



# Effects of Mycorrhizae on Phytoremediation of Soil Contaminated with Small-Scale Gold Mine Tailings Containing Mercury

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**Abstract** – A study that was aimed to explore the effects of mycorrhizae inoculation on the potential of local tree species (*Duabanga moluccana*, *Paraserianthes falcataria*, and *Erythrina orientalis*) for phytoremediation of soils contaminated with small-scale gold mine tailings containing mercury was conducted in a glasshouse. Each of the plant seedlings was planted in a plastic pot containing 10 kg of tailing-contaminated soil. Treatments tested were three plant species, and doses of mycorrhizal inoculation, i.e. 0 and 30 spores/ plant. At harvest of nine weeks, shoots and roots were analyzed for mercury concentration. The remaining soils in the pots were used for growing maize for eight weeks. The results showed that the most potential plant species for phytostabilization was *P.falcataria*, while the most mercury tolerant plant was *D.moluccana*. Without mycorrhizal inoculation, the highest accumulation of mercury (73.51mg/kg) was found in the root of *P.falcataria*. If the mycorrhizae were inoculated, the highest accumulation of mercury was found in the root of *D. moluccana*. Results of the second experiment proved that the growth and biomass production of maize after myco-phytoremediation by the three plant species were higher than those of maize grown on media without myco-phytoremediation.

**Keywords** – Mycorrhizae, Local Tree Species, Myco Phytoremediation, Phytostabilization, Soil Contaminated.

## I. INTRODUCTION

Indonesia is considered as the main location for the small gold mining activities (ASGM). In 2010, there were about 900 ASGM spots in Indonesia, which cover approximately 250,000 miners and about 1 million populations depend on this sector [1]. In most of the ASGM in Indonesia, generally amalgamation process with mercury followed by cyanidation process is used to recover gold [2]. One of ASGM sites is located in Sekotong District of West Lombok. Wastes of the amalgamation and cyanidation processes in the form of sludge that still contain Hg and various other heavy metals, are generally discharged to agricultural land and water bodies. Results of a survey conducted by [3] at ASGM locations in Sekotong District of West Lombok showed that on average the amalgamation tailings contains 3,002 mg Hg / kg, while the gold cyanidation tailings contain 1,628 mg Hg / kg. These high Hg contents in the tailings led to the increasing Hg content in soils contaminated by small-scale gold mine tailings. A sustainable technology that promises to restoration of metal contaminated soil is phytoremediation [4].

Phytoextraction and phytostabilization are the most widely used technique in phytoremediation of heavy metal contaminated soil. Because the long-term accumulation of metals in aboveground plant biomass may pose a risk of

transfer to the food chain, phytostabilization can be a more viable approach than phytoextraction for the management of heavy metal contaminated sites [5]. The use of native plants is the focus of phytostabilization. Because of many species of native plants that have adapted to the contaminated conditions, then the best way for the selection of the best species is through observation of native plant species that can grow near heavy metal contaminated area [6]. Previous studies reported that in areas contaminated by tailings generated from gold cyanidation processes at Sekotong District of West Lombok there were at least 28 species of trees that have long to adapt and survive in extreme conditions (high metal concentration) [7]. Among them, three species (*Duabanga moluccana*, *Paraserianthes falcataria*, and *Erythrina orientalis*) are candidates for phytostabilization strategy based on the tolerance to heavy metals (Cd, Pb, Cu, and Zn).

Actually, the plant does not solely do phytostabilization since there is always the interaction between microorganisms in the rhizosphere that led to increased activity associated with the remediation [8]. Utomo et al. [9] reported that *Duabanga moluccana*, *Paraserianthes falcataria*, and *Erythrina orientalis* found in the ASGM locations at Sekotong District of West Lombok were in association with *Glomus aggregatum*, *Glomus deserticola*, *Glomus geosporum*, *Glomus leptotichum*, and *Glomus mossaeae*. This suggests that the association of mycorrhizae with the three plant species can be further developed for myco-phytoremediation. Arbuscular mycorrhizal fungi (AMF) are important endophytes that live in the roots of most terrestrial plants. This symbiosis directly benefits plant growth through the acquisition of phosphorus and other nutrients from the soil. In addition, the fungus can also increase plant resistance to biotic and abiotic stresses [10]. Arbuscular mycorrhizal fungi also play an important role in reducing the influence of heavy metal stress on plants [11].

Arbuscular mycorrhizal fungi (AMF) can reduce metal stress on host plants or improve plant growth through a variety of ways. Production and excretion of organic compounds (e.g. citrate and oxalate) can improve the dissolution of phosphate mineral, which is one of essential nutrients for plants [12]. On the other hand, the increased solubility of metals or metal complexation through mycosphere acidification can enhance the uptake of metals by plants that it is very important in phytoextraction. Metal complexation occurs through glomalin, i.e. metal absorber glycoprotein produced by the AMF and biosorption into the cell wall constituent such as chitin and chitosan [12].



External mycelium of AMF cause more breadth exploitation of the soil volume that can be reached by the roots [13] [14], thus increasing access to heavy metals in the rhizosphere. In addition to the above, AMF can improve plant growth on heavy metals contaminated soil [15] due to improved supply of nutrients [16] [17], the availability of water [18] and the improvement of soil aggregation [19] [20]. This study was aimed to explore the effects of mycorrhizae inoculation on the potential of tree species (*D. moluccana*, *P. falcataria*, and *E. orientalis*) for phytoremediation of soils contaminated with small-scale gold mine tailings containing mercury at Sekotong District of West Lombok, Indonesia.

## II. MATERIALS AND METHODS

### A. Experiment 1: Effects of Mycorrhizae Inoculation on Phytoremediation of Hg by Three Plant Species

The study was conducted in a glasshouse STPP Malang from June to December 2014. The three dominant tree species tolerant of contamination of gold cyanidation tailings reported by Utomo et al. [9], namely *D.moluccana*, *P.falcataria*, and *E. orientalis*, were planted in plastic pots containing a mixture of cyanidation tailings and compost (50%: 50% by weight) referring to the method of Mendez et al. [21]. The tailings were collected from tailing disposal sites at Sekotong District of West Lombok (115<sup>o</sup>.46'-116<sup>o</sup>.20' E and 8<sup>o</sup>.25'-8<sup>o</sup>.55' S). The characterization of tailings that included texture, pH, as well as organic C, total-N, total P, and Hg was performed by standard laboratory methods of Soil Laboratory, University of Brawijaya. Hg concentration was determined using a F732-S Mercury Cold Vapor Atomic Absorption analyzer (Shanghai Huaguang Instrument Company) based on the reduction of mercury by stannum chloride (SnCl<sub>2</sub>). Results of the analysis of tailing samples showed the tailing characteristics as follows: sandy loam texture, pH 8.73, 0.47% organic C, 0.02% N, 5 mg P / kg, and 357.75 mg Hg/kg. Compost used in this study was obtained from Brawijaya University Composting Unit with a composition of 1.2% N, 1.4% P, 0.63% K, pH 5, C/N ratio of 12-13 and 30% water. Results of the chemical analysis of the tailings and compost mixture were as follows: pH 7.83, 1.73% organic C, 0.07% N, 17,68mg P / kg, and 130.39 mg Hg / kg.

The treatment tested in this study was a combination of three species of trees, mycorrhizal inoculation, and without mycorrhizal inoculation. Dose of mycorrhizal inoculation was 30 mycorrhizal spores per plant. Two seeds of each species of plants that have been germinated were planted on 10 kg growing media described above and grown for 63 days (maximum vegetative). Before planting, each pot received basal fertilizers of 100kg N / ha (in the form of urea), 50kg P<sub>2</sub>O<sub>5</sub> / ha (in the form of SP36) and 50kg K<sub>2</sub>O / ha (in the form of KCl). Six treatments were arranged in a completely randomized design with three replications. The water content of the soil medium was maintained at a water holding capacity. During the experiment, the supply of water was done every day to maintain a sufficient water supply for plant growth. Plant

height was measured every 7 days, while the shoot biomass, root biomass, and number of mycorrhizal spores were measured at harvest (63 days). The shoot biomass and root biomass were dried in an oven at 40 ° C for 48 hours for the analysis of Hg using a F732-S Mercury Cold Vapor Atomic Absorption analyzer (Shanghai Huaguang Instrument Company). Data were obtained were subjected to analysis of variance followed by least significant difference test at 5%.

### B. Experiment 2: Effects of Myco-Phytoremediation on Growth of Maize

Pots that still contained the growing media after the experiment 1 described above was then used for growing maize (NK33 variety from Board of Agriculture of Malang) for 84 days. Treatments consisted of seven treatments (six former treatments of experiment 1, and one control, the planting media without phytoremediation). Before planting, each pot received basal fertilizers of 100kg N / ha (in the form of urea), 50kg P<sub>2</sub>O<sub>5</sub> / ha (in the form of SP36) and 50kg K<sub>2</sub>O / ha (in the form of KCl). Seven treatments were arranged in a randomized complete block design with three replications. During the experiment, the supply of water was done every day to maintain a sufficient water supply for plant growth. At harvest (84 days), maize shoot dry weight, maize root dry weight, maize cob dry weight, and Hg contents in maize shoot and root were measured as in the experiment 1. Data were obtained were subjected to analysis of variance followed by least significant difference test at 5%.

## III. RESULTS AND DISCUSSION

### A. Mychorrhizal Density

Of the three types of mycorrhizae found, *Glomus* was the most widely population colonizing the plant roots (Figure 1). The highest number of indigenou smycorrhizae of *Glomus* was observed at *P.falcataria* (PfM1) treatment and the lowest was at *E.orientalis* (EoM1) treatment. *P.falcataria* was a better host plant for mycorrhizae than *E.orientalis*. Determinants of the effectiveness of mycorrhizal inoculation in addition to placement, a placement and soil conditions/environment, is host plant species. Comparison of in digenou smycorrhizal density in each treatment with mycorrhizae presented in Figure1showthat *Glomus* was the most compatible mycorrhizae against the three types of host plants studied.

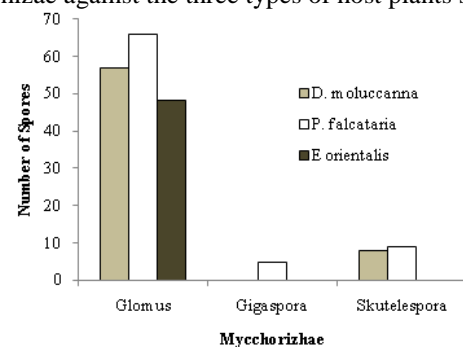


Figure 1. Density of indigenous mycorrhizae.



### B. Growth of Phytoremediator Plants

*D.moluccana* without mycorrhizae (DmM0) had the fastest growth, while *E.orientalis* without mycorrhizae (EoM0) had the slowest growth (Figure 2). Since the beginning of the growth period *D.moluccana* showed a high level of adaptation. Mycorrhizal inoculation did not significantly affect plant growth and biomass (shoot and root) weight of the three tested plant species (Figure 3). In line with the growth, the highest shoot and root dry weights were found in *D.moluccana* without mycorrhizae (DmM0), while the lowest was observed for *E.orientalis*. This indicates that *D.moluccana* is more tolerant to Hg than the other tested plant species.

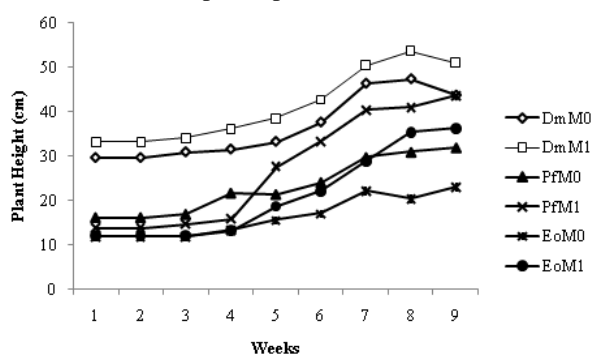


Figure 2. Growth of *P.falcataria* (Pf), *D.moluccana* (Dm), and *E.orientalis* (Eo) with mycorrhizal inoculation (M1), and without mycorrhizal inoculation (M0) for 9 weeks

### C. Mercury Accumulation by Phytoremediator Plants

The highest Hg accumulation (73.51mg/kg) was observed in the roots of *P.falcataria* without mycorrhizae inoculation (PfM0), while the lowest Hg accumulation (1.58mg/kg) was found in the shoots of *E.orientalis* without mycorrhizae inoculation (EoM0) at (Figure 4). The highest total mercury accumulation (shoot and root) was found in *D.moluccana* with mycorrhizae (DmM1) of 86.93 mg / kg and the lowest in *E. orientalis* without mycorrhizae (EoM0) of 28.31 mg / kg. Results of statistical analysis, however, showed that mycorrhizal inoculation did not significantly affect the accumulation of mercury. This might indicate a loss of Hg which can be attributed to Hg volatilization as a result of mycorrhizae influence [25].

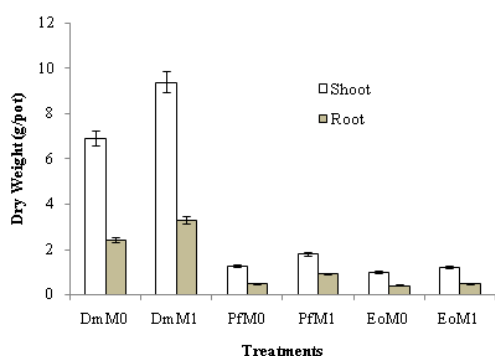


Fig. 3. Shoot and root dry weights of *P.falcataria* (Pf), *D.moluccana* (Dm), and *E.orientalis* (Eo) with mycorrhizal (M1), and without mycorrhizal (M0) inoculation grown for 9 weeks

All treatments posed TF values of less than 1, i.e. DmM1 = 0.47, DmM0 = 0.23, PfM0 = 0.18, PfM1 = 0.11, EoM0 = 0.06, EoM1 = 0.03. This indicates that all tested plants are potential plants for phytostabilization strategy [22]. It is argued that the bioavailability of mercury in the rooting zones of the three plant species declined into a form that was less soluble as absorbed by organic compounds released by plant roots, or absorb the metal into the root surface, and then accumulated the metal in the root tissues [23] [24]. Outside the roots, the hyphae and root surface could adsorb Hg so that Hg translocation into roots could be inhibited, and inside the roots, it change cell wall components of plant, hence possibly enhancing the sequestration of Hg [25]. In line with this, buffering heavy metal-stress had been assigned, at least partly, to selective immobilization of heavy metals in those root tissues that contain fungal structures [26] or to the high metal sorption capacity of the extra radical mycelium of AMF [27].

Overall, the tested three plant species could be used for phytostabilization of soils contaminated with small-scale gold mine tailings, but its interactions with mycorrhizae did not significantly affect the accumulation of mercury. AM fungi have generally such a strong influence on plant biomass that the mycorrhizal effect on phyto-extraction remains positive [28]. The highest potential for mercury accumulator was *P. falcataria* without mycorrhizae inoculation, but *D.moluccana* inoculated with mycorrhizae also posed as the best potential plant for phytostabilization of mercury. Supriyanto [29] who tested to grow *Duabanga moluccana* and *Paraserianthes falcataria* on gold mine tailings reported that the two plant species were able to grow well with few additional inputs such as compost.

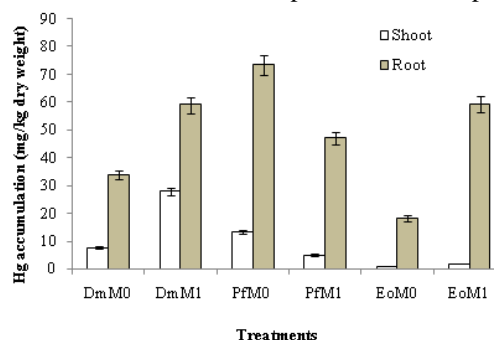


Fig. 4. Accumulation of mercury in shoots and roots of *P.falcataria* (Pf), *D.moluccana* (Dm), and *E.orientalis* (Eo) with mycorrhizal (M1), and without mycorrhizal (M0) inoculation grown for 9 weeks

### D. Growth and Biomass of Maize After Myco-Phytoremediation

The fastest growth rate of maize was initially observed in the media previously grown with mycorrhizae inoculated *P. falcataria* (PfM1) (Figure 5). However, in the last week of observation, the growth rate of maize in the media previously grown with mycorrhizae inoculated *D. moluccana* (DmM1) exceeded that of PfM1 treatment.

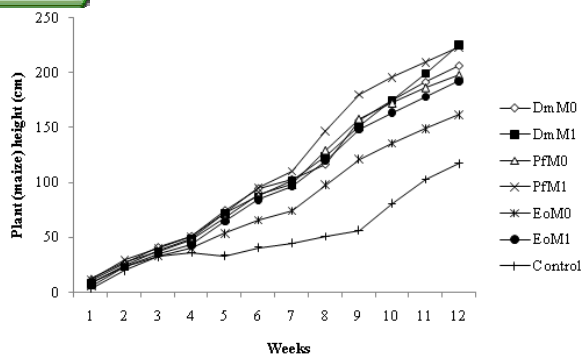


Fig. 5. Growth of maize on growing media previously grown with *P.falcataria* (Pf), *D.moluccana* (Dm), and *E.orientalis* (Eo) with mycorrhizal (M1), and without mycorrhizal (M0).inoculation

Compared to the maize growth rate at the control treatment (media with no phytoremediation treatment), the rate growth rate at all treatments were better. The rate of maize growth in the treatments with mycorrhizae inoculation was higher than that of without mycorrhizae. This indicates the role of mycorrhizae in improving environmental conditions for plant growth against stresses. Harm et al. [12] pointed out that mycorrhizae can reduce stress against metal to enhance plant growth. Faramarzi et al. [30] reported mycorrhizae application increased biomass, yield and yield component of corn. The highest maize plant biomass was also observed in the media previously treated mycorrhizae inoculated *P.falcataria* (PfM1) and the lowest was in the control treatment (media with no phytoremediation treatment). Data presented in Figure 6 show that treatment of three species both with and without mycorrhizae, did not significantly affect weights of shoot, root, and maize seed. This is because the maize plant is tolerant of extreme conditions, such as heavy metal stress and lack of water.

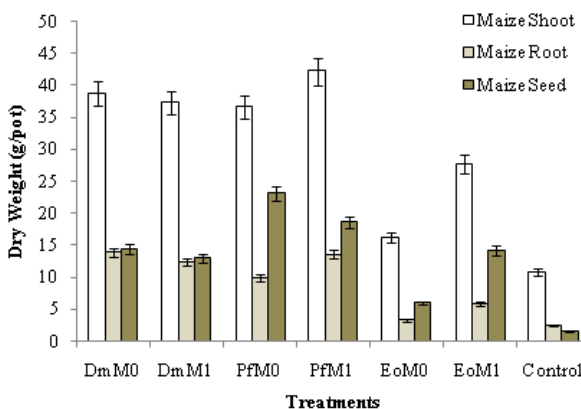


Fig. 6. Dry weights of shoot, root, and seed of maize on growing media previously grown with *P.falcataria* (Pf), *D.moluccana* (Dm), and *E.orientalis* (Eo) with mycorrhizal inoculation (M1), and without mycorrhizal inoculation (M0).

### E. Mercury Accumulation by Maize

Results of analysis of variance proved that among the treatments significantly affected the accumulation of mercury in maize. The highest mercury accumulation

(239.43 mg/kg) was found in the roots of maize on the media previously planted with mycorrhizae inoculated *D.moluccana* (DmM1), while the lowest mercury accumulation (0.36mg/kg) was found in the maizecorn on media previously cultivated with mycorrhizae inoculated *P.falcataria* (PfM1) (Figure 7). If the maize is to be used for human consumption, the optimal treatment is PfM1. However, if the maize shoot is to be used for animal feed, the optimal treatment is DmM1 because of the lowest content of mercury compared with other treatments. The overall results of experiment 2 showed that the accumulation of mercury in maize grown on soil previously remediated by three types of plants (experiment 1) was lower when compared to the accumulation of mercury in maize grown on soil that previously was not remediated soil with the tested three plant species. Thus the process of phytoremediation of mercury (in this case was phytostabilization, based on the TF values) by three species of plants had a good potential for the improvement of the quality of soils contaminated with small-scale gold mine tailings containing mercury.

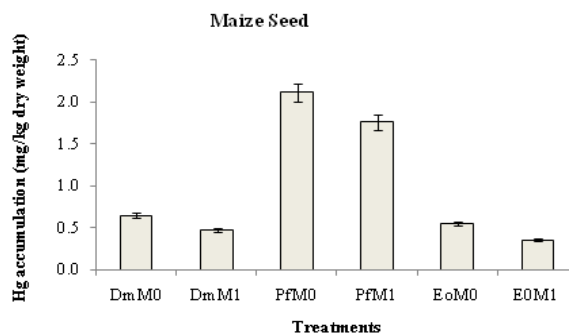
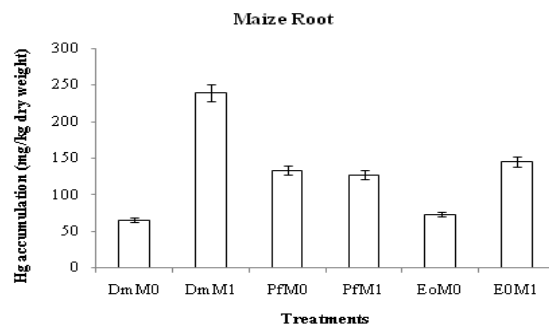
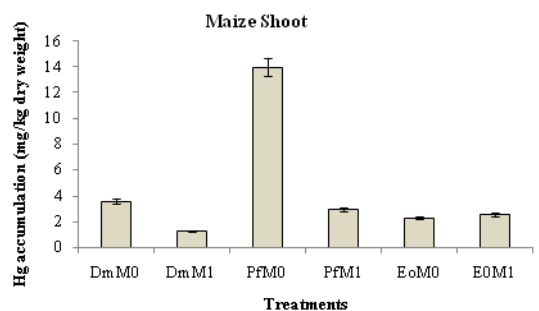


Fig. 7. Accumulation of mercury in shoot, root, and grains of maize grown on media previously used for growing *P.falcataria*, *D.moluccana*, and *E.orientalis* with and without mycorrhizae inoculation



#### IV. CONCLUSION

Mycorrhizae were commonly found in the rooting zone of various indigenous plants grown near the contaminated with small-scale gold mine tailings at Sekotong District of West Lombok. Mycorrhizaegenus were *Glomus*, *Gigaspora* and *Skutelespora*. *Glomus* was the mostcolonizing the plant roots of *P.falcataria*, *D.moluccana*, and *E.orientalis*. The most potential local plant species for phytostabilization was *P.falcataria*, while the most mercury to lerant local plant was *D.moluccana*. Without mycorrhizae inoculation, the highest accumulation of mercury was found in the root of *P.falcataria* of 73.51mg/kg. If the mycorrhizae were inoculated, the highest accumulation of mercury was found in the root of *D.moluccana*. Results of experiment 2 proved that the growth and biomass production of maize after phytoremediation by three species of plants above were higher than those of maize grown on media without phytoremediation.

#### V. ACKNOWLEDGMENT

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