Utilization of plants colonized by mycorrhizal fungi to reduce Lead in the soil: Greenhouse study

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Abstract. Utilization of plants and soil microbes in rejuvenating heavy metal-polluted soil is one of interesting topic and environmental-friendly technology. The study aimed to evaluate the role of arbuscular mycorrhiza (Glomus intraradices) in promoting growth of Vetiver zizanioides and Cynodon dactylon under various concentration of Lead (0, 100, 400 and 800 mg.kg⁻¹). All fungal cultures were grown in greenhouse for ten weeks. Several plant growth promoting parameters and Lead content were examined. Tested plant s were able to grow under all lead concentrations while Vetiver zizanioides showed the highest growth than C. dactylon. Lead content decreased in all treatments while Vetiver zizanioides also showed the highest accumulation of lead than C. dactylon. The results showed that inoculation of mycorrhiza were able to lower negative impacts of lead to plants with varying degree.

1. Introduction

Lead (Pb) is one of heavy metals polluting the soil with negative impact to human and organismal health. Various kinds of anthropogenic activities such as mining, metal smelting, electroplating, exhaust gas, energy and fuel production, waste, agricultural fertilization and use of pesticides produce pollutants, including leads. Agricultural fertilizers are categorized as one of the sources of pollutants since the content of particular elements and compounds is harmful to the environment. For example phosphate fertilizer contains Pb between 7–225 ppm. Lead is a low-insoluble metal, with a low translocation power from the roots to other plant organs [1].

Rejuvenation and remediation are needed to restore the contaminated land. Mitigation of heavy metals is still based on physico-chemical methods which require expensive instruments and monitoring systems. Therefore, it is necessary to apply a more applicative method which are easy, inexpensive and effective with regards of environmental-friendly. Phytoremediation is a method which utilize plants whether to remove, stabilize or even remediate pollutants in the form of organic or inorganic compounds. The basis of phytoremediation is to screen certain species with the highest capability in removing pollutants.[2].

Many plant families are categorized as hyper-accumulators ranging from ornamental plants, grasses, shrubs to tree species. Particular characteristics for phytoremediative plants are fast growers, high biomass and intensive root systems. Ornamental plants are proved to be an applicative group of phytoremediators. One species known as lead-accumulating plant is Cordyline fruicoisa from ornamental group while from grass group, Vetiver zizanioides L. Nansh. The species Vetiver zizanioides L. Nansh is a plant species commonly used in phytoremediation of heavy metal-contaminated areas [3].
The phytoremediation of heavy metal-contaminated soil can be accelerated by inoculation of arbuscular mycorrhizal fungi (AMF) because mycorrhiza may provide a protection to host plants from the absorption of toxic elements through feature of filtration, complexation and accumulation. Mycorrhiza can also act as biocontrol of heavy metals absorption which help plants to avoid heavy metal poisoning such as from As, Cr and Pb poisoning [4,5]. Through mycorrhizal symbiosis, plants are also resistant to extreme drought and hypoxia. One of mycorrhizal genus, Glomus associated with plants has proven as an effective agent in absorbing heavy metals, namely Cd, Zn, and Pb [5-7]. In this study Glomus intraradices is used as inoculant to V.zizanioides in proving the enhanced lead accumulation by the plant. In addition, selected species was used based on its high adaptability and fast reproduction.

2. Materials and methods

2.1. Soil preparation
The soil used as a planting medium is sieved (4 mm) and sterilized 1 hr with steam (100 °C) for 3 (three) sequential days to eliminate natural AMF propagules. Lead (Pb) treatment is prepared in the form of lead-nitrate solution [Pb(NO$_3$)$_2$] with a concentration of 100, 400 and 800 mg Pb/kg soil. Control soils were prepared without any lead-nitrate solution. Soils are mixed with treatment solution followed by watering with bidistilled water until reaching field capacity. The soils are arranged and plotted into plastic bags for 15 days and regularly stirred every 3 days to improve uniformity of soil conditions.

2.2. Arbuscular mycorrhizal fungi (AMF) culture
Species of AMF used in this study is Glomus intraradices. The AMF inoculum used was a mixture of zeolites, spores and colonized root pieces of 20 g/kg of soil to obtain a high level of root colonization. Inoculation of AMF was performed once the plants are treated with lead contamination in culture pots.

2.3. Experimental design
The study used completely randomized factorial design. Treatments consisted of two plant species (V.zizanioides and C.dactylon) with four different concentrations of lead-nitrate solutions with five replicates. Plants and cultures are maintained for 8 (eight) weeks.

2.4. Data analysis
Parameters observed in this study are: percentage of root colonization [8,9], dry weight of shoots and monitoring of lead concentration in soils using atomic absorption spectroscopy (AAS). Analysis of variance (ANOVA) was used to test significances among treatments followed with Duncan’s multiple range test (DMRT) at 5% of significance level for each concentrations applied.

3. Results and discussions

3.1. Mycorrhizal colonization of roots
The percentage of G.intraradices colonization in the roots of V.zizanioides and C.dactylon at various lead concentrations is presented in Figure 1. In general, the percentage of mycorrhizal colonization in both plants increased with increasing Pb concentration for up to 400 mg/kg and decreased at concentration of 800 mg/kg. In V. zizanioides the percentage of colonization increased from 39.6 to 61.3% at 400 mg/kg of Pb but declined to 28.5% at 800 mg/kg Pb. In C. dactylon the percentage of colonization increased from 31.8 to 56.4% at 400 mg/kg Pb and declined to 19.6% at 800 mg/kg Pb. Based on these results, there is a difference in the degree of G. Intraradices colonization in both plant species at the same lead concentration. Colonization of V.zizanioides is higher than that of C.dactylon. Our result is supported by previous work which explained the different characteristics of AMF.
towards plant hosts [10]. Another explanation is that certain species of AMFs are attracted to certain signal produced by plant roots through exudates signaling system [11].

Figure 1. Root colonization of mycorrhizal plants (V. zizanioides and C. dactylon) under different lead concentration. (Different letter above the bars indicate significant different at p<0.05 according to DMRT)

Furthermore, the rate of G.intraradices colonization was not significantly different for both plants in each Pb concentration except, at 400 mg/kg Pb, where the percentage of colonization in V. zizanioides was significantly different from C.dactylon. However, G intraradices still thrived at low Pb concentrations in the soil. These results indicated that Pb concentrations of up to 400 mg/kg are not considered as limiting factor to mycorrhizal fungi, G.intraradices. The result is supported by previous study which reported that sensitivity of AMF towards heavy-metal contamination may alter the growth of AMFs through reduced spore germination, delayed hyphal elongation and decreased root colonization [12,13].

3.2. Dry weight of shoots
The growth response in terms of dry weight of shoots of V.zizanioides and C.dactylon at various Pb concentrations is presented in Figure 2. In general, the dry weight decreased with increasing Pb concentrations. Decrease in dry weight of C.dactylon is greater than that of V.zizanioides. In V.zizanioides, a decrease in dry weight is obtained from 17.2 to 14.7 g at concentration of 800 mg/kg, while C.dactylon decreased from 15.1 to 10.6 g. At concentration of Pb 100 mg/kg Pb, the dry weight of V.zizanioides shoots was not significantly different from C.dactylon. The increase in Pb concentration at 400 and 800 mg/kg produced a greater dry weight of V.zizanioides shoots and was significantly different from C.dactylon.

The difference in shoots dry weight of V.zizanioides and C.dactylon is related to the ability of two plants to grow in unfavorable conditions. In general, lead contamination in soils affected the dry weight of shoots for both plants although V.zizanioides is seemed to be more tolerant. Other study reported that V.zizanioides has a good tolerance towards certain environmental stressors [14]. In addition, the massive and deep root system of V.zizanioides provides advantages in better absorption of nutrients to prolong the growth of plants under stress conditions [15].

3.3. Lead (Pb) concentration in soil
The analysis of Pb content in the shoots and roots of both species is presented in Table 1. The increase of Pb concentration was followed by accumulation of Pb in both plants. In general, the Pb content in the shoots and roots between *V. zizanioides* and *C. dactylon* is relatively similar at Pb concentration of 400 mg/kg in soil. Different result is observed in the Pb concentration of 800 mg/kg in which Pb content is higher in *V. zizanioides* and significantly different than *C. dactylon*.

The Pb content in both plants is higher in root tissue than shoots. Following the general result, the Pb content in the root of *V. zizanioides* is higher than that of *C. dactylon*. This difference might illustrate the difference in the ability between two species in reducing Pb contamination in soil. These results may indicate that Pb content in shoots are also regulated by mycorrhizal fungi. Existence of high Pb concentrations in both plants may be supported by the mechanisms offered by inoculated AMFs leading to a more efficient translocation of nutrients by fungal hyphae [16].

![Figure 2. Shoot dry weights of mycorrhizal plants (*V. zizanioides* and *C. dactylon*) under different lead concentration. (Different letter above the bars indicate significant different at p<0.05 according to DMRT)](image)

| Lead treatments (mg/kg) | Species          | Lead (Pb) content | Shoot | Root   |
|-------------------------|------------------|-------------------|-------|--------|
| 0                       | *V. zizanioides* | 2.21 a            | 32.13 a |
|                         | *C. dactylon*    | 4.43 a            | 25.18 a |
| 100                     | *V. zizanioides* | 4.95 a            | 76.28 a |
|                         | *C. dactylon*    | 6.28 a            | 67.52 a |
| 400                     | *V. zizanioides* | 10.12 a           | 81.24 a |
|                         | *C. dactylon*    | 9.85 a            | 58.24 b |
| 800                     | *V. zizanioides* | 32.14 a           | 193.26 a |
|                         | *C. dactylon*    | 16.94 b           | 126.02 b |
Mycorrhizal symbiosis within plants can increase plant tolerance to heavy metals, including Pb, by increasing the nutritional status of plants and changing the chemical properties of heavy metals [17]. However, the exact role of AM fungus in the accumulation and distribution of Pb in plants is still not described. By looking from table 1 result, it was found that the Pb content of plants is abundant in the root tissue to protect the canopy from damage [12,18]. Furthermore, AM fungus prevents the transport of Pb to the plant canopy by localizing Pb inside the hyphal cell wall, hyphal membrane, vesicular membrane and vacuole [19].

Lead (Pb) content in plant roots of V. zizanioides is higher than that of C. dactylon. It is thought that this is related to the difference in root systems between two species, where V. zizanioides has more and deeper roots than C.dactylon [14,21]. This condition caused V. zizanioides to be able to accumulate Pb more than C.dactylon. These results indicate that under the same Pb concentration, V.zizanioides is capable of binding Pb more than C. dactylon. Thus efforts to reduce the concentration of Pb in the soil will be more effective by using V. zizanioides inoculated with AMF.

4. Conclusion

Based on the results, it can be concluded that inoculation of AM fungus can protect plants against the negative effects of high Pb concentration in the soil, despite a decrease in colonization G. intraradices in roots. Utilization of V. zizanioides inoculated with AMF gave better results than C. dactylon in terms of Pb reduction in the soil. The accumulation of Pb is more in the root tissue than the shoot which allows metabolic processes to occur normally to sustain the plant growth under lead contamination in soils.

Acknowledgements

The authors would like to express the highest gratitude to the Institution of Research of Universitas Sumatera Utara for financial aids and support in the form of TALENTA Research Program with contract number : 2590/UN5.1.R/RPM/2018

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