, Lampung.

2.2 Procedures

2.2.1 Testing of Hg content in tailings

Tailings were taken from the artisanal gold mining area. Hg content was tested using the Direct Thermal Decomposition-Gold Amalgamation-CVAAS.

2.2.2 Germinating of S. macrophylla seeds

S. macrophylla seeds were germinated on merang paper media. *S. macrophylla* seedlings were weaned 14 days after germination.

2.2.3 Weaning and AMF Inoculation

S. macrophylla seedlings were weaned on planting media in pots. The experimental design used in this study was a split-plot design. The main factor is AMF, which consists of six levels: Without AMF, *Acaulospora* sp.1, *Acaulospora* sp.2, *Acaulospora* sp.3, *Acaulospora* sp.4, and *Acaulospora* sp.5 (Fig. 1).

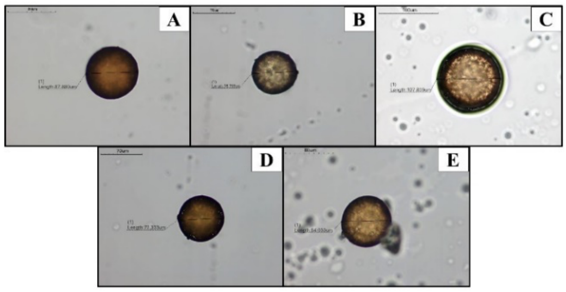

Fig. 1. AMF spores inoculated to plants *S. macrophylla*. A. *Acaulospora* sp. 1, B. *Acaulospora* sp. 2, C. *Acaulospora* sp. 3, D. *Acaulospora* sp. 4, and E. *Acaulospora* sp. 5.

The AMF spores used come from the roots of plants in artisanal gold mining areas. The five spores belong to the genus Acaulospora because they have a single arrangement, a round shape, a size of 40 – 400 μm, and the interior reacts with Melzer. The characteristic that distinguishes these five types of AMF found is the color of their spores, allowing us to determine that these five types of AMF are different species (molecular identification needs to be carried out). *Acaulospora* sp. 1 has an orange color, *Acaulospora* sp. 2 has a hyaline color, *Acaulospora* sp. 3 has a yellowish-brown color, *Acaulospora* sp. 4 has a brown color, and *Acaulospora* sp. 5 has a yellow color.

The planting medium used is tailings from gold mine processing, which has a Hg content of 42.61 ppm. Each treatment was repeated 3 times. AMF inoculation was done by placing 50 AMF spores of each type near the seedling roots. A tray was placed at the bottom of the pot so that Hg would not be washed away, and AMF contamination of other plants would not occur. The seedlings were then placed in the greenhouse for 3 months.

2.2.4 Plant maintenance

Seedling maintenance is done by manual watering and pest control. Watering was done every 2 days as much as 100 ml.

2.2.5 *Observation of AMF colonization on seedlings*

AMF colonization was observed using the root staining method [14]. The stages of the method include: 1) Fresh roots are selected from each growing vegetation, 2) The roots were then washed thoroughly using distilled water, 3) The roots were soaked in 20% KOH solution for 48 hours, 4) The roots were then washed using a sieve under running water until clean, 5) The roots were soaked in 0.1 M HCl solution without washing, 6) The roots were then soaked in aniline blue dye solution for 48 hours, 7) Roots were soaked in destaining solution for 24 hours, 8) Roots were cut along 1 cm as many as 10 then arranged parallel to the object glass and covered with a cover slip. Preparations that have been made are then observed using a compound microscope. The presence of hyphae, vesicles, or arbuscules indicates colonization. Percent colonization is determined based on the category [15].

$$\% \text{ colonized roots} = \frac{\sum colonized\ field\ of\ view}{\sum total\ field\ of\ view} \times 100\% \quad (1)$$

2.2.6 *Testing of Hg content in leaves*

Hg content was tested in plant tissues after the seedlings were 3 months old. Hg content testing was carried out using the Direct Thermal Decomposition method.

3 Result and Discussion

3.1 Hg content in tailings

Tailings were taken at the location of the artisanal gold mining area. Based on the test results, the Hg content in the tailings was 42.61 ppm. This content is much higher than the threshold, which, according to PP No. 22 of 2021 concerning the Implementation of Environmental Protection and Management, the Hg threshold in the soil is 0.05 ppm [16]. The release of heavy metal Hg into the environment has negative health impacts, such as damage to the nervous system in adults and neurodevelopmental disorders in infants and children through consuming contaminated foodstuffs [17]. High Hg content can also reduce soil fertility and cause plant poisoning [18].

3.2 Hg Uptake in *S. macrophylla* Leaves

Table 1 shows the Hg content in *S. macrophylla* leaves inoculated with FMA types *Acaulospora* sp. 1, *Acaulospora* sp. 2, *Acaulospora* sp. 4, and *Acaulospora* sp. 5 absorbed greater Hg than plants that were not inoculated with AMF. This is in accordance with the statement that AMF inoculation to phytoremediation plants can accelerate the remediation through increasing plant biomass and improving soil physical, chemical and biological properties. The symbiosis of *Lolium perenne* with mycorrhiza helps the absorption of Hg, proven by the greater accumulation of Hg in the root tissue than in the soil [19]. The application of mycorrhiza in the process of phytoremediation of land polluted with heavy metals is also known as mycorrhizoremediation [20].

Table 1. Hg content in *S. macrophylla* leaves

Treatment	Hg content (ppm)	Percentage increase (%)
A0	0,13999	0,00
A1	0,21441	0,53
A2	0,24954	0,78
A3	0,07708	-0,45
A4	4,15140	28,65
A5	2,36192	15,87

A0: Without AMF, A1: *Acaulospora* sp. 1, A2: *Acaulospora* sp. 2, A3: *Acaulospora* sp. 3, A4: *Acaulospora* sp. 4, A5: *Acaulospora* sp. 5.

Hg can accumulate in leaves through a root translocation process [21]. Translocation from the roots will absorb Hg from contaminated soil. Heavy metals are absorbed by plants in the form of water-soluble ions [22]. Plant roots will form regulatory proteins in the form of phytocelatins as binding compounds. When this regulatory protein binds to Hg, a complex compound will be formed that causes Hg to be translocated into plant tissues. This Hg translocation is through the generating tissues, namely the xylem and phloem. The process of Hg translocation to all plant tissues causes the concentration of Hg in the roots to decrease and the accumulation of Hg in the leaves to increase. Leaves exposed to mercury change color from green to yellow to brown because the element Mg has been replaced by Hg, damaging the chloroplast structure [21]. Leaves can also absorb heavy metals from atmospheric deposition in the plant canopy [23].

The leaves of *S. macrophylla* plants inoculated with FMA with the type of *Acaulospora* sp.4 have the ability to absorb Hg the most compared to plant leaves inoculated with other types of FMA. Plant leaves inoculated with FMA with *Acaulospora* sp.4 were able to absorb 28.65% more Hg than the control treatment. This is because FMA has the ability to accumulate heavy metals and increase the availability of nutrients for plants [24, 25]. FMA is able to increase the availability and translocation of various nutrients, especially P, due to the presence of an external hyphal network [26, 27]. Phytoremediation carried out through plant association with FMA can be done by two methods, namely phytoextraction and phytostabilization [24]. Phytostabilization is the absorption, deposition, and reduction of heavy metals in the root system without accumulation in the shoots [28, 29]. Phytoextraction is a phytoremediation method that facilitates heavy metal uptake by roots and transport to the shoots. Heavy metals can be lost with the harvesting of the plants. The ability of FMA to remediate heavy metals can occur through the following mechanisms [24]: 1) Immobilization of metals in the mycorizosphere with glomalin, 2) Metal accumulation in fungal structures such as vesicles or arbuscules, and 3) Metal adsorption by extraradical hyphae.

Hg-containing leaves, if they fall to the ground, can cause the soil to be contaminated with Hg. Animals that eat fallen leaves can also accumulate Hg. This is because Hg can move to higher trophic levels in the food chain. Based on this, leaves and other parts of plants used for phytoremediation need to be harvested and then biologically treated to break down contaminants before disposal. In addition, harvesting and burning of phytoremediation plants can also be done.

3.3 Percentage of AMF Colonization on *S. macrophylla* Roots

Table 2 shows the percentage of AMF colonization. Observation of the percentage of mycorrhizal colonization can be determined by the presence of internal hyphae, vesicles, and arbuscules in plant roots [30, 31]. Internal hyphae are hyphae in cortex cells that will develop into vesicles and arbuscules. Vesicles are places to store food. Arbuscules serve as a place of contact and transfer of mineral nutrients. Figure 2 shows the structure of AMF: 1. Internal hyphae, 2. Vesicles, and 3. Arbuscule inside the colonized root.

Table 2. Percentage of AMF colonization

Treatment	Percentage of AMF colonizatio n (%)	Category
A0	0,00	Not colonized
A1	6,67	Low
A2	13,33	Low
A3	6,67	Low
A4	16,67	Low
A5	13,33	Low

A0: Without AMF, A1: *Acaulospora* sp. 1, A2: *Acaulospora* sp. 2, A3: *Acaulospora* sp. 3, A4: *Acaulospora* sp. 4, A5: *Acaulospora* sp. 5.

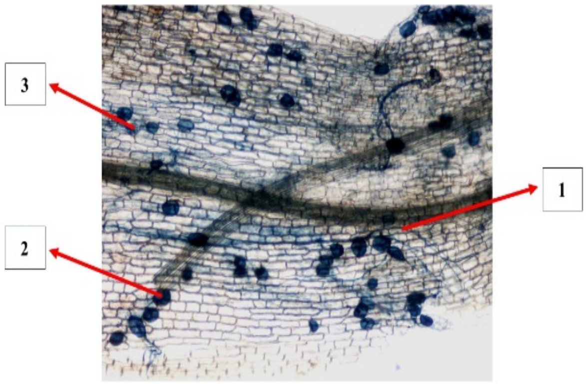

Fig. 2. Structure of AMF. 1. Internal hyphae, 2. Vesicles, and 3. Arbuscule.

The observation of the percentage of FMA colonization showed that *S. macrophylla* plants without FMA treatment produced a colonization percentage of 0.00% (not colonized). This shows that in this study, there was no contamination from the treatment with FMA to the treatment without FMA. *S. macrophylla* plants inoculated with FMA with the types of *Acaulospora* sp. 1, *Acaulospora* sp. 2, *Acaulospora* sp. 3, *Acaulospora* sp. 4, and *Acaulospora* sp. 5 produced a low percentage of colonization. Heavy metal content in soil can affect FMA infection in roots because high heavy metal content will inhibit FMA mycelia diffusion and spore germination [32]. This is in accordance with the results of the study, which stated that the greater the concentration of Hg, the lower the FMA colonization. This is because Hg will inhibit root development, reducing the contact area between roots and FMA [33]. The higher the heavy metal concentration, the less root length and root development [34]. *S. macrophylla* plants inoculated with FMA type *Acaulospora* sp. 4 produced the highest percentage of colonization at 16.67%. This indicates that *Acaulospora* sp. 4 has a better symbiotic ability compared to other types of FMA inoculated.

4 Conclusion

S. macrophylla plants have the ability to be used in Hg phytoremediation. The leaves of plants inoculated with AMF with *Acaulospora* type 4 were able to absorb 28.65% more Hg than the treatment without AMF inoculation. *S. macrophylla* plants inoculated with AMF with *Acaulospora* sp. 1, *Acaulospora* sp. 2, *Acaulospora* sp. 3, *Acaulospora* sp. 4, and *Acaulospora* sp. 5 produced a low percentage of colonization. *Acaulospora* sp. 4 produced the highest percentage of AMF colonization compared to the control treatment and other types of AMF, namely 16.67%.

The authors expressed their gratitude to Indonesia Endowment Funds for Education (LPDP) and Center for Higher Education Funding (BPPT) for funding support. The authors also would like to thank The Chief of Bunut Village, Pesawaran Regency, Lampung for the permission to do research in the artisanal gold mining area.

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