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Potential of Two Vegetable Plants in Reducing Lead Contamination in Soil

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ABSTRACT

Phytoremediation is the technique of using green plants to remove toxic pollutants from heavy metal contaminated soil through degradation and detoxification mechanisms. Therefore, this research examines the potential of two types of vegetable crops, namely *Crassocephalum crepidioides* and *Amaranthus* sp., in reducing Pb contamination in polluted soil. The treatments tested were planting media in polybags dosed with 0.3 and 6 g/polybag of Pb 1 week before planting. The method used was a randomized block design, with each treatment being repeated three times. Furthermore, each treatment consists of three sample plants. The growth evaluation started 6 days after planting and was performed every 6 days. The evaluation was conducted on plant height, leaf area, leaf chlorophyll content, fresh and dry weight of roots and shoots, Pb levels in roots and shoots, and Translocation Factor (TF). The results showed that the higher the Pb in the media, the lower the rate of change in plant height, leaf area, biomass, and chlorophyll content. Additionally, *C. crepidioides* and *Amaranthussp*. absorb Pb in the soil with a phytoextraction mechanism, thereby remediating heavy metal contaminated soil, as indicated by the TF value > 1. In conclusion,soil remediation should be performed using *C. crepidioides*, considering that it is less popular as a vegetable in Indonesia.

Keywords: Amaranthussp., C. crepidioides, lead, phytoremediation, vegetable.

INTRODUCTION

Heavy metal pollution is increasing yearly, not only in Indonesia but in almost all countries of the world. Organic and inorganic sources of pollutants have been widely reported (Saxena and Bharagava, 2017). Heavy metals cause a decrease in crop quality and agricultural production (Lei et al., 2016). Furthermore, it poses a severe threat to human health by accumulating in the body through the food chain and respiration (Yadav, 2010; Maszenan et al., 2011; Dixit et al., 2015; Sarwar et al., 2017). Lead (Pb) is a heavy metal well-known as a pollutant because of its high presence and non-biodegradable nature.

Pb is a minor element and has no biological function in the body. However, it can cause serious health hazards and is listed as a significant pollutant by many environmental protection agencies worldwide (Jaishankar et al.,2014; Dixit et al.,2015; Sarwar et al.,2017). Therefore, removing Pb from contaminated soil is an urgent means of protecting the environment and human health. This is performed through phytoremediation, an effective, low-cost, and environmentally friendly solution for preventing and controlling Pb pollution (Muthusaravanan et al.,2018; Chandra et al.,2015; Ali et al.,2013; Mahar et al.,2016).

Phytoremediation uses green plants to remove toxic pollutants from the contaminated soil and water/waste through degradation and detoxification mechanisms (Bharagava et al., 2017; Ali et al., 2013). It is a non-intrusive and environmentally friendly technology to remove heavy metal pollutants from contaminated sites (Lee, 2013; Chandra et al., 2015; Chirakkara et al., 2016). Therefore, this research aims to prove the potential of hyperaccumulator plants such as *C. crepidioides* and *Amaranthus* sp. on a field scale to remove heavy metals from polluted soil. Amaranthus sp.is a vegetable plant commonly consumed by humans, while C. crepidioides is better known as a weed, especially for the people of Indonesia. Cadmium (Cd) has been the focus of the majority of research on C. crepidioides, while lead (Pb) has received less attention (Lestari, 2021; Cheng et al., 2021; Xie et al., 2021). Plants have different abilities to accumulate heavy metals (Kacalkova et al., 2015). Therefore, the selection of its species for heavy metal phytoremediation depends on the capacity and biomass of the selected plant (Rezania et al., 2016). The higher the biomass produced, the more accumulation of heavy metals in plant tissues. This research aimed to examine the potential of C. crepidioides and Amaranthus sp. in remediating Pb in soil. It is expected that C. crepidioides can absorb more Pb than Amaranthus sp. This is because it isnot yet popularly used as a vegetable in Indonesia, reducing the hazardous risk of heavy metals to human health.

METHODOLOGY

Time and place

The research was conducted from April to July 2021 at the Greenhouse of the Faculty of Agriculture, the University of Islam Malang, with an altitude of 540 meters above sea level and a temperature of $21-30^{\circ}$ C.

Materials

Seedlings of *C. crepidioides* (C) and *Amaranthus* sp. (A) were sown and transferred after two weeks into polybags containing a mixture of soil and compost in 1:1. Before planting, the Pb content in the media was analyzed to ensure that it was contaminant free. This heavy metal was added 1 week before planting at a dose of 0.3 and 6 g/polybag. Furthermore, it was mixed with the media and stirred evenly as well as each polybag was planted with 1 plant. ZA, TSP, and SP-36 fertilizers were administered twice, namely 2 g/ polybag ZA, TSP, and SP-36 at 7 DAT and 1 g/ polybag ZA, TSP, and SP-36 at 14 DAT.

Parameters measured

The growth evaluation started 6 days after planting and was performed every 6 days. This

was conducted on the variables such as plant height, leaf area, leaf chlorophyll content, fresh and dry weight of roots and shoots, Pb levels in roots and shoots (was determined using an Atomic Absorption Spectrophotometer), and Translocation Factor (TF). It is calculated as follows:

$$\frac{\text{Pb on crop}}{\text{Pb on root}} \tag{1}$$

Experimental design

This study used a randomized block design, whereby each treatment was repeated 3 times, with each consisting of 5 sample plants.

Statistical analysis

SPSS was used to conduct the statistical analysis. The data were reported as means with standard errors, and the means were compared statistically at the 0.05 percent level using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Plant growth

The common symptoms of Pb poisoning are stunted plant growth, root growth, blackening of the root system, and chlorosis of the leaves. This heavy metal can cause inhibition of photosynthesis and enzyme activity (Sharma and Dubey, 2005). Therefore, there were morphological differences between Pb-treated and control plants. Table 1 shows that C. crepidioides and Amaranthus sp. experienced increased plant height from the beginning to the end of the observation. However, it was lower at 30 DAT except for the control treatment. The higher the Pb dose in the soil, the lower the increase in plant height. Meanwhile, the leaf increase until 12 DAT. Table 2 shows that the increase in leaf area was not significant between treatment and harvest.

Biomass is an excellent indicator to characterize plant growth performance due to the presence of Pb. This research shows that plant growth rate and overall biomass production increase as Pb dose decreases. Similar results were observed in various plants (Brunet et al., 2008). Furthermore, the decrease in plant growth is related to inhibiting the mitotic index due to Pb treatment (Dalla et al., 2005). On the other hand, low Pb

Treatment	Average plant height (cm) at various ages of observation (DAT)					
	6	12	18	24	30	
C0	6.42 f	8.36 d	11.28 cd	19.24 e	24.18 c	
C3	5.77 e	7.13 bc	9.15 ab	13.52 bcd	14.99 ab	
C6	5.17 de	6.18 a	8.10 a	9.21 a	10.92 a	
A0	4.35 bc	8.32 d	13.04 d	21.84 e	27.28 c	
A3	4.42 c	7.25 c	11.95 d	16.87 de	19.92 bc	
A6	3.54 a	6.10 a	9.91 bc	13.62 cd	14.63 a	

Table 1. The average height of two vegetable plants due to Pb contamination in the soil

Table 2. Average leaf area (cm²) of two vegetable plants due to Pb contamination in soil

Treatment	Average leaf area (cm ²) at various ages of observation (DAT)					
	6	12	18*	24*	30*	
C0	8.21 d	23.21 d	33.98	44.40	48.09	
C3	7.85 cd	17.10 abcd	22.52	29.01	38.29	
C6	2.25 a	8.57 a	19.09	32.26	35.52	
A0	9.77 d	23.38 d	35.96	54.47	65.53	
A3	7.08 bcd	18.79 cd	28.29	54.21	62.81	
A6	5.35 abc	17.52 bcd	26.69	32.47	34.06	

Note: * means not significant.

concentrations regulate the absorption and transport of Fe, Zn, and N in plants to increase photosynthesis, increasing plant height and biomass (Soares et al., 2019; Carvalho et al., 2020). Therefore, *C. crepidioides and Amaranthus* sp. plants continuously increased plant height and leaf area at a dose of 6 g Pb/polybag.

Plant biomass

The highest fresh and dry weight of roots and shoots was discovered in the control treatment for *C. crepidioides* and *Amaranthus sp.* Figure 1 & 2 shows that the higher the Pb content in the soil, the lower the variable. *Amaranthus sp.* produced a significantly higher fresh and dry weight of roots and shoots than *C. crepidioides*. This indicates that the biomass formed by *Amaranthus* sp. was the highest. The Pb toxicity levels in plants depend on the species involved (Khan et al., 2018). Plants that produce higher biomass facilitate the phytoextraction process, hence, they have a higher absorption rate of heavy metals (Ho et al., 2008). The results are consistent with some other research which stated that on high Pb concentrations, root, shoot, and leaf growth, fresh and dry biomass was significantly reduced in *Pisum Sativum* (Kevresan et al., 2001) and *Zea mays* (Cimrin et al., 2007).

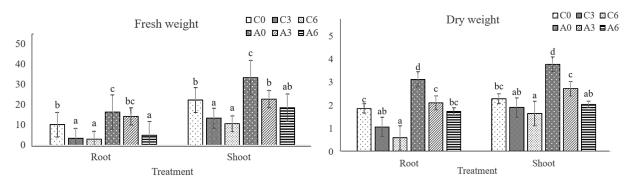


Figure 1. The fresh and dry weight of roots and shoots in Crassocephalum and Amaranthus

Chlorophyll content

Based on observations, the chlorophyll content decreased with increasing Pb dose in C. crepidioides and Amaranthus sp. (Figure 2). High Pb concentrations affect plant growth due to damage to chlorophyll synthesis (Xin et al., 2018). A decreased chlorophyll content due to Pb contamination has also been reported in many plants (Choudhury & Panda, 2004). Therefore, chlorophyll pigment appears to be one main symptom of Pb damage in plants. This heavy metal can indirectly cause stress by interfering with the electron transport of photosynthesis. The chlorophyll content decreases due to a striking change in the fine structure of chloroplasts and damage to the marginal membrane of meristematic cells (Fargasova, 2001). This is consistent with changes in the biomass of C. crepidioides and Amaranthus sp. because Pb inhibits photosynthesis, leading to a decrease in biomass. Saygideger et al. (2004) stated that the mechanism of inhibition of the chlorophyll biosynthesis process due to Pb is by blocking the action of enzymes needed. Changes in chlorophyll content due to increasing Pb concentration damage the chloroplast structure. Furthermore, the formation of this structure is strongly influenced by Mg and Fe. The excessive intake of Pb in plants reduces the absorption of Mg and Fe, causing changes in the volume and number of chloroplasts.

In many plant species, the photosynthetic pigments, including chlorophyll-a, b, and carotenoids, decrease after exposure to Pb (Singh et al., 2010). Furthermore, changes in photosynthetic activity and essential nutrient distribution reduce plant growth. This is attributed to changes in the composition of pigments in the photosynthesis process which have lower light-harvesting chlorophyll proteins (LHCPs) (Gill et al., 2012). Decreased levels of LHCPs are an adaptive defense mechanism of leaves and plants, which helps them to survive in adverse conditions. For example, photosynthesis in higher plants is more sensitive to Pb treatment, affecting the biosynthesis of chlorophyll and accessory pigments (Gill et al., 2012; Iqbal et al., 2010). Therefore, it is assumed that the heavy metal inhibits the biosynthesis of chlorophyll by interfering with the absorption of important photosynthetic pigment elements, such as magnesium, potassium, calcium, and ions (Piotrowska et al., 2009).

Pb uptake in roots and shoots

The results showed that the higher the dose of Pb in the soil, the higher the uptake in the roots and shoots. The highest Pb concentration was in Amaranthus roots treated with 6 g/polybag, which differed from other treatments (Figure 3). According to Gupta and Sinha (2008), plants actively have their mechanism to prevent movement from roots to shoots by sequestering metals in the roots, especially in the vacuoles or cell walls. Additionally, Yoon et al. (2006) stated that roots sometimes have a metal transport stop system to the leaves, hence there is accumulation in the roots. Therefore, Pb, one of the non-essential metals for plants, tends to be piled up by the roots rather than transferred to the canopy.

This is in line with the report by Li et al. (2016) that the cell wall plays an essential role in the detoxification and accumulation of Pb in the roots of T. sacchariflora seedlings. The cell wall is mainly composed of polysaccharides and proteins with carboxyl, hydroxyl, amino, and other functional groups, which provide massive binding sites with metal ions, limiting their transport across membranes, and decreasing their content in protoplasts (Zhang et al., 2014). Meanwhile, Pb can increase pectinase activity and low-twisted pectin content on the cell wall. Also, it induces the rearrangement of pectin in the cell wall chamber (Krzeslowska, 2011). Furthermore, root exudates, such as soluble sugar, free amino acids, and citric acid, combine with metal ions to form complexes or change the pH around the root system, encouraging the absorption and accumulation of heavy metals by cell walls (Zu et al., 2015).

Cenkci et al. (2010) also showed that Pb accumulation was higher in the roots than in the canopy of *Brassica Rapa*. The ability of *Amaranthus sp*.plants in accumulation and tolerance to Pb ions shows efficient hyperaccumulation mechanisms for removing the heavy metal ions from contaminated soil.

Translocation factor (TF)

This shows the efficiency of plants in the translocating metal accumulation from roots to canopy (Padmavathiamma and Li, 2007), which is a ratio between the concentration of Pb in the canopy and at the root.

Table 3 shows the value of TF*C.crepidioides* and *Amaranthus* > 1 in the control

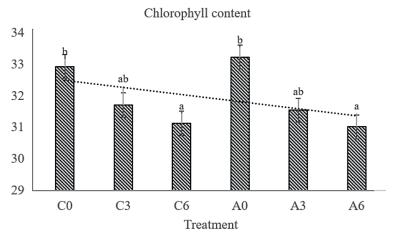


Figure 2. Chlorophyll concentration in C. crepidioides and Amaranthus

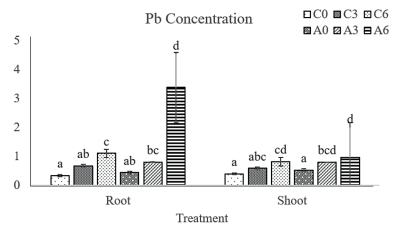


Figure 3. Pb concentration in the roots and shoots of C. crepidioidesand Amaranthus sp.

crassocepharum and amarantitus				
Treatment	The average TF value (ppm)			
CO	1.18 bc			
C3	0.87 ab			
C6	0.74 abc			
A0	1.20 c			
A3	0.99 abc			
A6	0.28 a			

 Table 3. The average value of translocation factor in crassocephalum and amaranthus

treatment and decreases with the increase in the dose of Pb. According to Yoon et al. (2006), only plant species with TF> 1 are used for phytoex-traction. This research shows that *C.Crepidioides* and *Amaranthus sp.* function as phytoextraction because their TF value is > 1 at 1.15 ppm in *C. crepidioides* and 1.34 ppm in *Amaranthus* sp. Phytoextraction, also known as Phytoaccumulation/

Phytoabsorption, is the absorption of contaminants from the soil or water by plant roots and translocated to as well as accumulated in biomass above the ground, canopy (Yoon et al., 2006). Metal translocation to the shoot is an essential and desirable biochemical process for effective phytoextraction since root biomass harvesting is not generally possible (Guerin et al., 2011).

CONCLUSIONS

C. crepidioides and *Amaranthus sp.*have a phytoextraction mechanism, hence, they can be used for heavy metal remediation, especially Pb. These plantsare classified as vegetables, however *Amaranthus* sp. is known to most people, while *C. crepidioides* is considered a weed in Indonesia, and a small number of people use it as vegetables. Therefore, *C. crepidioides* should be used to treat soil contaminated with Pb.

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