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Abundance of arbuscular mychorrizal fungi in rehabilitation area of nickel post-mining land of Sorowako, South Sulawesi

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Abstract. Acceleration management of land rehabilitation in nickel post-mining in Sorowako has been main attention of Vale Indonesia. This acceleration can be done by utilizing of natural resources, especially indigenous endomycorrhiza. Endomycorrhiza also called arbuscular mycorrhizal has got a lot of attention for its ability to form a mutualistic symbiosis with 80% - 96% of plant species. This study aims to determine the dominance of indigenous endomycorrhiza spores and its potential to accelerate the management of land rehabilitation post-mining of nickel, which is carried out in three stages; sampling rhizosphere, trapping spores, isolation and identification of the arbuscular mycorrhizal spores types. The results showed that the dominance of indigenous endomycorrhiza were *Acalauspora* sp (75.1%), *Gigaspora* sp (19.4%) and *Glomus* sp (5.6%). Research on the effectiveness of indigenous endomycorrhiza using *Acalauspora* sp in land rehabilitation of nickel post-mining is still ongoing.

1. Introduction

Vale Indonesia is a nickel-mining company located in Sorowako village, Malili sub-district, Luwu Timur district, South Sulawesi Province, Indonesia. This company has contracted land for mining in Sulawesi with total area of 218,528.99 hectares. Those areas are spread across Sulawesi, such as Sorowako in South Sulawesi (118,387.45 hectares), Pomala in Southeast Sulawesi (635,060.18 hectares) and Bahodopi in Central Sulawesi (36,635.36 hectares) [1]. There are 4,973.15 hectares of area in South Sulawesi that have been explored by the company. Moreover, there are 3,975.91 hectares of the area that are rehabilitated and reclaimed at the end of 2014 [2].

The twelve-year revegetation process has brought positive impacts on both physical and chemical parameters of soil, which are increased significantly [3]. Besides, the growth rate of plants in the revegetation area is around 95-99%. Most of the plants that are planted in this area are pioneer type such as *Paraserianthes falcataria*, *Eucalytus eurograndis*, *Enterolobium macrocarpum*, also local species namely *Melochiaumbellata, Sandoricum kacappeae* and *Elmerelia* sp. [4].

In general, the majority of various plants are involved in a symbiotic relationship with another organism. For instance, plants with arbuscular mycorrhizal (AM) are benefitted by each other through mutualistic symbiosis. Specifically, 80% of plant's roots are associated with the fungi [5]. There are 2

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types of this association, namely endomycorrhiza and ectomycorrhiza [6]. Furthermore, the major genera found in nickel post-mining land were *Glomus* sp., *Acaulospora* sp., dan *Gigaspora* sp. [7]. However, their abundance level and population growth rate in Sorowako as nickel post-mining area are not revealed yet.

2. Materials and Methods

This qualitative research was carried out in three stages. The first stage was the taking of rhizosphere samples from rehabilitation area in nickel post-mining land owned by Vale Indonesia in Sorowako Village, that have been rehabilitated for less than 2 years in Sumasang 1; 2 to 4 years in Sumasang 2 block A and more than 4 years in Sumasang 2 block B. The sampling method was done by developing the method undertaken by [8, 9]. Mycorrhizal spores were isolated from the host plant rhizosphere using the wet screening method of Gerdemann and Nicolson (1963) which has been compared with other methods by [12]. The second stage was trapping cultures of spora were performed to obtain high spore viability, using trapping culture methods from [10] and [11] in nursery agroplastid in Parepare. Finally or third stage, the population of AM spore from *Glomus* sp., *Acaulospora* sp. and *Gigaspora* sp. were observed in Laboratory of Microbiology of Research and Development Center for Environment and Forestry Makassar. Microscopic characters of spore were matched with identification guide from [13] to identify genus of mycorrhizal fungi. Data are presented in a pie chart and bar chart.

3. Results and Discussion

Root is a suitable environment for a microbe to live, which interaction could benefit for both sides. According to scientists, the contact areas for a microbe in the plant are divided into phyllosphere (above-ground) and rhizosphere (under-ground) [14]. On the other side, the microbes that interact with plants are divided into phyllosphere (on the surface of plant organs) and epiphyte (colonialize the inner tissue of the plant) [15]. Moreover, microbes that interact with plants under the ground are further divided into rhizoplane (attach to root) and endophyte (live inside root cells) [16]. Those microbes help the plant to supply the nutrition or act as an anti-pathogenic agent that can be harm for the host. Next, the host plant provides the habitat and food supplies for microbes to live. In other words, this interaction is mutualistic for both sides.

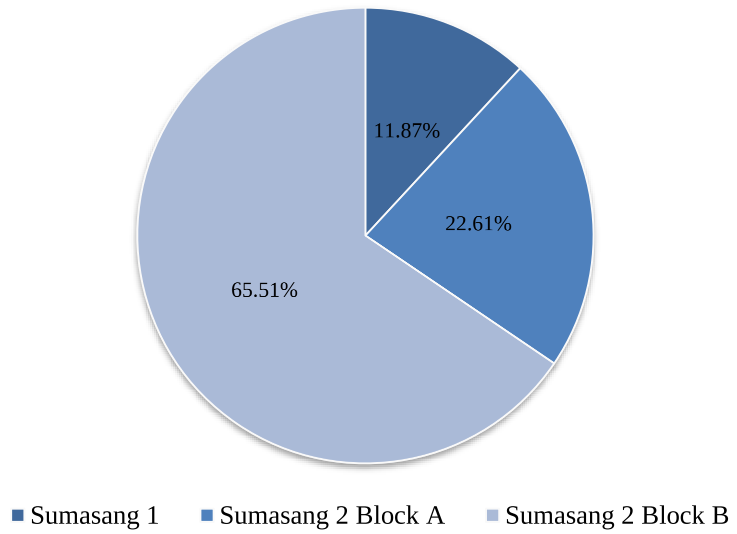

Figure 1. Population abundance of arbusculas mycorrhizal spores at various ages of nickel post-mining rehabilitation land.

Arbuscular mycorrhizal spore was found abundance in the area with more than 4 years of rehabilitation. The abundance level of the three genera was 65.51% (figure 1). As a result of crop adaptation to the environment, the energy supply needed for the survival of fungi is very suitable (figure 2) so that the development of mycorrhizal may increase 189.7% in rehabilitated area with more than 4 years. According to Hermawan [3], twelve-year revegetation process has improved the physical (texture, bulk density, porosity, water concentration, and penetration resistance) and chemical (pH, organic carbon, minerals, exchangeable cation, and exchange capacity) variables of soil.

Figure 2. The vegetation of trees and shrubs in nickel post-mining rehabilitation land in Sorowako (In Sumasang 1 (a); Sumasang 2 block A (b); and Sumasang 2 block B (c)).

The rhizosphere is suitable habitat for microbes due to the availability of organic matter. This condition allows microbes to grow and develop that are called as rhizosphere effect. This effect is centrifugal and tends to increase as the root branching system become more complicated. Besides, there are also several factors that determine the rhizosphere effect, namely soil type, soil humidity, pH, Temperature, plant's age, and plant's condition [17].

In general, there are two major types of the rhizosphere, namely endo rhizosphere including stele, cortex, endodermis, and root cap also recto rhizosphere that covers the area surrounding the root such as soil contact zone [17]. [18] Stated that rhizosphere includes space surrounding root, in which there are complex biological and ecological processes take place. It mediates biological interaction inside the soil [19].

Root secretes several organic matters in the form of exudate needed by microorganism as their energy resources and substrate for their metabolism such as sugar, amino acid, organic acid, fatty acid, sterol, nucleotides, flavonom, enzyme, and miscellaneous [20]. There are several factors that determine exudate composition and quantity such as plant species, plant age, environmental condition (temperature, radiation, soil humidity, soil type, plant nutrition, and plant stress), and microorganism existence [17]. Beside exudate, roots actively pump out several materials called as root secrets [21]. This can be physiological response toward external environment, such as organic acid that is secreted by rice as the response to Aluminum existence [22]. Another material that is secreted by root is root lysate such as protein, fat, and amino acid which are passively produced during autolysis of root cells [21]. Next material is musigel as the form of polysaccharide synthesized by golgi apparatus inside the cells of root cap, which moves through the cytoplasm to plasmalemma [23]. For instance, musigel can be formed as root secets, epidermis cell debris, and microbial cell fuse with root cap cell debris, a metabolic product, organic colloid, and an organic colloid [21]. At last, the main enzymes produced by root are oxidoreductase, hydrolase, lyase, and transferase. On the other hand, microbes secrete cellulose, dehydrogenase, urease, phosphatase, and sulphatase in the rhizosphere [24].

In succession process, vegetation is initially formed by the invasion of the new plant into the area through stages. This plant along with the other plants then adapting to the environmental condition that is called as aggregation stage. Following this, those plants competing for each other in order to reach the ecosystem balance or called as stabilization stage [25].

On the other side, the abundance of spore population on the land that has been rehabilitated for less than 2 years shows insignificant growth (figure 3). This can be expected as the effect of stress caused by backfilling activity, in which the spore has not been adapted to the new host. Sundari *et al.* [26] added that the existence of AM is determined by an environmental factor. Moreover, Delvian [27] deduced several theories about AM growth and development. The germination process of AM spore is divided into 4 phases, namely hydration, activation, sprout channels growth, and hyphae growth. In the first phase, water enters into spore for hydrating the organelles and macromolecules such as ribonucleic acid and enzymes to activate metabolism. Spore becomes active in the second until the tenth day of hydration. It follows by the formation of sprouts channel and hyphae growth.

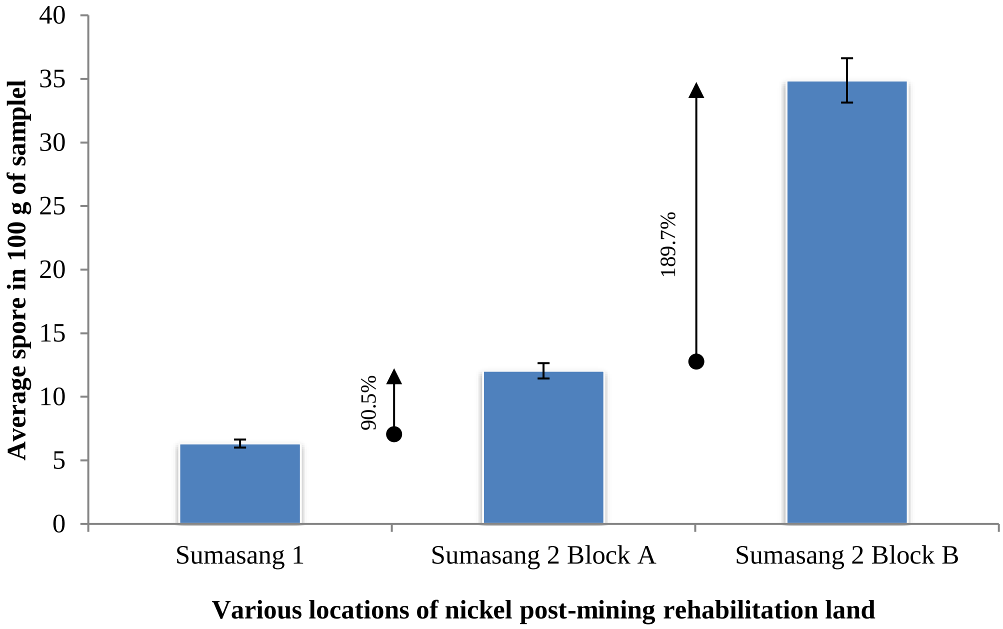

Figure 3. Increased population of AM Spores in various locations of nickel post-mining rehabilitation land in Sorowako.

There is several factors influence spore infection. Specifically, there are two steps of infection namely primary and secondary infection. The primary infection is mainly influenced by the other fungi spore growth, hyphae growth in soil. Besides, some AM also depends on sprout channel growth that is possibly influenced by root exudate, soil fertility, water supply, and enter spots in the root. These factors can be limitations for AM formation. In addition, secondary infection is entirely determined by host physiology due to it provides energy from photosynthesis for hyphae spread, which is transferred from plant to fungi through arbuscular or internal hyphae.

The characteristics of spores obtained from observation are varied. The classification was done based on their shape and colors. There are three genera identified in this study, *Glomus* sp, *Acalauspora* sp, and *Gigaspora* sp. Analysis of spore abundance shows that *Gigaspora* sp. least dominating the area. Moreover, each spore shows specific character. For instance, thick wall and ornamental spore are shown by *Acaulospora*. Following this, *Gigaspora* has bulbous suspenser in its hyphae base and has no sprouts layer. At last, *Glomus* spore has subtending hyphae (figure 4).

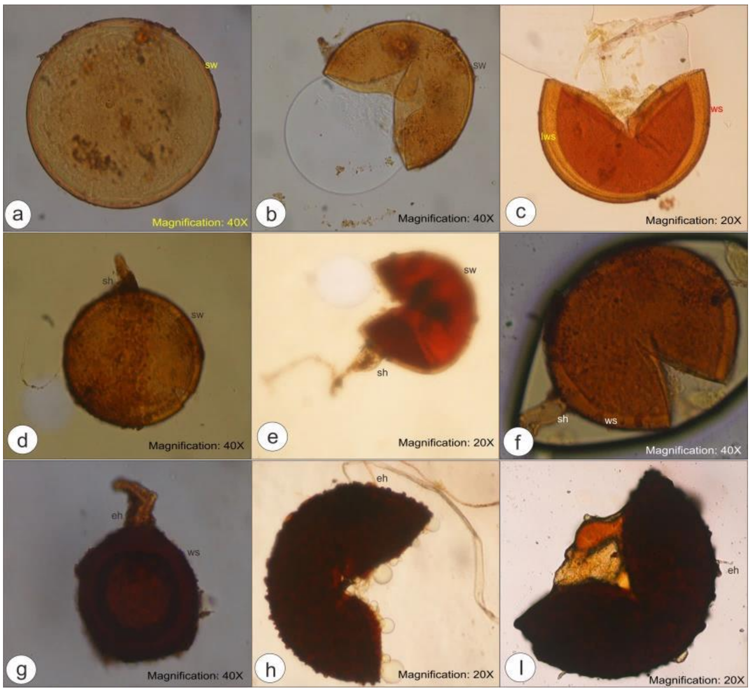

Figure 4. Spores of arbuscular mycorrhiza in the nickel post-mining rehabilitated land (*Acaulospora* sp. (a - c), *Gigaspora* sp. (d - f), *Glomus* sp. (g - i)).

4. Conclusion

There is a significant improvement in the biological characteristic of soil in rehabilitation area of nickel post-mining land in Sorowako through time. Arbuscular mycorrhizal form *Acauolospora* sp. and *Gigaspora* sp. are found dominating the area.

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