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Research Article

Utilization of biochar and *Trichoderma harzianum* to promote growth of shallot and remediate lead-contaminated soil

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Abstract

This study aimed to determine the effect of biochar and *Trichoderma harzianum* toward lead removal in soil, lead absorption, lead content in plant tissue also growth and yield of shallot cultivated on lead-contaminated soil. The experimental design used was a completely factorial randomized block design consisting of two factors. The first factor was corn cobs biochar which was applied 1 week after basic fertilizer treatment and consisted of 4 levels, namely B0: without biochar, B1: 2.5 t ha^{-1}, B2: 5 t ha^{-1}, and B3: 10 t ha^{-1}. The second factor was the dosage of liquid of *Trichoderma harzianum*, namely TR0: *without T.harzianum*, TR1: 10 mL L^{-1}, and TR2: 20 mL L^{-1}, which was applied three times at 14, 28 and 42 days after planting. Data were analyzed using the F test and continued with DMRT (Duncan Multiple Range Test) at $p = 0.05$ level. The results showed that the application of 5 t biochar ha^{-1} was able to remove lead and decreased lead uptake in plants. Application of *T. harzianum* could remove and decrease absorption in plant tissue biochar was not able to increase the growth of shallot while *T. harzianum* increased the number of leaves and the number of tubers.

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Introduction

Shallot (*Allium ascalonicum* L.) is one of the important horticultural commodities widely consumed by Indonesians (Basuki, 2014). Shallot has high economic value and has attractive market prospects. Shallot has many benefits, as flavouring spice related to the aroma and used as cough medicine (phlegm spray), shortness of breath, fevers, colds and appetite enhancers (Nurhasanah et al., 2015). The problem of shallot fulfilment in Indonesia is low domestic shallot production of 10.22 t ha^{-1} compared to other countries such as Thailand and the Philippines, with an average production of 12 t ha^{-1} of dry tubers (Taufik, 2015). The low production is caused by conventional cultivation techniques and the presence of pests and diseases (Rahayu et al., 2018). The use of fertilizers and pesticides to provide cultivation inputs can increase yields. However, the application of fertilizers and pesticides that tends to be excessive in terms of dosage and concentration for a long time can cause environmental pollution such as contamination in the soil (Karyadi et al., 2012; Bahar, 2016). The threshold for the accumulation of lead element in soil and shallot are 12.75 ppm and 2 ppm, respectively (Hartini, 2011).

Environmental pollution is a significant problem that must be resolved. The agricultural sector contributed heavy metal residues such as lead (Pb) which is used in a mixture of various types of synthetic pesticides and chemical fertilizers to increase the concentration of heavy metals in soil and plants (Hidayat, 2015). Lead is reported to be highly toxic for human health and found to be involved in oxidative stress, bringing about the creation of unevenness between the generation of free radicals and the capacity of natural framework to promptly detoxify oxygen responsive species (Flora et al., 2007). Heavy metals pose a risk to human health because of their persistence, toxicity, non-biodegradable nature and widespread occurrence in the natural and human-altered environment (Aslam et al., 2008). They enter the human body by consuming heavy metals, contaminated food crops, water and dust (Murtaza et al., 2010). Efforts to reduce heavy metals that have accumulated in the soil can be held by adding soil amendments, such as biochar and microorganisms, which have the ability to remove contaminants and accumulate them as biosorbents. This method is known as bioremediation (Kurniawan and Nuraeni, 2016).

Biochar that is obtained by the pyrolysis of various organic sources can provide a new solution to soil pollution problems (Wang et al., 2010; Hidayat, 2015; Hussain et al., 2017). Biochar has the capacity to exchange ions and is alkaline in nature (Paz-Ferreiro et al., 2014). Park et al. (2011) reported a decrease in metal uptake by plants and better plant growth when the soil was amended by biochar. Another study reported that lead accumulated in the roots of plants grown on biochar-amended soil, at 10 mg L^{-1} was 40% less than that in plants grown on non-biochar-amended soil (Ali et al., 2019). Haryati and Erfandy (2019) reported that the addition of 5 t ha^{-1} increased the yield of shallot by 56%.

Trichoderma sp. is a fungus that is generally found in soil and plant roots. *Trichoderma sp*. can attack pathogens that cause plant disease; also, it can survive on agrochemicals residue (Tripathi, 2013). *Trichoderma* is found commonly in all types of soils, and some of them have the ability for cleaning polluted environments and can be applied as effective microorganisms for bioremediation of pollutants (Vankar and Baipai, 2008). *Trichoderma* also can stimulate plant growth up to 300% and protect them from stress factors, especially pathogenic organisms (Mohsenzadeh and Shahrokh, 2014). The study reported that the application of secondary metabolites *T. harzianum* in spinach effectively reduced Cd by 63.81% and increased growth by 18%, and increased dry canopy weight by 23% (Herliana et al., 2018). Based on the description, it is important to explore the potential of biochar and *T.harzianum* for remediation of lead-contaminated soil and its effect on the growth of shallot.

Materials and Methods

Planting media preparation

This research was conducted from March to August 2020 at the screen house of the Faculty of Agriculture, Universitas Jenderal Soedirman, Karangwangkal, Purwokerto (110 m above sea level). The planting medium used was an alluvial soil taken from shallot cultivation land in Brebes, where shallot cultivation has been carried out for generations by frequently applying agrochemicals (fertilizers and pesticides) with high doses. The soil media was dried, sieved using a 2 mm sieve and weighed as much as 6 kg per polybag. The Pb content in the soil based on the analysis results from Balingtan (Center for Agricultural Environment Research of Indonesia) was 29.99 ppm, which exceeded the threshold Pb content in the soil of 12.75 ppm and 2 ppm in shallot bulbs (Hartini, 2011).

Experimental design

Pot experimental was arranged in a completely randomized block design consisting of two factors. The first factor was biochar application (0, 2.5, 5, and 10 t ha^{-1}), and the second factor was *Trichoderma* sp application (0, 10, and 20 mL L^{-1}) which was applied three times at 7, 28, and 42 days after planting. Each treatment combination was subjected to three replications.

Biochar preparation

The biochar was made by pyrolysis process of corn cobs. The corn cobs were put in a reactor to a slow-burning process (carbonation) at a temperature of 300 - 400°C for about 5-6 hours with the absence of oxygen. After the combustion, cool corncob charcoal was taken from the combustion reactor, then crushed and sieved using a 100 mesh sieve size (Nurhidayati and Mariati, 2014). The process yielded approximately 35% of the pyrolyzed product (Ali et al., 2019). Biochar was applied 7 days before planting on the growing medium (Ratnasari et al., 2020)

Trichoderma harzianum preparation

T. harzianum isolate obtained from the Laboratory of Plant Protection, Faculty of Agriculture, Universitas Jenderal Soedirman was rejuvenated on PDA (potato dextrose agar) media and incubated for 7-10 days. The liquid used for bioremediation experiment was prepared by mixing coconut water media that was filtered from husk was then added with 20 g of sugar in a 1000 mL beaker glass. The mixture was then sterilized in an autoclave. The sterilized media was then transferred into a 500 mL bottle and inoculated with the starter of *T. harzianum* for one ose and incubated in a fermentor for 7 days. Potato extract media was prepared by boiling 200 g of potato cuts 1 L of water for ± 20 minutes, filtered and added 20 g of sugar, stirred until dissolved. The potato extract was put in a 1000 mL beaker glass and sterilized in an autoclave. The sterilized potato extract was then transferred into a 500 mL bottle and inoculated with the starter of *T. harzianum* as much as one ose and incubated in a fermentor for 7 days (Nurhidayati, 2015)

Shallot cultivation

Seedlings of Bima Brebes shallot variety used in this work were coated with fungicide directly on pots and planted in the soil as deep as 2/3 part of the tuber. Urea fertilizer at a rate of 150 kg ha^{-1} (equivalent to 0.6 g pot^{-1}) was supplied two times when the plants were 7 and 20 days old. Irrigation was held for two days by giving 500 mL of water. Weeding was carried out every ten days. During planting, there was the *Spodoptera litura* attacked was controlled by spraying with Decis insecticide. Harvest was carried out when the plant was 65 days old, marked by plant leaves that began to wilt and turned yellow.

Variables observed

The variables observed were shallot growth components (plant height, number of leaves, leaf chlorophyll content, leaf width), shallot yield components (number of bulb, wight of bulb and diameter of bulb), lead contents in soil and shallot bulb, and soil pH. Lead content in the soil was determined by using 0.01 N $CaCL_2$ solvent, shaking for 2 hours and filtering the extract. Soil extract was measured by AAS (Atomic Absorption Spectrometer), and then the lead concentration was calculated using a calibration curve. The calibration curve was made by removing the absorbance and the standard concentration of lead. Lead concentration was calculated with the regression equation obtained from the calibration curve (Wulandari et al., 2014). Lead content in the shallot plant tissue was analyzed by preparing a dried plant sample then added to the digestion flask with 5 mL of H_2SO_4 solution. For the digestion process, heating was carried out gradually, starting from the lowest heat then gradually increasing it until the solution was black. After that, 10-20 drops of concentrated HNO_3 solution were added until a yellowish solution was obtained. The digestion results were then cooled and diluted with distilled water to a volume of 25 mL and filtered with Whatman filter paper No. 42. Lead content in the sample solution was measured using AAS (Priandoko et al., 2012).

Effectiveness of plant absorption and removal efficiency

The effectiveness of plant absorption (EPA) in metal absorption illustrates the ability of plants to absorb heavy metals. The EPA value was calculated using the following formula (Prayudi et al., 2015),

$$\text{EPA}\ (\%) = \frac{\text{CMAP}}{\text{DCMS}} x\ 100\%$$

where:

EPA	=	effectiveness of plant in metal absorption
CMAP	=	concentration of metal in plant
DCMS	=	decreased concentration of metal in soil

Removal efficiency (RE) of Pb from the soil by the plant was calculated using the following formula (Hardiani, 2008),

$$\text{RE}\ (\%) = \frac{\text{IMC}-\text{FMC}}{\text{FMC}} x\ 100\%$$

where :

RE	=	removal efficiency
IMC	=	initial metal concentration in soil
FMC	=	final metal concentration in soil

Data analysis

Data were analyzed using the F test followed by the DMRT (Duncan's Multiple Range Test) at $p = 0.05$.

Results and Discussion

Soil pH

Generally, farmers in Buara, Ketanggungan District, Brebes used pesticides by mixing 3-5 types of pesticides, spraying almost every day, especially during the rainy season, to reduce yield losses from plant pests. Lead (Pb) and mercury (Hg) in various types of pesticides were reported to contribute the increasing their concentrations in soil and plants (Ogunlade and Agbeniyi, 2010). Incepticol pH decreased from 7.4 to 6.8. The decrease in pH occurs due to the release of heavy metals in the soil so that their mobility increases. Low pH indicates high mobility of metals in the soil so that the availability of metals in the soil is low (Widiyatmoko, 2011)

The effectiveness of plant absorption, removal efficiency and Pb content in plant tissue

The results of the analysis of variance showed that the application of biochar increased the effectiveness of Pb removal. Non-biochar treatment showed the lowest Pb removal effectiveness percentage (49,77%) that was significantly different from that of the application of 2.5 t biochar ha^{-1} (59.86%), but it was not significantly different to that of 5 t biochar ha^{-1} (60.01%) and that of 10 t biochar ha^{-1} (62.97%) (Figure 1). This is inversely proportional to the effectiveness of plants in Pb absorption and Pb content in Shallot plant tissue. Non-biochar treatment showed the highest percentage of Pb plant absorption (62.04%), which was significantly different from that of 2.5 t biochar ha^{-1} treatment (53.73%). Increasing biochar dose could decrease the percentage value of Pb removal effectiveness. The Pb removal effectiveness due to the application of 5 t biochar ha^{-1} was 60.01%, while that due to the application of 10 t biochar ha^{-1} was 62.97% (Figure 2). The addition of biochar reduced Pb accumulation in the soil and plant tissue (Figure 3). Pb content in the soil without biochar application was 3.17 ppm that was significantly different from the addition of 2.5, 5, and 10 t biochar ha^{-1} of 3.01, 2.87 and 2.87 ppm, respectively. According to Hartini (2011), the Pb threshold for shallot is 2 ppm. The result showed that Pb content in shallot was unsafe for human health if shallot is consumed in the long term. Biochar amendment can increase soil productivity through adsorption processes and physicochemical reactions (Zheng et al., 2010). With its alkaline nature, biochar can increase soil pH and stabilize heavy metals

(Yuan et al., 2011). Various studies have shown that biochar can improve soil productivity and absorb heavy metals (Sukartono and Utomo, 2012; Fellet et al., 2014).

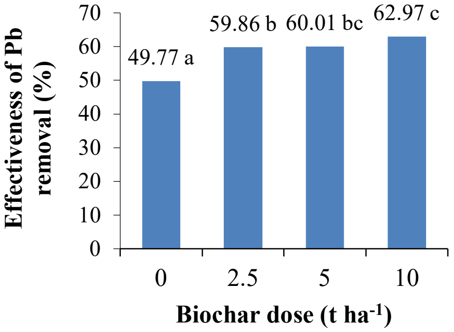

Figure. 1. Biochar treatment on effectiveness of Pb removal.

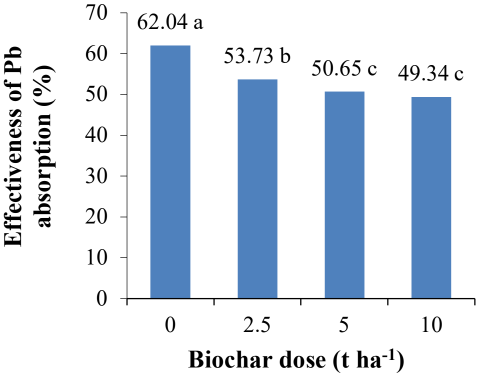

Figure 2. Biochar treatment on effectiveness of Pb absorption.

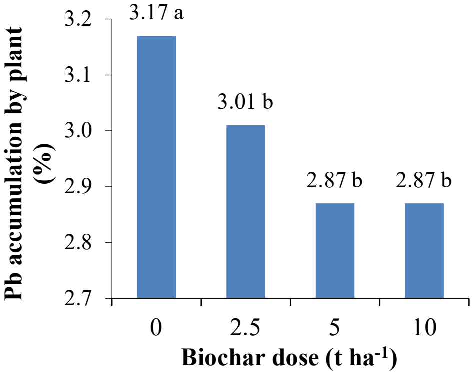

Figure 3. Biochar treatment on Pb accumulation in shallot plant tissue.

Biochar application can increase the removal of heavy metals and reduce their absorption in plant tissue (Puga et al., 2015). The addition of biochar has the potential to reduce the toxicity of heavy metals on polluted land because it has the ability to stabilize metals by significantly reducing the absorption of heavy metals by plants and can improve their quality by improving the physical, chemical and biological properties of soil (Ippolito et al. 2012; Komarek et al. 2013). Biochar that has a large surface area and a high capacity to absorb heavy metals can potentially be used to reduce bioavailability and leaching of heavy metals as well as organic pollutants in the soil through adsorption and other physicochemical reactions (Park et al., 2011). *T. harzianum* application increased the effectiveness of Pb removal. Non-treatment showed the lowest percentage of the effectiveness of Pb removal (49.46%).

The doses of 10 and 20 mL L^{-1} were significantly different from non *T.harzianum* application of 59.42% and 62.32% (Figure 4). The plant absorption of Pb decreased due to *T.harzianum* treatment. The highest value of plant absorption on Pb was 63.89% without application of *T.harzianum.* The percentage of plant absorption on Pb decreased to 52.82 % and 48.37% on 10 and 20 mL L^{-1} dose (Figure 5). That decreasing value of Pb absorption was in line with Pb content in plant tissue (Figure 6). Non-*T.harzianum* treatment showed the highest Pb content of 3.53 ppm that was significantly different from 10 and 20 mL L^{-1} treatments of 2.99 and 2.92 ppm, respectively.

It is known that the members of *Trichoderma* genus have a wide range of economic aspects that are owned applications for various biotechnology uses, especially in agriculture (Shovan et al., 2008), industry and environmental biotechnology (Rincon et al., 2009). *Trichoderma* species show ecological capacity because of their ability to produce various kinds of enzymes to degrade substrates. Some of the principles use of microorganisms from the fungal group such as *T. harzianum* to reduce heavy metals are biosorption, bioaccumulation, bioprecipitation, bioreduction, and bioleaching (Rao et al., 2002; Damodaran et al., 2011; Chaturvedi et al., 2015; Dixit et al., 2015) which is done through chemical modification or by influencing chemical bioavailability (Harms et al., 2011). According to Gelagutashvili (2013), biosorption is interpreted as a metal removal process weight through passive binding to biomass that does not live from a solution, and this reduction mechanism is not controlled metabolically.

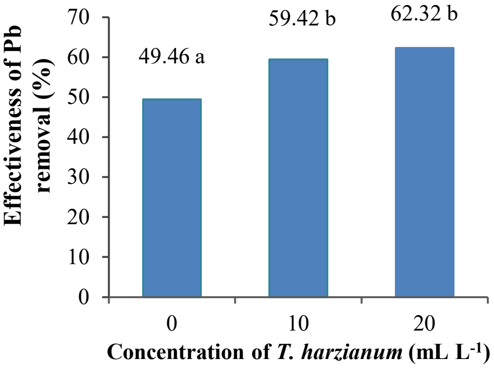

Figure 4. *T.harzianum* treatment on effectiveness of Pb removal.

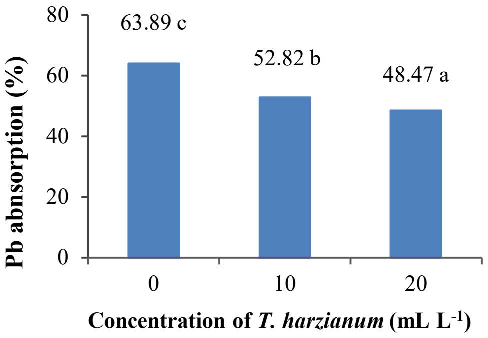

Figure 5. *T.harzianum* treatment on effectiveness of Pb absorption.

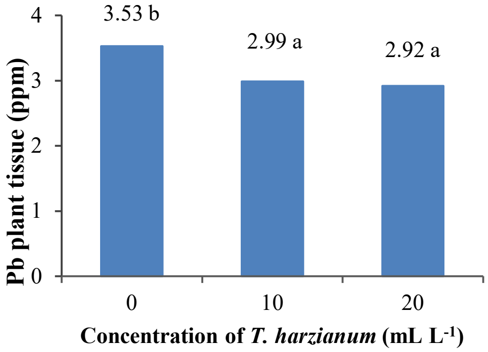

Figure 6. *T.harzianum* treatment on Pb content in plant tissue.

Table. 1. Effects of biochar and *T.harzianum* treatment on growth and yield of shallot cultivated on lead-contaminated soil.

Variable	PH (cm)	LA (cm^2)	NL	PFW (g)	PDW (g)	CC (mg L^{-1}	NB	BW (g)	BD (mm)
				Biochar (t ha^{-1})					
0	35.74	52.12	27.42	35.13	4.640	3.410	9.28	26.75	35.13
2.5	36.82	54.86	28.44	35.32	5.206	3.778	10.15	27.80	35.33
5	37.35	54.20	28.81	35.19	5.087	3.690	8.97	28.59	35.19
10	37.02	63.91	30.73	34.26	4.954	3.848	9.77	26.79	36.23
CV	13.29	17.03	12.79	14.94	21.15	12.42	19.79	15.76	13.62
				***Trichoderma harzianum* (mL L^{-1})**					
0	36.19	53.84	23.88 a	34.73	4.75	3.699	8.53 a	27.67	34.73
10	36.84	56.40	25.09 a	34.62	5.05	3.648	9.27 ab	26.63	34.61
20	37.11	59.32	28.90 b	35.56	5.17	4.697	10.83 b	28.15	35.56
CV	13.29	17.03	12.79	14.94	21.15	12.42	19.79	15.76	13.62

Remarks: PH = plant height, LA = leaf area, NL = number of leaf, PLW = plant fresh weight, PDW = plant dry weight, CC = chlorophyll content, NB = number of bulb, BW = bulb weight, BD = bulb diameter. The average numbers followed by different lowercase letters in the same column show significant different effect based on $p = 0.05$ level of DMRT.

Bioaccumulation of heavy metals in living organisms is described as a process and pathway for the migration of pollutants from one trophic level to another, including through the food chain so that it can accumulate in tissues to organs of an organism at a certain level (Akan et al., 2012; Alia et al., 2015). Bioprecipitation, in principle, is a chemical reaction against precipitation heavy metals to form precipitates that do not dissolve, and then the precipitate was separated through a sedimentation process or filtration (Fu and Wang, 2011). Bioreduction occurs immediately by involving enzymatic activity, while the mechanism is indirect, involving metabolic products (reductants or oxidants) through a reduction reaction to chemical oxidation (Wani and Ayoola, 2015). In the bioleaching process, heavy metals will undergo processes of solubilization, cation exchange, precipitation, adsorption, complexation, and other reactions (Chen et al. 2005).

Growth and yield of shallot

The response of growth and yield of shallots to the application of *Trichoderma sp*. and biochar in the efforts to remediate lead contamination showed varying results. Heavy metal toxicity causes negative effects on plants. It has been reported that lead stress can cause decreased root growth, damaged cell walls, disruption of cell division (Kopittke et al., 2007; Kumar and Tripathi, 2008; Ghelich et al., 2013). The application of biochar and *Trichoderma sp* as soil amendment agents is expected to suppress the adverse effects on plant growth. The results of the analysis of variance presented in Table 1 show that the application of biochar at all doses (2.5, 5, and 10 t ha^{-1}) did not have significant effects on plant height, leaf area, number of leaves, fresh plant weight, dry plant weight, number of tubers, tuber weight and tuber diameter, compared to without biochar application. This shows that plants have several defence mechanisms against heavy metal stress. This defence is demonstrated by not disrupting plant growth, such as root growth, photosynthetic metabolism and others (Rosidah, 2014).

Several previous studies reported various plant defence mechanisms, among others 1) chelating metal peptides such as phytocellin and metallothionein, 2) immobilization, and 3) compartmentalization of metal ions in vacuoles (Cobbet, 2000). In soil conditions gripped by heavy metals Pb, plants can still grow well because biochar can improve the physical and chemical properties of soil. *T. harzianum* application had a significant effect on the number of leaves and tubers (Table 1). The addition of 10 and 20 mL *T. harzianum* L^{-1} increased the number of leaves, but it was not significantly different between the two treatments a 10 mL dose of *T. harzianum* yielded 25 leaves, and a 20 mL dose yielded 29 leaves. The number of tubers also experienced a significant difference with the application of *T. harzianum*; the application of 20 mL L^{-1} yielded 11 tubers, an increase of 26.96% compared to those without *T. harzianum* and a dose of 10 mL L^{-1} with 6 and 9 tubers, respectively. Shallot plants cultivated on land with high heavy metal content have the ability to adapt (Cho et., 2009). In sub-optimal soils having poor nutrients or under drought conditions, shallot plants can grow with the addition of *T.harzianum* (Hadiawati, 2020). Increased growth is due to the ability of *Trichoderma* sp. in symbiosis with the shallot plant roots to increase early seed growth and subsequent growth (Darsan et al., 2016). *T. harzianum* is reported to be able to increase plant growth, increase the absorption of active minerals and other nutrients from the soil (Latifah et al., 2011). Santoso et al. (2007) reported that *T. harzianum* and *T. koningii* were able to stimulate the growth of tomato and tobacco plants with dry weight increase around 213-275% and 259-318%, respectively. It is assumed that the extract of antagonistic fungi can have a PGPF (Plant Growth Promoting Fungi) mechanism. *Trichoderma* can protect the plant, increase growth, help nutrient absorption, improve fertilizer use efficiency, and stimulate plant defences against biotic and abiotic damage (Shoresh et al., 2010).

Conclusion

The application of 5 t biochar ha^{-1} was able to remove lead with Pb removal effectiveness level of 62.97% and decreased lead uptake in plants by 50.65%. The lead content in shallot was 2.87 ppm. of the application of 10 mL *T. harzianum* L^{-1} could remove Pb by 59.42%, and the application of 20 mL *T. harzianum* L^{-1} decreased absorption of Pb in plant tissue by 48.73%. However, the application of biochar was not able to improve plant growth, while the application of *T. harzianum* increased the number of leaves and tubers of shallot.

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