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# Phytoremediation of Mercury-Contaminated Soil Using Three Wild Plant Species and Its Effect on Maize Growth

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**Abstract** In West Lombok- Indonesia, gold amalgamation tailings are commonly discharged to agricultural lands resulting in reduced maize yield in the area. Phytoremediation can represent a low-cost alternative to traditional techniques such as soil removal. This study was aimed to elucidate the potential of Lindernia crustacea (L.) F., Paspalum conjugatum L., and Cyperus kyllingia Endl., for phytoremediation of mercury-contaminated soils in conjunction with the ammonium thiosulphate to phytoextract mercury and its effect on maize growth. Each of the plant seedlings was planted in a plastic pot containing 15kg of mercury-contaminated soil for 9 weeks. Treatments tested were three plant species), and two rates of ammonium thiosulphate application, i.e. 0 and 8g / kg of soil. Ammonium thiosulphate was applied one week before harvesting the plants. At harvest (9 weeks) shoots and roots were analyzed for mercury concentration. The remaining soils in the pots were used to grow maize for 8 weeks. The results showed that on average, the addition of ammonium thiosulphate increased the accumulation of mercury in plant shoots and roots by 82% and 47%, respectively, compared to the media without addition of ammonium thiosulphate. In comparison to the control treatment, the average increase of dry weight of maize (shoot+root) grown on media previously remediated with three plant species with addition of ammonium thiosulphate was 40%, while that grown on media previously remediated with three plant species without addition of ammonium thiosulphate was 62%.

***Keywords:*** *lindernia crustacea, paspalum conjugatum, cyperus kyllingia, phytoremediation, mercury, ammonium thiosulphate*

## 1. Introduction

Indonesia is regarded as a major location for artisanal and small-scale gold mining (ASGM). Aspinall [1] reported that there are 713 small-scale mining sites throughout Indonesia, including West Lombok. Miners generally use a mercury amalgamation method as it is considered efficient and requires only a small investment. Despite the assumed efficiency of mercury amalgamation, the ability of mercury to recover gold from ore is highly dependent upon the size and geochemistry of a gold particle [2]. A research trial conducted at an ASGM location in the Philippines reported that the gold recovered by amalgamation was only 10% [3]. There is also a loss of mercury into the environment through discharge of the water used for grinding, and tailings. In West Lombok the discharge of gold amalgamation tailings to agricultural lands gives negative impacts on the production of maize as the main food crop in the area. Chlorosis is the main symptom of mercury toxicity in crops. Therefore, a remediation technique for metal removal toward the rehabilitation and reclamation of heavy-metal polluted sites is needed [4].

During the past decade, there has been increasing interest in the possibility of using vegetation for remediating heavy-metal contaminated sites. This technique that is commonly defined as phytoremediation, can represent a low-cost alternative to traditional techniques such as soil removal and capping [5]. Among the different areas in the field of phytoremediation, special interest has been devoted to the phytoextraction of metals from contaminated soils. In this method, plants take up heavy metals from soils. Harvesting and disposal of plant biomass allows removal of the metal from the soil [6,7]. Muddarisna et al. [14] reported that from six wild plant species evaluated for their phytoremediation potential there were three species, i.e. *Lindernia crustacea* (L.) F., *Paspalum conjugatum* L., and *Cyperus kyllingia* Endl., that were potential for phytoextraction of Hg since they were efficient to take up and translocate mercury from roots to shoots [14].

The success of phytoextraction depends on the availability of the metal in soil for plant uptake [8]. For example, Hg that is mostly found in soils as an uncharged complex in soil solutions has limited solubility in soils [9], and thus low availability for plant uptake. Therefore, uptake of Hg by plant will depend on the ability of plant to control the processes that can enhance the concentration of Hg in the soil solution [10]. The partitioning of Hg from the solid phase into soil solution will occur as a consequence of coordinative reactions where Hg ions are exchanged with water molecules for some preferred ligands [11]. Mercury will precipitate as insoluble cinnabar (HgS) in the presence of other metal sulfides or sulfhydril groups, [12]. The strong affinity of Hg to organic matter that influences Hg solid phase speciation is regarded as one of the major driving forces for Hg adsorption by soil particles [13]. Solution containing sulfur has been used to stimulate the accumulation of Hg in plant tissues [19]. For example, *Brassica juncea* can concentrate Hg to 40 mg / kg in the plant canopy tissue after application of ammonium thiosulfate ($[NH4]_2S_20_3$) on mine waste contaminated with 2.8mg Hg/kg. Therefore ammonium thiosulfate is often used by some researchers as a potential strategy for the remediation of polluted environments Hg. Application of this method, however, has never been used in Indonesia because of the limited information of phytoremediation of mercury contaminated soil using wild plants.

This study was aimed to elucidate the potential of the three wild plant species for phytoremediation of mercury-contaminated soils in conjunction with the thiosulphate to phytoextract mercury and its effect on maize growth.

# 2. Materials and Methods

This study was carried out in a shade house belonging to farmers having agricultural lands contaminated with small-scale gold mine tailing containing mercury. The site is located at Sekotong District of West Lombok, Indonesia (115°.46'-116°.20'E and 8°.25'-8°.55'S). Pot experiments were conducted from July to December 2012. Samples of soil contaminated with gold mine tailing were collected at 0-30 cm depth. The samples were air dried at room temperature for two weeks, crushed and ground to pass through 2mm sieve for analyses of texture (Bouycous hydrometer method), pH (1:2.5 soil-water suspension), and N (Kjeldahl method), P (Olsen method), C organic (Walkley and Black method) and Hg (Cold Atomic Absorption Mercury Vapor analyzer) contents. Results of soil sample analyses showed the soil characteristics as follows: sandy loam texture pH 7.1, 1.3% C organic, 0.2% N, 20.5mg P $kg^{-1}$, and 88.9mg Hg $kg^{-1}$. The value mercury content in the soils was much higher than the tolerable mercury concentration of 0.002mg $kg^{-1}$ regulated by the Indonesian Ministry of Environment. *Lindernia crustacea* L., *Paspalum conjugatum* L., and *Cyperus kyllingia* Endl. used for this study were two-week old acclimatized seedlings previously collected from areas nearby the gold amalgamation process facilities in West Lombok.

## 2.1. Phytoextraction of Mercury

Each of the three seedlings was planted in a plastic pot containing 15kg of mercury-contaminated soil for 9 weeks. Treatments tested were (1) plant species (three species), and (2) rates of ammonium thiosulphate ($[NH_4]_2S_2O_3$) application, i.e. 0 and 8g ammonium thiosulphate/ kg of soil [15]. To ensure plant growth, all pots received basal fertilizers of N, P and K with rates equivalent to 100, 50 and 20kg $ha^{-1}$, respectively. Ammonium thiosulphate ($[NH_4]_2S_2O_3$) was applied at 8 weeks after planting. Six treatments (combinations of three plant species and two rates of ammonium thiosulphate application) were arranged in a randomized block design with three replicates. The plants were grown for 9 weeks. During the experiment, water was regularly supplied to ensure that water did not limit plant growth. At harvest (9 weeks), shoots and roots were separated, washed, weighed and oven dried at 40°C for 48 hours for mercury analysis using the method describe above. The concentration of mercury was determined using a F732-S Cold Atomic Absorption Mercury Vapor analyzer (Shanghai Huaguang Instrument Company) that works on the reduction of mercury by stannum chloride ($SnCl_2$). Data obtained were subjected to analysis variance followed by 5% last significance different test.

## 2.2. Growth and Biomass Yield of Maize

After harvesting the phytoremediation plants, the remaining soils in the pots were used for growing maize. Six treatments similar to those of experiment 1 and one control treatment (mercury contaminated soil with no phytoremediation treatment) were arranged in a randomized block design with three replicates. Each pot received basal fertilizers equivalent to 100kg N $ha^{-1}$ (supplied as Urea), 50kg P $ha^{-1}$ (supplied as SP36), 50kgK $ha^{-1}$ (supplied as KCl), and 10kg compost $ha^{-1}$. During the experiment, soil moisture was maintained at 80% of field capacity by adding water periodically. Maize was harvested at maximum vegetative period (70 days). Maize shoots and roots were separated, washed, weighed and oven dried at 40°C for 48 hours for mercury analysis. Mercury concentration in the maize shoot and roots was analyzed using the method similar to that of experiment 1. The data obtained were subjected to to analysis of variance followed by 5% least significant difference test [22] using Minitab 13 software.

# 3. Results and Discussion

## 3.1. Plant Biomass

The test results of three plant species tolerance capabilities suggested that all plants showed high tolerance to soil contaminated with gold mine tailings containing mercury. This was demonstrated by the absence of inhibition of plant growth and no visible physical damage that showed toxicity symptoms at all plants. The shoot and root dry weights produced by *P.conjugatum* were not significantly different from those of *C.kyllingia* and *L.crustacea* (Figure 1 and Figure 2).

Based on the statistical analysis it was known that in general the addition of ammonium thiosulphate exerted a significant effect on the dry weight of shoots on all plants tested. However, the addition of ammonium thiosulphate did not significantly ($p<0.05$) increase root dry weights.

A type of plant to be classified as heavy metal accumulator group must meet the criteria in addition to having the ability to withstand high concentrations of metals in the soil, the level of uptake and translocation of metals in tissues with a high rate should ideally also have a high potential for biomass production [16]. Figure 1 and Figure 2 show that at 9 weeks, *P.conjugatum* has the highest potential to produce biomass followed by *L.crustacea* and *C.kyllingia*. In terms of biomass production, *P. conjugatum* seemed to be the best plant species for phytoremediation of mercury-contaminated soils.

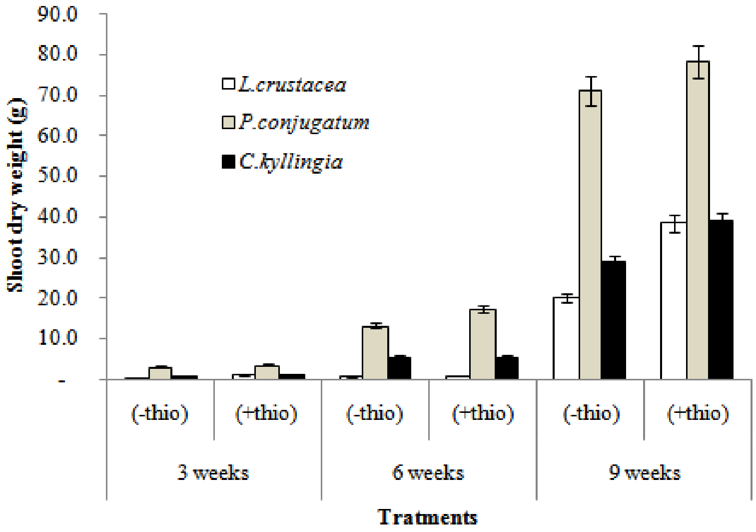


**Figure 1.** Shoot dry weight of three plant species grown for 9 weeks

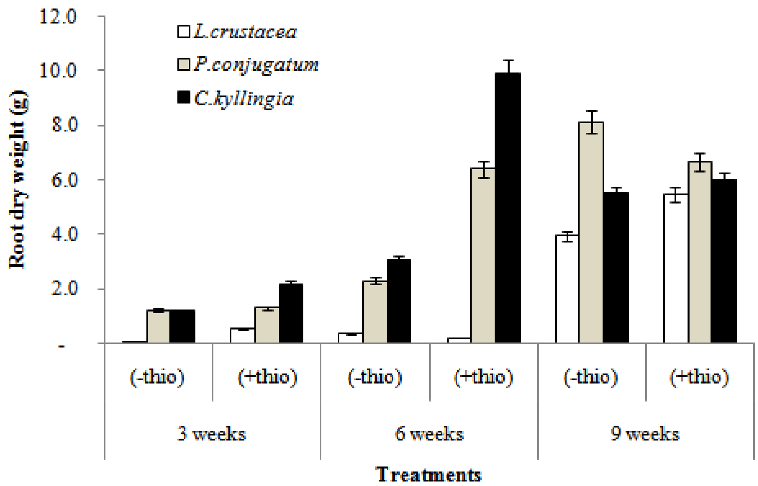


**Figure 2.** Root dry weight of three plant species grown for 9 weeks

## 3.2. Mercury Accumulation in Plants

The highest concentration of mercury was found in *P.conjugatum* followed by *L.crustacea* and *C.kyllingia*, both with and without addition of ammonium thiosulphate. Addition of ammonium thiosulphate in media did not significantly increase Hg content in the shoots of all plant tested, but significantly improved the content of Hg in the root. Plants develop some effective mechanisms to tolerate high levels of metals in the soil [17]. Accumulator plants did not prevent the metal into the roots but develop specific mechanisms to detoxify heavy metals in soils with high levels in the cell that allows the bioaccumulation of metals in high concentrations [5]. High accumulation in plant species reflects the high concentration of metals in the rhizosphere. Plants can naturally accumulate metals exceeding a threshold value of 1% (Zn, Mn), 0.1% (Ni, Co, Cr, Pb and Al), 0.01% (Cd and Se), 0.001% (Hg) or 0.0001% (Au) of the weight of dry biomass without showing any symptoms of poisoning [16].

The calculation of the content or the accumulation of Hg and comparison of accumulation of Hg in each plant species presented in Figure 3 shows a difference in ability to accumulate Hg. Hg accumulation was high in P*aspalum conjugatum*, followed by *L.crustacea* and *C.kyllingia*. Highest root Hg accumulation was observed in *L.crustacea* followed by *P.conjugatum,* and *C.kyllingia*. High biomass production provides a meaningful influence on the accumulation of Hg (Hg yield per plant dry weight). Addition of ammonium thiosulphate at planting media significantly increased the accumulation of Hg in the plant shoots and roots. With no addition of thiosulphate, accumulation of mercury by *L.crustacea*, *P.conjugatum,* and *C.kyllingia* shoots at 9 weeks ranged from 9.0mg $kg^{-1}$ (*L.crustacea*) to 32.5mg $kg^{-1}$ (*P.conjugatum*). These values were significantly ($<p0.05$) lower than that of ammonium thiosulphate treatment that ranged from 21.0mg $kg^{-1}$ (*C.kyllingia*) to 39.1mg $ha^{-1}$ (*P.conjugatum*) (Figure 3). A study conducted previously on soil contaminated with gold cyanidation tailing showed that the three plant species accumulated 9.06, 10.36 and

15.65mg Hg $kg^{-1}$, respectively [14]. This figure exceeded the threshold value of mercury concentration of 0.001% or 10mg $kg^{-1}$ of total dry weight [7]. Previous workers suggested that there is a relationship between the levels of heavy metal pollution in the soil by absorption by plants [17]. Accumulation occurs because there is a tendency of heavy metals to form complex compounds with inorganic substances found in the body of organisms [18].

Addition of ammonium thiosulphate at planting media also significantly influenced the accumulation of mercury in the roots that ranged from 2.6mg $kg^{-1}$ (*L. crustacea*) to 3.7mg $kg^{-1}$ (*P.conjugatum*) (Figure 3). With no addition of ammonium thiosulphate the accumulation of Hg in the roots ranged from 1.3mg $kg^{-1}$ (*L. crustacea*) to 4.0mg $kg^{-1}$ (*P.conjugatum*) (Figure 3).

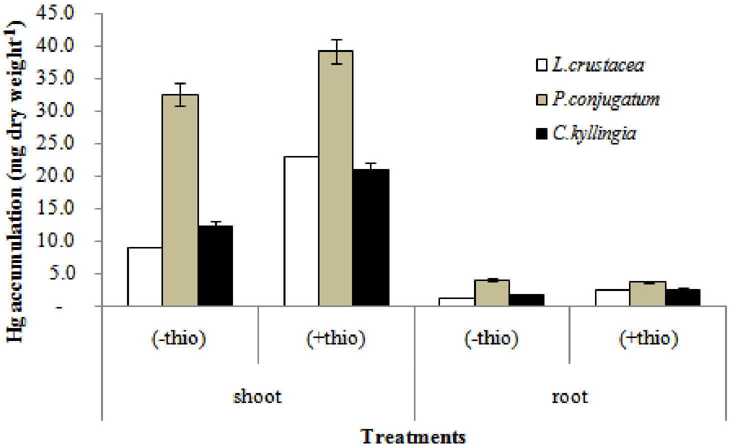


**Figure 3.** Accumulation of mercury in shoot and root of three plant species grown for 9 weeks

On average, the addition of thiosulphate increased the accumulation of Hg in plant shoots and roots by 82% and 47%, respectively, compared to the media without addition of thiosulphate. This occurred because mercury has a strong affinity with thiol groups, especially complex sulphide and bisulphide [18,19]. *B.juncea* has been shown to be able to concentrate Hg to 40mg $kg^{-1}$ in plant tissue after application of ammonium thiosulphate in mining waste contaminated with 2.8mg Hg $kg^{-1}$ [19].

Accumulated Hg ratio shoot / root on three plant species showed that all plants have Hg shoot / root ratios of more than one, both for ammonium thiosulphate treated and untreated pots (Figure 4). The ratio of Hg shoot/root of *L.crustacea* treated with ammonium thiosulphate was the greatest, followed by *P.conjugatum*, and *C.kyllingia*. With no addition of ammonium thiosulphate, however, the greatest ratio was observed for *P. conjugatum* followed by *L. crustace* and *C. kyllingia* (Figure 4)

The difference in the ratio of Hg shoot/root on all plants showed differences in the effectiveness of each type of plant in transporting mercury from the root system of the shoot (as a place of accumulation) [18].

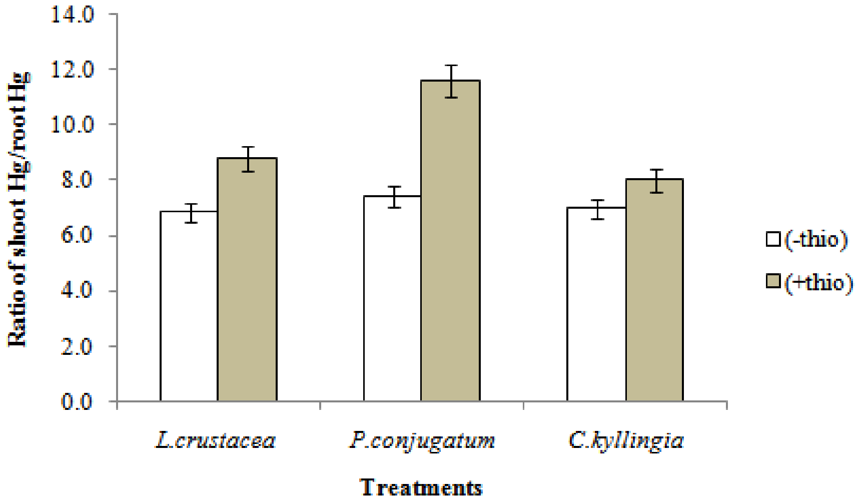


**Figure 4.** Ratio of Hg accumulation in shoot and root of three plant species grown for 9 weeks

### 3.3. Growth and Biomass Yield of Maize

At harvest (8 weeks), maize plant height varied from 16.13 cm (control) to 24.90cm (*P.conjugatum* treatment) in media without addition of ammonium thiosulphate (Figure 5). In the media with addition of ammonium thiosulphate, plant height varied from 16.13cm (control) to 31.21cm (*P.conjugatum* treatment) (Figure 5). Overall, in comparison to the control treatment, the average height improvement of maize grown on media previously remediated with three plant species without addition of ammonium thiosulphate was 49%, while that grown on media previously remediated with three plant species with addition of ammonium thiosulphate was 83%.

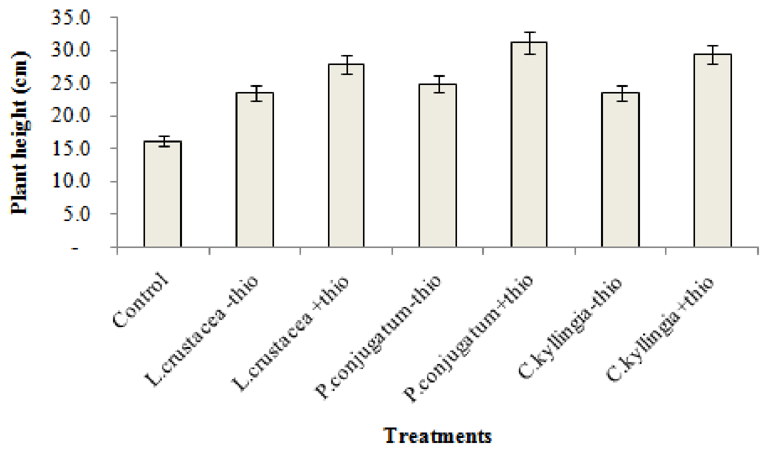


**Figure 5.** Height of maize grown on post-phytoremediation soil for 8 weeks

Shoot and root dry weight of maize also increased (compared to control) after phytoremediation of mercuty contaminated soil with three plant species. Consistent with the highest ability of *P.conjugatum* to accumulate Hg, the highest increase of maize shoot and root dry weights occurred on the *P.conjugatum* treatment (Figure 6).

On average, the biomass dry weight increase of maize (shoot + root) grown on media previously remediated with three plant species without addition of ammonium thiosulphate was 40%, while that grown on media previously remediated with three plant species with addition of ammonium thiosulphate was 62%. The lower increase in plant growth and biomass production of maize grown on post-phytoremedation without addition of ammonium thiosulphate compared to that grown on post-phytoremediation soil with addition of ammonium thiosulphate related to the removal of mercury by three wild plant species (Figure 3).

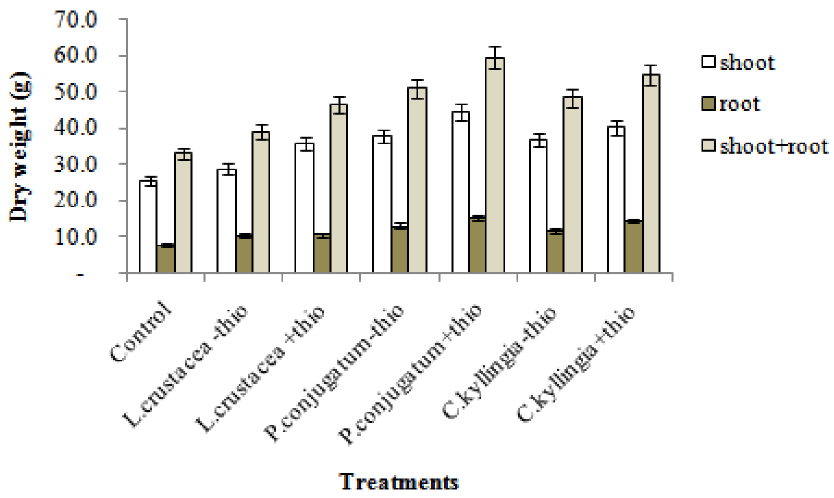


**Figure 6.** Shoot and root dry weight of maize grown on post-phytoremediation soil for 8 weeks

The remaining mercury in media without addition of ammonium sulphate was higher than that on media with addition of ammonium thiosulphate, thus inhibiting the growth of plants, in the plant. Mercury poisons and causes damage to the enzyme, polynucleotide, nutrient transport system and disrupts cell membrane integrity [17]. Roots elongation is often used as a first indication that the plants were poisoned elemental Hg [20]. Mercury toxicity symptoms in general are stunted growing seeds and roots, and inhabitation of photosynthesis process which in turn reduces crop production. Additionally mercury accumulated in root tissue can inhibit K uptake by plants [13]. Hg absorbed by plants can lead to inactive several enzymes because mercury incorporation into sulfhydril of peroxide through the formation of reactive oxygen compounds, such as superoxide ($O_2$), hydroxyl radical (OH-) and hydrogen peroxide ($H_2O_2$) [21].

## 4. Conclusion

*P.conjugatum*, *C.kylingia* and *L.crustacea* are potential for phytoremediation of mercury-contaminated soil. Addition of ammonium thiosulphate to the mercury-contaminated soil increased mercury accumulation in plants. Growth and biomass production in maize grown on remediated soil increased 74% and 67%, especially after phytoremediation with *P.conjugatum*.


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