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Pattern of Lead Accumulation in Two Vegetable Plants Due to EDTA Treatment

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ABSTRACT

Phytoextraction and phytostabilization are the most consistent patterns or mechanisms of action of phytoremediation. One of the elements influencing the mechanism of action of heavy metal absorption by plant species is Ethylene Diamine Tetraacetic Acid (EDTA). Therefore, this study aimed to determine the pattern of phytoremediation in water spinach and spinach due to the addition of EDTA in the soil. The treatments tested by factor 1 were water spinach (T1) and spinach (T2), and factor 2 was the concentration of EDTA consisting of 3 levels, 0, 3, and 6 g/polybag. Each treatment was repeated three times on five sample plants. Furthermore, growth evaluation was carried out in the first six days after planting and conducted every 3 days. It was carried out on variables such as changes in plant height, leaves area, total root length, Pb content in the soil, fresh and dry weight of shoots and roots, shoot, seeds, and Translocation Factor (TF). The results showed that water spinach and spinach had different mechanisms of action due to the application of EDTA in Pb-contaminated media. Furthermore, water spinach and spinach have a mechanism of phytoextraction and phytostabilization, respectively. Therefore, spinach is safer than water spinach when grown in Pb-polluted land.

INTRODUCTION

Lead (Pb) is one of the numerous heavy metals that can easily contaminate agricultural soil. This contaminant can come from garbage, liquid waste, and other pollutants from agricultural activities, such as using fertilizers and pesticides. Heavy metal pollution remains a world problem (Sigua et al. 2005, Fu & Wang 2011) because of its effect on crop yields, biomass, and soil fertility, resulting in bioaccumulation of metals in the food chain (n.d.) (Rajkumar et al. 2009, Wong 2003 and Sivarajasekar & Baskar 2014). Therefore, heavy metal needs to be removed from the soil for agro-ecological sustainability and the carrying capacity of human health, Mittal et al. 2016, Alqadami et al. 2017, Bushra et al. 2017, Daneshvar et al. 2017, Kumar et al. 2017, and Naushad et al. 2017).

Various soil removal methods are expensive, labor intensive, and cause soil disturbance (Khan et al. 2004, Bhargava et al. 2012). Scientists are more interested in phytoremediation methods, which clean the soil of heavy metal pollutants using plants (Chaudhary et al. 2016). An example is phytoremediation technology, which has been a cheaper option until now. Its mechanism consists of several basic concepts, such as phytoextraction, phytovolatilization, phytodegradation, phytostabilization, rhizofiltration, and interaction with pollutant-degrading microorganisms (Marques et al. 2009, Glick 2003). Furthermore, it depends on several factors, including the accumulation of plants for various pollutants and concentrations.

Many studies demonstrated that water spinach and spinach absorb heavy metals from the soil. Therefore, they were classified as hyperaccumulators because they can accumulate potentially phytotoxic elements at 50 to 500 times higher than the average plants (Gupta et al. 2004, Yang et al. 2006, Reeves et al. 2018, Gratão et al. 2005).

The absorption of metal by plants can be increased by inducing a phytoextraction process using chelate compounds. The application of this compound to the soil can stimulate the availability and transfer of metal from the roots to the canopy. In the chelating mechanism, it is believed that plants absorb metal elements as metal-chelate complexes, which are more easily absorbed by roots and translocated to the canopy. A chelating compound commonly used to optimize phytoremediation is EDTA (*Ethylene Diamine Tetraacid*). This compound has a strong chelating ability for different heavy metals, specifically for Pb (Katoh et al. 2012, Zou et al. 2009, Finzgar et al. 2014, Hu et al. 2014, García et al. 2017).

Many studies also discussed various aspects of phytoremediation in water spinach and spinach. However, there is conflicting evidence regarding whether water spinach and spinach have the same phytoremediation mechanism and how the presence of EDTA in the soil influences the phytoremediation pattern.

MATERIALS AND METHODS

This study was conducted from September to December 2020 at the Greenhouse, Faculty of Agriculture, Islamic University of Malang, with an altitude of 540 meters above sea level and a temperature of 21-30^{o}C. The media used include soil and sand at a ratio of 4:1 and then a 10 kg polybag. The soil was analyzed to ensure that the Pb content in the ground was low. Afterward, Pb was added to each polybag at 350 mg.kg^{-1} soil (1.75 g.polybag). It was applied 1 week before planting and mixed with the prepared media until homogeneous. EDTA was then dissolved with 100 ml of distilled water, mixed with 1 kg of media, and combined with other substances. EDTA was applied 1 week before planting. Furthermore, 15-day-old Spinach and water spinach seedlings were planted in polybags containing Pb and EDTA.

This study adopted the randomized block design. The treatments tested in factor 1 were water spinach (T1) and spinach (T2), and factor 2 was the concentration of EDTA consisting of 3 levels, namely 0 (K1), 3 (K2), and 6 (K3) g/polybag. Each treatment was repeated three times with five sample plants. Growth evaluation was carried out every three days for the first six days after planting. Furthermore, the variable evaluated includes changes in plant height, leaves area, fresh and dry weight of shoots and roots, total root length, Pb levels in soil, shoots, roots, and Translocation Factor (TF).

RESULTS AND DISCUSSION

Plant Growth

Several abiotic stress factors severely limit plant growth, including heavy metal contamination. This study showed different growth responses on water spinach and spinach grown on Pb-contaminated media. Furthermore, water spinach plant media without EDTA tended to give the best plant height gain value at various ages of observation, as shown in Fig. 1. The average plant height decreased from 21 DAT, but the leaf area declined to 24 DAT. Water spinach planted on media supplemented with 6 g/polybag EDTA experienced the greatest decrease in plant height. Those planted on media supplemented with 6 g/polybag EDTA experienced the highest rate of reduction in plant height, accounting for 71%. Meanwhile, in spinach, the rate of decrease in plant height was not significantly different between all treatments.

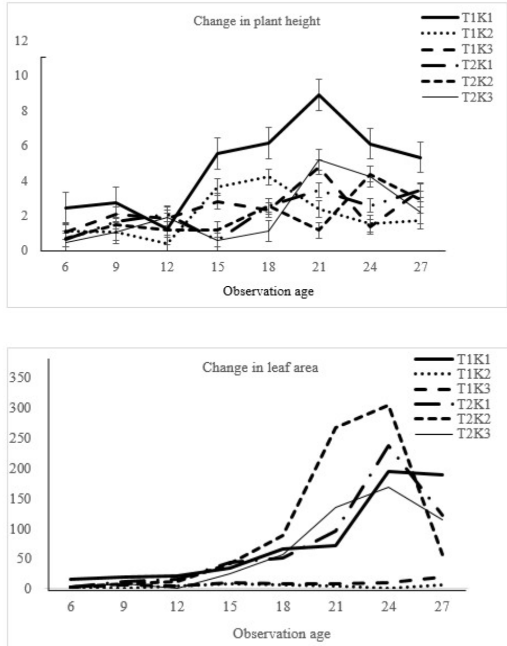

Fig. 1: Changes in plants' height and leaves area.

The highest decrease in leaf area occurred in spinach treated with EDTA at a 3 g/polybag dose. The rate of change in leaves area on water spinach was insignificant among all treatments. Furthermore, Pb is tightly bound to minerals and soil organic matter, making it difficult for plants to absorb through the roots. It is mainly absorbed through the roots. Hence, it will easily form complex bonds with nutrients, limiting plants' ability to translocate it to the canopy. Pb accumulator plants managed to translocate no more than 30% to the shoot. The application of EDTA facilitates this translocation from roots to shoots, accumulating as Pb-EDTA complex bonds resulting in a higher Pb. In addition, it induces physiological and morphological changes in certain concentrations, reducing plants' height and leaf area.

Water spinach and spinach were grown on Pb-contaminated media without EDTA and had the highest fresh and dry weight of roots and shoots, as shown in Fig. 2. The administration of EDTA at 3 and 6 g/polybag reduced the fresh and dry weight of roots and shoots. Furthermore, the reduction rate in the new weight of roots and shoots in water spinach was 89% and 93%, respectively, while in spinach, it was 49% and 25%. The rate of roots and shoots dry weight reduction in kale was 89% and 94%, respectively, while in spinach, it was 23% and 19%.

Water spinach and spinach productivity is related to the mass content of the plant, both fresh and dry weight. According to Fattahi et al. (2019), plants dry weight decreased in response to increasing lead concentrations at 0,

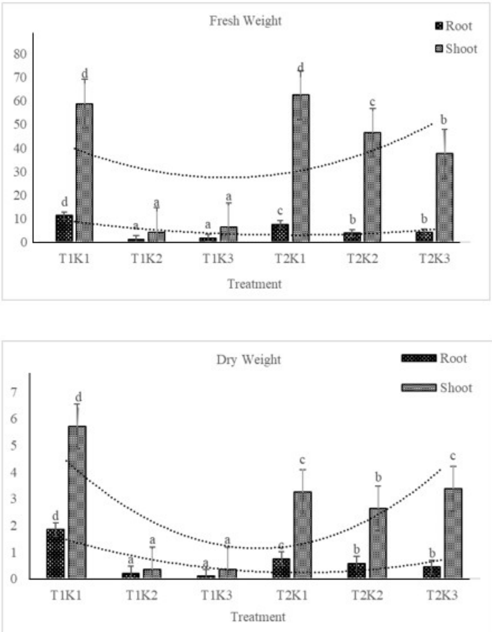

Fig. 2: Fresh weight and dry weight of plants in various treatments.

100, 200, and 400 mg.kg^{-1} soil. Therefore, it is concluded that physiological reactions related to the primary metabolism of plants, which are responsible for growth and development, are affected by Pb stress.

The roots length of water spinach decreased to 77% due to the administration of EDTA at various doses in the media, while spinach had no significant changes, as shown in Fig. 3. The roots are the first organs in plants that are in contact with the different rhizosphere environments. They are one of the most important plant organs to absorb nutrients and heavy metals (Li et al. 2009). The roots also play an important role in accumulating heavy metals in plants' organs. In line with the results of (Xia et al. 2016), the correlation between root surface area, volume, and heavy metal concentrations in mustard shoots was statistically significant.

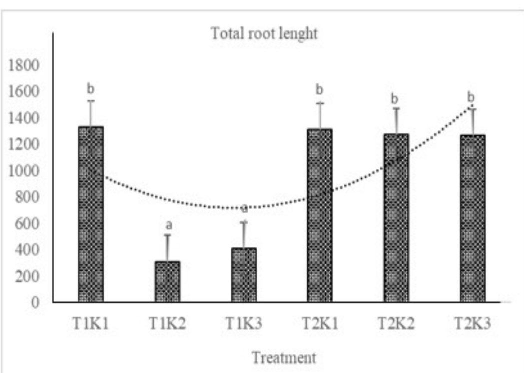

Fig. 3: Total root length.

Some commonly used growth performance indicators for plants exposed to heavy metals such as Pb include total plant biomass, growth rate, root, and shoot length. In addition, the presence of Pb in the environment can act as a stress-triggering factor that induces physiological changes or total plant growth inhibition. Every plant is different in its ability to accumulate heavy metals. Therefore, it determines the mechanism of action or pattern of accumulation.

Phytoextraction is the process where plants absorb pollutants from water or soil through the roots, which are then stored in the plant's canopy (Jacob et al. 2018, Yoon et al. 2006). Translocation of metals to shoots is an important and desirable biochemical activity, but it is ineffective for phytoextraction because harvesting root biomass is generally impossible (Tangahu et al. 2011).

Phytostabilization uses metal-tolerant plant species to immobilize heavy metals underground and reduce their bioavailability. This prevents their migration into the ecosystem and reduces the possibility of metals entering the food chain (Wong 2003, Marques et al. 2009). Furthermore, phytostabilization occurs through the deposition of heavy metal or reduction of valence in the rhizosphere, absorption, and sequestration in root tissues or onto the cell walls (Ginn et al. 2008, Kumpiene et al. 2012, Gerhardt et al. 2017).

EDTA is one of the most potent chelating agents to artificially increase the solubility, absorption, and complexation of metals, including Pb ions, in soil (Guo et al. 2014, Najeeb et al. 2017). Its application produces a synergistic effect in Pb uptake and increases roots' uptake and translocation. This study showed that water spinach and spinach decreased the plant's height, leaves area, fresh and dry weight of both shoots and roots, as well as total root length. This is due to the application of EDTA, which increases Pb absorption by plants, thereby causing stress.

Pb Content in Soil and Plant

One of the key factors for the success of phytoremediation in metal-contaminated soils is the high accumulation of metal in plants and its availability in the soil. However, the increased tolerance of the selected plants can ensure normal growth. To compensate for the ineffectiveness of metal in the soil, organic chelating chemicals such as EDTA can be used to increase metal uptake and delivery to plant parts (Seth et al. 2011, Guo et al. 2014, Shahid et al. 2014).

Fig. 4 shows a significant difference in the concentration of Pb in the soil after water spinach, and spinach were harvested at 28 days. The media used for water spinach contains Pb concentrations, which decrease with the addition of EDTA. The higher the dose of EDTA, the less Pb is left in the soil. On the other hand, the administration of EDTA at

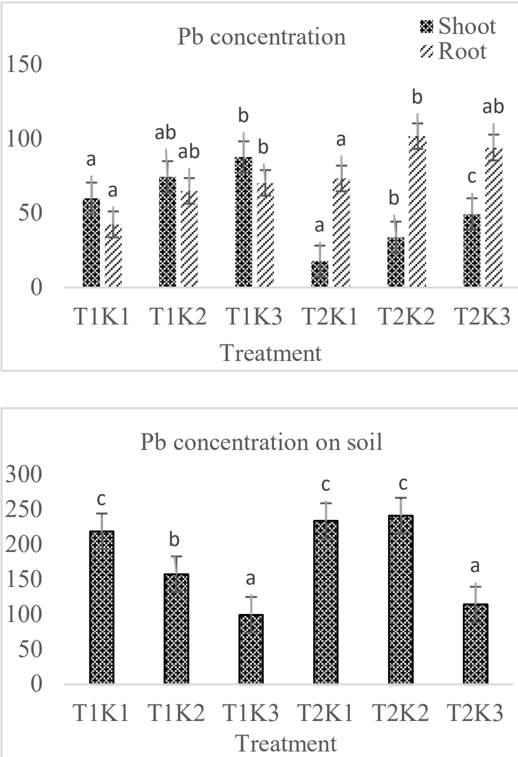

Fig. 4: Pb content in soil, roots, and shoot.

a dose of 3 g/polybag in spinach did not show a significant change in the concentration of soil Pb. At a quantity of 6 g/polybag, EDTA can reduce the concentration of soil Pb. This shows that the application of EDTA in Pb-polluted soil can increase the absorption of Pb by plants. Hence, only a small amount is left.

Fig. 4 shows that the administration of EDTA significantly affected the total Pb of the plant's roots and shoots. In water spinach, the concentration of Pb at the shoot and root is 125.3 and 111.32 ppm, respectively. Similarly, spinach has values of 61.57 ppm and 161.08 ppm. Therefore, it can be concluded that water spinach accumulates Pb in the shoots, while spinach is in the roots. The difference in Pb concentration indicated that both plants had different Pb absorption mechanisms.

Pb stress has direct and indirect effects on plant development, metabolism, and several physiological processes (Hasanuzzaman et al. 2011). Furthermore, plant biomass and height are the best indices to evaluate response to Pb toxicity (Zulfiqar et al. 2019). Reduction of biomass results in lower nutrient absorption, changes in hormonal status and cell membrane permeability, impaired respiration, inhibition of cell division at root tips, and decreased photosynthetic activity. These negative effects of EDTA are due to the high mobility of metal in soil solution (Epelde et al. 2008). EDTA also dissolves Pb attached to soil particles (Katoh et al. 2012) and increases Pb transfer to roots by diffusion or mass flow. Israr & Sahi (2008) reported a significant reduction in the growth of *Sesbania drummondii* with EDTA. According to (Barrutia et al. 2011), the application of lower doses of EDTA to avoid a considerable decrease in biomass effectively increased the phytoremediation tolerance of water spinach and spinach.

Translocation Factor (TF)

TF shows the efficiency of plants in translocating accumulated metals from underground parts, such as roots and rhizomes, to stems and leaves (Bonanno & Vymazal 2017). The TF value generally characterizes the ability of phytoremediation (Srivastava et al. 2006, Yoon et al. 2006, Usman & Mohamed 2009). Furthermore, plants with a TF value greater than 1 are classified as highly efficient for metal translocation from roots to shoots. In this study, the highest TF value was found in water spinach grown on media with EDTA 6 g/polybag (1.25) and was significantly different from the control treatment (1.05). This value increased in response to the addition of EDTA. TF value of spinach was not significantly different between the treatment and control groups, as shown in Fig. 5 (Chunilall et al. 2005) stated that the accumulation of metals varies greatly among plant species. Element uptake depends on plant species and the quality of growth.

The low level of translocation from roots to shoots indicates that spinach is a good candidate for Pb phytostabilization. Meanwhile, EDTA stimulated Pb translocation from the soil to the roots (Wang et al. 2007), as indicated by TF = 0.32 <1 for the treatment without EDTA at a dose of 3 g/polybag. The analysis of Pb concentrations in plants showed that spinach stored higher Pb in the roots. The EDTA treatment effectively increased the Pb content in spinach roots. These results are consistent with (Tammam et al. 2021) on *Glebionis coronaria* L.

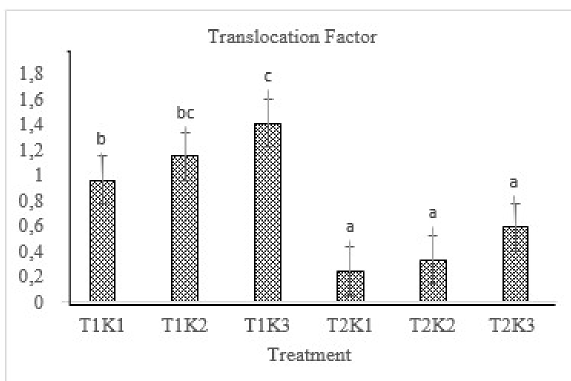

Fig. 5: Translocation factor.

TF value > 1 indicates that the plants translocate the absorbed contaminants to their upper organs. Furthermore, TF is defined by the ratio of the contaminant number in the upper organs of the plants to the roots (Maldonado-Magaña et al. 2011, Branzini & Zubillaga 2013). The results of this study indicated that water spinach had a TF > 1, which is consistent with the analysis of Pb concentrations in the shoots and roots, which is higher in the canopy of kale. This is thought to be due to the role of EDTA in increasing the absorption of Pb ions by plant roots and increasing their transport, which may be due to the formation and action of metal-EDTA complexes (Doncheva et al. 2013, García et al. 2017).

CONCLUSIONS

The results indicated that water spinach and spinach are potential phytoremediators in Pb-contaminated soil due to their strong tolerance and ability to accumulate Pb. However, these plants have different working mechanisms due to the application of EDTA. The concentration of Pb absorbed by the roots increased significantly for water spinach and accumulated in the canopy. Therefore, it possesses a phytoextraction mechanism of action. In spinach, the concentration of Pb is higher in the roots, indicating that it has a phytostabilizing mechanism. Therefore, when grown on Pb-polluted land, spinach is safer than water spinach.

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