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Bioremediation of Pb and Cd contaminated soil by mycorrhiza and biochar treatment and its effect on growth and yield of shallot

Abstract. The contamination of shallots in the food chain by heavy metals such as lead (Pb) and cadmium (Cd) is caused by chemical fertilizers and pesticides. The study aimed to determine the growth and yields of shallot cultivated on soil contaminated with Pb and Cd using mycorrhiza and biochar. The study was conducted in the screen house at Jenderal Soedirman University, Faculty of Agriculture, from April to September 2020, and it was carried out using a factorial Randomized Completely Block Design that involved three replications and two factors. The first factor of mycorrhiza dosage comprised 0, 1, and 2 g/pot, and the second factor of biochar dosage comprised 0, 2.5, 5, and 10 t/ha. The plant height, leaf area, growth rate, number of leaves, total root length, net assimilation rate, leaf chlorophyll, the percentage of root infection, P uptake by plant tissue, tuber weight, harvest index, the effectiveness of absorption and removal of heavy metals were the variables recorded. The results showed that applying biochar at 2.5, 5, and 10 t/ha and mycorrhiza at 1 and 2 g/pot could increase plant height and the percentage of root infection. The application of mycorrhiza at 1 and 2 g/pot increased P uptake by plant tissue.

Keywords: Biochar · Bioremediation · Heavy metal · Mycorrhiza · Shallot

Submitted: 5 February 2024, Accepted: 13 November 2024, Published: 15 December 2024
DOI: <https://doi.org/10.24198/kultivasi.v23i3.57709>

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Introduction

Shallot (*Allium ascalonicum* L.) is a horticultural commodity with high economic value. Furthermore, it is used as a cooking spice and raw material for medicine and the food industry (Saptana et al., 2021). Shallots contain Fe, antioxidants, antifungal, and anticancer properties (Abdelrahman et al., 2017). Therefore, a key challenge in vegetable production and marketing is raising public knowledge of the importance of food safety and quality. Vegetable cultivation, particularly of shallots, heavily relies on agrochemical inputs, often resulting in varying crop quality and potential heavy metal contamination in the bulbs. Furthermore, the main contributor to contaminated food is the buildup of heavy metals in soil, water, or plants. Rapid industrial development and excessive use of pesticides and fertilizers have increased heavy metal deposition in the environment. (García-Díaz et al., 2017; Zarcinas et al., 2004). These events have raised a global concern about soil pollution. Plants produce a substance known as thiols, which has a low molecular weight and thus, has a high affinity for toxic metals. Metal ions would signal to plants to produce thiols as a defense mechanism against heavy metal stress by accumulating heavy metals and synthesizing low molecular weight metal-binding proteins (Borisova et al., 2016). The plant root system is suitable for absorbing heavy metals such as Pb and Cd when found in the soil (Ali et al., 2019).

Contamination of soil with Cd and Pb can have negative effects on soil quality, plant growth, and soil microorganisms. Effects of Cd and Pb on soil properties include Decreased metabolic activity of bacteria and other soil microorganisms, disrupted plant development, Reduced fertility and plant nutrient uptake Soil pollution (Esringü et al., 2021; Rolka & Wyszowski, 2021). The effect of potentially toxic elements can reduce soil microbial activity, soil microbial community composition, soil enzyme activity, and soil physiochemical properties thereby reducing soil fertility (Bansal, 2018).

Lead (Pb) accumulates in plant parts in the form of leaves, stems, and roots. Lead absorption by roots occurs when water-insoluble compounds convert into water-soluble forms, allowing lead to bind with root mucus carboxyl groups, which limits the root's protective heavy metal bonds. The absorption of lead in the leaves is through a passive absorption process in the

stomata of the leaves, which causes the amount of lead levels to be greater due to absorption from the air. If the plant is consumed by humans, it can cause lead poisoning in the form of nausea, vomiting, severe abdominal pain, brain dysfunction, severe anemia, and kidney damage (Almaroai & Eissa, 2020). The threshold value for lead is 1.0 ppm in soil and 0.2 ppm in plants, while cadmium in shallots ranges from 0.135 to 0.285 ppm, exceeding the threshold limit of 0.1 ppm. (Nadir et al., 2018).

Cadmium (Cd) generally comes from factory waste and household domestic waste. If plants contaminated with cadmium are consumed by humans, it can cause lung obstruction, slow growth, osteoporosis, and disturbances in the balance of calcium and phosphate content in the kidneys which results in a deficiency of B vitamins resulting in softening of the bones (Yusuf et al., 2016). The threshold value for cadmium in soil is 0.24 ppm, while the threshold for cadmium in plants is 0.1 ppm (Nadir et al., 2018).

However, a solution is needed to overcome and rehabilitate soils contaminated with heavy metals, such as the use of an amendment substance called biochar. Biochar is produced by the pyrolysis of waste from different sources, such as forests or agriculture (Hussain et al., 2017) and it is used as an alternative for restoring and remediating soil contaminated with synthetic fertilizers and pesticides. The absorption mechanism of heavy metals Pb and Cd by biochar is dominated by complexation with functional groups, cation exchange, and precipitation. The hydrogen atom in the -COOH carboxyl group can be released as an H⁺ ion or undergo deprotonation, so that it has the opportunity to form complexes with metal ions such as lead and cadmium (Cui et al., 2020). Furthermore, biochar also binds to some elements such as N, Ca, and K (Puga et al., 2015).

The use of biofertilizers is an alternative way of suppressing synthetic fertilizer usage and restoring microorganisms in the soil. An example of biological fertilizer is the Vesicular-arbuscular mycorrhizal fungi which is known to boost the uptake of P supplements through its roots by expanding the absorption system of the hyphae which extends into the soil to absorb P ions from the soil minerals or other organisms, and translocate them to the roots of the host plant (Susanti et al., 2018). Arbuscular mycorrhizal fungi function to retain these heavy metals so

they are not absorbed into plant tissues, or reduce heavy metal levels so that plants are safe for consumption (Nadir et al., 2018).

Based on the previous studies and literature on the role of mycorrhiza and biochar in the process of heavy metal abandonment, this research is interesting to carry out. This study aims to determine the role of biochar and mycorrhiza on the yield of shallots and its effect on Pb and Cd remediation.

Materials and Methods

Preparation of media. The experimental pot was prepared in April - August 2020 at the Screen House, in Jenderal Soedirman University, Faculty of Agriculture, Purwokerto, Karangwangkal (110 masl). Alluvial soil was the medium used for the experiment and it was obtained from the Brebes area at an initial pH of 7.27. Heavy metals such as Pb and Cd were present in the soil at a concentration of 29.05 ppm and 3.66 ppm. Furthermore, the media obtained from the soil was dried to remove moisture and filtered using a filter of size 2 mm and weighed to obtain 6 kg per pot.

Research design. Randomized factorial complete block design (RCBD) was used for this study and it consisted of 2 factors. The first was mycorrhizal which was made up of 3 levels while the second, was Biochar which comprised 4 levels. To achieve 36 experimental units, 12 treatments were combined and repeated thrice. The experimental unit was made up of 4 plants which were used to obtain 144 pots.

Biochar application. Corn cob, as the main material in this study, was collected from maize fields in Jakenan Village, Jakenan District, Pati Regency, Central Java Province, Indonesia. Corn cob biochar is produced by the Center for Agricultural and Environmental Research. Furthermore, the Biochar was applied to the growth medium and left for seven days before planting was conducted. A temperature range of 100-300°C was utilized for 3-5 hours, followed by the removal of the combustion temperature after the pyrolysis process. The material was thereafter allowed to cool for 12 hours. Then, mash until granule 0.2 mesh is ready for application (Ratnasari et al., 2020). The chemical analysis of corn cob biochar shows it contains 15.9% fixed carbon, 9.2% ash, and has a C/N ratio of 4.39. It

consists of 76% carbon (C), 3.2% hydrogen (H), 3% nitrogen (N), and 17.8% oxygen (O).

Mycorrhiza application. The mycorrhiza used in this study was *Glomus* sp type, which is explored from corn fields. Then propagation is carried out and coated with a carrier material in the form of zeolite 1 g of mycorrhiza biofertilizer, containing 7 spores of *Glomus* sp, was added to the growth medium and left for 7 days before planting the tuber of shallot.

Plant cultivation and crop management. The used shallot seeds were the Bima Brebes variety. Two seedlings coated with fungicide were planted in the pot, with 2/3 of the tuber deeply inserted into the soil. 0.6 grams per pot of urea fertilizer was used to fertilize the soil. The fertilizers were added twice after planting (Day 7 and Day 20). 500 ml of water was used for watering every 2 days, while weeding was carried out every 10 days. The plant was harvested when it attained a 65 DAP, the leaves began to turn yellow and wilt, and the tubers started sticking out to the surface of the planting medium.

Variable observed. The variable determined was the number of leaves, leaf area, total root length, plant growth rate, net assimilation rate, chlorophyll content, P uptake, number of bulbs, weight of bulb, harvest index, root infection of mycorrhizae, Pb and Cd in tissue plant of shallot, effectiveness of absorption and removal of Pb and Cd.

The net assimilation rate. This variable was measured based on the dry weight and leaf area of the plant per unit time with the following formula (Shon et al., 1997):

$$\text{NAR} = \frac{1}{A} \times \frac{\Delta W}{\Delta t} \\ = \frac{(\text{Log } A2 - \text{Log } A1)}{(A2 - A1)} \times \frac{(\text{Log } W2 - \text{Log } W1)}{(t2 - t1)}$$

NAR = Net Assimilation Ratio

A1 = leaf area on measurement time 1

A2 = leaf area on measurement time 2

W1 = plant biomass on measurement time 1

W2 = plant biomass on measurement time 1

t1 = measurement time 1

t2 = measurement time 2

Leaf chlorophyll content (mg/L). Its variable is determined by the following formula (Dharmadewi, 2020):

Chlorophyll a = 1.07 (OD 663) - 0.094 (OD 644)
 Chlorophyll b = 1.77 (OD 644) - 0.28 (OD 663)
 Total Chlorophyll = 0.79 (OD 663) + 1.076 (OD 644)

Remark:

OD = Optical Density of spectrophotometer.

P uptake. The calculation of P uptake is determined by the following formula (Eviati & Sulaeman, 2009):

P content (%) = ppm curve x ml extract/1,000 ml x 100/mg sample x B.A. P/B.M. PO₄ x fp x fk
 = ppm x curve 50/1,000 x 100/250 x 31/95 x fp x fk
 = ppm x curve 0.02 x 31/95 x fk

Remark:

ppm curve = sample rate obtained from the relationship curve between series rates standard with its reading after being corrected by the blank.

100 = conversion factor to %

1,000 = conversion factor to ppm (mg kg⁻¹)

fp = dilution factor (10)

fk = moisture content correction factor = 100/(100 - % moisture content)

Percentage = % P in plants

P absorption = Dry weight of the plant x P content of the plant

Root infection. The measurement of the percentage of root infections uses the following formula (Adetya et al. (2019):

(%) Infection = $\frac{(\text{Number of infected roots})}{(\text{Total number of roots})} \times 100\%$

Removal efficiency. The effective absorption rate of heavy metals is also known by the Environmental Protection Agency EPA. It refers to the ability of plants to absorb heavy metals. The EPA was obtained with the formula below (Herliana et al., 2021):

$$RE (\%) = \frac{IMC-FMC}{FMC} / \times 100\%$$

Remark:

RE = removal efficiency

IMC = initial metal concentration in soil

FMC = final metal concentration in soil

Data analysis. All data were evaluated using analysis of variance (ANOVA). The effectiveness of treatment was obtained using a 95% confidence level. The variance obtained was significantly different. Therefore, it was compared with treatments using a follow-up test called Duncan's Multiple Range Test (DMRT) at the 95% confidence level.

Results and Discussion

Plants have been able to adapt to heavy metal stress, however, the addition of biochar and mycorrhizal brought a change in the growth, yield, and removal of this heavy metal (Table 1).

Table 1. Effect of mycorrhiza and biochar application on shallot growth and yield.

Treatment	Variables								
	NL (g)	LA (cm ²)	TRL (cm)	PGR (g/day)	NAR (g/cm ² /day)	CC (mg/l)	P uptake (ppm)	NB	WB (g)
Mycorrhiza									
0 g/pot	26.33	47.24	295.40	0.096	0.048	13.12	132.48 b	7.048 b	2.53
1 g/pot	24.62	52.52	292.52	0.085	0.044	11.84	167.68 a	8.181 a	2.67
2 g/pot	25.84	68.66	335.65	0.129	0.018	12.80	177.25 a	8.362 a	2.75
Biochar									
0 t/ha	20.93 b	52.32	307.29	0.071	0.040	12.70	139.00	6.444 c	2.19a
2.5 t/ha	25.73 a	59.53	296.76	0.106	0.020	12.50	179.27	7.463bc	2.60b
5 t/ha	28.67 a	62.78	293.34	0.145	0.029	12.66	155.47	7.806bc	2.81bc
10 t/ha	26.60 a	65.25	334.04	0.090	0.058	12.48	162.80	9.741a	3.02c
CV (%)	17.23	8.25	27.19	25.40	21.32	14.42	22.07	21.46	24.55

Remarks: NL: Number of leaves, LA: Leaf Area, TRL: Total root length, PGR: Plant Growth Rate, NAR: Net Assimilation Rate, CC: Chlorophyll content, P Uptake, NB: Number of Bulbs, WB: Weight of Bulb. The number followed by the lowercase letter shows a significant difference based on the DMRT test with $p=0.05$

Effect of mycorrhiza on growth and yield of shallots on heavy metal contaminated soil.

The mycorrhiza used in this experiment was from the *Glomus* species. While it did not affect growth variables, it significantly increased the number of bulbs by enhancing nutrient, water, and mineral absorption through its root-associated hyphae, thereby optimizing bulb formation. The addition of 1 g mycorrhiza/pot or 2 g mycorrhiza/pot was able to increase P uptake in the tissue plant. When 1 and 2 g/pot of mycorrhizal were applied, the P absorption of plant tissue was 167.68 ppm and 177.25 ppm, respectively. However, there is no significant difference between the two values. However, there was a significant difference in the treatment when mycorrhiza was not applied, where the P uptake was 132.48 ppm (Table 1). Mycorrhiza is a beneficial fungus that forms symbiotic associations with many plants. They enhance the uptake of nutrients and water through increased root surface area and a wide hyphal system. Furthermore, the efficiency of AMF inoculation is regulated by several factors such as the type of mycorrhizal and plant, availability of nutrients for crops, climate, and stress factors (Chen et al., 2018). Mycorrhiza symbiosis with almost all plant roots contributes significantly to the availability of plant nutrients, especially to phosphorus absorption (Smith et al., 2003). First, due to its enormous surface area, the hyphal tissue of Arbuscular Mycorrhiza Fungi (AMF) is very efficient in nutrient absorption (Plenchette et al., 2005). Fungal partners form extensive extraradical mycelium in the soil, enhancing the root's absorption area (Lioussanne et al., 2009).

Furthermore, from this study, AMF inoculation was known to increase P uptake significantly. The role of mycorrhizal fungi in the acquisition of mineral nutrients, especially phosphorus was explained previously by Bolduc & Hijri, (2010) who demonstrated the increase in growth of *Allium cepa* as a result of inoculation with mycorrhiza fungi (Citterio et al., 2005). Mycorrhizal fungi improve plant growth by enhancing nutrient uptake under phosphorus-limiting conditions. (Bano & Ashfaq, 2013) also stated that the growth of the mycorrhizal inoculated plant has a positive relationship with the growth of the host plant, thereby, increasing nutrient uptake through an external mycelium by expanding the root absorption surface or by producing a chemical compound that causes the release of nutrient.

Effect of biochar on the growth and yields of shallots on soil contaminated with Pb and Cd.

Biochar has a significant effect on the number of leaves, bulbs, and weight. Furthermore, it was seen that soil without biochar gave a low yield of 20.926 cm, which is significantly different from soil with biochar treatment of 2.5, 5, and 10 t/ha, which gave a yield of 25.731, 28.667, and 26.602 cm respectively. In terms of bulb variables, soil without biochar gave a low yield of 6.444 but this was not significantly different from biochar treatment of 2.5 and 5 t/ha, which gave a yield of 7.463 and 7.806 number of bulbs per pot. The highest yield of 9.741 bulbs was produced by 10 t/ha of biochar.

The existence of biochar in various agricultural waste materials confirms the presence of different minerals such as phosphorus and nitrogen. These minerals increase soil productivity leading to a high yield of crops. Therefore, they are vital for plant growth and for boosting soil productivity. Nurida (2014) stated that the addition of biochar to the soil increases the N and P minerals present. Furthermore, it is known to have a high water-holding capacity which helps prevent nitrogen minerals from being washed away easily, making it more available to plants. Sohi, et al. (2010) found that the utilization of biological fertilizer and biochar had a notable impact on the vertical growth of the *Brahiaria decumben* grass species. This growth is affected by the rate of nitrogen uptake by the soil which increases after biochar application. According to Song, et al. (2018), adding biochar to land improves the available P and N-total. Satriawan & Handayanto (2015) explained that plants require phosphorus (P) nutrients for development, root and seed formation, faster flowering, and maturation. As a result, the amount of P element present in the soil determines the P nutrients needed by plant roots.

Effect of mycorrhiza to Cd and Pb on tissue plant and removal efficiency.

Figure 1 shows that the utilization of mycorrhiza significantly affects the absorption of Pb and Cd. In soil that was not inoculated, the uptake of Pb by plant tissue gave a high yield of 16.65 ppm, which is significantly different from the soil with 1 and 2 g mycorrhizae/pot, where the Pb uptake was 13.228 ppm and 11.269 ppm, respectively. The uptake of Cd gave a high yield of 1.433 ppm when no inoculation was conducted. However, this result was significantly different from the soil with 1 and 2 g mycorrhiza/pot, where the Cd uptake was 1.203 ppm and 1.07 ppm, respectively.

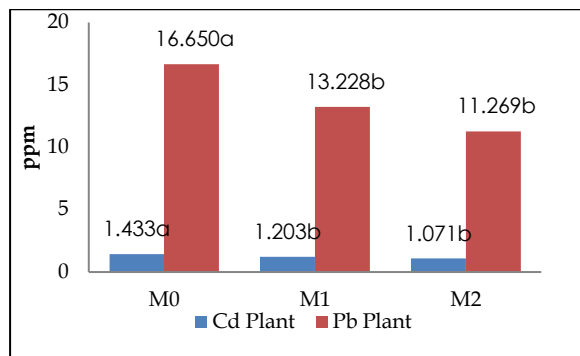


Figure 1. Effect of mycorrhiza application to Cd and Pb on plant uptake.

Notes: (*) M0 = 0 g mycorrhiza/pot; M1 = 1 g mycorrhiza/pot, M2 = 2 g mycorrhiza/pot

The application of mycorrhiza to the soil is very significant in the removal of heavy metal effectively as shown in Figure 2. Therefore, planting medium without mycorrhizae removes Pb at a small percentage of 33.74%. This value is significantly different from media inoculated with 1 and 2 g mycorrhizae/pot which remove Pb at 53.94% and 65.26%. Furthermore, the percentage of Cd removal in the media that was not inoculated with mycorrhizae was 29.74%. This result is significantly different from media inoculated with 1 and 2 g mycorrhiza/pot which remove Cd at 48.33% and 55.62%. The removal ability of heavy metals was highly classified refers to the EPA standard.

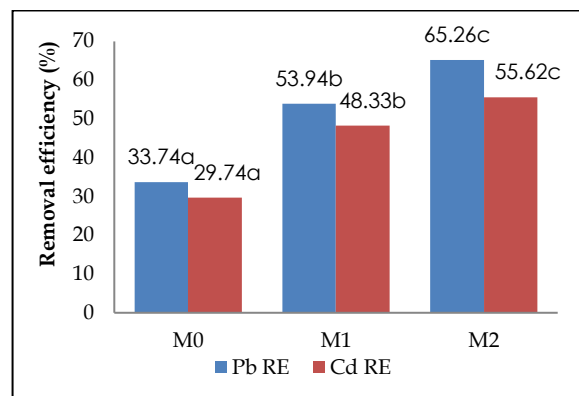


Figure 2. Effect of mycorrhiza application to Cd and Pb on Removal Efficiency (RE).

Notes: (*) M0 = 0 g mycorrhiza/pot; M1 = 1 g mycorrhiza/pot, M2 = 2 g mycorrhiza/pot

Mycorrhizae have the potential to aid in the bioremediation of heavy metal contamination in soil. Arbuscular mycorrhizal fungi reduce the threat of heavy metals by secreting several compounds, which affect the deposition of metals

in polyphosphate granules in the soil, adsorb metals to the fungal cell walls, and chelate heavy metals in the fungi. Organic acids and glomalin released from plants and fungi also play an important role in immobilizing heavy metals in the soil. Plant-colonized arbuscular mycorrhizal fungi release organic acids, which increase the sequestration and absorption of heavy metals, and organic acids are precipitated as chelated polyphosphate granules and heavy metals that are immobile in the soil. Soil management applications reduce the ability of mycorrhizal sporulation and colonization by disrupting the extra-radical mycelium network. Disturbance of the hyphal tissue reduces its surface area. To prevent stressful conditions in the environment, AMF grows a wider mycelium (Herath et al., 2021). The bioaccumulation and immobilization of hazardous heavy metals in soil is also facilitated by the endomycorrhizal relationship. Mycorrhiza is a symbiotic relationship between fungus and higher plant roots. Fungus infiltrates plant roots and produces hyphae, arbuscules, and vesicles. As a result of this interaction, nutrients, particularly phosphorus, are transported. Cadmium and lead, for example, interfere with several physiological and biochemical processes in plants, including photosynthesis, respiration, nitrogen, and protein metabolism.

Effect of biochar to Cd and Pb on tissue plant and removal efficiency. Biochar can decrease heavy metal uptake in plant tissue (Figure 3). Application of biochar 10 t/ha and 7.5 t/ha showed a significant effect on Pb in plant tissue were 9.849 ppm and 11.982 ppm lower than biochar 2.5 t/ha treatment and without biochar application with the value of 15.680 ppm and 16.684 ppm, respectively. Biochar application has not been able to significantly reduce Cd uptake, but its value tends to decrease.

Biochar also can remove heavy metals (Figure 4), the use of biochar has a significant effect on the effective removal of Pb and Cd. Pots that lack biochar removed Pb at 36.91%, which is low. This is significantly different from pots that contain biochar 2.5 and 5 t/ha, which removed Pb at 41.329% and 46.065%. The highest percent is seen when 10 t/ha biochar is applied to a pot. However, the application of 2.5 t/ha biochar was able to remove Cd at 36.82%, which is not significantly different from the 5 and 10 t/ha biochar treatments, which remove Cd at 42.54% and 49.73%, respectively.

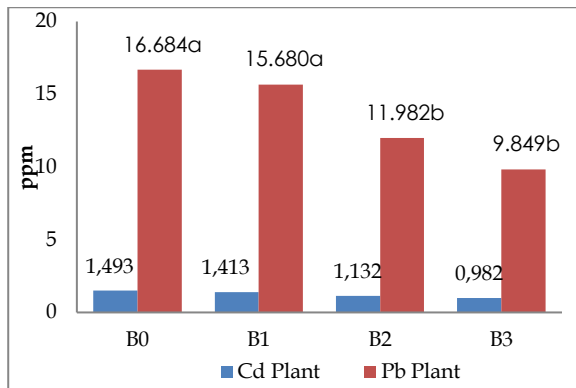


Figure 3. Effect of Biochar Application to Cd and Pb on Plant Uptake.

Notes: (*) B0 = 0 t/ha biochar, B1 = 2.5 t/ha biochar, B2 = 5 t/ha biochar, B3 = 10 t/ha biochar

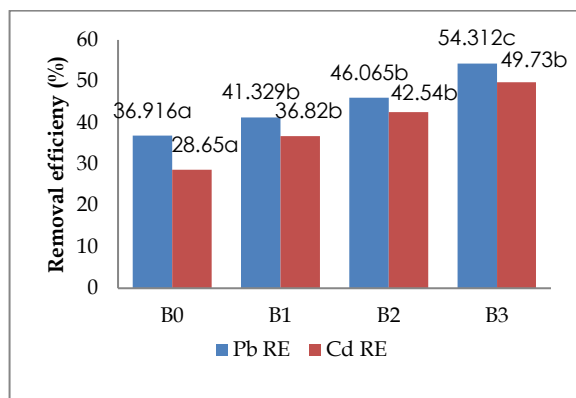


Figure 4. Effect of Biochar Application to Cd and Pb on Removal Efficiency (RE).

Notes: (*) B0 = 0 t/ha biochar, B1 = 2.5 t/ha biochar, B2 = 5 t/ha biochar, B3 = 10 t/ha biochar

The corn cob biochar used in the present study contained 69.937% cellulose, 17.797% hemicellulose, and 9.006% lignin. If the elements in corn cobs are used as biochar, they will be deformed which can increase the availability of nutrients and the amount of c-organic in the soil. Increasing C-organic indirectly provides a good habitat for soil microbes, which play a role in decomposing organic matter in the soil to increase nutrient availability and bind heavy metals that are harmful to soil and plants (Rodriguez et al., 2019).

Biochar can stabilize heavy metals in polluted soil, absorb heavy metals, and improve the physical, chemical, and biological qualities of the soil. The application of biochar can reduce the mobility of polluted heavy metals in the soil so that heavy metals in the form of radicals will not be absorbed by plants (Yao et al., 2011). Biochar can reduce the mobility of lead and cadmium radicals in soils with low pH and non-electrostatic absorption. Biochar can eliminate the activity of heavy metal ions in contaminated soil so that heavy metals do not enter the food chain system in living things because they will be immediately leached and precipitated (Tan et al., 2015).

The application of biochar resulted in a total lead concentration in shallot plantations of 17.53-22.59 mg/kg (Dewi et al., 2022). This concentration is far below the critical limit of heavy metals needed for agricultural land, namely 100-400 mg/kg of lead. This is because biochar can reduce lead in roots, tubers, and leaves. Total soil cadmium concentrations in shallot plantations that were applied with biochar were between 1.01-1.46 mg/kg, and this value was still below the critical limit of 3-8 mg/kg. The addition of biochar can change the bioavailability and mobility of cadmium in the soil. These steps are connected to many processes, including redox reactions, precipitation, adsorption, and complexation.

Interaction of mycorrhiza and biochar factors affect root infection and plant height.

There was a significant effect of interaction between biochar and AMF factor on the root infection (Figure 5) and plant height (Figure 6). The 81.7% root infection was recorded when 2 g mycorrhizae/pot were combined with 10 t/ha of biochar. Treatment of 1 g mycorrhiza/pot combined with 10 t/ha of biochar was shown to increase plant height by 38.06 cm. However, this has no significant difference with mycorrhizae 2 g/pot combined with biochar 1 t/ha which increases plant height by 37.89 cm.

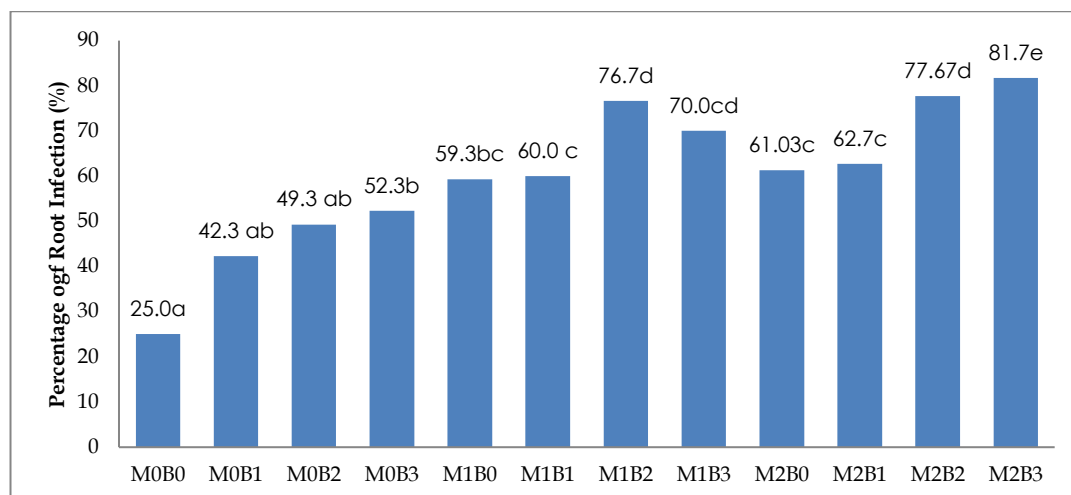


Figure 5. Interaction of Mycorrhiza and Biochar Factors Affect Root Infection.

Note: (*) M0B0 = no treatment M0B1 = 0 mycorrhiza and 2.5 t/ha biochar M0B2 = 0 mycorrhiza and 5 t/ha biochar M0B3 = 0 mycorrhiza and 10 t/ha biochar M1B0 = 1 g mycorrhiza/pot and 0 t/ha biochar M1B1=1 g mycorrhiza/pot and 2.5 t/ha biochar M1B2=1 g mycorrhiza/pot and 5 t/ha biochar M1B3=1 g mycorrhiza/pot and 10 t/ha biochar M2B0 = 2 g mycorrhiza/pot and 0 t/ha biochar M2B1= 2 g mycorrhiza/pot and 2.5 t/ha biochar M2B2= 2 g mycorrhiza/pot and 5 t/ha biochar M2B3= g mycorrhiza/pot and 10 t/ha biochar

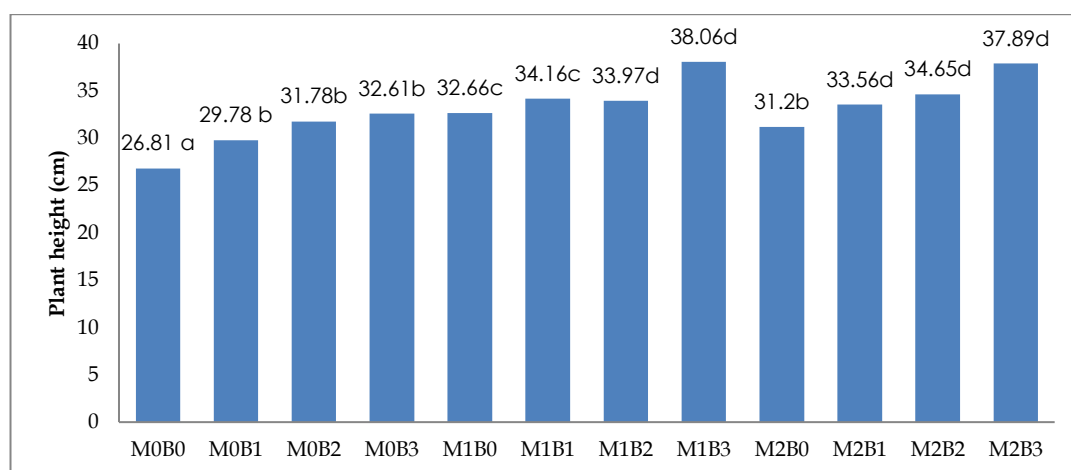


Figure 6. Interaction of Mycorrhiza and Biochar Factors Affect Plant Height.

Note: M0B0 = no treatment M0B1 = 0 mycorrhiza and 2.5 t/ha biochar M0B2 = 0 mycorrhiza and 5 t/ha biochar M0B3 = 0 mycorrhiza and 10 t/ha biochar M1B0 = 1 g mycorrhiza/pot and 0 t/ha biochar M1B1=1 g mycorrhiza/pot and 2.5 t/ha biochar M1B2=1 g mycorrhiza/pot and 5 t/ha biochar M1B3=1 g mycorrhiza/pot and 10 t/ha biochar M2B0 = 2 g mycorrhiza/pot and 0 t/ha biochar M2B1= 2 g mycorrhiza/pot and 2.5 t/ha biochar M2B2= 2 g mycorrhiza/pot and 5 t/ha biochar M2B3= g mycorrhiza/pot and 10 t/ha biochar

The use of biochar has helped improve the soil physical and biochemical properties, which has increased plant production (Hossain et al., 2020). It carries out these functions by increasing the nutrients and water content present in the soil, which aids in plant growth. According to Almaroai & Eissa, (2020), the nature of biochar includes the ability to retain and increase nutrients and water content, enhancement of soil biological, physical,

and chemical properties, and improvement of soil structure and productivity. Mycorrhizal inoculation has a significant effect on plant height due to its role in metabolism, which occurs in plant roots, leading to an increased growth rate. Its metabolic activity is 2 to 4 times higher than that of non-mycorrhizal roots because of its ability to aid the absorption of mineral salts by increasing the supply of hydrogen ions (Liu et al., 2021).

In addition, the application of biochar in mycorrhizal treatment has been found to have the potential to enhance the rate of root infection. However, the observed increase in root infection percentage did not reach statistical significance, as it ranged from 59.30 to 84.30%. (Yang et al., 2023) state that an increase in mycorrhizal doses in roots tends to increase the root infection. Mycorrhizae promotes plant growth, development, stress tolerance, soil remediation, carbon sequestration, food safety, and agricultural sustainability

Soil given biochar consistently has an increased C content which is more stable than soil without biochar. Its high surface area and porosity made it possible for plants to absorb or retain nutrients, water, and also act as a habitat for growth of beneficial microorganisms. Biochar is suitable for improving the fertility of both chemicals, physical and biological soil (Jeffries, et al. 2003). Since AMF is reported to enhance N uptake, largely due to improved phosphate availability (Smith et al., 2011; Sohi et al., 2010), biochar can also supports mycorrhizal growth by providing a favorable habitat for soil microbes, aiding nutrient breakdown and plant absorption. So that biochar and mycorrhiza synergize with each other in efforts to improve soil.

Conclusion

Applying 2.5, 5, and 10 t/ha biochar are suitable for increasing plant height and the percentage of root infection. Application of mycorrhiza increases the P uptake and the number of the bulb by 25.26%. It also decreases the Pb and Cd uptake by 32.31% and 25.08%, respectively, leading to an increase in heavy metal removal.

Acknowledgments

All thanks go to LPPM UNSOED who facilitated this study through the "Riset Dasar Unsoed" Research program from the funding of BLU UNSOED of 2020.

References

- Abdelrahman M, Mahmoud HYAH, El-Sayed M, Tanaka S, Tran LSP. 2017. Isolation and characterization of cepa2, a natural alliospiroside A, from shallot (*Allium cepa* L. Aggregatum Group) with anticancer activity. *Plant Physiology and Biochemistry*, 116: 167-173.
- Adetya V, Nurhatika S, Muhibuddin A. 2019. Pengaruh pupuk mikoriza terhadap pertumbuhan cabai rawit (*Capsicum frutescens*) di tanah pasir. *Jurnal Sains dan Seni ITS*, 7(2): 75-79.
- Ali Z, Nawaz I, Yousaf S, Naqvi STA, Mahmood T, Khan N, Iqbal M. 2019. Wheat straw biochar promotes the growth and reduces the uptake of lead, cadmium and copper in *Allium cepa*. *International Journal of Agriculture and Biology*, 21(6): 1173-1180.
- Almaroai YA, Eissa MA. 2020. Effect of biochar on yield and quality of tomato grown on a metal-contaminated soil. *Scientia Horticulturae*, 265: 109210.
- Bano SA, Ashfaq D. 2013. Role of mycorrhiza to reduce heavy metal stress. *Natural Science*, 5(12): 16-20.
- Bansal OP. 2018. The influence of potentially toxic elements on soil biological and chemical properties. *IntechOpen*, 11: 1-14.
- Bolduc AR, Hijri M. 2010. The use of mycorrhizae to enhance phosphorus uptake: A way out the phosphorus crisis. *Journal of Biofertilizers & Biopesticides*, 2(1): 1-5.
- Borisova G, Chukina N, Maleva M, Kumar A, Prasad MNV. 2016. Thiols as biomarkers of heavy metal tolerance in The aquatic macrophytes of Middle Urals, Russia. *International Journal of Phytoremediation*, 18(10): 1037-1045.
- Chen M, Arato M, Borghi L, Nouri E, Reinhardt D. 2018. Beneficial services of arbuscular mycorrhizal fungi—from ecology to application. *Frontiers in Plant Science*, 9: 1-14.
- Citterio S, Prato N, Fumagalli P, Aina R, Massa N, Santagostino A, Sgorbati S, Berta G. 2005. The arbuscular mycorrhizal fungus *Glomus mosseae* induces growth and metal accumulation changes in *Cannabis sativa* L. *Chemosphere*, 59(1): 21-29.
- Cui L, Li L, Bian R, Yan J, Quan G, Liu Y, Ippolito JA, Wang H. 2020. Short-and long-term biochar cadmium and lead immobilization mechanisms. *Environments*, 7(7): 1-15.
- Dharmadewi, AIM. 2020. Analisis kandungan klorofil pada beberapa jenis sayuran hijau
- Herliana O, Ahadiyat YR, Cahyani W. 2024. Bioremediation of Pb and Cd contaminated soil by mycorrhiza and biochar treatment and its effect on growth and yield of shallot. *Jurnal Kultivasi*, 23(2): 323-333.

- sebagai alternatif bahan dasar food supplement. *Emasains: Jurnal Edukasi Matematika dan Sains*, 9(2), 171-176.
- Dewi T, Martono E, Hanudin E, Harini R. 2022. Impact of agrochemicals application on lead and cadmium concentrations in shallot fields and their remediation with biochar, compost, and botanical pesticides. *IOP Conference Series: Earth and Environmental Science*, 1109: 1-9.
- Eviati, & Sulaeman. 2009. *Analisis Kimia Tanah, Tanaman, Air, dan Pupuk*. Balai Penelitian Tanah, Bogor
- Esringü A, Turan M, Cangönül A. 2021. Remediation of Pb and Cd Polluted Soils with Fulvic Acid. *Forests*, 12(11): 1-13.
- García-Díaz A, Bienes R, Sastre B, Novara A, Gristina L, Cerdà A. 2017. Nitrogen losses in vineyards under different types of soil groundcover. A field runoff simulator approach in Central Spain. *Agriculture, Ecosystems and Environment*, 236: 256-267.
- Herath BMMD, Madushan KWA, Lakmali JPD, Yapa PN. 2021. Arbuscular mycorrhizal fungi as a potential tool for bioremediation of heavy metals in contaminated soil. *World Journal of Advanced Research and Reviews*, 10(3): 217-228.
- Herliana O, Rahayu AY, Cahyani W. 2021. Utilization of biochar and *Trichoderma harzianum* to promote growth of shallot and remediate lead-contaminated soil. *Journal of Degraded and Mining Lands Management*, 8(3): 2743-2750.
- Hossain MZ, Bahar MM, Sarkar B, Donne SW, Ok YS, Palansooriya KN, Kirkham MB, Chowdhury S, Bolan N. 2020. Biochar and its importance on nutrient dynamics in soil and plant. *Biochar*, 2(4): 379-420.
- Hussain M, Farooq M, Nawaz A, Al-Sadi AM, Solaiman ZM, Alghamdi SS, Ammara U, Ok YS, Siddique KHM. 2017. Biochar for crop production: Potential benefits and risks. *Journal of Soils and Sediments*, 17(3): 685-716.
- Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea JM. 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology and Fertility of Soils*, 37(1): 1-16.
- Lioussanne L, Jolicœur M, St-Arnaud M. 2009. Role of the modification in root exudation induced by arbuscular mycorrhizal colonization on the intraradical growth of *Phytophthora nicotianae* in tomato. *Mycorrhiza*, 19(6): 443-448.
- Liu D, Ding Z, Ali EF, Kheir AMS, Eissa MA, Ibrahim OHM. 2021. Biochar and compost enhance soil quality and growth of roselle (*Hibiscus sabdariffa* L.) under saline conditions. *Scientific Reports*, 11(1): 1-11.
- Nadir M, Syamsia S, Laban S. 2018. Pemanfaatan cendawan mikoriza arbuskular untuk mereduksi kadar Pb dan Cd pada lahan sawah serta pengaruhnya terhadap pertumbuhan tanaman selada. *Jurnal Ecosolum*, 7(2): 61-66.
- Nurida NL. 2014. Potensi pemanfaatan biochar untuk rehabilitasi lahan kering di Indonesia. *Jurnal Sumberdaya Lahan Edisi Khusus*, 8(3): 57-68.
- Plenchette C, Clermont-Dauphin C, Meynard JM, Fortin JA. 2005. Managing arbuscular mycorrhizal fungi in cropping systems. *Canadian Journal of Plant Science*, 85(1): 31-40.
- Puga AP, Abreu CA, Melo LCA, Beesley L. 2015. Biochar application to a contaminated soil reduces the availability and plant uptake of zinc, lead and cadmium. *Journal of Environmental Management*, 159: 86-93.
- Ratnasari IFD, Hadi SN, Suparto SR, Herliana O, Rahayu AY. 2020. Phytoremediation of cadmium-contaminated soil using terrestrial kale (*Ipomoea reptans* Poir) and corncob biochar. *Journal of Degraded and Mining Lands Management*, 7(4): 2502-2458.
- Rodriguez A, Lemos D, Trujillo YT, Amaya JG, Ramos LD. 2019. Effectiveness of biochar obtained from corncob for immobilization of lead in contaminated soil. *Journal of Health and Pollution*, 9(23): 13-21.
- Rolka E, Wyszowski M. 2021. Availability of trace elements in soil with simulated cadmium lead and zinc pollution. *Minerals*, 11: 1-15.
- Saptana, Gunawan E, Perwita AD, Sukmaya SG, Darwis V, Ariningsih E, Ashari. 2021. The competitiveness analysis of shallot in Indonesia: A policy analysis matrix. *PLoS ONE*, 16(9): 1-19.
- Satriawan BD, Handayanto E. 2015. Effects of biochar and crop residues application on chemical properties of a degraded soil of South Malang, and P uptake by maize. *Journal of Degraded and Mining Lands*

- Management, 2(2): 271–280.
- Shon TK, Haryanto TAD, Yoshida T. 1997. Dry matter production and utilization of solar energy in one year old *Bupleurum falcatum*. Journal Faculty of Agriculture Kyushu University, 41, 133-140.
- Smith SE, Jakobsen I, Grønlund M, Smith FA. 2011. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: Interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiology, 156(3): 1050–1057.
- Smith SE, Smith FA, Jakobsen I. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiology, 133(1): 16–20.
- Sohi SP, Krull E, Lopez-Capel E, Bol R. 2010. A review of biochar and its use and function in soil. Advances in Agronomy, 105(1): 47–82.
- Song D, Jiwei T, Xi X, Shuiqing Z. 2018. Responses of soil nutrients and microbial activities to additions of maize straw biochar and chemical fertilization in a calcareous soil. European Journal of Soil Biology, 84: 1–10.
- Susanti A, Hidayat R, Prasetjono H. 2018. Implementasi mikoriza sebagai sarana pengetahuan konservasi mandiri lahan marginal di Kecamatan Kabuh Kabupaten Jombang. Jurnal Agoradix, 1(2): 9–17.
- Tan X, Liu Y, Zeng G, Wang X, Hu X, Gu Y, Yang Z. 2015. Application of biochar for the removal of pollutants from aqueous solutions. Chemosphere, 125: 70–85.
- Yang S, Imran Ortas I. 2023. Impact of mycorrhiza on plant nutrition and food security. Journal of Plant Nutrition, 46(13): 3247–3272.
- Yao Y, Gao B, Inyang M, Zimmerman AR, Cao X, Pullammanappallil P, Yang L. 2011. Biochar derived from anaerobically digested sugar beet tailings: Characterization and phosphate removal potential. Bioresource Technology, 102(10): 6273–6278.
- Yusuf M, Nurtjahja K, Lubis R. 2016. Analysis of Metallic Content of Pb, Cu, Cd And Zn On Vegetables Sawi Kangkung and Spinach In The Area Agriculture and Paya Rumpit Village Industry Titipapan Medan. BioLink, 3(1): 56–64.
- Zarcinas BA, Ishak CF, Mclaughlin MJ, Cozens G. 2004. Heavy metals in soils and crops in Southeast Asia: 1. Peninsular Malaysia. Environmental Geochemistry and Health, 26(4): 343–357.