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Research Article

Effect of arbuscular mycorrhizal fungi on the potential of three wild plant species for phytoextraction of mercury from small-scale gold mine tailings

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Abstract: A study that was aimed to explore the effects of arbuscular mycorrhizal (AM) fungi inoculation on the potential of wild plant species (*Paspalum conjugatum*, *Cyperus kyllingia*, and *Lindernia crustacea*) for phytoextraction of mercury from small-scale gold mine tailings was conducted in a glasshouse. Each of the plant seedlings was planted in a plastic pot containing 10 kg of planting medium (mixture of tailings and compost; 50%: 50% by weight). Treatments tested were three plant species and doses of AM fungi inoculation, i.e. 0 and 30 spores/plant. At harvest of 63 days, plant shoot and root were analyzed for mercury concentration. The remaining planting media in the pots were used for growing maize for 84 days. The results showed that the most potential plant species for phytoextraction of mercury was *Paspalum conjugatum*, while the most mercury tolerant plant was *Cyperus kyllingia*. Without AM fungi inoculation, the highest accumulation of mercury (44.87 mg/kg) was found in the root of *Paspalum conjugatum*. If AM fungi were inoculated, the highest accumulation of mercury (56.30 mg/kg) was also found in the shoot of *Paspalum conjugatum*. Results of the second experiment proved that the growth and biomass production of maize after mycophytoextraction by the plant species were higher than those of maize grown on media without mycophytoextraction of mercury.

Keywords: *C.kyllingia, gold mine tailings, L.crustacea, mercury, P.conjugatum, phytoextraction*

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 (Ismawati, 2010). In most of the ASGM in Indonesia, generally amalgamation process with mercury followed by cyanidation process are used to recover gold (Viega et al., 2006). 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 Krisnayanti et al. (2012) 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 (Padmavathiamma and Li, 2007).

Phytoextraction is the most widely used technique in phytoremediation of heavy metal contaminated soil (Mertens et al., 2004). The use of native plants is the focus of phytoextraction. 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 (Monica and Maier, 2008). 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 plants that have long to adapt and survive in extreme conditions (high metal concentration) (Handayanto et al., 2014). Among them, three species (*Paspalum conjugatum, Cyperus kyllingia*, and *Lindernia crustacea*) wee candidates for phytoextraction of mercury from soil contaminated with small-scale gold mine tailings.

The plant does not solely do phytoextracton since there is always the interaction between microorganisms in the rhizosphere that led to increased activity associated with the remediation (Compant et al., 2010). Utomo et al. (2014) reported that *Paspalum conjugatum, Cyperus kyllingia*, and *Lindernia crustacea* 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 mycophytoremediation.

Arbuscular mycorrhizal (AM) fungi 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 (Harrier and Sawczak, 2000). AM fungi also play an important role in reducing the influence of heavy metal stress on plants (Hildebrandt et al., 2007). AM fungi 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 (Harms et al., 2011). 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 AM fungi and biosorption into the cell wall constituent such as chitin and chitosan (Harms et al., 2011).

External mycelium of AM fungi cause more breadth exploitation of the soil volume that can be reached by the roots (Khan et al., 2000; Malcova et al., 2003), thus increasing access to heavy metals in the rhizosphere. In addition to the above, AM fungi can improve plant growth on heavy metals contaminated soil (Enkhtuya et al., 2002) due to improved supply of nutrients (Taylor and Harrier, 2001; Feng et al., 2003), the availability of water (Auge, 2001) and the improvement of soil aggregation (Kabir and Koide, 2000; Rillig and Steinberg, 2002). This study was aimed to explore the effects of AM fungi inoculation on the potential of three wild plant species (*Paspalum conjugatum, Cyperus kyllingia*, and *Lindernia crustacea*) for phytoextraction of mercury from small-scale gold mine tailings at Sekotong District of West Lombok, Indonesia.

Materials and Methods

Inoculation of arbuscular mycorrhizal (AM) fungi and phytoextraction of Hg

The study was conducted in a glasshouse STPP Malang from June to December 2014. Each of the three plant species (*Paspalum conjugatum, Cyperus kyllingia*, and *Lindernia crustacea*) that have been reported to be tolerant to gold cyanidation tailings (Handayanto et al., 2014), was planted in a plastic pot containing cyanidation tailing and compost mixture (50%: 50% by weight) referring to the method of Mendez et al. (2007). The tailings were collected from a tailing disposal site at Sekotong District of West Lombok (115^0. 46'-116^0.20' E and 8^0.25'-8^0.55' S). The characterization of tailings that included texture, pH, as well as organic C, total-N, total P, and Hg contents, was performed by standard laboratory methods of Soil Laboratory, Brawijaya University. Total mercury concentration was determined using a F732-S Mercury Cold Vapor Atomic Absorption analyzer (Shanghai Huaguang Instrument Company).

Results of tailing analysis 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 chemical analysis of the tailings and compost mixture were as follows: pH 7.83, 1.73% organic C, 0.07% N, 17.68 mg P / kg, and 130.39 mg Hg / kg. The treatments tested in this study were combinations of three plant species, AM fungi inoculation, and without AM fungi inoculation. Dose of AM fungi inoculation was 30 spores per plant. Two pre-germinated seeds of each plant species were planted on 10 kg of planting medium described above and grown for 63 days. Before planting, each pot received basal fertilizers of 100kg N / ha (urea), 50kg P_2O_5 / ha (SP36) and 50kg K_2O / ha (KCl). Six treatments were arranged in a completely randomized design

with three replicates. The water content of the growing medium was maintained at a water holding capacity. During the experiment, water was supplied every day to maintain a sufficient water supply for plant growth. Plant height was measured every 7 days, while shoot biomass, root biomass, and number of AM fungi 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 obtained were subjected to analysis of variance followed by least significant difference test at 5%.

Effect of mycophytoextraction of Hg on growth of maize

Pots that still contained the growing media after harvesting the first experiment above were then used for growing maize (NK33 maize variety from Board of Agriculture of Malang) for 84 days. Seven treatments (six former treatments of the first experiment, and one control - planting medium without phytoextraction of mercury) were arranged in a completely randomized block design with three replicates. Before planting, each pot received basal fertilizers of 100kg N / ha (urea), 50kg P_2O_5 / ha (SP36) and 50kg K_2O / ha (KCl). During the experiment, water was supplied 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 first experiment. Data obtained were subjected to analysis of variance followed by least significant difference test at 5%.

Results and Discussion

Arbuscular mycorrhizal (AM) fungi density

Of the three types of AM fungi found, *Glomus* was the most widely population colonizing the plant roots (Figure 1). The highest number of *Glomus* was observed at *P.conjugatum* (PcM1) treatment and the lowest was at *L.crustacea* (LcM1) treatment. *P.conjugatum* was a better host plant for AM fungi than *L. crustacea*. The determinant of the effectiveness of AM fungi inoculation in addition to placement and soil conditions / environment is host plant species. Comparison of AM fungi density in each treatment presented in Figure 1 shows that *Glomus* was the most compatible AM fungi against the three plant species studied.

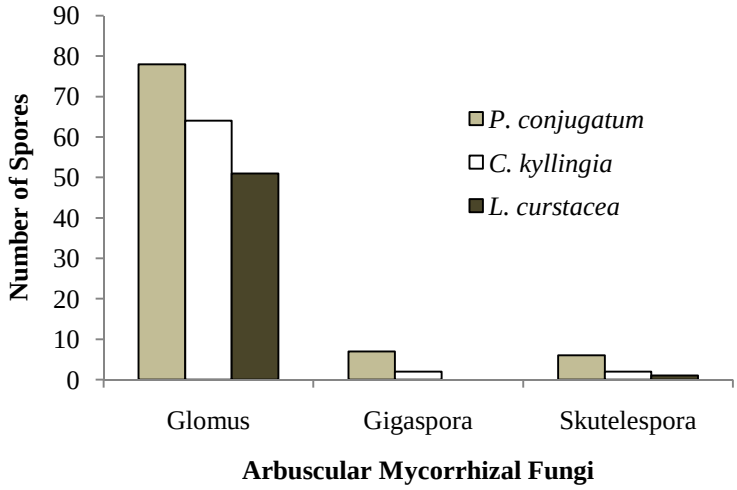

Figure 1. Density of arbuscular mycorrhizal (AM) fungi.

Growth of P. conjugatum, C. kyllingia, and L.crustacea

P.conjugatum with AM fungi (PcM1) had the fastest growth, while *L. crustacea* without AM fungi (LcM0) had the slowest growth (Figure 2). Since the beginning of the growth period, *P.conjugatum* showed a high level of adaptation. AM fungi inoculation significantly affected plant growth and biomass (shoot and root) weight of the three plant species (Figure 3). In line with the growth, the highest shoot and root dry weights were found in *P.conjugatum* with AM fungi (PcM1), while the lowest was observed for *L.crustacea*. This indicates that *P.conjugatum* was more tolerant to Hg than the other two plant species.

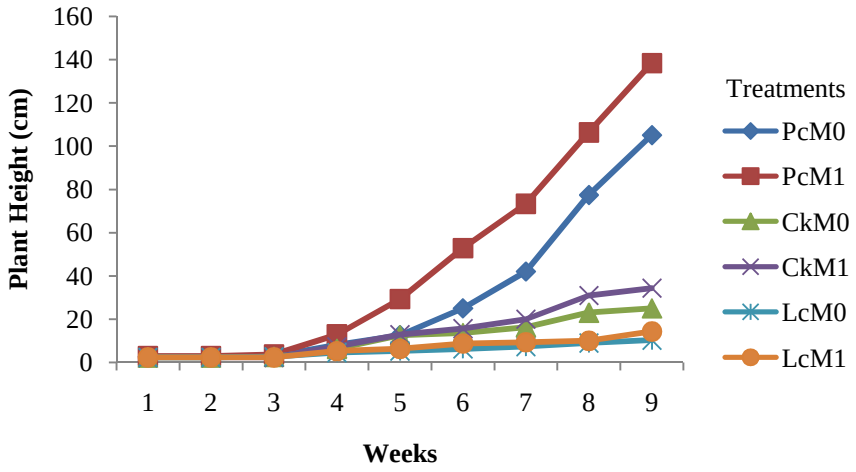

Figure 2. Growth of *P.conjugatum*, *C.kyllingia*, and *L.crustacea* with and without AM fungi inoculation. PcMO = *P. conjugatum* without AM fungi inoculation, PcM1 = *P. conjugatum* with AM fungi inoculation. CkMo = *C. kyllingia* without AM fungi inoculation, CkM1 = *C. kyllingia* with AM fungi inoculation, LcMo = *L. crustacea* without AM fungi inoculation, LcM1 = *L. crustacea* with AM fungi inoculation.

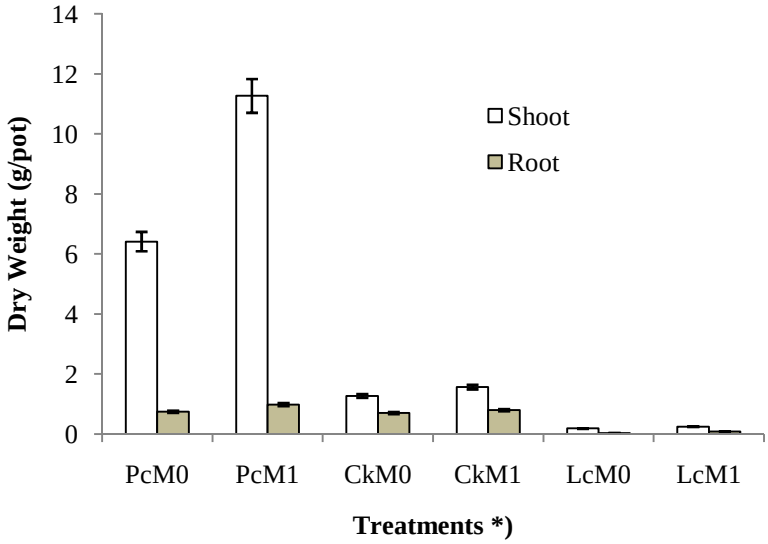

Figure 3. Shoot and root dry weights of *P.conjugatum*, *C.kyllingia*, and *L.crustacea* with and without AM fungi inoculation. *) see Figure 2.

Mercury accumulation by P. conjugatum, C.kyllingia, and L.crustacea

The highest Hg accumulation (56.3 mg / kg) was observed in the shoot of *P.conjugatum* with AM fungi inoculation (PcM1), while the lowest Hg accumulation (4.71 mg/kg) was found in the root of *L. crustacea* without AM fungi inoculation (LcM0) (Figure 4). The highest total mercury accumulation (shoot and root) was found in *P.conjugatum* with AM fungi (PcM1) of 76.53 mg/kg and the lowest was in *L. crustacea* without AM fungi (LcM0) of 11.34 mg/kg. Results of statistical analysis, however, showed that AM fungi 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 AM fungi influence (Yu et al., 2010). All treatments posed TF values of more than 1, i.e. PcM1 = 2.78, PcM0 = 1.96, CkM0 = 2.44, CkM1 = 1.80, LcM0 = 1.41, LcM1 = 1.62; indicating that all tested plants are potential plants for phytoextraction strategy (Brooks, 1998).

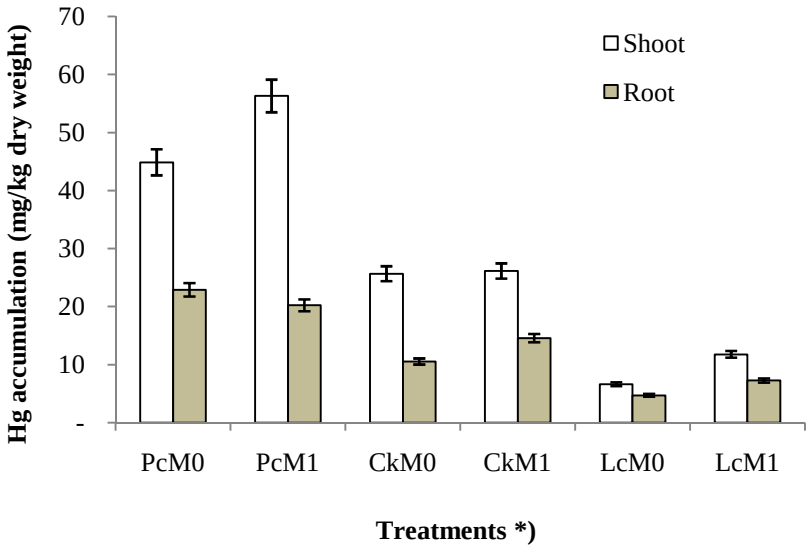

Figure 4. Accumulation of mercury in shoot and root of *P.conjugatum*, *C.kyllingia*, and *L.crustacea* with and without AM fungi inoculation. *) see Figure 2.

It is thought 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 plant tissues (Berti and Cunningham, 2000; Wong, 2003). Outside the roots, the hyphae and root surface can absorb Hg so that Hg translocation into roots can be inhibited, and inside the roots, it changes cell wall components of plant, hence possibly enhancing the sequestration of Hg (Yu et al., 2010).

In line with this, buffering heavy metal-stress has been assigned, at least partly, to selective immobilization of heavy metals in those root tissues that contain fungal structures (Kaldorf et al., 1999) or to the high metal sorption capacity of the extra radical mycelium of AM fungi (Joner et al., 2000). Overall, the tested three plant species could be used for phytoextration of mercury from small-scale gold mine tailings, but their interactions with AM fungi did not significantly affect the accumulation of mercury. AM fungi have generally such a strong influence on plant biomass that the mycorrhizal effect on phytoextraction remains positive (Wang et al., 2007). The highest potential for mercury accumulator was *P.conjugatum* with AM fungi inoculation, but *C.kyllingia* without AM fungi inoculation also posed as the potential plant for phytoextraction of mercury.

Growth and biomass of maize after mycophytoextraction of mercury

The fastest growth rate of maize was initially observed in the media previously grown with AM fungi inoculated *P.conjugatum* (PcM1) (Figure 5). Compared to the maize growth rate at the control treatment (media with no phytoextraction treatment), the growth rate at all treatments were better. The rate of maize growth in the treatments with AM fungi inoculation was higher than that of without AM fungi. This indicates the role of AM fungi in improving environmental conditions for plant growth against stresses. Harms et al. (2011) pointed out that AM fungi could reduce stress against metal to enhance plant growth. Faramarzi et al. (2012) reported that AM fungi application increased biomass, yield and yield components of maize.

The highest maize plant biomass was also observed in the media previously treated with AM fungi inoculated *P.conjugatum* (PcM1) and the lowest was in the control treatment (media with no phytoextraction treatment). Data presented in Figure 6 show that treatment of three plant species, both with and without AM fungi, 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.

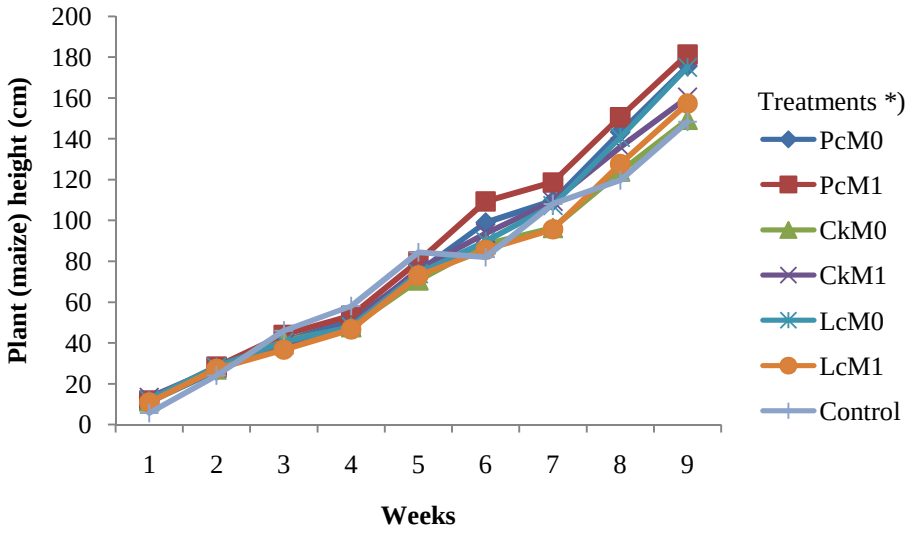

Figure 5. Growth of maize on growing media previously grown with of *P.conjugatum*, *C.kyllingia*, and *L. crustacea* with and without AM fungi inoculation. *) see Figure 2.

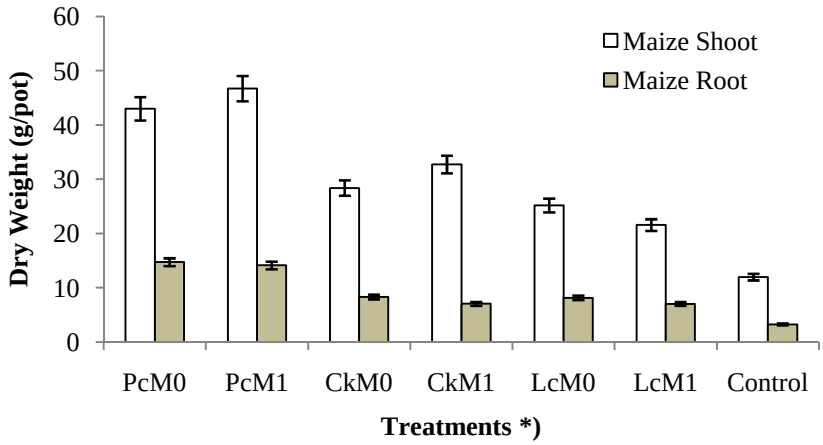

Figure 6. Dry weights of shoot and root of maize on growing media previously grown with of *P.conjugatum*, *C.kyllingia*, and *L.crustacea* with and without AM fungi inoculation. *) see Figure 2.

Mercury accumulation by maize

Results of analysis of variance proved that the treatments significantly affected the accumulation of mercury in maize. Mercury accumulation the maize root was higher than that in the maize shoot and seed (Figure 7). The highest mercury accumulation in maize shoot (2.34 mg / kg) was found on the media previously planted with AM fungi inoculated *P.conjugatum* (PcM1), while the lowest mercury accumulation in maize shoot (0.87 mg/kg) was found on media previously planted with non AM fungi inoculated *C.kyllingia* (CkMo) (Figure 7). If the maize is to be used for human consumption, the optimal treatment is PcM1 because of the lowest Hg accumulation in maize seed (0.09 mg/kg). However, if the maize shoot is to be used for animal feed, the optimal treatment is CkMo because of the lowest accumulation of mercury in the maize shoot (0.87 mg / kg). The overall results of the second experiment showed that the accumulation of mercury in maize grown on media previously remediated by three plant species (experiment 1) was lower than that in maize grown on non remediated planting media. Based on the TF (Translocation Factor) values, the three plant species were potential for phytoextraction of mercury from small-scale gold mine tailings.

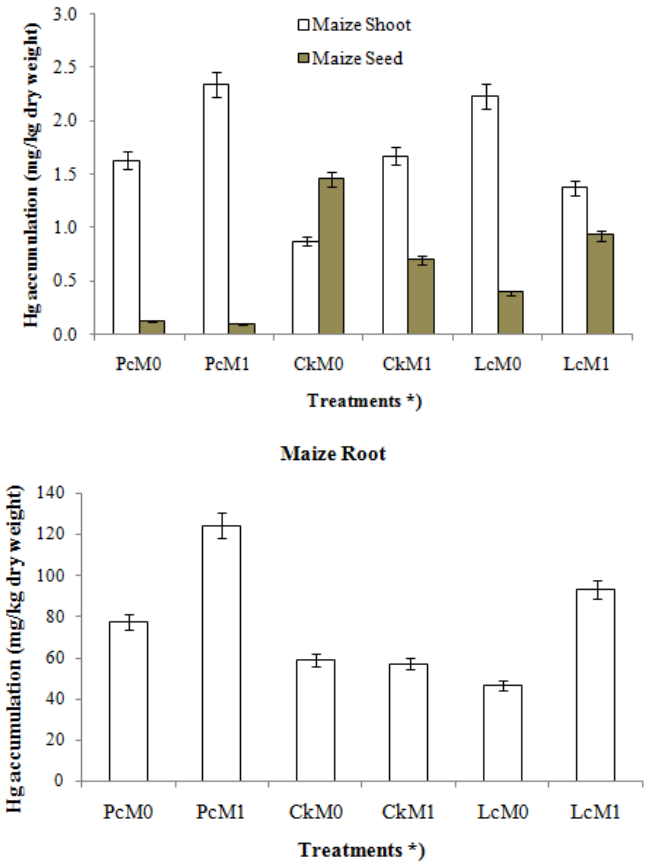

Figure 7. Accumulation of mercury in shoot, seed, and root of maize on growing media previously grown with of *P.conjugatum, C.kyllingia,* and *L.crustacea* with and without AM fungi inoculation.
*) see Figure 2.

Conclusion

AM fungi were commonly found in the rooting zone of various indigenous plants grown near the area contaminated with small-scale gold mine tailings at Sekotong District of West Lombok. AM fungi found were *Glomus*, *Gigaspora* and *Skutelespora*. *Glomus* was the most colonizing the plant roots of *P.conjugatum*, *C.kyllingia*, and *L.crustacea*. The most potential wild plant species for phytoextraction of mercury was *P.conjugatum*, while the most mercury tolerant local plant was *C.kyllingia*. Without AM fungi inoculation, the highest accumulation of mercury (44.87 mg/kg) was found in the shoot of *P.conjugatum*. If AM fungi were inoculated, the highest accumulation of mercury was also found in the shoot of *P.conjugatum* (56.30 mg/kg). Results of the second experiment proved that the growth and biomass production of maize after phytoextraction of mercury by the three plant species were higher than those of maize grown on media without phytoextraction of mercury.

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