UTILIZATION OF ARBUSCULAR MYCORRHIZAL FUNGI FOR THE EFFICIENCY OF LEAD METAL ABSORPTION AND YIELD OF HANJUANG PLANTS (*Cordyline fruticosa*)

ABSTRACT

Contamination of Pb metal into the soil is an environmental problem that has a serious impact on living things, so it needs to be addressed through phytoremediation, one of which is using hanjuang plants. Plant roots in symbiosis with arbuscular mycorrhizal fungi (AMF) can increase plant growth and the phytoremediation ability of plants growing in polluted soil. This research aims to determine the effect of AMF application on the growth of hanjuang plants and the ability of hanjuang roots to absorb Pb metal. The research design used a single treatment with a Completely Randomized Block Design (CRBD) of 4 doses of AMF with 6 replications, namely control; 10 g polybag^{-1}; 20 g polybag^{-1}; and 30 g polybag^{-1}. Planting was carried out for 8 weeks to see the growth and planting of hanjuang and the efficiency of Pb absorption in plant roots. The results showed that AMF had a significant effect on plant height aged 5 to 8 weeks, plant biomass, root wet weight, and root dry weight, but had no significant effect on the efficiency of Pb absorption in the roots of hanjuang plants. An AMF dose of 30 grams per polybag provides the best results for the growth of hanjuang plants.

Keywords: phytoremediation, arbuscular mycorrhizal fungi, hanjuang plant, Pb metal.

1. INTRODUCTION

Environmental pollution by heavy metals is a serious environmental problem because heavy metals are difficult to degrade and their toxic properties endanger human health and the environment. Likewise, if heavy metals pollute the soil, they can endanger the health and the ecosystem through direct contact with the soil, reducing the quality of the soil used for agricultural production, and through the food chain. Soil contaminated with heavy metals must be restored so that it can be used for cultivation and prevent greater ecological impacts. Land as a production factor has an important role.

However, remediating land contaminated with heavy metals using conventional methods is expensive and difficult to carry out. Lead (Pb) is one of the most dangerous heavy metals for living creatures after arsenic (As). Pb metal that exceeds the threshold will endanger metabolism and inhibit the enzyme activity of living things [1]. The source of Pb pollution is industrial activities through waste that is not managed properly, dust accumulation, rain, and soil erosion.

Phytoremediation is a method for remediating soil contaminated with heavy metals using plants to accumulate heavy metals. These plants are tolerant and capable of accumulating heavy metals in large amounts. Phytoremediation plants can translocate heavy metals to plant tissues including roots, stems, and leaves without disrupting plant growth. One of the plants that can be used in phytoremediation of the heavy metal Pb is hanjuang (*Cordyline fruticosa*). Hanjuang is an ornamental plant and is not consumed so the Pb metal it absorbs does not have the potential to endanger human and animal health. This plant can absorb Pb metal better than other plants, such as sambang dara (*Excoecaria cochinchinensis*) and mother-in-law's tongue (*Sansevieria trifasciata* Prain) [2].

Hanjuang roots are the part of the plant that accumulates the highest amount of Pb metal compared to other plant parts, however, the ability of hanjuang plants to absorb heavy metals will be lower in soil with a high Pb metal content [3]. One way that can be done to increase the ability of plants to absorb heavy metals is to use arbuscular mycorrhizal fungi (AMF). AMF is a fungus that has a symbiotic relationship with plant roots which can increase plant growth. Phytoremediation using mycorrhiza is more effective than just treating plants [4]. Mycorrhizae can increase the absorption of heavy metals by contributing to the immobilization of heavy metals in the rhizosphere of plant roots in the soil.

This research aims to determine the effect of AMF on the growth of hanjuang plants in soil contaminated with Pb metal and the efficiency of absorption of Pb metal in the roots of hanjuang plants.

2. RESEARCH METHODS

2.1. Time and Place

This research was carried out from February to May 2023 in Kembang Janggut Village and the Soil Science Laboratory, Faculty of Agriculture, Mulawarman University for Pb metal analysis.

2.2. Materials and Tools

Materials and Equipment Research materials include soil, hanjuang plants, lead chloride ($PbCl_2$), AMF, sulfuric acid (HCl) 25% and 37%, and distilled water (H_2O). The tools used include polybags measuring 30 cm x 30 cm, polybag coasters, sieves, analytical scales, ovens, meters, atomic absorption spectrometers (SSA), mortals and pestles, desiccators, volumetric flasks, test tubes, and centrifuges.

2.3. Experimental Design

The research was structured using a Completely Randomized Block Design (CRBD) consisting of four mycorrhizal dose treatments (P) and six replications, namely as follows: p0 = 0 g polybag^{-1} as control, p1 = 10 g polybag^{-1}, p2 = 20 g polybag^{-1} and p3 = 30 g polybag^{-1}

2.4. Research Activity

2.4.1. Application of Pb Metal to Soil

The air-dried soil was sieved, weighed 5 kg, added 0.67 g of $PbCl_2$ metal, and stirred in a special container, then put in a polybag to leave for one day.

2.4.2. Arbuscular Mycorrhizal Fungi (AMF) Application

The AMF application is given simultaneously when transplanting according to the treatment by placing it in the planting hole around the roots of the hanjuang plant.

2.4.3. Planting Hanjuang Plants

The hanjuang plants used in the research were uniform with a plant age of 2 months, an average plant height of 29 cm, and a total of 5 leaves per plant. Plants were planted in soil that had been contaminated with Pb metal in polybags and AMF was applied according to the treatment dose.

2.4.4. Hanjuang Plant Maintenance

Watering is done once a day in the afternoon. Weeding is done manually if weeds are growing in the polybag.

2.4.5. Harvest

When the plants are 8 weeks old after planting. Plants will be dismantled from polybags and cleaned from the soil to collect the required data.

2.5. Data collection

2.5.1 Plant data

Plant data collection consisted of plant height (cm) at the age of 1 to 8 weeks after planting, plant wet weight (g), longest root length (g), root wet weight (g), and root dry weight (g). Pb metal analysis was carried out on the initial soil and roots of Hanjuang plants using the atomic absorption spectrometer method.

2.5.2. Lead Metal Data (Pb)

Done on the initial soil and roots of the hanjuang plant. The dried soil and plant roots, weighing 1 gram, are crushed until smooth and then put into a measuring flask. After the sample is ground, add 1 ml of 25% HCl into the test tube. Next, add 5 ml of 37% HCl into the test tube, and desiccator at 200℃ until the color changes to brown. The sample is heated until it becomes clear yellow, the time required is approximately ± 6 hours. After that, the digestion results were transferred into a 5 ml test tube which would be put into a centrifuged at a speed of 2,500 rpm for 5 minutes. Next, the Pb metal value was reading the Pb metal value using an atomic absorption spectrometer (SSA) in ppm units.

The absorption efficiency of Pb metal in hanjuang roots is obtained by the following equation:

Pb Absorption Efficiency = [(Pb in plant roots)/(Pb in initial soil)] x 100 %

2.6. Data Analysis

Data analysis uses variance at the 5% level and if they are significantly different, continue with the Least Significant Difference Follow-up Test (LSD) at the 5% level.

3. RESULTS AND DISCUSSION

3.1. Plant Growth of Hanjuang Plant

The results of variance analysis showed that FMA application had no significant effect in soil contaminated with Pb metal on plant height at the age of 1 to 3 weeks after planting, but had a significant effect on plant height at the age of 4 to 8 weeks after planting. The results of the research on average plant height at the age of 1 to 8 weeks after planting are presented in Table 1.

Table 1. Effect of AMF application on soil contaminated with Pb metal on Hanjuang plant height

Dose of AMF	Plant Height (cm)

(g)	1 ns	2 ns	3 ns	4*	5 *	6*	7*	8*
Week After Planting								
0	28,18	28,48	30,35	32,00[a]	34,08[a]	36,72[a]	39,60[a]	42,67[a]
10	30,62	31,25	33,33	35,03[a]	37,30[a]	40,58[b]	43,55[a]	47,23[a]
20	30,37	31,62	33,62	37,53[b]	40,13[b]	43,68[b]	46,85[b]	51,72[a]
30	29,00	30,10	32,42	37,25[b]	40,35[b]	45,08[b]	49,22[c]	54,37[b]

Note: ns = non-significant; * = affected significantly; The average value of one column followed by the same letter means it is not real based on the 5% LSD test.

The variance analysis showed that AMF application had no significant effect on root length but had a significant effect on root wet weight, root dry weight, and plant wet weight. The research results on average root length, root wet weight, root dry weight, and plant wet weight are presented in Table 2.

Table 2. Effect of AMF application on wet weight, root length, root wet weight, and root dry weight of hanjuang plant

Dose of AMF (g)	Wet weight of plant (g)*	Root length (cm)	Wet weight of roots (g)*	Dry weight of roots (g)*
0	51,83[a]	24,00	11,00[a]	3,12[a]
10	51,50[a]	27,25	13,17[a]	3,42[a]
20	60,17[ab]	30,75	13,33[a]	3,68[a]
30	66,67[b]	31,42	20,67[b]	6,78[b]

Note: ns = non-significant; * = affected significantly; The average value of one column followed by the same letter means it is not real based on the 5% LSD test.

Figure 1. Roots of hanjuang plants in soil contaminated with Pb metal from each treatment (p0= without AMF application, p1= 10 g polybag^{-1}, p2 = 20 g polybag^{-1}, p3 = 30 g polybag^{-1})

The results showed that the application of AMF for 8 weeks after planting (WAP) on soil contaminated with Pb metal had a significant effect on the height of hanjuang plants aged 4 WAP to 8 WAP (Table 1). The influence of mycorrhiza on the height of hanjuang plants aged 1 to 3 WAP had no significant effect. AMF application did not have a significant effect on plant root length, but there was an increase in root length compared to the control. AMF provides benefits for plants in absorbing nutrients, especially phosphorus and water. So plant growth increases compared to plants without mycorrhizal application [5]. The application of mycorrhiza increases plant growth by forming hyphae, namely tissue that is a place for carbon exchange from plants to mycorrhiza and nutrients in the soil [6].

The influence of mycorrhiza on the height of hanjuang plants aged 1 to 3 WAP had no significant effect. It is suspected that mycorrhiza needs time to infect plant roots so that its effect is only visible at 4 WAP, where plant height at 4 WAP and onwards is significantly different. Plant height can be a benchmark for increasing the absorption of heavy metals by plants, as in the results of research by [2] which states that the growth rate of hanjuang plants increases in proportion to the absorption of Pb metal in hanjuang plants. The effect of Pb metal on plant height is influenced by chlorophyll which plays an important role in the process of photosynthesis and plant growth, however, plant tolerance to heavy metals and high levels of Pb metal can inhibit plant growth, including plant height [7]. The symbiosis of

mycorrhiza with plant roots provides benefits for the host plant by absorbing nutrients so that plant growth increases [5].

Mycorrhiza applied to soil contaminated with Pb had a significant effect on the fresh weight of hanjuang plants. Mycorrhiza increased the wet weight of hanjuang plants compared to the control. Plant wet weight is related to the plant's ability to absorb water and nutrients. Even though Pb reduces the ability of plants to absorb water and nutrients [8], mycorrhizae can help hanjuang roots absorb nutrients and water thereby increasing the plant's wet weight, where the highest wet weight is achieved at a dose of 30 g. Mycorrhiza also affects the wet weight of plants. Hanjuang plants with AMF application had better plant growth compared to those without AMF application. Mycorrhiza can help improve root performance in absorbing nutrients and water [9]. This is by research by [10], that mycorrhiza can improve plant growth for the better by increasing the area of nutrient absorption.

Plant root growth is an indicator of water shortages and the physical condition of the soil, including contamination in response to heavy metals in the soil. Mycorrhizal application did not have a significant effect on plant root length, but there was an increase in root length compared to the control. In this study, there was an increase in root length with AMF application with the highest results in the P3 treatment of 31.42 cm. Even though the hanjuang roots were infected with mycorrhiza, the short testing time meant that the root length was not optimal. Hanjuang is a hyperaccumulator plant of Pb metal so root growth is not hampered even without AMF application. In this research, plants were planted in polybags so that the root reach to get a water source was not too far, in addition to the availability of sufficient water for the plant so that the growth in root length adjusted to the distance to reach the water source available to the plant. However, hanjuang roots infected with mycorrhiza in soil contaminated with Pb metal could grow well and had the highest wet root weight in the P3 treatment as shown in Figure 1. This is [11] that hanjuan roots infected with mycorrhiza will grow and develop faster so that the root volume becomes wider.

3.2. Logan Pb Content and Pb Uptake Efficiency

The results of the analysis of various AMF applications had no significant effect on the metal Pn content and the efficiency of Pb uptake by the roots of hanjuang plants. The results of the research on the average Pb metal content and the efficiency of Pb uptake by the roots of hanjuang plants are presented in Table 3.

Table 3. Pb Metal Content in Hanjuang Plant

Tabel 3. Kandungan Logam Pb di Perkaran Hanjuang

Dosis FMA (g)	Timbal (ppm)	Efisiensi (%)
0	48,69	48,01
10	31,55	31,11
20	49,66	48,96
30	53,25	52,50

The highest value was obtained for heavy metals in plant roots at a dose of 30 g AMF, namely 53.25 ppm. However, the results of the analysis of variance showed that the dose of AMF had no significant effect on the metal Pb content in the roots of hanjuang plants. This is thought to be because AMF takes time to infect plant roots, if the planting time is longer then absorption efficiency increases in all AMF treatments.

In the treatment without mycorrhiza, the roots of the hanjuang plants were able to absorb Pb metal as indicated by the quite high efficiency of absorbing Pb metal, which means this plant is a hyperaccumulator. Although the heavy metal content in plant roots does not differ, the increasing root weight when AMF is applied shows that more Pb metal has been absorbed and localized in the roots. The symbiosis between mycorrhiza and plant roots increases plant growth in conditions of land contaminated with Pb due to mycorrhiza's ability to increase the absorption of heavy metals and immobilize and store them in hyphae, thereby reducing the absorption of excess heavy metals directly into plant cells [12]. This can reduce plant stress levels towards the heavy metal Pb, increase the accumulation of Pb metal in the root zone, and inhibit the accumulation of Pb metal in stems and leaves.

The effectiveness of Pb absorption in the roots of hanjuang plants did not have a significant effect but increased with the addition of AMF doses. The initial Pb metal content in the soil was 101.42 ppm with a dose of 30 g polybag^{-1} of AMF, the absorption efficiency was 52.50%. Even though it did not have a real effect on increasing absorption compared to without AMF application, root growth with the application of

30 grams of AMF was better than other treatments. This is thought to be because mycorrhiza takes time to infect plant roots. Increasing the efficiency of Pb metal absorption is supported by plant growth rates, namely plant height, plant fresh weight, and root wet weight. So the length of time needed for plants to absorb Pb metal is not yet maximum because the height of plants using AMF is significantly different compared to without using AMF.

In research using other phytoremediation plants, such as sunflowers, the absorption of Pb metal by mycorrhiza was not effective [13], as was the case with sengon plants, but had a significant effect on the growth of sengon plants [14]. Mycorrhizae have a symbiotic relationship with plant roots to increase the ability of plants in polluted environments to remediate soil contaminated with heavy metals [15]. The phytostabilization process that occurs can be caused by the plant roots being directly in contact with soil contaminated with Pb metal. Pb metal is classified as the slowest metal to move to the top. Pb metal tends to be accumulated by roots rather than being transferred to the shoot because Pb is a non-essential metal [16].

The level of metal absorption by plant roots is different for each type of plant. Factors that influence plants in absorbing metals include exposure time, number of roots, plant weight, temperature, growing media, and plant age. As the age of the plant increases, the size of the nucleus also becomes larger so it can form phytochelatin to bind more metals which affects the number and size of the endoplasmic reticulum and Golgi apparatus which can speed up the phytochelatin reaching the cell surface. Apart from that, it also affects the size of mitochondria and chloroplast cell membranes which help bind metals that enter the plant system [17].

4. CONCLUSION

Based on the results of the research and discussion, it can be concluded as follows:

1. Application of AMF to soil contaminated with Pb increased the growth of hanjuang plants and had no significant effect on increasing the absorption of Pb metal in the roots of hanjuang plants within 8 weeks.
2. Application of AMF at a dose of 30 g polybag^{-1} is best in increasing the growth of hanjuang plants on soil contaminated with Pb metal.

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