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Indigenous endomycorrhizal fungus in the area contaminated Fe and Mn in South Sulawesi, Indonesia

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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 indigenous 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, namely; rhizosphere sampling of *Polypodium glycyrrhiza*, Sumasang sp (local name) and *Spathoglottis plicata* at coordinates 2º31'57.6"S and 121º22'50.7"E, Sorowako, South Sulawesi, 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 genus Acaulospora was more dominantly found in areas contaminated with the metal of Fe and Mn, and was able to adapt and survive compared to other genera.

1. Introduction

Endomycorrhizal a form of symbiotic mutualism association between root endodermis of higher plants with fungal hyphae, which have reached 80% of plant species. Some researchers have shown the positive role of endomycorrhizal on plant growth in stressful environments of salinity [1], nutrients [2], drought [3], pathogens [4] and heavy metals [5] in order to maintain the productivity of farming [6] and reducing complaints of local people to the negative impact of agriculture and livestock environment [7].

Research on the use of endomycorrhizal to reduce heavy metal contamination is a phenomenon that has received the attention of phytorhizoremediation researchers. Arisusanti and Purwani [8] infect *Glomus fasciculatum* on the roots of Dahlia sp to reduce lead metal (Pb). *Rhizophagus clarus* and *R. irregularis* can provide protective effects on vines on land contaminated with Copper (Cu) [9]. *Glomus versiforme* and *Rhizophagus intraradices* can reduce the absorption of Cadmium (Cd) on *Lonicera japonica* [10]. Alam et al. [11] using endomycorrhizal species obtained from International of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM), West Virginia University (WV), the USA, inoculated on Lens culinaris to reduce Arsenic (As), but the endomycorrhizal used are not indigenous endomycorrhizal from areas that contaminated with heavy metals.

The chemical elements iron (Fe) and manganese (Mn), respectively, have a specific gravity of 7.86 g.cm^{-3} and 7.21 g.cm^{-3}, so that classified as heavy metals [12,13]. Fe and Mn, including as micro-essential heavy metals that can cause metabolic disorders for soil microorganisms if those are in high concentrations [14]. Some research results to show that heavy metal pollution gives out a detrimental effect on the life cycle of soil microorganisms [15] and causes changes in the soil microorganisms community structure [16], but ultimately causes soil microorganisms to become tolerant and resistant [17]. However, the level of tolerance and resistance between groups of fungus is largely determined by the type of fungus and environment [18].

Researchers have found several strains of endomycorrhizal fungus that are tolerant of heavy metal stress, namely, *Gigaspora albida* and *Glomus clarum* tolerant of zinc (Zn), cadmium (Cd), lead (Pb) and copper (Cu) [19], *Glomus intraradiaces* and *Glomus mosseae* tolerant of Pb and Cd [20], *Acaulospora morrowiae* tolerant of Cu [9], *Acaulospora mellea* tolerant to nickel (Ni) and chromium (Cr) [21] and *Scutellospora pellucida* tolerant of Zn [22].

Endomycorrhizal tolerant and resistant to heavy metals with high concentrations can be a biotechnology agent for the success of post-mining land phytorhizoremediation programs in Sorowako, Indonesia. So that exploring the indigenous endomycorrhizal fungi spores from areas contaminated with Fe and Mn is the objective of this study, which also provides an opportunity for other researchers to inoculate on an endemic plant of location.

2. Methodology

The study is conducted in two phases. The first phase, rhizosphere collection of *Chromolaena odorata*, *Melastama affine,* and *Spathoglottis plicata* at coordinated of 2^{O}31'57.6 "S and 121^{O}22'50.7" E, Sorowako, South Sulawesi, Indonesia. The rhizosphere collection method uses a diagonal system [23]. In the second phase, endomycorrhizal fungus spores are isolated from the rhizosphere using the wet sieving technique [24] and the sucrose gradient centrifugation method [25] at Microbiology Laboratory, Research, and Development Center for Environment and Forestry, Makassar, Indonesia. Spore morphology identified using a manual book from the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungus (https://invam.wvu.edu) and Handbook Work with AMF [26]. Concentration Fe and Mn in soil were measured while in the laboratory of chemistry, Polytechnic of Ujung Pandang, Makassar, using X-Ray Florence Spectrophotometer/Bruker/S2 Ranger, concentration Fe and Mn in soil (figure 1).

3. Results and discussion

Concentrations of Fe and Mn in nickel post-mine area of Sorowako have exceeded the critical limit for the environment and plants (Figure 1), some researchers suggest that critical limit for Fe in the soil were 100,000 ppm [27] and in plants were 1,000 ppm [13,28], while for Mn, the critical limit in the soil were 1,500 ppm [29] and in plants was 400 ppm [13,30]. Fe concentration and high Mn is likely caused by mining activities, especially when returning overburden so that it can have a toxic effect on the activity of soil microorganisms to develop and reproduce, but according to Ivshina et al. [31] that strategy to adaptation and tolerance also owned by each organism in an unfavorable environment.

Endomycorrhizal also has defense strategy to heavy metal stress. The strategy is likely to involve one of the mechanisms, including (i) new expression of fungus gene [32], (ii) metal of quarantined and deposited at extracellular [33], (iii) produce of metal-binding protein [34], (iv) reduce of metal absorption [35], (v) increase efflux [36], (vi) formation of complexes outside the cell [37], (vii) release of organic acids [38] and (viii) ligand syntheses such as polyphosphates and metallothionein [32,39].

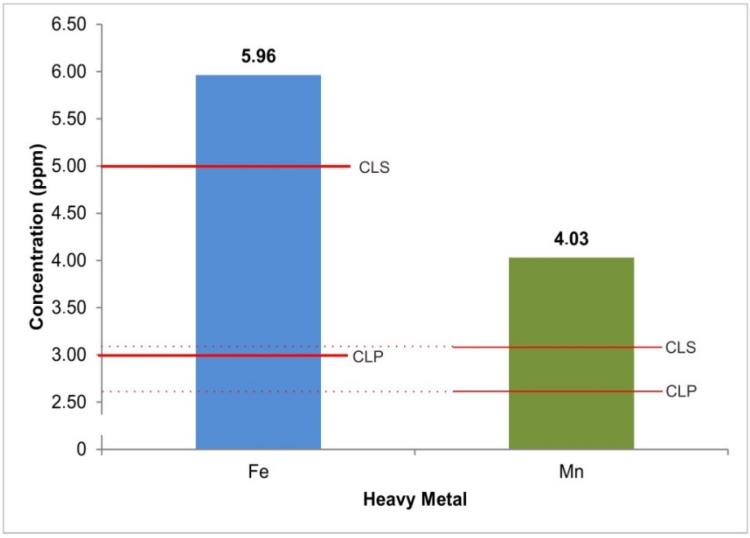

Figure 1. Concentrations of Fe and Mn in the nickel post-mine area of Sorowako, Indonesia. (CLS, Critical limit in the soil; CLP, Critical limit in the plant). Data is displayed in logarithmic form.

Indigenous endomycorrhiza spores identified in various plant rhizosphere obtained two genera of spores that are able to adapt in areas that have high concentrations of Fe and Mn, namely Acaulospora sp and Gigaspora sp. Acaulospora sp. was the genus mycorrhiza, which belongs in the family Acaulosporaceae. This genus has several characteristics including having 2-3 spore walls, spores formed on the side of the neck of the sporiferous saccule, globose to elliptical, hyaline, yellow, or yellowish red, spore diameter between 74-289 µm (https://invam.wvu.edu), however, the diameter of the spores found by Akib et al. [40] on nickel-contaminated land ranged from 60 - 80 µm.

Gigaspora sp. was the mycorrhizal genus that belongs to the Gigasporaceae family. This genus has characteristics, among others, the spore is produced singly in the soil, haven't a layer of inner spore walls, have bulbous suspensors, globose, or sub-globose shaped, creamy to yellow in color, 206-358 µm in diameter (https://invam.wvu.edu). The research results of Akib et al. [40] in the nickel post-mine area found Gigaspora sp spores with a diameter of 203 - 235 µm.

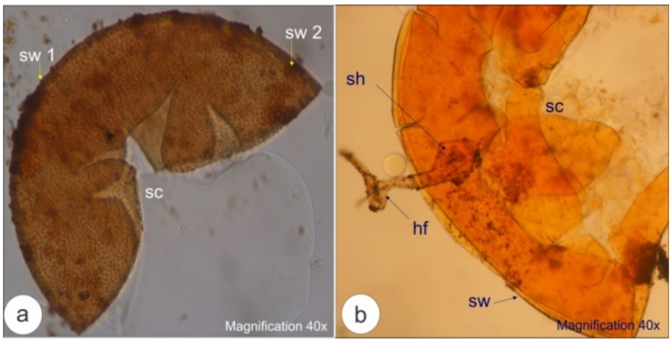

Figure 2. Spores morphology of *Acaulospora* sp (a) and *Gigaspora* sp (b), which isolated from the area contaminated with Fe and Mn. (Note: sw, cell wall; sc, saccule; sh, sub standing hyphae; hf, hyphae).

The presence of indigenous endomycorrhizal spores in the environment contaminated with Fe and Mn allegedly do one of the strategic defense, as has explained by researchers. However, according to Herrera et al. [41], the ability of adaptation and resistance of indigenous endomycorrhizal to be tolerant, it is possible to follow the intracellular metal-binding mechanism through ligand synthesis (metallothionein, polyphosphate), and/or accumulate metals to in vacuoles.

Table 1. The number of indigenous endomycorrhizal spores per 1000 mg of rhizosphere sample.

Family	Rhisosfer of Pioner Plant	Number of Spora	
		GS	AC
Asteraceae	*Chromolaena odorata*	0	24
Melastomataceae	*Melastama affine*	1	1
Nephrolepidaceae	*Nephrolepis exaltata*	0	13

Note: GS, *Gigaspora* sp; AC, *Acaulospora* sp.

Calculation of spore's indigenous endomycorrhizal number per 1000 mg of rhizosphere contaminated with Fe and Mn were found in different amounts and dominated by the genus Acaulospora sp. (Table 1). This is probably due to Acaulospora sp having more than one cell wall, so it has a stronger defense against heavy metal stress than Gigaspora sp. According to Upadhyaya et al. [42] that endomycorrhizal can store heavy metals in hyphal cell walls, specifically stored in crystalloids in the mycelium, this explanation reinforced by Chen et al. [43] who show that in roots of plants that are contaminated with heavy metals, it is seen that the endomycorrhizal extraradical mycelium is able to absorb and accumulate heavy metals outside the mushyel wall of hyphal wall zone, in the cell wall, and in the cytoplasm of fungus hyphae, while the research results of Tuheteru et al. [44] show that utilization Acaulospora tuberculata can increase Mn, Fe, Cr and Ni uptake of Nauclea orientalis L.

Several studies have reported that indigenous endomycorrhizal effectively and efficiently infection endodermis of plant roots in areas that are contaminated with heavy metals [45]. According to Zadehbagheri et al. [46] that indigenous endomycorrhizal isolates that develop and reproduce naturally in areas contaminated with heavy metals are more tolerant than isolates originating from non-polluted areas. Thus, filtering tolerant indigenous endomycorrhizal isolates needs to be done to ensure the effectiveness of symbiosis between endomycorrhizal and endodermis of plant roots. Therefore, it is very important to infect endodermis endemic plant roots with indigenous endomycorrhiza isolates in future studies.

4. Conclusions

Indigenous endomycorrhizal of genus Acaulospora sp and Gigaspora sp that are able to adapt in areas contaminated with Fe and Mn have been found in Sorowako and can be used as sources of inoculum in phytorhizoremediation programs combined with the endemic plant of locations.

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