Growth performance and biomass production of Eleusine indica and Rorippa sylvestris on heavy metal contaminated soil after biochar application

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Research Article

Growth performance and biomass production of *Eleusine indica* and *Rorippa sylvestris* on heavy metal contaminated soil after biochar application

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Abstract: Heavy metal contamination was an environmental and human health problem all over the world. Cadmium is the most hazardous heavy metals due to its high mobility and toxicity at low concentrations. Lead (Pb) also belongs to the hazard element caused by its prolonged persistence in the soil. This study aimed to develop the remediation techniques on polluted land, i.e. a combination of biochar and indigenous plant. The biochar was produced by slow pyrolysis method. This experiment was conducted at the farmland at Sumber Brantas, Malang, East Java. We used rice husk and tobacco waste biochar, and *Eleusine indica* (L.) Gaertn and *Rorippa sylvestris* (L.) Bess. as remediator plants. The results showed that rice husk biochar had a significant effect on pH value and potassium content (p = 0.0001; p=0.0004). On the contrary, the soil nitrogen content, soil organic-C content, and soil cation exchange capacity applied with tobacco waste biochar application were higher than that applied with rice husk biochar (p = 0.03; p=0.00001; p = 0.00001). The improvement of soil characteristics increased the growth of *Eleusine indica* and *Rorippa sylvestris* as indicated by the plant height and biomass. The addition of biochar could promote the growth of remediator plant and enhanced the accumulation of Pb and Cd in the plants. Mixtures of rice husk biochar and tobacco waste biochar caused *Eleusine indica* more effectively absorbed heavy metals than *Rorippa sylvestris* on all types of biochar treatments; *Eleusine indica* absorbed Pb and Cd higher than *Rorippa sylvestris* as shown by Pb and Cd contents in the soil.

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***Keywords:** biochar, heavy metal contaminated soil, phytoremediation*

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Introduction

Heavy metal contamination has become a common environmental and human health problem all over the world, due to biomagnification and their difficulties to degrade. Industrialization and technology advances have led to an increase in anthropogenic activities that are responsible for heavy metals input into the soil such as smelting, mining, use of fertilizers, pesticides, and sludge. The addition of fertilizer and sewage sludge in agricultural practices can increase heavy metals, especially Cd and Pb, contamination in the soil. Application of phosphate fertilizer that is rich in Cd will lead to Cd contamination. Cadmium is categorized as the most common heavy metal in the environment. According to Devi and Bhattacharrya (2018), Cd is the most mobile and high risk for the environment. Lead (Pb) also belongs to the hazard element caused by its prolonged persistence in the soil (Wani et al., 2012). Some technologies included conventional methods has been used for decades to remediate the hazardous heavy metals (Khalid et al., 2017). Unfortunately, conventional remediation methods are expensive and environmentally damaged. One of the innovative, eco-friendly and low-cost alternative technologies is phytoremediation. This technique uses heavy metal tolerant plants to clean up the contaminants by absorbing, accumulating and detoxifying pollutants from the site through their metabolic processes (Bhat et al., 2016).

Phytoremediation is a low-cost, non-destructive, and aesthetically sound. It has caused an increasing interest to exploit the ability of plants to remediate pollutants from contaminated soil. The efficiency of phytoremediation is dependent on soil physical and chemical properties, metal bioavailability of plant, and plant capacity to uptake, accumulate and detoxify metals. The effectiveness of phytoremediation mechanisms depends on the plant biomass, heavy metal level in the plant tissues, and the availability of the heavy metals in the soil (Garba et al., 2013). The plant capability in absorbing heavy metals depends on the ability of the plant in producing biomass (Conesa et al., 2012). Many studies showed that grasses are the most common preferable plants for phytoremediation because of their capability to grow rapidly, produce a large amount of biomass, and adapt stress in the environment (Elakes, 2014). Brassicaceae family also contains a high number of species which can hyper accumulate heavy metals (Babula et al., 2012; Dar et al., 2014). Hyperaccumulator plant that belongs to grass species is *Eleusine indica* (L.) Gaertn, and that from Brassicaceae family is *Rorippa sp*. Anarado et al. (2018) showed that *Eleusine indica* (L.) Gaertn is the best hyperaccumulator plant for zinc and cobalt; while *Rorippa sylvestris* (L.) Bess has significant potential in accumulating uranium on its shoot (Cordeiro et al., 2016).

Generally, heavy metals will cause oxidative stress by formatting free radicals which will cause a reduction in plant growth and biomass (Panda and Patra, 2016); it is thus important to enhance the plant resistance against heavy metal stress. One of the techniques is using organic amendments, such as biochar. According to Chirakkara and Reddy (2015), the biomass of plants can be improved by adding biochar and compost amendments. By adding biochar, metals will be immobilized so that the essential nutrients can be released; soil water holding capacity, porosity and soil structure will be improved, and finally, it promotes plant growth. Biochar application on contaminated soils will also allow the plant to sequester C then storing it into their part of the biomass. However, studies on phytoremediation heavy metal contaminated soil using biochar amendment are still limited. The objectives of this study were to assess the effect of biochar addition on the biomass production of remediator plants.

Materials and Methods

Study site

The study site is located at Sumber Brantas village, Bumiaji Sub District, Batu, East Java. Sumber Brantas village is one of the horticulture areas located in Batu, East Java. This area is a cultivated area that is fertilized intensively with inorganic fertilizer containing cadmium (Cd) and mercury (Hg). The previous studies showed that the content Cd and Hg in the soil had already exceeded the Cd and Hg threshold value, i.e. 1.96 mg/kg and 0.5 mg/kg, respectively; thus these elements were categorized as toxic. According to Ministry of Environment of Finland (2007), the threshold value of Cd = 1 mg/kg; Hg = 0.5 mg/kg; Pb= 60 mg/kg).

Experimental design

The experimental design used for this study was a completely randomized block design comprised two factors (types of biochar and types of plant) with three replicates. There were four types of biochar and two types of remediator plant. The plot size used in this study was 3 m x 4 m. Biochars used in this experiment were derived from tobacco and rice husk. These materials were burned by a slow pyrolysis process at 300-550^{0}C and residence time between 45-90 minutes (Gezae and Chandraratne, 2018), and named as RHB (rice husk biochar) and TWB (tobacco waste biochar). The chemical composition of the biochars used in this study is presented in Table 1. The treatments tested in this study research were biochar addition and types of remediator plant. A control treatment (no biochar addition) was also included in this study. There were four treatment combinations, i.e. B0 = no biochar addition; B1 = RHB (rice husk biochar); B2 (TWB biochar), and (3) B3 = RHT (50% of rice husk biochar + 50% of tobacco waste biochar); and

Table 1. The characteristics of the biochar used in this study.

Type of Biochars	pH (H_2O)	C (%)	N (%)	P (%)	K (%)	CEC (cmol/kg)
Rice Husk Biochar (RHB)	8.44	34.57	1.10	1.19	1.61	19.64
Tobacco Waste Biochar (TWB)	8.26	38.15	1.59	0.68	1.06	51.89

two types of remediator plants T1 (*Eleusine indica* L. Gaertn) and T2 (*Rorippa sylvestris* L. Bess.). The biochar dosage used in this research was 20 kg/ha. The biochar was applied one week before planting by mixing it thoroughly on the 20 cm of the topsoil. Remediator plants were planted with a distance of 20 cm x 25 cm. During this experiment, the plant growth and soil parameters were measured.

Sampling techniques of the remediator plant

The remediator plants were taken from the previous studies, which are attributed to finding the best practices to remediate the contaminated soil due to excessive fertilizer. This study was an experimental plot that was conducted to obtain (1) the biochar characteristics and also (2) indigenous plant that capable of absorbing heavy metal. The research showed that biochar was not effective enough to immobilize heavy metal; it was thus needed to combine with other remediation technique such as phytoremediation.

The previous experiment showed that both of the indigenous plants (*Eleusine indica* L. Gaertn and *Rorippa sylvestris* L. Bess.) grew well on the experimental sites contaminated by heavy metals. Plant growth was an indicator of their potential as a remediator plant (Ahammad, 2018). According to Nejad and Jung (2017), a combination of remediator plants and amendment such as biochar can be more effective in the remediation process. This experiment was conducted to explore how the biochar can affect the indigenous plant biomass and increase heavy metal uptake. During this experiment, plant height was measured every week, while plant biomass (root weight, shoot, and leaf, dry weight), and root length were recorded at harvest time (90 days after planting).

The plant sample was cut 5 cm above the soil surface, stored in a plastic bag, then collected and separated carefully into roots and shoots. The total of plant biomass was air-dried, and weigh their dry weight. All samples were collected in the morning to maintain the freshness of the plant samples. At harvest, root, shoot and leaf samples were washed by tap water, then sterilized by distilled water; ground into fine powdered, then oven-dried at 60°C for 72 hours for analyzing heavy metal content on plant tissue. Hg and Pb concentration in the shoots and roots of the plants were determined by extracting the 250 mg of plant material with 10 ml of HNO_3 and $HClO_4$ acid.

Soil sampling preparation and analysis

Soil samples were taken two times randomly at the experiment sites, at the early stage of this research (before treatment), and the end of the experiment (after plant harvest). Soil samples were air-dried at 60°C, ground, and sieved pass through 2 mm then analyzed their soil nutrient content. Soil physical characteristic analysis consisted of soil texture; aggregate stability, bulk density, and soil water content. Soil chemical characteristics analyzed were pH (H_2O); organic-C, total nitrogen, total phosphorus, total potassium, cation exchange capacity (CEC); and also heavy metal contents (Cd and Pb). Soil samples passed through sieves less than 2 mm fraction was used to determining soil pH (1:5) soil/water extract.

Heavy metal analysis

Two grams of soil sample was taken then extracted in a mixture of 10 mL of HNO_3 and $HClO_4$ and was heated until 2 mL of liquid left. This solution then was mixed by distilled water step by step and heated until the solution became clear. The extracts then were analyzed by the atomic absorption spectrophotometer. Then, for analysis of heavy metal content, air dried-soil was extracted in a mixture of concentrate HNO_3 acid, HCl and 27,5% H_2O_2 (USEPA Method). According to this method, 0.3 g of soil sample was added with 10 mL of concentrate HNO_3 for at least 10 minutes. The mixture was then heated at 175°C and 1 atmosphere until the fluid was clear enough. The filtrate was then put into a 100 mL volumetric flask and diluted with distilled water to the boundary markers. Filtered was conducted through a Whatman filter paper.

The total concentration of heavy metal was measured by Atomic Absorption Spectrometer. The translocation factor (TF) was calculated to evaluate the rate of transfer of heavy metals between roots and stems of plants, as well as bio-concentration factors (BCF) to determine the indigenous plant's ability in absorbing heavy metals.

$$TF = \frac{\text{concentration of heavy metals in stems}}{\text{concentration of heavy metals in roots}}$$

$$BCF = \frac{\text{concentration of heavy metals in stems}}{\text{concentration of heavy metals in soils}}$$

Statistical analysis

The data obtained were analyzed using SPSS 16.0 using $P<0.05$ as the threshold of significance. Two-way ANOVA, followed by the Least Significance Difference test (LSD) at the 5% significance level ($\alpha = 0.05$) was used to test the differences among treatments. Specific parameters that could not be analyzed statistically were analyzed descriptively. The figures in this study were plotted using Microsoft Excell 2010.

Results and Discussion

Soil characteristics

The addition of biochar affected soil characteristics (Table 2). The effect depended on the source and types of biochar. The magnitude of the effects biochar types on the soil properties determined by soil types, biochar types, and incorporation rate (Dai et al., 2014), whereas swine manure biochar > pineapple peel biochar > rape straw biochar > reed straw biochar. Overall, the results of this study showed that the application of biochar induced changes in soil chemical characteristics, in terms of N, organic-C, K, and CEC. The soil pH value, potassium content and soil organic-C content before and after application of rice husk biochar were not different ($p = 0.25$; $p = 0.15$; $p = 0.34$). For the total Nitrogen and CEC, biochar application had a significant effect ($p = 0.003$; $p = 0.003$), which was shown by the increase of their contents in the soil due to biochar application. The same phenomenon also happened in tobacco waste biochar addition (Table 3). Application of tobacco waste (TW) biochar did not influence the value of pH and organic C ($p = 0.099$; $p = 0.17$), however, the application affected the nitrogen and potassium contents and also the soil CEC ($p = 0.011$; $p = 0.041$; $p = 0.001$). Most of the studies showed that biochar increases soil pH, but our results showed the opposite. Biochar amendment may decrease or increase the soil pH, depending on the pyrolysis processes, biochar type, and incorporation rate (Dai et al., 2014). Microorganisms can decrease the soil pH by producing organic acid, SO_2, and release ammonia content, while bacterial hydrolysis of protein which is releasing the NH_4^+ will increase soil pH. The different effects of biochar were attributed to the nature of biochar, and how is the biochar is produced (Obia et al., 2015).

Table 2. Effects of rice husk (RH) biochar on soil characteristics.

Biochar Application	pH	N (%)	K (mmol/k)	Organic-C (%)	CEC (mmol/kg)
Before	7.40±0.231	0.33±0.006	13.8±0.033	34.25±0.149	305.6±0.097
After	7.42±0.012	1.11±0.041	16.0±0.007	35.12±2.944	395.8±0.041

Note: Values represent the mean ± standard error (n=3).

Table 3. Effects of tobacco waste (TW) biochar on soil characteristics.

Biochar Application	pH	N (%)	K (mmol/kg)	Organic-C (%)	CEC (mmol/kg)
Before	6.00±0.058	0.30±0.023	21.1±0.076	31.81±5.423	281.3±0.151
After	6.28±0.012	1.52±0.051	10.7±0.024	48.13±0.033	518.7±0.025

Note: Values represent the mean ± standard error (n=3).

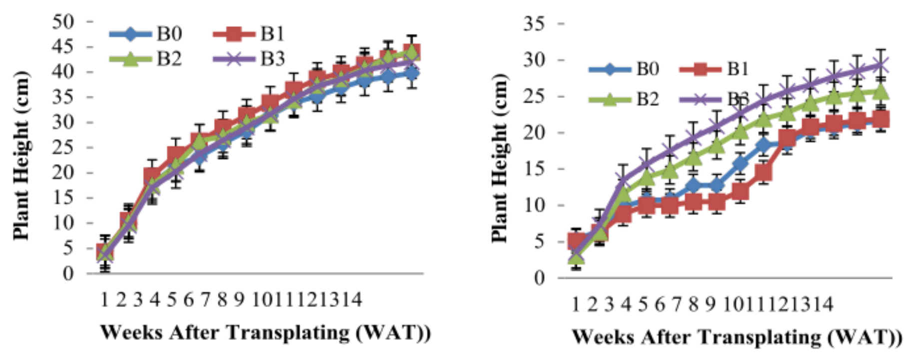
The nature of biochar will show the different effect on soil pH. Generally, biochar application improved soil characteristics (Tables 1 and 2) and affected all soil parameters measured. But the statistical analysis showed different effect between rice husk biochar (RH) and tobacco waste biochar (TWB)on related soil properties. Rice husk biochar (RH) had a significant effect on pH value and potassium content ($p = 0.0001$; $p = 0.0004$). On the contrary, the addition of tobacco waste biochar served more available of soil nitrogen, soil organic-C, and CEC ($p = 0.03$; $p = 0.00001$; $p = 0.00001$) than rice husk biochar. This difference was attributed to the alkalinity of biochar which is determined by the pyrolysis process and the soil types. Biochar alkalinity has a large contribution to the soil pH changes by changing the soil pH buffering (Dai et al., 2014). Rice husk biochar and tobacco waster biochar differed in alkalinity. Biochar amendment will increase the soil pH on acidic soil, just as already shown by Zhang et al. (2019). Obia et al. (2015) showed the difference effect of rice husk biochar and cacao shell biochar. Their study showed that rice husk biochar addition resulted in only 0.2 soil pH increase, whereas the cocoa shell will increase the soil pH by 2.3 units. It shows the alkalizing effect of biochar.

Growth and biomass of remediator plants

Generally, the research results showed that both *Eleusine indica* and *Rorippa sylvestris* are potential metal tolerant- plants because they could grow well on the contaminated land. The addition of the biochar amendment had a significant effect on plant growth, as shown by increasing plant height (Figures 1a. and 1b.). Biochar has a positive effect on plant growth, due to improvement in soil physical, chemical, and biological properties

(Cornelissen et al., 2013; Jalal et al., 2020; Ding et al., 2016), as also shown in Tables 2 and 3. The beneficial effects of biochar addition for the availability of C, N, Ca, Mg, P, and K are largely due to biochar capability in absorbing and releasing the nutrients (Rawat et al., 2019). Improving soil physical characteristic is caused by the porous structure of biochar, and also the presence of functional groups in the organic compounds of biochar. These soil characteristics indicate the sorption properties, included the sorption water capacity; then it will enhance the sorption on the nutrient. Biochar will also affect the activities and population of soil microorganisms, fungi and bacteria; and soil chemical properties, by creating a favourable environment for the microorganism, so it increases the soil fertility and productivity. Biochar addition improved soil fertility, especially soil C and N (Jalal et al., 2020), sufficient amount of nitrogen availability will lead to increase in plant growth (Akhtar et al., 2018).

The beneficial effects of biochar addition for the availability of nutrients are largely due to the higher content of potassium, phosphorus, and zinc availability, and to a lesser extent, calcium and copper (Lehmann et al., 2003).



(a) (b)

Figure 1. The influence of biochar on the height of *Eleusine indica* (a), and *Rorippa sylvestris* (b).

Biochar application will improve soil physicochemical characteristics such as water holding capacity (WHC) and bulk density. According to Kätterera et al. (2019) biochar addition significantly decreased soil bulk density, increased WHC, increased available P, and also available K (Wang et al., 2018; Prawito, 2019). Biochar has a high surface area with high porosity (Jien and Wang, 2013), variable charges, cation exchange capacity (19.21 cmolc/kg), and surface sorption area (Sun et al., 2018). Biochar also contains more labile compounds of organic matter that support carbon for microbes activities. Microbial biomass was attributed to biochar mineralization, and responsible for organic matter content which is essential in serving available substrates for soil microbes. Ghorbani et al. (2019) showed that biochar added will affect the soil microbial abundance and community composition, then improve the soil organic content. The ratio of soil carbon and nitrogen will also change; thus, the nitrogen mineralization will increase. Saletnik et al. (2018) stated that biochar addition increases other biogenic compounds such as phosphorus, potassium, magnesium, and nitrogen. The role of biochar as a soil agent in improving soil properties could provide benefits for plant growth and development. Generally, the results of this study showed that *Eleusine indica* and *Rorippa sylvestris* are potential metal tolerant plants because they could grow well on the contaminated land. The addition of the biochar amendment had a significant effect on plant growth, as shown by the increase of plant height (Figures 1a. and 1b.). Biochar application affected the remediator plant growth. For all treatment, the plant growth increased polynomially from 3 weeks after transplanting (WAT) until 14 WAT. The addition of biochar could promote the remediator plant growth at the early growth stage. This phenomenon can be associated with the biochar characteristics, which categorizes as slow-release material. There were no significant differences between all of the biochar types added to the *Eleusine indica* height (Figure 1a)). However, a different pattern occurred on the growth of the *Rorippa sylvestris* plant. Figure 1 (b) shows that the mixture of tobacco and rice husk biochars (B3) had the highest rate of

Rorippa sylvestris height. Overall, the average height of *Eleusine indica* was higher than *Rorippa sylvestris*. *Eleusine indica* has greater adaptability on the contaminated land, which can be seen from the tillers and leaves amount. Results of this study showed that the addition of tobacco waste biochar (B2), as well as the mixture of tobacco waste with rice husk biochar (B3), gave a higher number of tillers and leaves compared with no biochar (B0) and rice husk biochar (B1) treatments. It seemed that the addition of a mixture of rice husk biochar and tobacco waste biochar was more effective in affecting the plant remediator growth compared with the rice husk biochar (B3)and tobacco waste biochar (B2). The total dry weight of leaf and root biomass increased due to the addition of biochar compared with no biochar treatment. Addition of biochar affected the dry weight of leaves and root biomass ($p<0.1$). Videgain-Marco et al. (2020) reported the increase of the dry weight of plant root biomass and grain weight due to biochar addition. Physico-chemical parameters changes caused by biochar adding can affect the plant biomass (Lehmann et al., 2011). Biochar affects the bulk density and soil porosity so that the roots can grow more easily. Biochar releases nutrients slowly and enhances the activities of soil microorganisms; therefore, the soil nutrients available will be longer, and the plant growth will improve. In this study, biochar types had no significant effect on the biomass dry weight ($p <0.05$), either the below-ground biomass (root) or above-ground biomass (leaves). Effectof the biochar on plant growth was strongly dependent on biomass feedstock derived biochar, pyrolysis method, and soil types (particle size, soil texture, mineralogy) (Videgain-Marco et al., 2020). The influence of biochar more clearly show on acidic sandy texture soils (Liu et al., 2013; Bi et al., 2019). Soil nutrient content such as hydrogen, nitrogen, and oxygen fraction decreased with increasing pyrolysis temperature. The specific area and pore volume also increased with rising pyrolysis temperature. Biochar produced at low temperature also leads to higher biological yield.

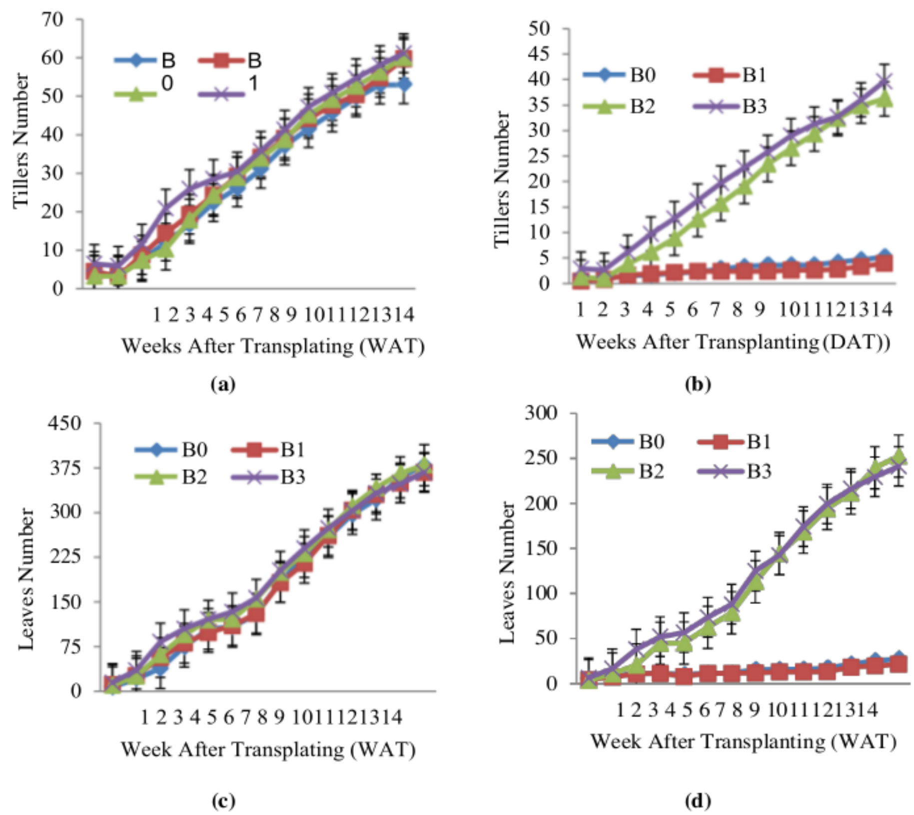


Figure 2. The influence of biochar on tiller and leaves of *Eleusine indica* (a), and *Rorippa sylvestris* (b).

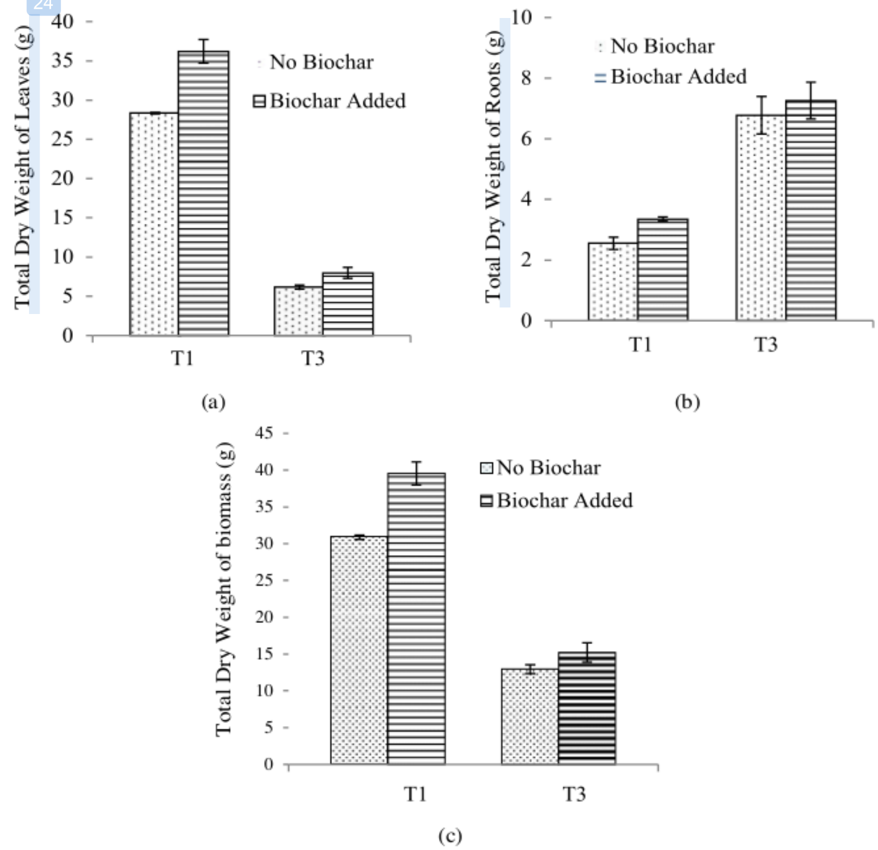


Figure 3. The dry weight of (a) leaves,(b) root, and (c) biomass of remediator plants due to the application of biochar, T1 = *Eleusine indica*, and T3 = *Rorippa sylvestris*.

Extracted Pb and Cd in soil

Pb and Cd contents in the soil due to the phytoremediation process by *Eleusine indica* was different from *Rorippa sylvestris* for all the types of biochar addition (Figure 4). Overall, *Eleusine indica* seemed to be more effective in absorbing heavy metals; the availability of soil Pb content was lower than that of *Rorippa sylvestris*. Figure 4 also shows that combining *Eleusine indica* with tobacco waste biochar mix with rice biochar (B3) was more effective than rice husk biochar (B1) or tobacco waste biochar (B2). Heavy metal phytoremediation by *Eleusine indica* and *Rorippa sylvestris* on polluted agricultural land significantly improved the soil characteristics. The reduction of soil heavy metals content is an indicator of the succeeds of soil characteristics improvement. The diminishing of soil heavy metal content by each plant is presented in Figure 4. The combination of two types of remediator plants and biochar seemed effective in reducing heavy metals as shown in the B3 treatment (a mixture of rice husk biochar and tobacco waste). The application of rice husk biochar or tobacco waste biochar alone was less significant in reducing soil Pb and Cd content, but the combination of two kinds of biochar and remediator plants was more effective in absorbing heavy metal (Figure 4). This indicates that combining *Eleusine indica* and *Rorippa sylvestris* with the mixture of rice husk biochar and tobacco waste can diminish Pb and Cd heavy metals in polluted soils. Different types of biochar have a different effect on heavy metal extractability and enzymes activity. Rice straw biochar more potential in increasing the urease and catalase activity (Yang et al., 2016), which is important in soil productivity. Biochar has high reactivity of organic compounds that are formed during the pyrolysis process. The pyrolysis process resulted in the forming of surface functional groups.

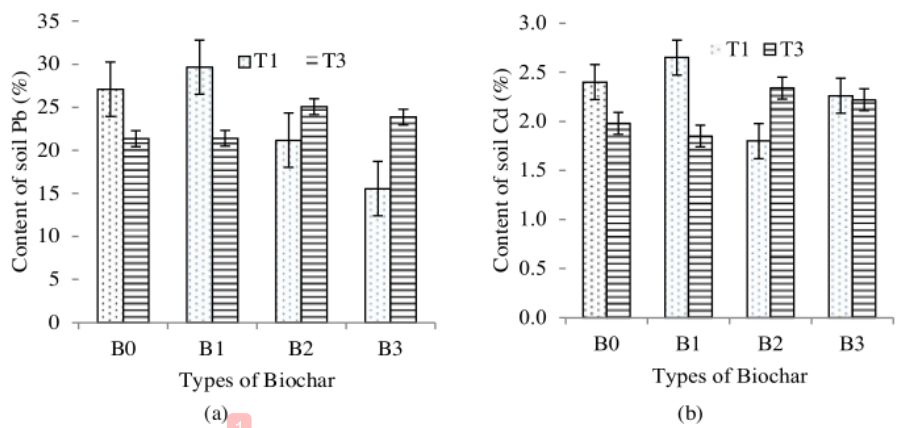


Figure 4. Soil Cd content on different types of biochar (B0 = without biochar; B1 = rice husk biochar; B2 = tobacco waste biochar; B3= mixture of B1 and B3) on *Eleusine indica* (T1) and *Rorippa sylvestris* (T3).

Thus it has potency in improving degraded soil, especially soil contaminated heavy metals. An organic acid is a polymer, an end product of a plant or living organism decomposition. Organic acids have the potential to enhance metal mobility in soil profiles by reducing soil pH and forming complexes with heavy metals. The high affinity and functionality of soil organic acid bind heavy metals that are related to their molecular weight will diminish heavy metals availability by chelating mechanism (Ahmed et al., 2019). The organic acids will form complexes with heavy metals organometallic or chelate complexes with their functional group (Adeleke et al., 2017). Biochar characteristics, i.e. porous structure, high charge surface area, and surface functional groups (carboxyl, hydroxyl, phenolic hydroxyl, and carbonyl groups) were an important factor that will affect the bioavailability of soil contaminant (Nartey and Zhao, 2014). The functional group at the biochar surface determined the heavy metals immobilization; thus, the availability of the heavy metals will be decreased (Chibuike and Obiora, 2014). The addition of soil amendment such as biochar is important in heavy metals immobilization on polluted soils (Liu et al.,2020). Biochar will enhance the absorption of heavy metals that in turn, improve soil physical, chemical and biological properties . According to Atkinson et al. (2010), biochar application will improve soil characteristics by (1) increasing nutrient availability, nutrient retention, and water retention, and (2) creating suitable habitat for symbiotic microorganisms. Besides their positive effect on soil properties, biochar application in acid soils increases crop productivity (Jeffery et al. 2011; Spokas et al. 2011). Gaskin et al. (2010) revealed that no significant effect of biochar application on soils with neutral pH in Mid-West USA. The application of biochar on agricultural land reduces the rate of CO_2 and N_2O emissions and contributes to increasing carbon stocks (52.8%), meaning that biochar can store carbon in a long time and large enough quantities (Chen et al., 2015).

Heavy metal content on shoots and root of remediator plants

Figure 5 illustrates the effects of different types of biochar on the accumulation of heavy metals in the leaves and roots of the two remediator plants. The metal concentration of heavy metal was higher on roots than shoots. This result coincided with Huang et al. (2018), they showed that biochar increased the root Pb, Zn, Cu, Cd, and As concentration, but no significant differences of the shoot of plants. Among the biochar types treatment, both *Eleusine indica* and *Rorippa sylvestris* have the same capability in heavy metal accumulation. However, Cd accumulation was lower than Pb accumulation. In this study, the rice husk biochar and tobacco waste biochar improved soil characteristics. Soil improvement would enhance the growth of the remediator plant, so the accumulation of Pb and Cd (Figure 5) would increase that in turn, affected the growth of *Eleusine indica* and *Rorippa sylvestris*. The plants would accumulate the heavy metals in their biomass, and soil heavy metals availability would be diminished and recovering the polluted soils. Planting the food crops in the next season by measuring the accumulation of heavy metal in their plant tissue will make sure whether this technique succeeded or not.

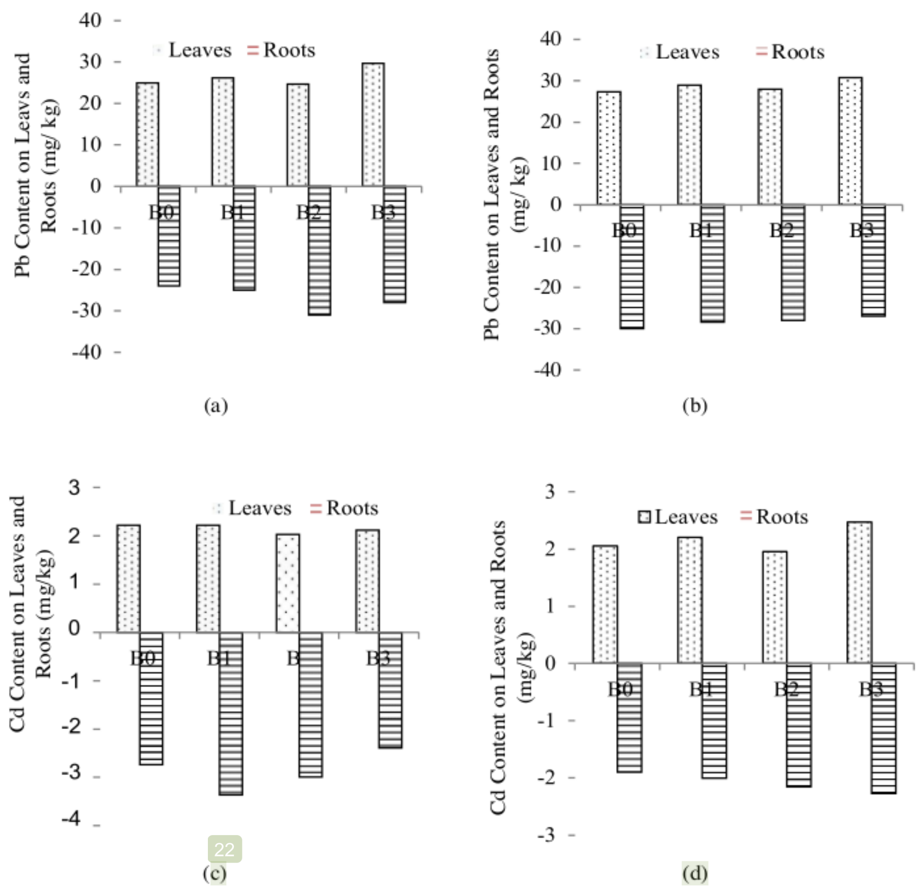


Figure 5. Effects of biochar types on Pb content in shoot and leaves of (a) *Eleusine indica* and (b) *Rorippa sylvestris*; and Cd content in shoot and leaves of (c) *Eleusine indica* and (d) *Rorippa sylvestris*.

The results showed that both *Eleusine indica* and *Rorippa sylvestris* were able to reduce heavy metals in the soil until 75%. *Eleusine indica* combined with rice husk biochar was able to reduce Pb until 78.36%, while tobacco waste combined with biochar could reduce Pb up to 84.15%. *Rorippa sylvestris* plant combined with rice husk biochar could reduce up to 82.49%, and the combination of rice husk biochar and tobacco waste biochar could reduce up to 82.74%. Results of this study showed that the combination of *Eleusine indica* (and tobacco waste biochar was the highest (85%) in absorbing heavy metals Cd, and the lowest was the combination of rice husk and tobacco waste biochar (79.03%). A different pattern was found with the uses of *Rorippa sylvestris*. Tobacco waste biochar with *Rorippa sylvestris* combination had the lowest heavy metals accumulation. A combination of rice husk biochar and tobacco waste biochar reduced Cd by 81.57%

and 80.40%, respectively, while tobacco waste biochar alone reduced Cd by 76.12%. Overall, the application of both types of remediator plants and rice husk and tobacco waste biochar was able to reduce Pb and Cd up to > 80%. This occurred because both plant species well adapted on polluted soil.

This study showed that *Rorippa sylvestris* had a higher capacity on Cd or Pb absorption than *Eleusine indica* as shown by their TF and BCF values for Cd and Pb. Both plant species had TF and BCF values for Cd and Pb higher than 1. This means that those plants can be categorized as Cd and Pb hyperaccumulators. The translocation factor value ranged for Cd and Pb from 1.91 until 2.14 for *Eleusine indica* and 1.79 until 2.06 for *Rorippa sylvestris* (Figure 6). Bioconcentration factor (BCF) represents the content of heavy metal in an organism and their potency to remove from the soil.

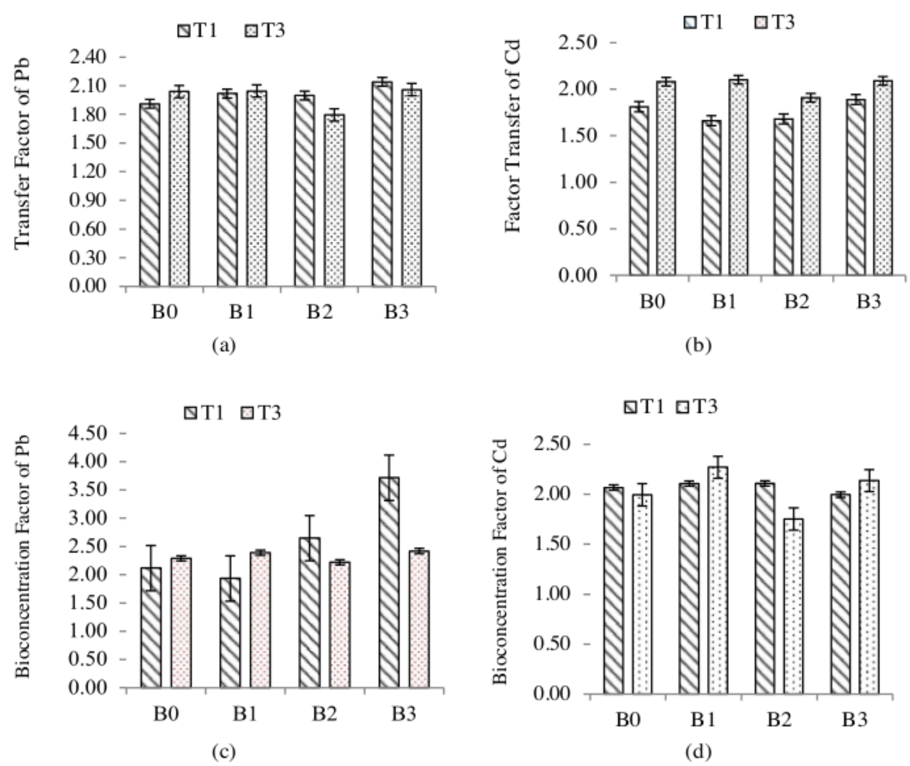


Figure 6. Translocation Factor (TF) of *Rorippa sylvestris* (T1) and *Eleusine indica* (T3) for Pb (a) and (b) Cd (b), and Bioconcentration Factor (BCF) of *Rorippa sylvestris* (T1) and *Eleusine indica* (T3) for Pb (c) and Cd (b).

The translocation factor (TF) is value refers to the ability of heavy metals to be translocated from roots to shoots or stems. These value refers to the plant ability to translocate heavy metals (Takarina and Tjiong, 2017). Each plant has different ways of storing heavy metals in stems, roots, or leaves (Ndeda and Manohar, 2014). BCF value is a common index that is usually used in determining and evaluating the ability of a plant to extract heavy metals. Plants with the BCF value higher than 1 are categorized as hyperaccumulators, while plants with BCF value lower than 1 are considered as accumulators (Mellem et al., 2009; 2012).The remediator plants have different ways of storing heavy metals in their body. Some plants store heavy metals in its roots, but others translocate heavy metals into their shoot and or leaves. The translocation factor (TF) value is commonly used to evaluate the heavy metals storage in plant tissue. TF>1 indicates that the plants are effective in translocating heavy metals from roots to shoot (Fayiga and Ma, 2006; Rezvani and Zaefarian, 2011). This result is in line with Cordeiro et al. (2016) who reported that *Rorippa sylvestris* showed significantly higher concentration of heavy metals in their upper parts of their body. The translocation factor value above 1 suggests better partitioning in the aerial parts, as also has been shown in Figure 5.

Conclusion

Eleusine indica (L.) Gaertn and *Rorippa sylvestris* (L.) Bess are potential remediator plants in Cd and Pb polluted soil based on their growth and high Pb and Cd accumulation in leaves. The application of biochar increased growth of the plants and decreased the accumulation of heavy metals in leaves. The application of the mixture of rice husk biochar and tobacco waste biochar showed the best improvement on plant growth, dry biomass of leaves and roots, plant height, and tiller numbers. The biochar application also increased pH, C, N, exchangeable K, and CEC of the soil.

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