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Mycophytoextraction of Mercury from Small-Scale Gold Mine Tailings Contaminating Agricultural Land and Its Effect on Maize Growth

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Abstract

An experiments aimed to explore the effects of mycorrhizae inoculation on the potential of local plant species (*Paspalum conjugatum, Cyperus kyllingia*, and *Lindernia crustacea*) for phytoextraction of mercury from small-scale gold mine tailings contaminating agricultural land has been conducted in a glasshouse. The first experiment was set up as three plant species, and doses of mycorrhizal inoculation, i.e. 0 and 30 spores/plant. At harvest of 63 days, shoots and roots were analyzed for mercury concentration, consisted of 6 treatments (PcM0; PcM1; CkM0; CkM1; LcM0; LcM1), and the second experiment using the remediated soils of the first trials consisted of treatments (six treatments previous and one control) were used for growing maize 84 days. Each of the plant seedlings was planted in a plastic pot containing 10 kg of tailing and compost mixture. The results showed that *Glomus* was the most compatible mycorrhizae against the three types of host plants studied. Mycorrhizal inoculation significantly affected plant growth and biomass weight of three plant species. The highest Hg accumulation (56.3 mg/kg) was observed in the shoot of PcM1. Overall, the tested three plant species could be used for phytoextration of mercury from small-scale gold mine tailings contaminating agricultural land, but its interactions with mycorrhizae did not significantly affect the accumulation of mercury. Myco-phytoextraction of mercury significantly enhanced maize growth and biomass.

Keywords: Cyperus kyllingia, Lindernia crustacea; Paspalum conjugatum; phytoremediation; gold mine tailings.

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1. 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 [12]. In most of the ASGM in Indonesia, generally amalgamation process with mercury followed by cyanidation process is used to recover gold [26]. 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 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 [17]. 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 [22].

Phytoextraction is the most widely used technique in phytoremediation of heavy metal contaminated soil [20]. 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 [21]. 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) [8]. Among them, three species (*Paspalumconjugatum, Cyperus kyllingia*, and *Lindernia crustacea*) were 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 [4]. Reference [25] reported that *Paspalumconjugatum, 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 myco-phytoremediation. Arbuscular mycorrhizal fungi (AMF) are one of the important endophyte that lives 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 [9]. 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 [9].

External mycelium of AMF cause more breadth exploitation of the soil volume that can be reached by the roots [16]; [18], thus increasing access to heavy metals in the rhizosphere. In addition to the above, AMF can improve plant growth on heavy metals contaminated soil [5] due to improved supply of nutrients [7, 24], the availability of water [1] and the improvement of soil aggregation [14, 23]. This study was aimed to explore the effects of myccorhizae inoculation on the potential of three plant species (*Paspalumconjugatum, Cyperus kyllingia*, and *Lindernia crustacea*) for phytoextraction of mercury from soil contaminated with small-scale gold mine tailings at Sekotong District of West Lombok, Indonesia.

2. Materials and Method

2.1. Experiment 1: Mycchorizae inoculation and phytoextraction of Hg by three plant species

The study was conducted in a glasshouse STPP Malang from June to December 2014. The three dominant plant species tolerant of contamination of gold cyanidation tailings reported by Reference [25], namely *Paspalum conjugatum, Cyperus kyllingia*, and *Lindernia crustacea*, were planted in plastic pots containing a mixture of cyanidation tailings and compost (80%:20% by weight) referring to the method of Mendez et al. (2007). The tailings were collected from tailing disposal sites 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 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 (SnCl2). 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, 17.68 mg P/kg, and 130.39 mg Hg/kg.

The treatments tested in this study were combinations of three plant species, mycorrhizal inoculation, and without mycorrhizal inoculation. Mycorrhizae used for this was indigenous mycorrhizae previously identified in Reference [25]. 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 100 kg N/ha (in the form of urea), 50 kg P_2O_5/ha (in the form of SP 36) and 50 kg K_2O/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 mycorrhizae 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%.

2.2. Experiment 2. Effect of myco-phytoextraction of Hg 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_2O_5/ha (in the form of SP_{36}) and 50 kg K_2O/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 seed weight, and Hg contents in maize shoot, root, and seed were also measured. Data were obtained were subjected to analysis of variance followed by least significant difference test at 5%.

3. Results and Discussion

3.1. Mycorrhizae density

Of the three types of mycorrhizae found, *Glomus* was the most widely population colonizing the plant roots (Figure 1). The highest number of indigenous mycorrhizae *Glomus* was observed at *Paspalum conjugatum* (PcM1) treatment and the lowest was at *Lindernia crustacea* (LcM1) treatment. *Paspalum conjugatum* was a better host plant

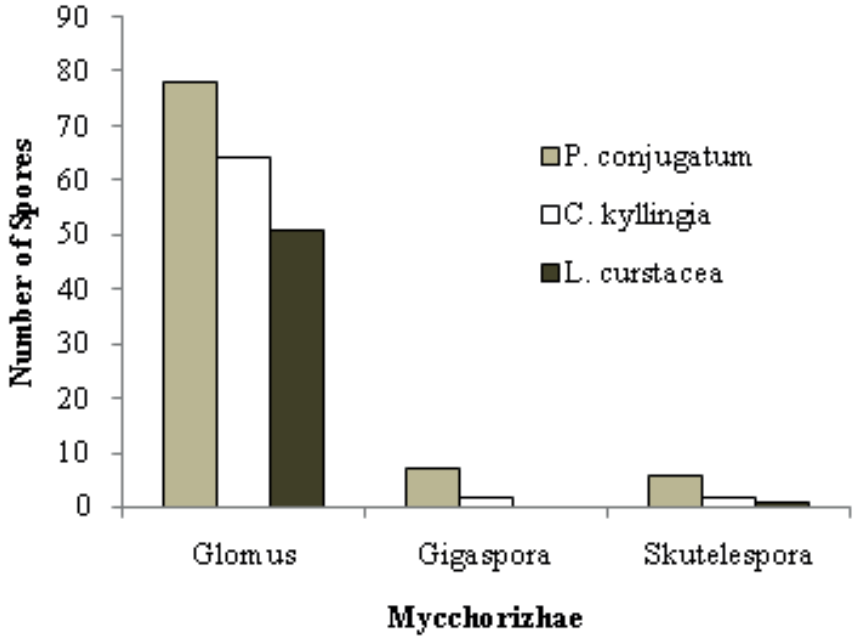

Figure 1: Density of indigenous mychorrizae.

for mycorrhizae than *Lindernia crustacea*. Determinants of the effectiveness of mycorrhizal inoculationin addition to placement and soil conditions/environment, is host plant species. Comparison of indigenous mycorrhizal density in each treatment with mycorrhizae presented in Figure1 shows that *Glomus* was the most compatible mycorrhizae against the three types of host plants studied.

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

Paspalum conjugatum with mycorrhizae (PcM1) had the fastest growth, while *Lindernia crustacean* without mycorrhizae (LcMo) had the slowest growth (Figure 2). Since the beginning of the growth period *Paspalum conjugatum* showed a high level of adaptation. Mycorrhizal inoculation significantly affected growth of *Paspalum conjugatum,* but not for *Cyperus kyllingia* and *Lindernia curstacea* (Figure 3).

In line with the growth, the highest shoot and root dry weights were found in *Paspalum conjugatum* with mycorrhizae (PcM1), while the lowest was observed for *Lindernia crustacea*. This indicates that *Paspalum conjugatum* is more tolerant to Hg than the other tested plant species.

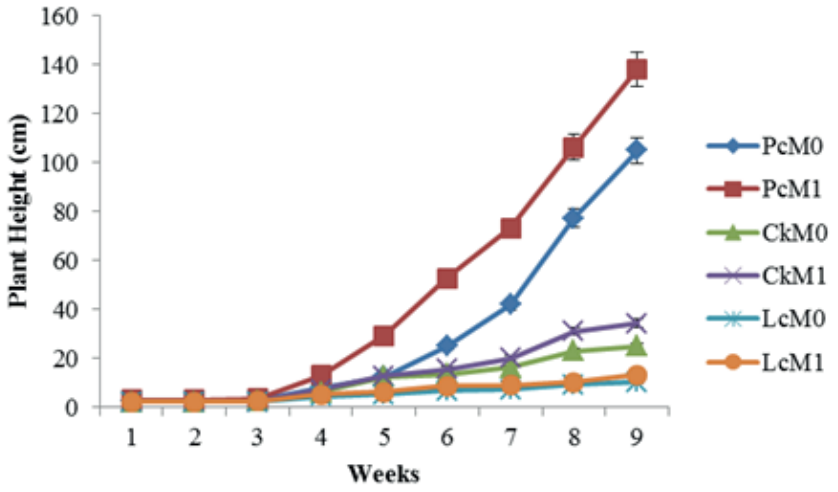

Figure 2: Growth of *Paspalum conjugatum, Cyperus kyllingia*, and *Lindernia crustacea*, with and without mychorrizae inoculation.

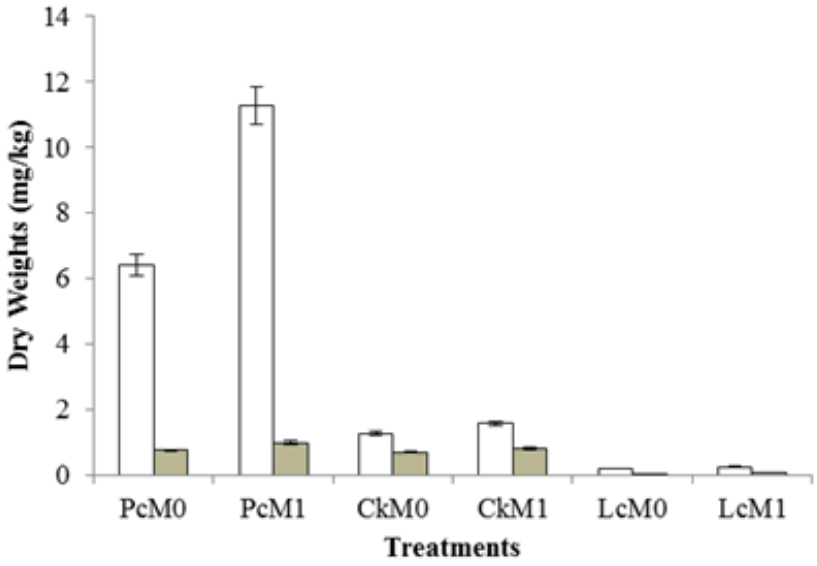

Figure 3: Shoot and root dry weights of *Paspalum conjugatum, Cyperus kyllingia*, and *Lindernia crustacea* with and without mychorrizae inoculation.

3.3. Mercury accumulation by *P. conjugatum, C. kyllingia*, and *L. crustacean*

The highest Hg accumulation (56.3mg/kg) was observed in the shoot of *Paspalum conjugatum* with mycorrhizae inoculation (PcM1), while the lowest Hg accumulation (4.71mg/kg) was found in the root of *Lindernia crustacea* without mycorrhizae inoculation (LcMo) (Figure 4). The highest total mercury accumulation (shoot and root) was found in *Paspalum conjugatum* with mycorrhizae (PcM1) of 76.53 mg/kg and the lowest in the root of *Lindernia crustacean* without mycorrhizae (LcMo) of 11.34 mg/kg.

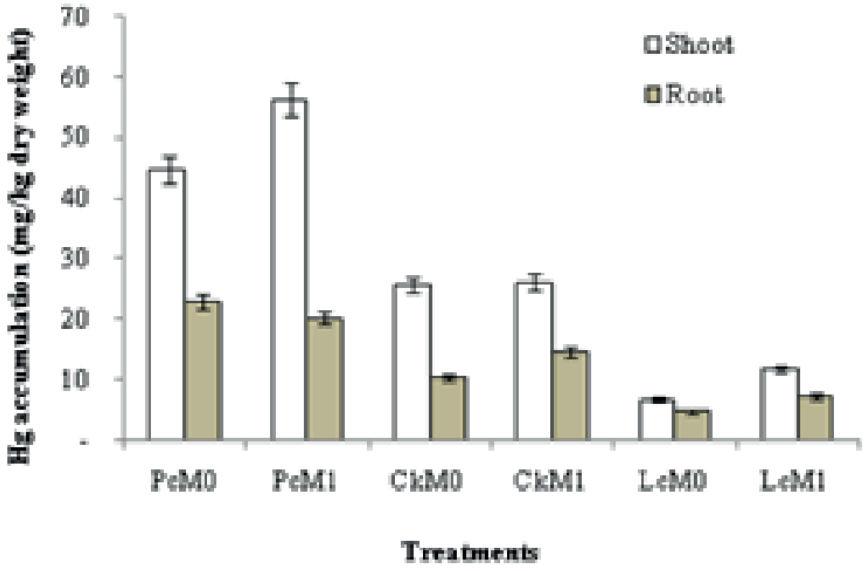

Figure 4: Accumulation of mercury in shoot and root dry weights of *Paspalum conjugatum, Cyperus kyllingia*, and *Lindernia crustacea* with and without mychorrizae inoculation.

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 mychorrizhae influence [29]. All treatments posed TF values of more than 1, i.e. PcM1 = 2.78, PcMo = 1.96, CkMo = 2.44, CkM1 = 1.80, LcMo = 1.41, LcM1 = 1.62. This indicates that all tested plants are potential for phyto-extraction strategy [3]. 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 plant tissues [2, 28]. Outside the roots, the hyphae and root surface could absorb 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 [29].

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 [15] or to the high metal sorption capacity of the extra radical mycelium of AMF [13]. Overall, the tested three plant species could be used for phytoextration of mercury from small-scale gold mine tailings contaminating agricultural land, 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 [27]. The highest potential for mercury accumulator was *Paspalum conjugatum* with mycorrhizae inoculation, but *Cyperus kyllingia* inoculated with mycorrhizae also posed as the potential plant for phyto-extraction of mercury.

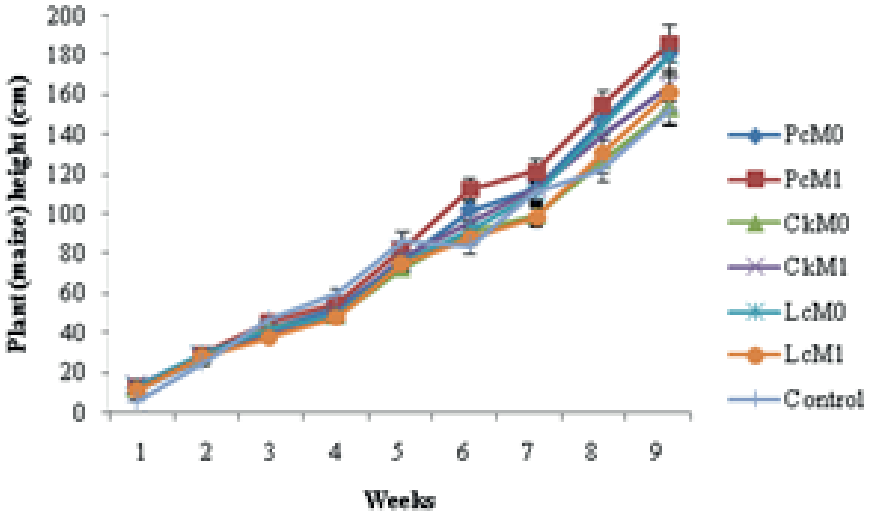

Figure 5: Growth of maize on growing media previously grown with of *Paspalum conjugatum, Cyperus kyllingia*, and *Lindernia crustacea* with and without mychorrizae inoculation.

3.4. Growth and biomass of maize after mycophyto extraction of mercury

The fastest growth rate of maize was initially observed in the media previous grown with mycorrhizae inoculated *Paspalum conjugatum* (PcM1) (Figure 5). Compared to the maize growth rate at the control treatment (media with no phytoextraction 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. Reference [9] pointed out that mycorrhizae can reduce stress against metal to enhance plant growth. It was reported mycorrhiza application increased biomass, yield and yield component of maize [6].

The highest maize plant biomass was also observed in the media previously treated mycorrhizae inoculated *Paspalum conjugatum* (PcM1) 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.

3.5. 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

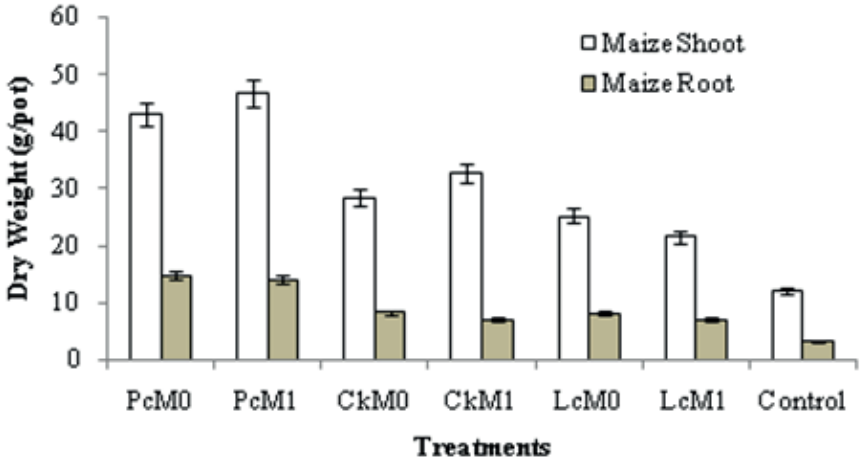

Figure 6: Dry weights of shoot and root of maize on growing media previously grown with of *Paspalum conjugatum, Cyperus kyllingia*, and *Lindernia crustacea* with and without mychorrizae inoculation.

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.

4. 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. Mycorrhizae genus were *Glomus, Gigaspora* and *Skutelespora*. *Glomus* was the most colonizing the plant roots of *Paspalum conjugatum, Cyperus kyllingia*, and *Lindernia crustacea*. The most potential local plant species for phyto-extraction of mercury was *Paspalum conjugatum*, while the most mercury tolerant local plant was *Cyperus kyllingia*. Without mycorrhizae inoculation, the highest accumulation of mercury was found in the shoot of *Paspalum conjugatum* of 44.87 mg/kg. If the mycorrhizae were inoculated, the highest accumulation of mercury was also found in the shoot of *Paspalum conjugatum* (56.3 mg/kg). All treatments posed TF values of more than 1 indicating thatall tested plants are potential for phyto-extraction strategy. Overall, the

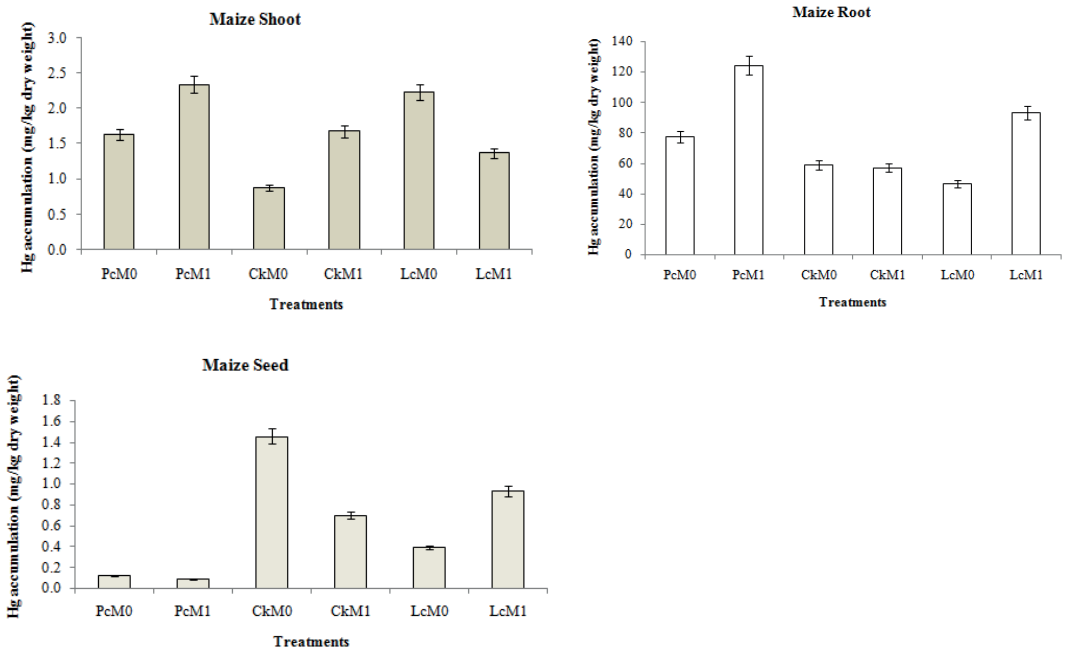

Figure 7: Accumulation of mercury in shoot, root, and grains of maize on growing media previously grown with of *Paspalum conjugatum, Cyperus kyllingia*, and *Lindernia crustacea* with and without mychorrizae inoculation.

tested three plant species could be used for phyto-extration of mercury from small-scale gold mine tailings contaminating agricultural land, but its interactions with myc-orrhizaedid not significantly affect the accumulation of mercury. Results of experiment 2 proved that the growth and biomass production of maize after phyto-extraction of mercury by three plant species above were higher than those of maize grown on media without phyto-extraction of mercury.

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