JOURNAL OF DEGRADED AND MINING LANDS MANAGEMENT
ISSN: 2339-076X, Volume 2, Number 1 (October 2014): 251-258
DOI:10.15243/jdmlm.2014.021.251

Research Article

Application of organic matter to enhance phytoremediation of mercury contaminated soils using local plant species: a case study on small-scale gold mining locations in Banyuwangi of East Java

N. Muddarisna[1*], B.C. Siahaan[2]

[1] Wisnuwardhana University, Jl. Danau Setani No 99, Malang 65139, Indonesia
[2] Former student of the Department of Soil Science, Faculty of Agriculture, Brawijaya University, Jl. Veteran, Malang 65415, Indonesia

* Corresponding author: nurulmuddarisna@yahoo.co.id.

Abstract: The discharge of small-scale gold mine tailing to agricultural lands at Pesanggaran village of Banyuwangi Regency caused soil degradation as indicated by reduced crop production. This soil degradation is mainly due to the toxicity of mercury contained in the tailing. The purpose of this study was to explore the potential of three local plant species, i.e. *Lindernia crustacea*, *Digitaria radicosa*, and *Cyperus kyllingia* for phytoremediation of agricultural land contaminated gold mine waste containing mercury, and its influence on the growth of maize. Six treatments (three plant species, and two levels of organic matter application) were arranged in a randomized block design with three replicates. Maize was grown on soil after phytoremediation for 8 weeks. The results showed that among the three plant species tested, *Cyperus kyllingia* was the potential candidate plant species for phytoremediation of soil contaminated with gold mine tailing containing mercury because of its ability to accumulate mercury from 32.06 to 73.90 mg / kg of soil in 60 days. Phytoremediation of mercury contaminated soil using *Cyperus kyllingia* using increased maize yield by 126% compared to that the biomass yield of maize grown on soil without phytoremediation. Induce phytoremediation needs to be carried out to accelerate the process of remediation of mercury contaminated soils.

Keywords: *indigenous plant species, mercury contaminated soil organic matter, phytoremediation*

Introduction

Gold mining sector in Indonesia consists of a large-scale gold mining, gold mining medium scale and small-scale gold mining (ASGM). Pesanggaran Village, District tile, Banyuwangi regency is one of ASGM locations in East Java that has been operating illegally since 2009. Miners generally using mercury amalgamation method because it is considered an efficient method and requires only a small investment. However, in addition to the assumption of the efficiency of mercury amalgamation, the ability of mercury to bind the gold from gold ore is highly dependent on particle size and geochemistry of gold (Viega et al., 2006). A study conducted at ASGM locations in the Philippines reported that gold obtained from the mercury amalgamation method is only 10% (Hylander et al., 2007). In addition, there is always mercury lost to the environment through the disposal of wastewater from amalgamation treatment processes.

At Pesanggaran Village, Genteng District of Banyuwangi Regency, the discharge of gold amalgamation waste to agricultural land gave a negative impact on maize production. Chlorosis is a major symptom of mercury toxicity in plants. As reported by head of Pesanggaran Village, illegal gold mining activities in the village reduced maize production by 70%. According to Fitter and Hay (2004), metal ions react specifically with enzymes, which in turn interfere with the metabolic processes in plants. Therefore, remediation technologies are needed for reclamation and remediation of soil contaminated with metals, including mercury (Wuana and Okieimen, 2011).

The last few years, concern about the use of plants for remediation environments contaminated with heavy metal is increasing. The technology known as phytoremediation can be used as a cheaper alternative than the physical method (Fasani, 2012). From various phytoremediation techniques, most attention has been focused phytoextraction of metals. In this method, the plants absorb heavy metals from soil contaminated with heavy metals. Harvesting and disposal of plant biomass also means cleaning up metal elements from soil (Banuelos et al., 2011; Pedron et al., 2011). Muddarisna et al. (2013) reported that *Digitaria radicosa*, *Cyperus kyllingia*, and *Lindernia crustacea* potential are potential for use in phytoextraction mercury because these plant species efficiently absorb and transport mercury from the roots to the shoots. Hidayati et al. (2009) reported several species of plants at ASGM locations in West Java that are able to accumulate mercury up to 20 mg/kg, i.e. *Lindernia crustacea*, *Digitaria radicosa*, and *Cyperus kyllingia*. Accumulator plants and the availability of metals in the soil for the plant uptake determine the success of phytoextraction (Lin et al., 2010).

Hg discharged to soils is generally retained by the soil through absorption on sulphide, clay particles and organic matter (Evans, 1989). The form of Hg is not soluble, so it is relatively not mobile in the soil. Mercury has a strong affinity with thiol groups, especially sulphide and bisulphide complexes (Morel et al., 1998). In addition, humic compounds that make up 50% of soil organic matter contain functional groups containing S in large enough quantities (Wallschlager et al., 1998a). Humic compounds that are composed of humic acid and fulvic acid are Hg chelate (Wallschlager et al., 1996). Therefore, Hg humic-fulvic acid complexes are mobile in the soil (Wallschlager et al., 1998b). Humic-fulvic acid has been shown to stimulate the availability of Hg in soil and uptake by organisms Hg (Hinton, 2002).

Based on the above findings, application of organic matter into small-scale gold processing waste containing mercury are expected to enhance the solubility of mercury that can be absorbed by the metal accumulator plants. The purpose of this study was to examine the study the effects of organic matter application on phytoremediation of soils contaminated with small-scale gold mine tailings using *Lindernia crustacea*, *Digitaria radicosa*, and *Cyperus kylingia*, and its effect on the growth and biomass production of maize.

Materials and Methods

A pot experiment was conducted in October 2011 to November 2012 in the glasshouse of the Faculty of Agriculture, Brawijaya University. Materials used in this study were (1) plant species, *Lindernia crustacea*, *Digitaria radicosa*, and *Cyperus kyllingia*, (2) waste of gold mining process with mercury amalgamation (hereafter referred to as amalgamation tailing), (3) topsoil (0-30 cm) that is not contaminated with amalgamation tailing containing mercury, and (4) maize seeds of NK33 variety. Seeds of three plant species (*Lindernia crustacea*, *Digitaria radicosa*, and *Cyperus kyllingia*), amalgamation tailing and uncontaminated soil were all obtained from the ASGM locations at Pasanggaran Village, Genteng District of Banyuwangi Regency.

Samples of soil and tailing were air dried for two weeks, then sieved to pass through a 2 mm sieve for analyses of pH (1:2.5 water suspension), and N, P_2O_5, organic C, Hg contents. The results of the analysis were as follows: (a) uncontaminated soil: pH 6.27, organic C 0.65%, 0.16% P_2O_5, and 0.67% total N, (b) amalgamation tailing: pH 7.02, organic C 0%, P_2O_5 0:06%, 0.22% total N, and 190.03 mg Hg / kg. The Hg concentration was measured by Cold Vapor Atomic Absorption Mercury analyzer. Samples of soil and amalgamation tailing were mixed with the proportion of 80% soil + 20% tailing. Seeds of each *Lindernia crustacea*, *Digitaria radicosa*, and *Cyperus kyllingia* that had been germinated for two weeks were then planted on 5 kg of the soil-tailing mixture, each of which was supplied with 10 t organic matter / ha, and without the addition of organic matter. Six treatments (three plant species and two doses of organic matter), were arranged in a randomized block design with three replications.

Organic matter used for this experiment was compost produced by Composting Unit of the Faculty of Agriculture, Brawijaya University. During the experiment, water was supplied every day to maintain a sufficient supply of water for plant growth. After 60 days, the plants were harvested and analyzed Hg concentrations in the plant biomass (shoots and roots) and soil-tailing mixture in the pot. The ability of plants to transport mercury from roots to shoots was evaluated by using the Translocation Factor (TF) (Yoon et al., 2006; Sarawet and Rai, 2007; Zacchiini et al., 2008). TF is the ratio of metal concentration in the plant shoot with metal concentrations in plant roots. Plants with TF values> 1 could potentially be used to phytoextraction, whereas plants with TF values <1 could potentially be used to phytostabilization (Yoon et al., 2006). Phytoremediation efficiency was expressed by RF (remediation factor), which is the metal uptake in the plant shoot compared with the amount of metallic elements in the soil (Sun et al., 2009).

The results of the statistical analysis were used as the basis for selecting the best plant species (highest Hg accumulation), for phytoremediation of mercury contaminated soils. The remaining soil-tailing mixture in the pot (post-phytoremediation), was then used for growing maize for 60 days. For comparison, one control treatment was included for each plant species. The control treatment was the soil-tailing mixture without phytoremediation treatment. Each pot was supplied basic fertilizers equivalent of 100kg N / ha, 50kg P / ha and 50kg K / ha. At the time of harvest (60 days), plant height and dry weight of maize shoot and roots were measured. Statistical analysis was performed using the F test (analysis of variance) with a significance level of 5% to determine the effect of treatment on the observation of parameters followed by Least Significant Difference at 5% significance level.

Results and Discussion

Pant biomass

The results of the evaluation of three herbaceous plant species showed that the three species were tolerant to soil contaminated with amalgamation tailing containing mercury (Figure 1). This indicates that the growth of the plants for 60 days did not show symptoms of toxicity and damage to plant morphology. Shoot and root dry weight of all plants without the addition of organic matter showed no significant differences. In the treatment of organic matter addition, addition dry weight was achieved by *C. kyllingia* that was significantly higher than *L. curstacea* and *D.radicosa*, while the shoot dry weights of *L. crustacea* and *D. radicosa* were not significantly different. However, the addition of organic matter did not significantly affect root dry weight of three of herbaceous plant species.

A type or plant species that can be classified as a heavy metal accumulator must meet the criteria in addition to survival in the conditions of the soil medium with high concentration of heavy metals, the rate of uptake and translocation of metals in plant tissues and, biomass yield should also high (Rascio and Navari-Izzo, 2011). Figure 1 shows that at the age of 60 days, *C. kyllingia* had the highest potential to produce biomass, followed by *D. radicosa* and *L. crustacea*. In terms of biomass production, Cyperus kyllingia can be expressed as best candidate species for phytoremediation of soil contaminated gold amalgamation tailing containing mercury.

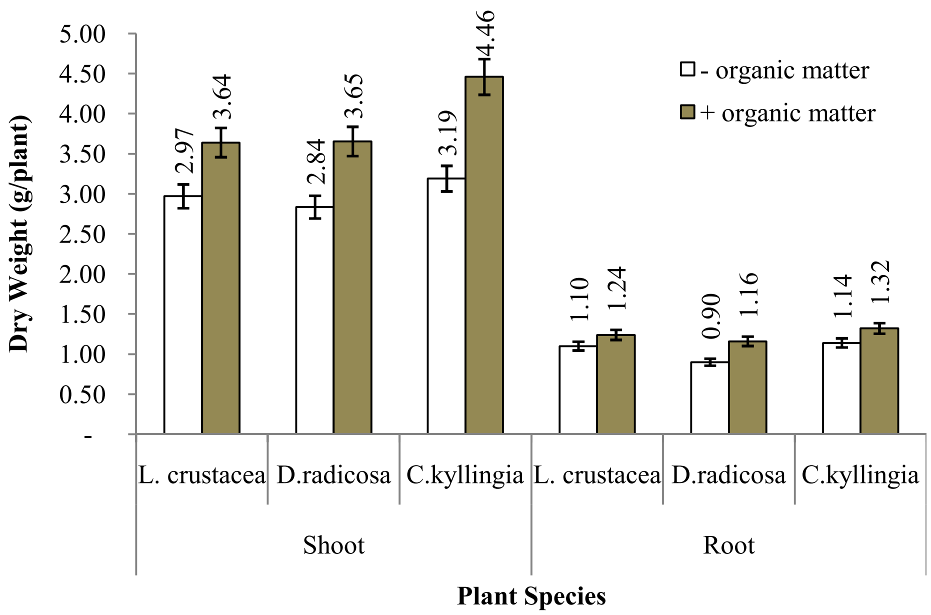

Figure 1. Biomass dry weight of *L.crustacea, D.radicosa* and *C.kyllingia* grown on soil contaminated with amalgamation tailing for 60 days, with and without addition of organic matter

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Accumulation of mercury in plants

The highest Hg concentration was found in *C. kyllingia* followed by *L. crustacea* and *D. radicosa*, either with or without the addition of organic matter. The addition of organic matter to the planting medium did not significantly increase the concentration of Hg in the plant roots, but significantly increased the concentrations of Hg in the shoots, especially for *C. kyllingia*. Plants develop some effective mechanisms in order tolerant to high concentrations of metals in the soil (Nagajyoti et al., 2010). Accumulator plants do not prevent the entry of metal into the root element, but develop specific mechanisms to detoxify the metal element inside the cell to bioaccumulation of metal elements (Fasani, 2012). The accumulation of heavy metal elements in plant species reflects the high metal concentrations in the rhizosphere. By nature, plants can accumulate metallic elements that exceed the tolerance threshold of 1% (Zn, Mn), 0.1% (Ni, Co, Cr, Pb and Al), 0.01% (by Cd and Se), 0.001% (Hg) or 0.0001% (Au) of the dry weight of the plant without showing symptoms of toxicity (Navari-Izzo and Rascio, 2011).

Calculation of the concentration of Hg and comparison of Hg concentration ratio in each plant species presented in Figure 2 show the differences in the ability of plants to accumulate Hg. The highest accumulation of Hg was observed for *C. kyllingia* followed by *L. crustacea* and *D. radicosa*. The highest Hg concentration in the roots was also found in *C. kyllingia* followed by *L.crustacea* and *D.radicosa*. High biomass production affected the accumulation of Hg. The addition of organic matter to the planting medium significantly increased Hg accumulation in the plant shoots and roots. Without the addition of organic matter, the accumulation of Hg in the shoots of the three plant species at 60 days ranged from 14.43 mg / kg (*L.crustacea*) to 32.06 mg / kg (*C.kyllingia*). This value was significantly lower than that of treatments with the addition of organic matter that ranged from 37.69 mg / kg (*L. Crustace*) to 73.90 mg / kg (*C. kyllingia*) (Figure 2). This value exceeded the threshold concentration of 10 mg Hg / kg dry weight of plants (Pedron et al., 2011).

According to Nagajyoti et al. (2010), there is a relationship between the levels of heavy metal contamination in soils with uptake by plants. Accumulation of metal elements occurs because there is a tendency to form a heavy metal compound complex with inorganic compounds found in the body of organisms (Selin, 2009). The addition of organic matter to the planting medium also significantly affected the accumulation of mercury in the roots that ranged from 33.87 mg / kg (*L. crustacea*) to 43.18 mg / kg (*C. kyllingia*) (Figure 2). In the treatments without addition of organic matter, the accumulation of Hg in roots ranged from 22.73 mg / kg (*L. crustacea*) to 28.07 mg / kg (*C kyllingia*) (Figure 2).

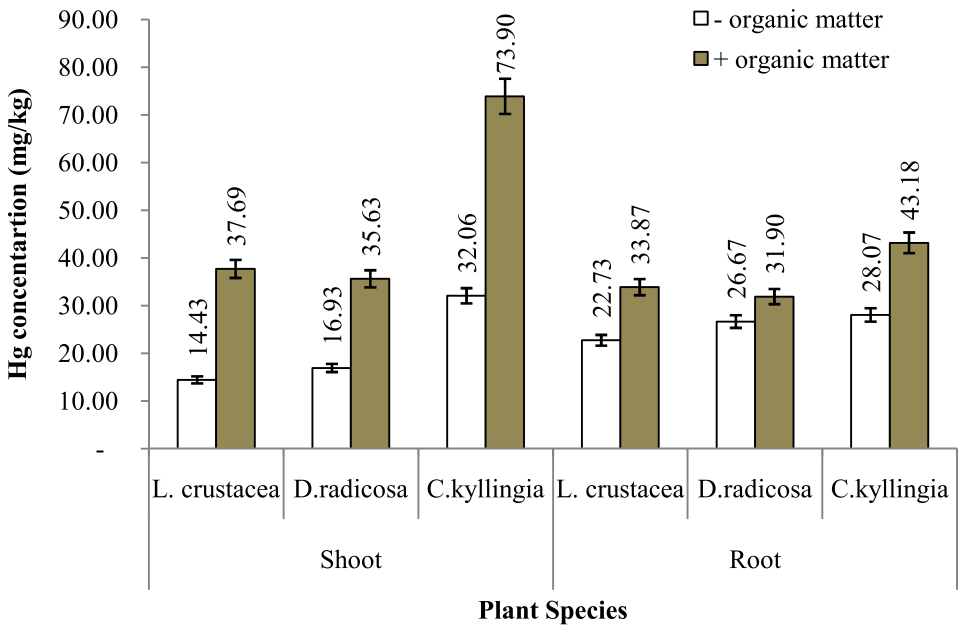

Figure 2. Concentration of Hg in the shoots and roots of *L. curstacea, D. radicosa* and *C. kyllingia* grown on soil contaminated with amalgamation tailing for 60 days, with and without addition of organic matter

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The addition of organic material in an average increased accumulation of Hg in the shoots and roots of the plants by 132% and 41%, compared to the media without the addition of organic matter. This is thought to occur because of the role of humic compounds in the organic matter (Wallschlager et al., 1998a). Humic compounds that composed of humic acid and fulvic acid chelate are Hg chelates (Wallschlager et al., 1996). Therefore, Hg humic-fulvic acids complex is mobile in the soil (Wallschlager et al., 1998b). Humic-fulvic acid has been shown to stimulate the availability of Hg in soil and uptake of Hg by organisms (Hinton, 2002).

The ability of plants to move metal elements from the root of the shoot can be measured by the value of TF (Translocation Factor), which is defined as the ratio of metal concentration in the shoot with metal concentrations in roots (Yoon et al., 2006). A plant species having a TF value of <1 is less suitable for phytoextraction (Fitz and Wenzel 2002). TF values> 1 indicate the effectiveness of moving the metal elements from the plant roots to the shoot (Zhang et al., 2002, Fayiga and Ma 2006). Results of this study showed that value of TF or the ratio of the concentration of Hg in the shoots and the roots of the addition of organic matter treatment resulted in were > 1 (Figure 3). However, the treatment with no organic material, the value of TF three plant species were all <1. This suggests that the addition of organic matter changes the role of the plant formerly as phytostabilizators (TF value of <1) to plant metal phytoextractor plants (TF values> 1) that is able to move Hg from the roots to the shoot.

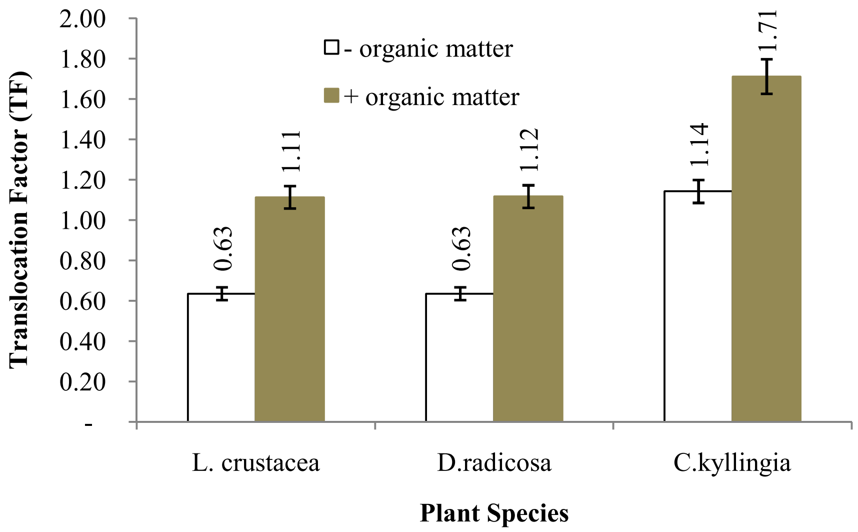

Figure 3. Translocation Factor (TF)

The highest TF value was found in *C. kyllingia* with or without the addition of organic matter (Figure 3). The differences in RF values on three plant species showed differences in the effectiveness of each plant species in the transport of mercury from the shoot to the root system as a place of accumulation (Selin, 2009). This difference is also thought to be related to the modification of plant growth under conditions of heavy metal was seized as a result of the absence of certain amino acids in plants (Ashraf et al., 2011). The efficiency of Hg phytoremediation of *C. kyllingia* Hg was higher than *D. radicosa* and *L.crustacea*, while the efficiency of phytoremediation of *D. radicosa* and *L. crustacea* were not significantly different (Figure 4). Application of organic matter significantly improved the efficiency of Hg phytoremediation by three plant species tested, with the highest increase was achieved by *C. kyllingia*.

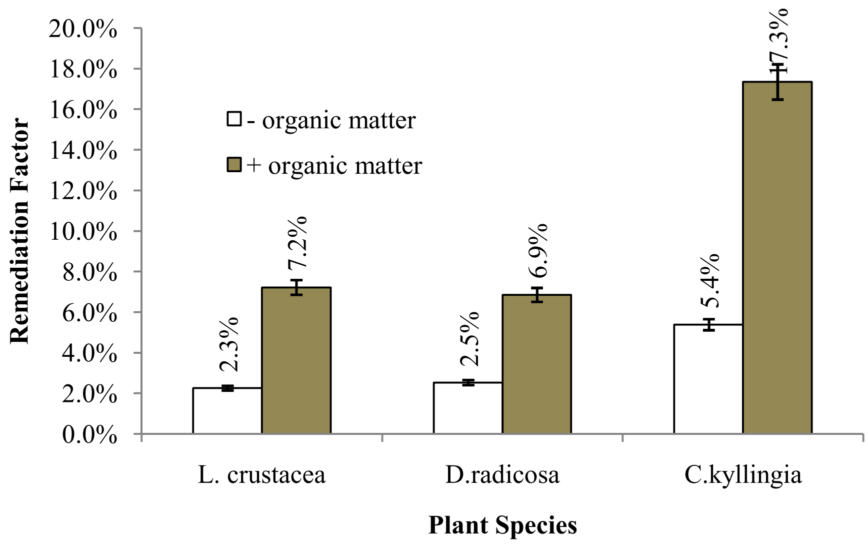

Figure 4. Phytoremediation efficiency of Hg by *L.crustacea*, *D. radicosa,* dan *C.kyllingia* grown on soil contaminated with amalgamation tailing for 60 days, with and without addition of organic matter

Growth of maize on post-phytoremediation soil

At the time of harvest (60 days), maize plant height ranged from 33.58 cm (control, without phytoremediation) to 116.10 cm (post-phytoremediation using *C.kyllingia*) in the planting medium without application of organic matter (Figure 5). At the growing media with application of organic matter, plant height varied from 36.29 cm (control) up to 158.31cm (post-phytoremediation using *C.kyllingia*) (Figure 5). Overall, compared with controls, the average height of maize plants grown in the media that had been remediated with three plant species without application of organic matter increased 151%, while the treatment with the application of organic materials the plant height increased by 230% .

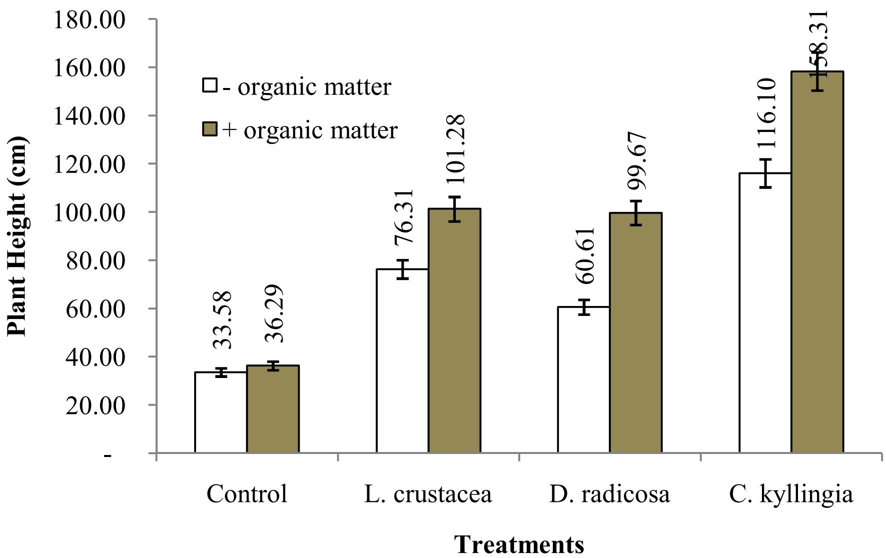

Figure 5. Maize plant height at 60 days on post-phytoremediation of soil contaminated with amalgamation tailing containing mercury.

Maize shoot dry weight also increased (compared to control) after soil phytoremediation by three species of herbaceous plants. Consistent with the ability of *C. kyllingia* in accumulating the highest Hg, the highest increase in dry weight of maize shoot was also observed for *C.kyllingia* treatment (Figure 6).The average dry weight biomass (shoot) of maize grown on previously growing media previously remediated with three herbaceous plant species without application of organic matter increased 65%, while that of grown on media previously remediated with three herbaceous plant species with application of organic matter increased 102%. The lower growth and biomass of maize grown on post-phytoremediation media without application of organic matter compared to the application of organic matter treatment, was related to the reduction in mercury content in the media due to uptake by the three accumulator plant species in the phytoremediation activities.

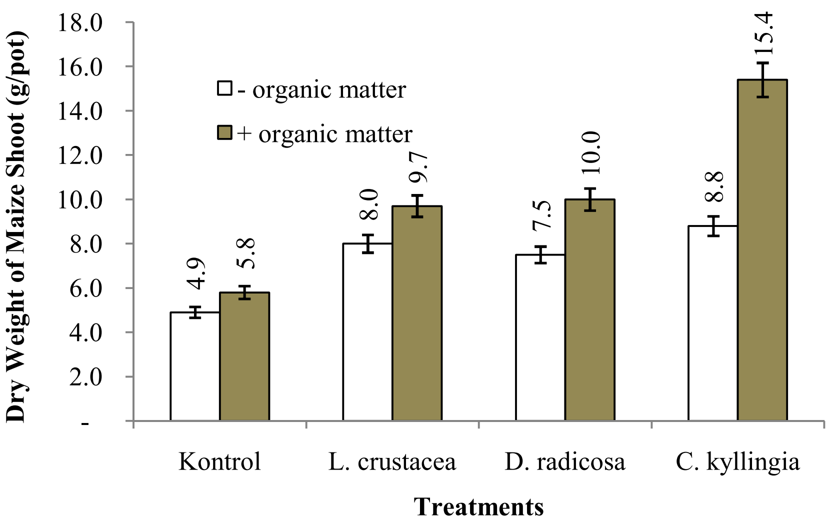

Figure 6. Dry weight of maize shoots at 60 hari on post-phytoremediation of soil contaminated with amalgamation tailing containing mercury.

Mercury remaining in the planting medium without application of organic matter was higher than the mercury remaining in the planting medium with the application of organic matter, thus inhibiting plant growth. In the plants, mercury is toxic and cause damage to enzymes, polynucleotide, nutrients transportation system, and destroy integrity of the cell membrane (Nagajyoti et al., 2010). Extension of the root is often used as a first indication of Hg toxicity (Moldovan et al., 2013). Symptoms of mercury toxicity in general are stunted growth of roots and seeds, and inhibition of photosynthesis which in turn reduces crop production. In addition, mercury accumulated in root tissue can inhibit K uptake by plants (Hooda, 2010). Mercury absorbed by the roots can cause some enzymes become inactive due to the inclusion of mercury in the form of sulfihidril peroxide through formation of reactive oxygen compounds, such as superoxide (O_2), hydroxyl radicals (OH-) and hydrogen peroxide (H_2O_2) (Chen and Yang, 2012) .

Conclusion

Digitaria radicosa, *Cyperus kylingia* and *Lindernia crustacea* are potential herbaceous plant species that van be used for phytoremediation of soil contaminated with gold amalgamation tailing containing mercury. The ability of the plant roots to transport Hg to the plant shoot was in the following order, *Cyperus kyllingia*> *Lindernia crustacea*> *Digitaria radicosa*. Application of organic matter in soil contaminated gold amalgamation tailing containing mercury increased the concentration of Hg and Hg uptake by plants accumulator. Phytoremediation of soil contaminated with gold amalgamation tailing containing mercury amalgamation increased growth and yield of maize.

Acknowledgements

Authors wish to thank the Indofood Riset Nugraha and Brawijaya University for financial support to undertake this study.

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