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Native Mycorrhizal Fungi in Land Contaminated Cr, Co and Cu

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ABSTRACT

Mycorrhizal fungi that are capable of adapting and resistant to heavy metal contaminated environments have received special attention for phytorhizoremediation researchers. The aim of the study was to explore native mycorrhizal fungi from areas contaminated with heavy metals to be used as starter biological agents in the phytorhizoremediation program. This research was carried out in two phases, i.e. rhizosphere sampling of Polypodium glycyrrhiza, Sumasang sp (local name) and Spathoglottis plicata at coordinates 2°31'57,6"S and 121°22'50,7"E. Rhizosphere of Chromolaena odorata, Melastama affine and Nephrolepis exaltata at coordinates 2°31'53,5"S and 121°22'35,4"E, Sorowako, Indonesia; While the other phase is isolating and identifying mycorrhizal spores in the Microbiology Laboratory, Research and Development Center for Environment and Forestry in Makassar, Indonesia. The results showed that be discovered three genus of mycorrhizal fungi were able to adapt and resistant in areas contaminated with Cr, Co, and Cu, i.e. 44.44% to 75.86% Acaulospora sp; 9.52% to 44.44% Gigaspora sp, and 3.38% to 19.05% Glomus sp. which could be used as source of inoculum in Phyto-rhizoremediation program. We recommend using native mycorrhizal fungi combined with endemic plant of location to rehabilitation heavy metal contaminated soils.

Keywords:
Acaulospora; fungi; heavymetal; postmine; phytorhizoremediation.

1. Introduction

Arbuscula mycorrhiza has a very important role in soils contaminated with heavy metals, this is because arbuscula mycorrhiza associated with plant roots is known to play a role in rehabilitating lands contaminated with heavy metals (Asmelash *et al*, 2016). The mechanism of protection provided by arbuscula mycorrhizae against heavy metals and toxic elements can be through the effects of filtration (Bano and Ashfaq, 2013), accumulation of heavy metals into hyphae (Ferrol *et al*, 2016), deactivate chemically (Abu-Elsaoud et al, 2017) or mechanism of decomposition of heavy metals by secretion of external hyphal (Gong and Tian, 2019).

Concentration of heavy metals that exceed the threshold becomes a source of pollutants in post-mining land. Heavy metal pollution has occurred in post-mining areas of gold (Bashola et al, 2016), nickel (Hu et al, 2017), tin (Kurniawan, 2017) and coal (Bhuiyan et al, 2010). High concentrations of heavy metals will inhibit growth (Jaishankar et al, 2014), change morphology (Bini et al, 2012) and disrupts organism's metabolism (Singh et al, 2012). However, each type of organism also has a defense strategy against heavy metal pressure to carry out the adaptation process (Emamverdian et al, 2015).

Heavy metals can cause changes in the community of microorganisms, so microorganisms are more resistant to pressure from heavy metals (Igiri *et al*, 2018), essential and non-essential heavy metals show toxicity if they are above a certain concentration (Bansal and Asthana, 2018). This stress toxicity is limited by the threshold value (Ju et al. 2016), which varies depending on many factors, including the type of microorganism, physicochemical properties and metal concentration, and soil conditions (edafic) and the environment (Emamverdian *et al*, 2015).

Mycorrhizal fungi are one of the obligatory soil microorganisms. These fungi have ability mutualistic symbiosis with 80% of plant species (Berruti *et al*, 2016). However, very determined by the type of mycorrhizal fungi, plant species and environmental conditions (Tahat and Sijam, 2012). Mycorrhizal fungi which have extensive adaptability will have the ability to survive in variety of environmental conditions, especially in soils contaminated with heavy metals (Chen *et al*, 2018). However, the tolerance level of heavy metals varies between various groups of fungi (Anahid *et al*, 2011). Several strains of mycorrhizal fungi can tolerate heavy metal stresses, including, *Glomus intraradices*, *Glomus mosseae* and several other important Glomus species. Therefore, the selection of mycorrhizal fungi that are tolerant of heavy metals is an important step in obtaining healthy plants in areas contaminated with heavy metals (Bano and Ashfaq, 2013).

Evaluating the tolerance of microorganisms in soil contaminated with heavy metals, specialists have adopted the concept of community tolerance caused by pollutants (Giller *et al*, 2009). This perspective establishes that over time, in an ecosystem, exposure to heavy metal contamination increases tolerance to the community of microorganisms (Shade *et al*, 2012). Tiwari and Lata (2018) showing that long-term exposure heavy metals in put pressure on soil microbes and increases tolerance. They concluded that the long-term accumulation of heavy metals in the soil gave the time to community of microorganisms to adapt to heavy metals. This adaptation has been linked to two factors, namely, the gradual decline in metal availability due to the immobilization reaction that occurs in the rhizosphere, while another factor is the gradual change in microbial community structure, based on changes in phospholipid fatty acid profiles (Azarbad *et al*, 2013) that produce organisms that are more tolerant.

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In addition to these two things, mycorrhizal fungi such as organisms accumulate heavy metals in vesicles, hyphae, and arbuscula fungi (Rezvani *et al,* 2015).

Native mycorrhizal fungi isolate that have been adapt to environments that have abiotic stress can be potential biotechnology tools to be inoculated in plants for the successful restoration of degraded ecosystems. This needed because some rehabilitation activities land which contaminated with heavy metals, using non-native mycorrhizal fungi so that association of plant roots and mycorrhizal fungi becomes inhibited. So that an activity is needed that aims to explore indigenous mycorrhizal fungi from areas contaminated with heavy metals to be used as a starter for biological agents.

2. Materials and Methods

The study was conducted in two phases. First phase, the taking rhizosphere of *Polypodium glycyrrhiza, Sumasang* sp (local name) and *Spathoglottis plicata* at coordinates 2°31'57,6 "S; 121°22'50,7" E. Rhizosphere of *Chromolaena odorata*, *Melastama affine* and *Nephrolepis exaltata* at coordinates 2°31’53,5 "S; 121°22'35,4" E, Sorowako, Indonesia. Using method from Krishnamoorthy (2015) and Toh et al (2018). Another phase, mycorrhizal fungi spores in isolated from host plant of rhizosphere followed wet sieving techniques (Brundrett et.al, 1984) using multilevel sieves (mess size of 325, 40 and 50 µm) in Microbiology Laboratory, Center for Research and Development for Environment and Forestry Makassar, Indonesia. Spore morphology was identified using a manual from International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM, 2019)

Concentration of soil heavy metals was measured while in laboratory of chemistry, Polytechnic of Ujung Pandang, Makassar, using manual book of X-Ray Florence Spectrophotometer/Bruker/S2 Ranger, Heavy metal concentration in soil can be seen in table 1. The coordinate determination of host plant rhizosphere taking location was determined using the Global Positioning System (GPS) Coordinates Finder (Figure 1).

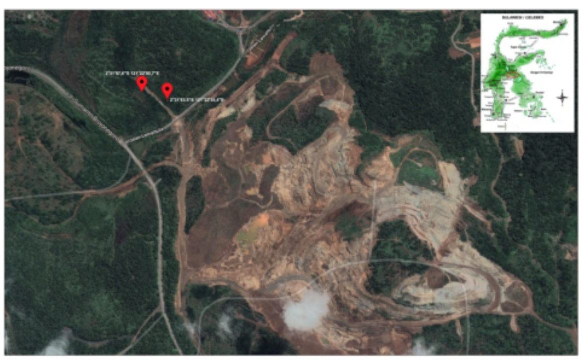

Figure 1. Location of rhizosphere taking of host plants on land contaminated Cr, Co and Cd.

3. Results and Discussion

Laboratory test results show that post-mining land has been contaminated with Cr, Co, and Cu metals which exceed the critical limit for soil and plants (Table 1), this will give stress to macroorganisms and soil microorganisms to complete their life cycle, but some organisms are able to adapt and tolerant to the environment contaminated with the metal.

Table 1 Elements of heavy metals in land contaminated with heavy metals in Sorowako, East Luwu district, South Sulawesi, Indonesia.

Heavy metal	**Coordinat**		**Critical limit**	
(ppm)	2°31'57,6" S 121°22'50,7" E	2°31'53,5" S 121°22'35,4" E	Soil	Plant
Chrome (Cr)	26.458	38.754	2,5*	5-30**
Cobal (Co)	1.578	3.005	10*	15-30**
Copper (Cu)	87.3	221	60-125*	20-100**

Source: *Ministry of State for Population and Environment Republic of Indonesia and Dalhousie University Canada. 1992; **Alloway (1995) and Shanab et al (2007).

A series of "classical" ecological principles that examine the process of increasing tolerance or resistance of microorganism community have been widely studied. The microorganism resistance, refers to the ability to resist the effects of pollutants which are usually effective against them, while microorganism resistance, refers to the ability to adapt to persistent pollutants (Bhalerao, 2013). Tiwari and Lata (2018) suggest that tolerance and resistance to the toxic effects of heavy metals depend on the mechanisms involved. In short, tolerance to heavy metals can be defined as a phenomenon where resistance microorganisms increase to again heavy metal stress which can lead to poisoning.

All organism, including microorganism, can achieve resistance to heavy metals by "avoidance" when organisms are able to limit metal absorption, or by "tolerance" when organisms survive in high internal metal concentration (Hossain et al, 2012; Khare et al, 2017; Ojuederie and Babalola, 2017). The first mechanism involved is reduced absorption or increase in efflux, formation and release of organic acids outside the cell complex. The second mechanism, metal is chelated intracellularly through the synthesis of ligands such as metallothionein, polyphosphate, and / or compartments in vacuoles (Ma et al, 2016; Singh et al, 2016). Individuals who are tolerant and sensitive to heavy metals can be distinguished by their growth performance on substrates contaminated with heavy metals (Glubiak et al, 2012, Singh et al. 2016).

Identification result of native mycorrhizal fungi spore obtained from various rhizosphere of host plants in land contaminated with heavy metals, were found three genera of mycorrhizal fungi spore that were adaptable and resistant in land with high concentrations of heavy metals, i.e. *Acaulospora* sp, *Gigaspora* sp and *Glomus* sp (Figure 2). Adaptability and resistance of fungi, possibly following the mechanism of metal chelating processes intracellularly through the synthesis of ligands such as metallothionein, polyphosphate, and / or compartments in vacuoles so that become tolerant.

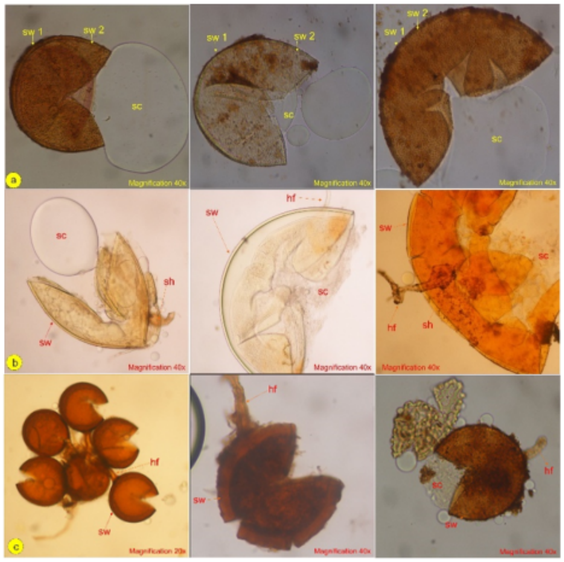

Figure 2. Morphology of native mycorrhizal fungi spores isolated from land contaminated with heavy metals. (a) *Acaulospora* sp., (b) *Gigaspora* sp., and (c) *Glomus* sp.

Glomus sp. is the genus mycorrhizal from family Glomeraceae. Some of the characteristics of this genus are spores formed singly or in pairs in the terminals of the nongamethangium hyphae which are undifferentiated in the sporocarp. When the adult spores are separated from the adhesive hyphae by a partition. Spores are globose, sub-globose, ovoid, or obovoid with spore walls consisting of more than one layer, hyaline to yellow, brownish, brown, and black, measuring between 20 - 400 µm (Lone et al, 2014; INVAM 2019).

Acaulospora sp. is the genus mycorrhizal which belongs to the family of Acaulosporaceae. This genus has several characteristics including having 2-3 spore walls, spores are formed on the side of the sporiferous saccule neck, shaped spores of globose up to ellipse, colored hyaline, yellow, or yellow red, measuring between 100-400 µm (Leno et al, 2014; INVAM 2019).

Gigasporaceae is a family of mycorrhiza which belongs to the genus Gigaspora sp. This genus has a characteristic, that is, spores are produced singly in the soil, spore do not have a inner layer wall, be found of bulbous suspensor, shaped of globose or sub-globose, colored of cream to yellow, size spore 125-600 µm (Leno et al, 2014 ; INVAM 2019).

Germination and formation process of mycorrhizal fungi spore, through 3 stages, i.e, (1) germination of spores in the soil, (2) penetration of hyphae into root cells and (3) development of hyphae in the root cortex (Pereira et al. 2015), and these third of stages possibility also be carried out by mycorrhizal fungi in the land contaminated heavy metals in to multiply themselves. The calculation results of the number of mycorrhizal fungi spores in 100 gr of heavy metals contaminated rhizosphere found in different amounts and dominated by the genus Acaulospora sp. (Table 2). This allegedly due beside to heavy metal stresses, also influenced by other abiotic factors. According to Jamiołkowska et al (2018) that abiotic factors which determine the abundance and development of mycorrhizal fungi spores among other temperature, pH, soil organic matter and soil water content.

Optimum soil temperature for germination of mycorrhizal fungi spores is very diverse and depends on the type. The best temperature for the development of mycorrhizal fungi is at ambient temperature of 30°C but for the best colonization of mycelia is at ambient temperature 28°C - 35°C (Sight, 2004). *Gigaspora* sp can grow and germinate well at ambient temperatures of 25°C - 35°C while *Glomus mosseae* originates from cooler regions. The best germination is ambient temperature 18°C - 20°C. Some scientific literature also suggests that colonization of plant roots by mycorrhizal fungi still occurs at soil temperatures as low as 5°C (Gavito and Aguilar, 2012).

Fungi are generally more resistant to changes in soil pH. However, the adaptability of each species of mycorrhizal fungi to soil pH varies greatly. Soil pH can also affect germination, development, and role of mycorrhiza to plant growth. The optimum pH for development of mycorrhizal fungi is ranging from pH 5.6-7 for *Glomus* sp. pH 4-6 for *Gigaspora* sp. pH 4-5 for *Acaulospora* sp, (Setiadi and Setiawan, 2011). According to Bertham (2003) *Glomus mosseae* usually in alkaline soils can germinate well at pH 6-9. *Gigaspora corallodea* and *G. heterogama* of more acidic species can germinate well at pH 4-6.

Soil organic matters also play a role in increasing the number of mycorrhizal fungi spores. The maximum number of spores found in soils containing organic material from 1 to 2 percent, while in soils containing organic matter less than 0.5 percent number of spores found is very low (Holste, et al. 2016).

Groundwater content also affects germination and the period of dormancy of mycorrhizal fungal spores. in wet soil, the dormancy period for *Glomus* sp and *Gigaspora* sp spores is longer than in dry soil. Whereas for *Acaulospora* sp spores the period of dormancy is generally not affected by soil water content (Tommerup, 1983; Juge et al, 2002).

Table 2. Number of mycorrhizal fungi spores per 1000 mg of rhizosphere samples

Coordinate	Family	Hots Plant Rhizosphere	Number of Spora		
			GL	GS	AC
2°31′57,6″S 121°22′50,7″E	Polypodiaceae	*Polypodium glycyrrhiza*	2	0	7
	-	*Sumasang sp (local name)*	2	2	8
	Orchidaceae	*Spathoglottis plicata*	0	1	1
2°31′53,5″S 121°22′35,4″E	Asteraceae	*Chromolaena odorata*	0	0	24
	Melastomataceae	*Melastama affine*	0	1	1
	Nephrolepidaceae	*Nephrolepis exaltata*	0	0	13

Note: GL, *Glomus* sp; GS, *Gigaspora* sp; AC, *Acaulospora* sp

Mycorrhizal fungi are very important in the heavy metal phytostabilization program that exceeds the critical limit. Plants that have been symbiosis with mycorrhizal fungi accumulate and store heavy metals in vesicles and hyphae of fungi on their roots, so that metal pollutants do not move and do not inhibit the growth and absorption of phosphorus and other micronutrients (Bano, 2013; Yang, 2016). Mycorrhizal fungi also release various organic acids which increase the solubility of phosphate compounds which are insoluble in soil (Bolduc dan Hijri, 2010; Johri *et al,* 2015; Jamal *et al,* 2018). Mycorrhizal fungi release glomalin which is a particular sorble metal glycoprotein that increases immobilization of toxic metals (Ambrosini, 2015; Hristozkova *et al*, 2016). The

Metallothionein protein released by certain mycorrhizal fungi, also reduces the toxicity of heavy metals in the soil (Bano dan Ashfaq, 2013). Certain external mycorrhizal myceliums also produce a type of protein called glycoprotein (Glomalin), which has a heavy metal binding area. (Trouvelot et al, 2015; Leal et al, 2016).

Several reports and reviews show that mycorrhizal from areas contaminated with heavy metals has developed tolerance to heavy metal toxicity and adapted well. Mycorrhizal has been proven to evolve tolerance to heavy metals, as stated by Lingua et al (2012) and French (2017), Some strains of mycorrhizal fungi tolerant develop in one or two years. However, to date, the potential interaction mechanism between mycorrhizal fungi and heavy metal, as well as cellular and molecular mechanisms about the tolerance of heavy metals by mycorrhizal fungi, is still poorly understood. Because mycorrhizal fungi cannot be cultivated without host plant, so more difficult to showed absorption of metals intrinsically by hyphae.

Isolate of native mycorrhizal fungi in area contaminated of heavy metals is more tolerant than isolate from non-polluted area, and it has been reported that native mycorrhizal fungi efficiently affect plant root growth in heavy metal stressed environments (Upadhyaya et al, 2010; Vaishaly et al, 2015; Burreti, et al, 2015). Thus, it is important to sieve native isolate that tolerant of heavy metal for ensure the effectiveness of symbiosis between mycorrhizal fungi and plant roots at area recovery programs contaminated heavy metal. Furthermore, suggested that phytoremediation potential for contaminated area can be increased by inoculated of hyperaccumulator plant roots with mycorrhizal fungi which are most suitable for contaminated sites. Therefore, very important for combine endemic plants with isolate of indigenous mycorrhizal fungi that adjusted for the type and concentration of heavy metals in future studies for the Phyto-rhizoremediation program.

4. Conclusion

The found three genus of native mycorrhizal fungi that ablel to adapt in area contaminated Cr, Co, and Cu which could be used as source of inoculum in Phyto-rhizoremediation program, we recommend for use native mycorrhizal fungi which combined with endemic plants of locations for more successful work in rehabilitating heavy metal contaminated soil.

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