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The Indigenous Arbuscular Mycorrhizal Fungi Consortium in Shallot Cultivation with Lead-Polluted Media

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ABSTRACT

This study aims to test a consortium of indigenous Arbuscular Mycorrhizal Fungi (AMF) in shallot cultivation with Pb contaminated media. The experiment was carried out in a greenhouse. A completely randomized design (CRD) was used in this study with one treatment factor. The treatment was the customary consortium of indigenous AMF from 17 locations of shallot land contaminated by Pb. The application of AMF showed no significant effect on fresh bulb fresh weight, leaf fresh weight, plant fresh weight, the total length of leaves, and the number of shallots. The percentage of infection in all treatments, except controls, showed a high percentage of AMF infection. AMF treatment from Slatri Land II showed the highest percentage of infection, that is 93.33%. The consortium of indigenous AMF from Kupu Land II was able to reduce the highest Pb accumulation of tubers compared to the control by up to 83.660%.

INTRODUCTION

One of the issues with shallot production is the use of too many pesticides and fertilizers, which results in pollution, a clogged drainage system, and unsupportive agroecological conditions, especially on sensitive and dry soil (Nendissa 2008). Previous research in the Tegal and Brebes areas has revealed that the heavy metal Pb level in the soil and shallots is relatively high, with the Pb content in the soil ranging from 12.33-19.74 ppm and the range in shallots ranging from 0.41-5.51 ppm (Badrudin & Jazilah 2013).

Heavy metals must be handled properly because they are harmful contaminants for living beings. One of the ways to deal with the toxicity of heavy metals in contaminated land and plants is bioremediation. An effective step to reduce the contamination is by making use of microorganisms to restore the land conditions (Gandjar et al. 2006). Heavy metals are also a pollution problem for the soil, and the use of organisms to overcome them is called bioremediation (Leyval et al. 2002).

Rejuvenation of the polluted land can improve its functions, nourish plants, and will have a positive impact on people's health. Bioremediation using AMF is considered as a solution to this problem. Mycorrhizal potential can increase phytoremediation in a polluted land. The AMF can absorb heavy metals in it, reducing toxicity and allowing plants to thrive much better (Gaur & Adholeya 2004). Fungi connected with the roots of live plants in the soil are known as AMF. AMF-inoculated roots were found to be effective at absorbing nutrients and water. In a root area in the soi, fungal hyphae threads have a wider range of nutrient use.

Bai et al. (2008) stated that AMF has an effect on metal uptake (movement and collection in plants) and host development. According to Sudová et al. (2007), the occurrence of excessive metal contamination in inoculation corn seedlings is a problem. As a result, plant growth is accelerated, and metals are delivered to the plant's roots and crown.

The issue is how successful the Fungi Mycorrhizal Arbuscula indigenous onion planting soil contaminated with Pb on media contaminated with Pb consortium is. It was also attempted to observe the influence on tuber growth and yield, as well as tuber Pb buildup.

MATERIALS AND METHODS

Materials and Tools

The materials and tools used in this study include the soils-contaminated with heavy metal Pb (24.75 ppm) from Larangan Village, Larangan District, Brebes Regency, indigenous AMF from 17 locations in Wanasari and Larangan District, the shallot seeds of Tuk Tuk variety, rockwool, UV plastic, fertilizer (SP36, KCL, urea and rice washing water), cheerful, hoes, shovels, drums, gas stoves, plastic trays, sprayers, 11 cm × 12 cm pots, trays, rulers and cameras.

Research Methods

For the treatment of indigenous AMF from 17 shallot areas affected with heavy metals, this study used a completely randomized design (CRD) with one component. They are: L0 = Control (without AMF treatment); L1 = Indigenous AMF, Wanasari Land I; L2 = Indigenous AMF, Wanasari Land II; L3 = Indigenous AMF, Wanasari Land III; L4 = Indigenous AMF, Sigentung Land I; L5 = Indigenous AMF, Sigentung Land II; L7 = Indigenous AMF, Kupu Land I; L8 = Indigenous AMF, Kupu Land II; L9 = Indigenous AMF, Kupu Land III; L10 = Indigenous AMF, Karangbale Land I; L11 = Indigenous AMF, Karangbale Land II; L12 = Indigenous AMF, Karangbale Land III; L13 = Indigenous AMF, Slatri Land I; L14 = Indigenous AMF, Slatri Land II; L15 = Indigenous AMF, Slatri Land III

L16 = Indigenous AMF, Larangan Land I; L17 = Indigenous AMF, Larangan Land II; L6 = Indigenous AMF, Larangan Land III. Each treatment was repeated 3 times, then 54 experimental units were obtained.

Observation Variables

The observation variables in this study are total leaf-length (cm), number of leaves (strands), number of tubers (piece) per plant, fresh tuber weight (gram) per plant, total root length (cm), analysis of Pb content in roots, tubers and leaves of the shallot (ppm) using Atomic Absorption Spectrophotometer (AAS), root infection intensity (%). The root infection intensity was measured by looking at the percentage of roots infected by AMF. It was carried out through staining techniques using the ink-vinegar solution. The formula for calculating the percentage of infected roots is (Nusantara et al. 2012) :

$$\%\ \text{Colonized roots} = \frac{\sum \text{mycorrhizal field of view}}{\sum \text{the observed field of view}} \times 100\ \%$$

The intensity of infection was classified according to the criteria of O'Connor et al. (2001).

Research Implementation

The steps in implementing this research are as follows:

The planting medium was soil contaminated with heavy metal Pb (24.75 ppm), taken from Larangan District, Brebes Regency. The soil was sterilized using a steam pressurized drum at a temperature of 80^{o}C for 8 h. It was stirred manually using a hoe. It was then placed in a pot measuring 11 cm × 12 cm with a volume fitting its size. It was coded, based on the respected treatment.

The seeds used are the Tuk-Tuk variety. Prepare a tray filled with soaked rockwool. The shallot seeds are put into the rockwool that has been perforated by two seeds in each hole. Put in a dark place for 2 days and after germination moves them into the sunlight. After 14 days, the seedlings are transferred to polybags with sterilized soil as the planting medium. Seedlings were moved after 40 days and have 3-5 leaves to pots with Pb-contaminated soil as the planting medium.

The AMF application was carried out directly during planting by placing it in a planting hole with 150 g per plant and then covered with thin soil.

Fertilization was done three times, that is before planting, using SP-36, N, and KCl. This initial fertilization dosage is SP-36 (250 kg.ha^{-1} or the equivalent of 4.80 g.pot^{-1}). It was applied three days before planting by spreading and stirring it evenly with the sterilized planting media. The second fertilization was carried out at 15 DAP; the fertilizers given were KCl and N with a dose of 200 kg.ha^{-1} or equivalent to 3.84 g.pot^{-1}. The third fertilization uses the same fertilizer, Urea, and KCl at 30 DAP with a dose of 100 kg.ha^{-1} or the equivalent of 1.92 g.pot^{-1}. Harvesting was done at 60 DAP.

Data Analysis

Hypothesis testing is carried out using the F test. The results of the F test show the real effect of the treatment carried out, so the Least Significant Difference Test (LSD) can be performed with an error rate of 5%. Previously, all observations were collected then organized and prerequisite tests were carried out (normality test and homogeneity test of variance) to fulfill the assumptions in the parametric statistical analysis. Because these two assumptions were not fulfilled, the Kruskal-Wallis non-parametric test was performed.

RESULTS AND DISCUSSION

The AMF Effects on Shallot Plants

The results showed that AMF did not significantly affect the number and total length of plant leaves (Table 2). The number and total length of the leaves are influenced by the availability of N in the planting medium. Based on the analysis of the soil as the planting medium, the total N content was 0.12% and it was in a low category (Table 1). In this study, N fertilization using urea was also carried out according to the dosage required for the shallot cultivation. Nitrogen elements belong to soluble nutrients for plants, so that they can be absorbed directly by their roots. The limited reliance on mycorrhiza is assumed to be due to the availability of sufficient N in the soil, which is a crucial factor for the formation of leaf organs.

According to Setiadi & Setiawan (2011), not all types of arbuscular mycorrhizae can significantly affect the growth of a plant. This effect can be determined by the effectiveness of the nutrient status of the media, isolates, and the level of dependence of plants on mycorrhizae. Wang et al. (2011) research also shows that co-inoculation with rhizobia and AM fungi significantly increased soybean growth under low P and/or low N conditions as indicated by increased shoot dry weight, along with plant N and P content. There were no significant effects of inoculation under adequate N and P conditions. Likewise, as stated by Saputra et al. (2015), environmental factors such as soil moisture, soil pH, rainfall, C-organic content, and NPK nutrient content affect mycorrhizal activity.

Mycorrhizal fungi are part of obligate fungi that combine to live with plant roots through spores. AMF, among other things, helps to increase the plant's nutrient uptake of P elements, can increase resistance to disease, drought, and other hazardous conditions. Based on the research finding of Moelyohadi et al. (2012), AMF can be used as a technology for fertilizing and plant-nutrients fertilizing and is also able to increase the corn yield.

The AMF treatment had no significant influence on plant root length, according to the results of the variance analysis, possibly due to environmental conditions that promote shallot growth. They include, among other things, sunlight and nutrient-dense foods.

Because it can improve root conditions, AMF treatment has a considerable impact on the percentage of root infections in shallots in this circumstance. It can also improve the ability to adjust to drought concerns relating to root growth, water absorption, a

This is due to the availability of nutrients and water in the planting medium. The fertilizers given to all shallot plants are SP36, KCL and Urea. According to Bangun et al. (2000), fertilization is a critical component of increasing crop yields. Furthermore, the shallot plants require favorable environmental circumstances to assist the tuber production process, one of which is water.

The results of the variance analysis indicated that mycorrhizal administration has a very significant effect on the intensity of plant root infections. The indigenous AMFs of the shallot land contaminated with Pb from 17 locations are a consortium of the genera of *Glomus, Gigaspora, Acaulospora,* and *Scutellospora* (Hajoeningtijas et al. 2019). According to Tamin et al. (2012), many factors contribute to the prevalence of mycorrhizal infections. Mycorrhizae of *Gigaspora margarita* type is a type having the ability to adapt to the surrounding habitat. *Gigaspora margarita, Acaulospora sp,* and *Glomus etunicatum* have various environmental conditions to survive. These three types of mycorrhizae are a type of *endomycorrhizae*. Many types of *endomycorrhizae* are found in nature. They can be found in most soils and do not have a specific host. The level of population and species composition varies due to plant characteristics and environmental factors such as temperature, soil pH, soil moisture, and phosphorus and nitrogen contents.

Table 1: The analysis results of soil and Pb in Larangan Village. Larangan District. Brebes Regency.

Parameter	Value		Criteria
Total N [%]	0.12	0.1-0.2 R	L
Organis C [%]	0.78	<1 SR	VL
C/N	6.31	5-10	L
Total P_2O_5 [%]	0.05	<15	VL
Total K_2O [%]	0.11	<10	VL
pH H_2O	6.60	-	-
KTK [me%]	45.45	>40	VH
KB [me%]	78.86	61-80	H
Ca [me%]	23.35	>20	VH
Mg [me%]	6.65	2.1-8.0	H
Na [me%]	5.00	0.4-0.7	M
K [me%]	0.87	0.6-0.10	H
Soil Pb tanah [ppm]	24.75*		
Texture	Clay		

Note : *= threshold value of 12.75 ppm (Balai Penelitian Tanah 2002)
VL = Very Low; L = Low; M = Medium; H = Height; VH = Very High

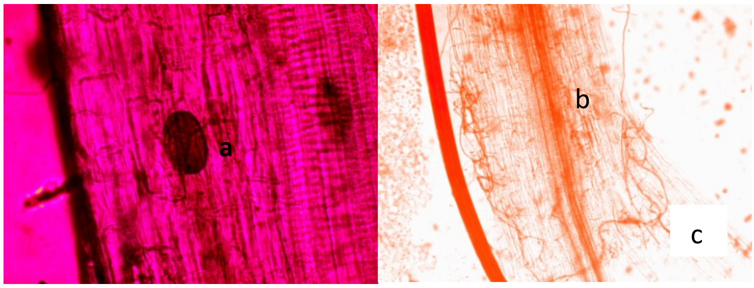

Fig. 1: The shallot roots infected by AMF show the presence of vesicles (a) (40/0.65), internal hyphae (b) and external hyphae (c) (5/0.10).

AMF will infect plant roots, thereby expanding the absorption area for nutrients and water. As stated by Prasasti et al. (2013), the higher the dose of AMF fertilizer, the more roots were infected with mycorrhizae, thus making the roots longer.

The workings of mycorrhizal infections, according to Novriani and Madjid (2009), occur when the infection is in the canal or root tissue, beginning with the spore germination process in the soil. There will be penetration of hyphae development and development in the cortex in the roots. The arbuscules, intracellular vesicles (Fig. 1 (a)), and internal hyphae (Fig. 1 (b)) between the cortical cells are generated at the afflicted section of the root. Hyphae penetration and development are most common in areas that are still going through a differentiation and growth phase. The formation of hyphae causes no harm to the cells.

The Effects of Land Characteristic in Wanasari and Larangan Districts on The Pb Levels of the Shallot

Based on Table 4, it is seen that the shallot tubers planted in contaminated media and given with AMF contain a heavy metal of Pb under its safe threshold. The threshold for Pb content in plant tissue, according to the Directorate General of POM, Ministry of Health, is a maximum of 0.2 ppm.

Table 2: The result matrix of various effects of amf application on the shallots growth (*Allium ascalonicum* L.) in the Pb-contaminated media.

Observation Variables	Analysis Result of Variety Scan
Fresh Plant Weight	ns
Fresh Leaf Weight	ns
Fresh Tuber Weight	ns
Total Root Lenght	ns
Number of leaves 15 DAP	ns
Number of leaves 30 DAP	ns
Number of leaves 45 DAP	ns
Number of leaves 60 DAP	ns
Number of leaves 67 DAP	ns
Height of Plant 30 DAP	ns
Height of Plant 45 DAP	ns
Height of Plant 60 DAP	ns
Height of Plant 67 DAP	ns
Percent of Root Infection	*

Note : ns = Not significant; * = Significant

Pb buildup in the tubers is considerable after L0 treatment. The roots were thought to absorb more Pb, which was then dispersed to all parts of the plant, including the tuber. AMF was able to collect and accumulate metals in the vesicles near the root in the AMF treatment plant, thus only a tiny fraction was transferred to other areas. As seen in Table 4., in the L0 treatment, the Pb content is 12.754 ppm and the L8 treatment shows the lowest Pb content, 2.084 ppm, a difference of 83.660% compared to the control. According to Hartini (2011), agricultural products may contain heavy metals, because the nutrients from the soil containing heavy metal Pb will be absorbed by plants in the growing process.

There are 2 types of heavy metals in the soil, free (mobile) and non-free (immobile). The difference between mobile and immobile metals is that free metals are toxic and absorbed by plants, whereas immobile metals are bound to nutrients, organic materials, or inorganic materials. In these circumstances, apart from having an effect on the amount of nutrient content in plants, they can also be contaminated by heavy metals. The heavy metals bound by the soil will combine in the soil, then be absorbed by plant roots, and then they will spread to other parts (Charlena 2004).

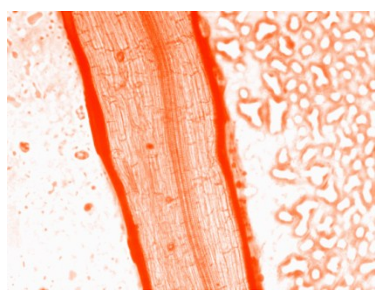

Fig. 2: Shallot roots uninfected by AMF (5/0.10).

The lead from the soil is mostly absorbed by the land plants and they retain the lead in large quantities in the roots. The leaves are also proven to contain lead (it is very likely that this lead is transferred to all other parts). To prevent the absorption of lead by the roots, calcium, and phosphorus can be applied to the soil (Asati et al. 2016). Pb is bound by the ions in the organophosphate in the center of shallot roots. Pb uptake in tubers is very low due to the minimal amount of Pb translocated from the roots to the top of the plant (Nurjaya et al. 2006). The rise in Pb uptake by plant roots rose with AMF infection (the maximum at L9, 52.041 ppm), then collected metal in the roots' vesicles, with only a small percentage being transported to other areas, including tubers, according to this study (6.748 ppm in leaves and 4.840 ppm in the tubers).

Fungi, yeast, bacteria, algae, and cyanobacteria are included in the types of microorganisms that can act as heavy-metal absorbers, as proved in several studies. Protonation, chelation, and chemical transformation are processes of program mobility by micro-organisms in the soil. Metals and phosphates in the soil will be easily dissolved by plant roots due to the excretion of microorganisms in the soil. This will undoubtedly have an impact on crops (Gonzalez-Chavez et al. 2002).

AMF is thought to be able to collect and absorb metals in host plant roots and biomass. Due to the influence of the extent and area of absorption in the soil, both AMF and ectomycorrhizae have the potential for metal absorption in both intracellular and extracellular mycelium (Jones et al. 2004). Cellulose, a cellulose derivative of chitin and melanin of the fungus AMF is a type of metal that binds to the wall components of the cells (Galii et al. 1993). Evidence has shown that AMF activity has a major role in removing metals bound by soil components in recent years (Gohre & Paszkowski 2006).

Other factors that influence metal absorption include (Joner et al. 2000, Leyval & Joner 2001): a.) Metabolite composition, b.) AMF type, c.) Fungal biomass cation exchange capacity, d.) metal pools, e.) Edaphic and environmental conditions, f.) Electrochemical properties of metals, g.) Competition between metals at absorption sites of mycorrhizal surfaces, h.) Natural host plants, i.) Root exudation patterns.

Bai et al. (2008) argued that AMF also affects metal absorption (translocation and accumulation in plant tissue) and the development of host plants. Sudová et al. (2007) discovered that mycorrhizal-inoculated corn absorbs Pb at high

Table 3: Mean matrix of each observation variable of AMF application in Pb-contaminated media in shallots.

Variety component	Total Root length[cm] (67 DAP)	Fresh Plant Weight [g] (67 DAP)	Fresh Tuber Weight [g] (67 DAP)	Fresh Leaf Weight [cm] (67 DAP)	Height of Plant (60 DAP)	Number of Leaves (60 DAP)	Per cent of Infection [%] (67 DAP)	
L0	24.33	4.67	8.33	4.33	10.83	12.33	0.00	a
L1	18.33	9.00	9.00	13.67	10.67	17.33	56.66	bcd
L2	45.33	47.50	34.67	38.33	32.83	29.50	73.33	bcd
L3	35.33	31.00	23.67	20.00	20.33	17.00	70.00	bcd
L4	31.00	30.00	32.33	30.67	30.83	15.67	40.00	b
L5	39.33	31.00	29.67	35.67	34.50	25.67	63.33	bcd
L7	24.00	32.33	34.67	28.33	36.83	34.50	46.66	bc
L8	38.00	34.67	32.33	35.67	34.33	34.83	46.66	bc
L9	7.00	16.50	21.33	25.00	16.50	22.17	63.33	bcd
L10	34.33	31.00	34.00	37.00	26.17	43.67	63.33	bcd
L11	20.00	36.83	33.67	39.33	35.33	40.83	60.00	bcd
L12	35.67	21.00	24.67	19.33	32.00	24.33	83.33	cd
L13	18.33	34.67	26.00	21.33	28.50	35.00	70.00	bcd
L14	20.67	27.00	27.67	31.67	30.83	28.33	93.33	d
L15	26.00	21.53	21.00	28.33	27.33	20.33	73.33	bcd
L16	21.33	23.17	31.00	22.33	22.33	35.33	66.66	bcd
L17	41.00	36.00	33.67	39.67	44.17	31.00	83.33	cd
L6	14.50	25.67	37.33	24.33	20.67	30.50	46.66	bc

Note: The different letters at the same column, of the same variable, represent significant differences

Table 4: Matrix of analysis results of Pb level in the roots. leaves. and shallots with AMF treatment in Pb-contaminated soil.

No.	Code	Pb Content [ppm]			
		Root	Leaf	Tuber	Decrease in the Accumulated Pb of Tubers [%]
1	L0	8.555	4.798	12.754	
2	L1	0.529	2.335	3.209	74.840
3	L2	9.076	3.240	5.546	56.516
4	L3	16.369	2.640	3.289	74.212
5	L4	29.335	4.276	3.331	73.882
6	L6	5.945	11.596	2.368	81.433
7	L7	12.365	3.027	2.654	79.191
8	L8	5.908	2.582	2.084	83.660
9	L9	52.041	6.748	4.840	62.051
10	L10	30.483	2.801	3.025	76.282
11	L11	-	2.755	2.645	79.261
12	L12	6.935	2.842	3.029	76.251
13	L13	22.385	3.445	3.368	73.593
14	L14	3.426	3.148	3.351	73.726
15	L15	46.965	3.452	3.095	75.733
16	L16	23.335	4.102	2.283	82.100
17	L5	4.648	2.195	2.919	77.113
18	L17	10.417	3.265	2.504	80.367

amounts. The plant has the benefit of growing well and accumulating and transporting metals. Increased development of phytochelatins, glutathione-derived peptides, which are only produced by higher plants, is another intriguing way by which mycorrhizal plants absorb heavy metals. Heavy metals and metalloids will thus be chelated by plants (Grill et al. 1987, Garg & Aggarwal 2011, Pollard et al. 2014). Glomalin, an insoluble glycoprotein, is synthesized in excess in AMF hyphae. Glomalin should be explored for biostabilization that leads to improved polluted soil conditions (González-Chávez et al. 2004, 2009).

Although the use of fungi is a suitable method for soil bioremediation, it is not the only method to apply. When it is combined with other bioremediation methods, it can work more effectively (Miransari 2017). Phytoremediation can utilize a modified AMF to support the accumulation of non-toxic heavy metals in plant roots and fungal mycelium (González-Chávez et al. 2002).

CONCLUSION

Based on the results of the analysis, it can be concluded that the application of indigenous AMF consortium from 17 locations with different Pb-contaminated land on the shallot varieties showed no significant effect on tuber weight, fresh leaf weight, fresh plant weight, total leaf length, and the number of leaves. The different applications of AMF show a good response to the per cent of infections in which the indigenous AMF consortium treatment from Slatri Land II shows the highest percentage of infections at 93.33%. The AMF treatment can reduce the Pb content of tubers compared to the control, up to 83.660%. It is necessary to carry out further research with single spores of AMF as a bioremediation agent on land contaminated with heavy metal Pb.

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REFERENCES

Asati, A.M. Pichhode, K. and Nikhil 2016. Effect of heavy metals on plants: An overview. Int. J. Appl. Innov. Eng. Manag., 5(3): 56-66

Badrudin, U. and Jazilah, S. 2013. Analysis of pesticide residue on shallots (Allium ascalonicum L.) in Brebes Regency. Pen J. Sci. Technol., 24(1): 75-86.

Bai, H.J., Zhang, Z.M., Yang, G.E. and Li, B.Z. 2008. Bioremediation of cadmium by rowing *Rhodobacter sphaeroides*: Kinetic characteristic and mechanism studies. Bioresour. Technol., 99: 7716-7722.

Balai, Penelitian Tanah 2002. Environmental Impact Management and Inventory Research. Final report. Part of the Research and Development Project on Soil Fertility and Climate. Bogor, pp. 1-5.

Bangun, E., Nur, M., Silalahi, H.I.,F.H. and Ali, J. 2000. Assessment of Shallot Fertilization Technology in North Sumatra. Proceedings of the National Seminar on Location-Specific Technologies Towards Decentralized Agricultural Development, 13-14, March 2000. Medan, pp. 338-342.

Charlena, K. 2004. Lead (Pb) and Cadmium (Cd) Heavy Metal Pollution in Vegetables. http://www.rudyct.com/pps702_ipb/09145/charlena.pdf.

Galii, U, Meier, M. and Brunold, C. 1993. Effect of cadmium on non-mycorrhizal and mycorrhizal fungus (*Laccasaria laccata* Scop.Ex.Fr) Bk and Br.: Sulphate reduction, thiols, and distribution of the heavy metal. New Phytol., 125: 837-843.

Gandjar, I., Sjamsuridzal, W. and Ariyanti, O. 2006. Basic and Applied Mycology. Indonesian Torch Foundation, Jakarta.

Garg, N. and Aggarwal, N. 2011. Effects of interactions between cadmium and lead on growth, nitrogen fixation, phytochelatin, and glutathione production in mycorrhizal *Cajanus cajan* (L.) Millsp. J. Pl. Growth Regul., 30: 286–300.

Gaur, A. and Adholeya. A. 2004. Prospect of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Cur. Sci., 86: 528-534.

Gohre, V. and Paszkowski, U. 2006. Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta, 223: 1115-1122.

Gonzalez-Chavez, M.C., D'Haen, J., Vangronsveld, J.J. and Dodd, J.D. 2002. Copper sorption and accumulation by the extraradical mycelium of different Glomus spp. (Arbuscular Mycorrhizal Fungi) isolated from the same polluted soil. Pl. Soil, 240(2): 287-297.

González-Chávez, M.C., Carrillo-González, R., Wright, S.F. and Nichols, K.A. 2004. Role of glomalin, protein produced by hypha of arbuscular mycorrhizal fungi in the sequestration of potentially toxic elements. Environ. Pollut., 130: 317–323.

González-Chávez, M.C., Carrillo-González, R. and Gutiérrez-Castorena, M.C. 2009. Natural attenuation in a slag heap contaminated withcadmium: The role of plants and arbuscular mycorrhizal fungi. J. Hazard. Mater., 161: 1288–1298.

Grill, E., Winnacker, E. and Zenk, M. 1987. Phytochelatins, a class of heavy metal-binding peptides from plants are functionally analogous to metallothioneins. Proc. Nat. Acad. Sci., 84: 439-443.

Hajoeningtijas, O.D., Mansur, I., Ekowati, N. and Tamad, K. 2019. Diversity of mycorrhizal fungi Arbuscula indigenous rhizosphere of shallots contaminated with heavy metal Pb. Research Results Report, Unpublished Bachelor Thesis, Universitas Muhammadiyah Purwokerto.

Hartini, E. 2011. Levels of Plumbun (Pb) in Shallot Bulbs in Kersana District, Brebes Regency. J. Visikes 10(1): 56.

Joner, E.J., Briones, R. and Leyval, C. 2000. The metal-binding capacity of arbuscular-mycorrhizal mycelium. Pl. Soil, 226(2): 227-234.

Jones, R., Sun, W., Tang, C.S. and Robert, F.M. 2004. Phytoremediation of petroleum hydrocarbons in tropical control soils. II: Microbial responses to plant roots and contaminants. Environ. Sci. Pollut. Res., 11: 340-346.

Leyval, C., Joner, E.J., Val, C. and Haselwandter, K. 2002. Potential of Arbuscular Mycorrhizal Fungi for Bioremediation. In: Gianinazzi, S., Schuepp, H., Barea, J.M. and Haselwandler, K. (eds), Mycorrhizal Technology in Agriculture, Burkhiluser Verlag, Switzerland, pp. 175-186

Leyval, C. and Joner, E.J., 2001. Bioavailability of Heavy Metal in the Mycorhizosphere. In: Gobran, G.R., Menzel, W.W. and Lombi, E. (eds), Trace Elements in the Rhizosphere, CRC. Boca Raton, FL, pp. 165-188.

Miransari, M. 2017. Arbuscular Mycorrhizal Fungi and Heavy Metal Tolerance in Plants. In. Wu, Q.S. (ed.), Arbuscular Mycorrhizas and Stress Tolerance of Plants, Springer, New York, pp.147-161. DOI 10.1007/978-981-10-4115-0_7.

Moelyohadi, Y., Harun, M.U., Munandar, Hayati, R. and Gofar, N. 2012. Utilization of various types of biological fertilizers in nutrient efficient corn (Zea mays L.): Cultivation in a marginal dry land. J. Subopt. Land 1(1): 31-39.

Nendissa, J.I. 2008. The influence of organic soil treatment (OST) and time of the landeto solution application on the growth and yield of shallots on Regosol. J. Budid. Pertan., 4: 122-131.

Nurjaya, K.M., Zihan, E. and Saeni, S. 2006. The effect of amelioration on soil Pb levels, uptake, and yield of shallots on inceptisols. Indones. J. Agric. Sci., 8(2): 110-119.

Nusantara, A.D., Bertham, Y.H. and Mansur, I. 2012. Working with Arbuscular Mycorrhizal Fungi. IPB Press, Seameo Biotrop.

Novriani, M. and Madjid, A. 2009. The Role And Prospects of Mycorrhizae. Palembang : Postgraduate, Sriwijaya University.

O'Connor, P. J., Smith, S.E. and Smith, F.A. 2001. Arbuscular mycorrhizal associations in the southern Simpson Desert. Aust. J. Bot., 49(4): 493. doi:10.1071/bt00014.

Pollard, A.J., Reeves, R.D. and Baker, A.J.M. 2014. Facultative hyper-accumulation of heavy metals and metalloids. Plant Sci., 217-218: 8–17. doi:10.1016/j.plantsci.2013.11.011.

Prasasti., O.H., Purwani, K. I. and Nurhatika, S. 2013. Effect of mycorrhiza glomus fasciculatum on vegetative growth of peanut plants infected by pathogen *Sclerotium rolfsii*. J. Sci. Arts, 2(2): 2337-3520. doi: 10.12962/j23373520.v2i2.3624.

Saputra, B., Riza, L. and Irwan, L. 2015. Vesicular arbuscular mycorrhizal fungus (MVA) in three types of rhizosphere soil of banana nipah (*Musa paradisiaca* L. var. Nipah): Plant in Pontianak Regency. Protobiont 4(1): 160-169.

Setiadi, Y. and Setiawan, A. 2011. Study on the status of arbuscular mycorrhizal fungi in the post-nickel mining rehabilitation area: A case study of PT INCO Tbk. Sorowako, South Sulawesi.. J Trop. Silvicul., 3(1): 88-95.

Sudová, R. and Vosátka, M. 2007. Differences in the effects of three arbuscular mycorrhizal fungal on P and Pb accumulation by maize plants. Plant Soil, 296: 77-83.

Tamin, R.P., Nursanti, M. and Albayudi, K. 2012. Identification of types and propagation of local endomycorrhizae in the Jambi University campus forest. J. Res. Univ. Jambi SAINS Ser., 14(1): 23-28.

Wang, X., Pan, Q., Chen, F., Yan., X. and Liao., H. 2011. Effects of co-inoculation with arbuscular mycorrhizal fungi and rhizobia on soybean growth as related to root architecture and availability of N and P. Mycorrhiza, 21: 173-181. https://doi.org/10.1007/s00572-010-0319-1