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# **Mycorrhiza Arbuscular's Morpho-Species Identification in The Post-Nickel Mining Soil**

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#### **ABSTRACT**

Using biological agent microorganisms such as Arbuscular Vesicular Mycorrhiza (AVM) is needed to improve post-mining soil fertility. This research aimed to explore and identify morpho species of AVM in the post-nickel mining areas. Purposive sampling was conducted to survey the soil on 25 blocks in nickel post-mining areas and one spot in a natural forest. Soil samples were taken from the tree rhizosphere at each representative point. The soil samples have been isolated using Brundrett's method of wet sieving and decanting; AVM spore density is determined for each 20 g soil sample; and spores are grouped based on morphological traits up to the morphogenic stage of the species. The results showed that there are four mycorrhizal species found in the rhizosphere of plants in the nickel post-mining soil of PT Vale Indonesia Tbk., namely *Gigaspora* sp, *Acaulospora* sp, *Glomus* sp, and *Scutellospora* sp. *Gigaspora* sp, *Acaulospora* sp, *Glomus* sp, and *Scutellospora* sp obtained 18, 8, 8, and 7 morpho species, respectively. The highest spore density is 34 spores of *Gigaspora* sp4 found in the rhizosphere of *Casuarina rumphiana*, 57 spores of *Acaulospora* sp1 in the rhizosphere of *Macaranga gigantea*, two spores of Glomus sp1, sp2, and sp6 each as many as two spores per 20 g of soil in rhizosphere of *Enterolobium cyclocarpum*, *Dillenia serrata*, *Maesopsis eminii*, 11 spores of *Scutellospora* sp6 in the *Shizigium* sp rhizosphere. One finding revealed that Gigaspora and Acaulospora predominated in the rhizosphere over other mycorrhizas. The results of this AVM identification will be the basis for the mass propagation of local AVM for broader use in the post-nickel mining land area in Sorowako. Widespread use of AVM is a form of post-nickel mining land management based on local resource potential, particularly the dominant trees rhizosphere and the most commonly found AVM types. This proves that AVM can colonize plant roots in this nickel post-mining soil and potentially be mass-propagated, especially *Gigaspora* and *Acaulospora* species.

Keywords: Mycorrhiza arbuscular; Gigaspora; Acaulospora; Glomus; Scutellospora; postnickel mining soil

### **1. INTRODUCTION**

Post-nickel mining soils have decreased in physical, chemical, and biological quality (Thomas et al., 2015). Therefore, technology is needed to improve soil properties to restore their function as a growing medium that supports plant growth and post-mining land rehabilitation activities. Post-mining soils experience damage to soil physical properties, especially the destruction of soil aggregates and the mixing of upper and lower layers (Setiadi & Setiawan, 2011; Ekawati et al., 2016). The destruction of soil aggregates will impact the destruction of pore space between aggregates, affecting the life of organisms in the soil. Sofo et al. (2020) stated that topsoil is a habitat for various microorganisms and soil fauna that support plant growth. These roles include helping to provide nutrients for plants, regulating nutrient cycling in the soil, synthesizing and breaking down soil organic matter, influencing soil water availability, and affecting plant health through parasitism, pathogenicity, or predators.

Plant growth requires sufficient nutrients, both macronutrients and micronutrients. One of the macro elements often not available in nickel post-mining soils is phosphorous (P). Plants obtain P entirely from the soil or fertilization, which results in the decomposition and mineralization of organic matter. Nickel post-mining soil from the reclamation area has low available P content, which is thought to be due to the high levels of exchangeable Al and exchangeable Fe (Neswati et al., 2020; Neswati et al., 2022; Husna et al., 2016). The speed of nutrient uptake by the plant root system depends on the speed at which the plant roots reach the nutrients. In the soil, P nutrients move by diffusion. All factors that play a role in determining the speed of P diffusion to the roots and root development in the soil will determine the availability of P to plants. In plants that are symbiotic with arbuscular vesicular mycorrhiza (AVM), the root absorption area is expanded by the mycelium of AVM so that nutrient absorption, especially P, becomes more available (Satria et al., 2023; Monreal et al., 2011; Huey et al., 2013; Chen et al., 2014; Soka and Ritchie, 2014; Cho et al., 2009; Scheublin et al., 2006; Scheublin et al., 2007). In addition to increasing P, mycorrhizal fungi release various organic acids that can improve the solubility of insoluble phosphate compounds in the soil (Jamal et al., 2018) and increase the capacity of phosphate absorption compared with non-mycorrhizal roots (Plassard and Dell, 2010). Unavailable forms of phosphorus are converted into available forms due to organic acids produced by fungi (Bolduc & Hijri, 2010). The advantage obtained by AVM is that it gets carbon from plants (Basuki, 2013). Based on the benefits of AVM, it is essential to identify morpho-species and numbers of AVM in nickel post-mining areas and then become the basis for developing and propagating AVM in specific areas because mycorrhizal diversity can vary due to environmental factors (Nursanti et al., 2012). Mycorrhiza is a symbiotic relationship between fungi and plants. Mycorrhiza can be found in most soils with plants on the surface. Endomycorrhiza is found in many crops and some forest plants, such as Hopea, Shorea, Eucalyptus, Albizia, Leucaena, and Acacia. This fungus is characterized by forming vesicles in the roots. The utilization of AVM as biological agents is an environmentally friendly biological approach and has been widely developed in the fields of forestry, agriculture,

and plantations (Souza et al., 2013). The advantages obtained by utilizing arbuscular mycorrhizal fungi are that they are safe to use (do not cause environmental pollution) and play an active role in the nutrient cycle. Plants that have been infected with AVM will benefit during the life of the plant.

#### **2. METHODOLOGY**

In the rhizosphere of the tree, soil samples were randomly taken at a depth of 0–25 cm and a radius of 20 cm from the plant, which is located in the block that has been reclaimed and the natural forest. One kilogram of soil samples were collected. After being allowed to air dry condition, the soil samples were brought to the lab for identification, and mycorrhizae was isolated. By combining 50 g of sample soil with 500 ml of water and stirring until the soil grains were suspended, spores were extracted using a pour-filter method based on the Pacioni (1992) method. The mixture was then filtered through a series of filters with filter sizes of 50  $\mu$ m, 150 µm, 250 µm, and 750 µm. A 50 µm, 150 µm, and 250 µm sieve was used to collect the soil. A centrifugation process based on Brundrett et al. (1996) procedure was then used after the filtercasting approach. After adding the filter results, 30 milliliters of distilled water were centrifuged for five minutes at 2,000 rpm. Next came the addition of 15 milliliters of 50% sucrose, followed by a minute-long 2,000 rpm centrifugation. The supernatant was examined under a microscope after being placed on a Petri plate. Mycorrhiza identification includes the number (population) of spores in 20 g of soil, the color of the spores, the ornament that can be observed on the surface of the spore walls, and the diameter of the spores (Figure 1).



Figure 1. Wet Sieving Decanting (from Huey et al., 2020)

In addition to AVM identification, soil samples taken from the research site were also analyzed for soil fertility chemical parameters, namely soil pH and organic C.

## **3. RESULTS AND DISCUSSION**

The presence of mycorrhiza in the rhizosphere area of plants in the nickel post-mining soil and soil from natural forest in the nickel mining concession area of Sorowako South Sulawesi was identified. Four genera of endomycorrhiza were identified: *Gigaspora* sp., *Acalauspora* sp., *Scutellospora* sp., and *Glomus* sp.

## **a.** *Gigaspora* **sp.**

Based on observations, Mycorrhiza in the Gigaspora genus is generally round spores. There is an appendage in the form of a bulbous suspensor. The size of the spore diameter is 230-600 μm. Based on its morphological characteristics, the genus Gigaspora is formed from the rounded end of the hyphae (bulbous suspensor); the color of the spores varies from clear to yellowish and dark yellow. A tiny sphere appears, which gets more extensive to reach the maximum size, which eventually becomes a spore. Gigaspora does not have a molded flexible germination wall (inner wall), and the suspensor is attached to the outermost surface of the spore wall. A distinctive characteristic is the bulbous suspensor without a germination shield. Gigaspora spores form 2 layers of wall, namely spore-wall and germinal-wall.

# **b.** *Acaulospora* **sp.**

Acaulospora spores, based on identification, are produced by a soporiferous saccule derived from the expansion of terminal hyphae. When the spore is fully formed, the contents of the saccule will be transferred into the spore, then the saccule thins, and over time, the saccule will degrade. Spores are usually round, slightly round, or oval. Mycorrhiza has two layers of spore walls and has a cicatrix. Spore diameter size 60-360 μm. The color of the spores when young is hyaline and brownish yellow to brownish-dark red after maturity. Acaulospora spore development starts at the end of hyphae (subtending hyphae) and enlarges like a spore called a hyphal terminus. A small sphere will appear between the hyphal terminus and the subtending hyphae, which gets bigger and bigger, forming a spore. The hyphal terminus will be damaged during its development, and its contents will enter the spore. The destruction of the hyphal terminus will leave a small hole called Cicatric.

# **c.** *Glomus* **sp.**

Based on observations, Glomus spores have round, slightly round, and oval shapes. Has several layers of spore walls. There is a straight, cylindrical hyphal holder (subtending hyphae). Does not have ornaments. Spore diameter size 50-162 μm. Spore color varies from hyaline, off-white, yellow, brownish, yellowish brown, light brown, brownish orange, to dark blackish brown. The

process of spore development is from the tip of the hyphae, which enlarges to the maximum size and forms spores. Spores originating from the development of hyphae are called chlamydospores, sometimes hyphae branches and each branch forms a chlamydospora and a sporocarp. At maturity, the spores are separated from the attached hyphae by a partition; spores are globose, subglobose, ovoid, or obovoid, with spore walls consisting of more than one layer. sp., which is commonly found on average, has a round to oval shape, has a brownish yellow, yellowish brown, dark brown, transparent yellow, and hyaline spore wall; the surface of the spore wall is relatively smooth and has a thick spore wall. However, each species has its characteristics ranging from spherical to oval spore shape.

# **d.** *Scutellospora* **sp.**

Spores are round, have no supporting hyphae, smooth spore surface, spore diameter size ranges from 55 - 600 µm or more, and have brownish yellow spores, hyaline, white, yellow, pink, or gray. sp. has round, slightly round, oval, and sometimes irregular spore shapes with yellow to brownish spore wall colors. The genus Scutellospora sp. has a sprout layer called the germination shield. Scutellospora sp. has a spore size of 120-400 μm.

Based on the results of the identification of mycorrhiza found in the rhizosphere of plants, there are differences in the diversity of spore types found in soil samples taken in the rhizosphere of plants (Table 1). Rhizosphere factors greatly influence the diversity of mycorrhizal genus and populations. This is due to differences in soil characteristics and environmental conditions of each location.

No	Morphology of VMA	Tree Rhizosphere	Spore/ 20 g
	Spores from the genus Acaulospora are yellowish in color and $180-210\mu m$ in diameter; spores form 2 layers of walls, namely spore-wall and germinal-wall. There is an ornament on the spore wall's surface	<b>Macaranga</b> gigantea; Calliandra calothyrsus	57
$\mathcal{D}_{\mathcal{L}}$	Spores of the genus Acaulospora are yellowish and have a diameter of $130-140\mu m$ . They form two layers of walls, namely the spore wall and the germinal wall. On the surface of the spore wall, there are ornaments typical of this genus.	Acacia auriculiformis	12
	Scutellospora genus with clear-colored spores in diameter $170-200\mu m$ . The character of Scutellospora is in the structure of the germination shield and bulbous suspensor.	Cassia siamea	

Table 1. Identification results of AVM species and their abundance in the study site







The observations of the presence of AVM in the rhizosphere showed that Gigaspora was found in 12 types of tree rhizosphere. Acaulospora and Glomus were found in 6 types of the tree rhizosphere. Scutellospora was found in 5 types of tree rhizospheres (Table 2). The genus Gigaspora sp1 was found in the natural forest area with nine spores per 20 g soil sample. This means 45 *Gigaspora* sp1 spores can be found in 100 g of soil. Thus, Gigaspora occupies the tree rhizosphere more dominantly than other mycorrhizas. Table 2 also shows that Scutellospora, although found in 5 types of tree rhizosphere, appears to have spores mostly found in *Eucalyptus urograndis* and *Shizigium* sp., Acaulospora seems dominant in the

rhizosphere of *Macaranga gigantea*, *Casuarina rumphiana*, and *Acacia auriculiformis*. Gigaspora is most commonly found in the rhizosphere of *Casuarina rumphiana* and *Shizigium* sp. This proves that AVM can colonize plant roots in this nickel post-mining soil and potentially be mass-propagated, especially Gigaspora and Acaulospora species. This result aligns with the research of Husna et al. (2017), who found that AVM can colonize plant roots on serpentine soils and various types of soils (Entz et al., 2004).

<b>Tree</b>	Gigaspora	Acaulospora	Glomus	Scutellospora	Number of
Rhizosfer					spores
Alstonia	12	14	$\overline{2}$		3
macrophylla					
Cassia siamea	14			$\mathbf{1}$	$\overline{2}$
Paraserianthes		4	$\overline{2}$		$\overline{2}$
falcataria					
Casuarina	60	29	$\overline{2}$	$\overline{2}$	$\overline{4}$
rumphiana					
Eucalyptus	3		$\mathbf{1}$	11	3
urograndis					
Macaranga gigantea		57			$\mathbf{1}$
Acacia		28			1
auriculiformis					
Calophyllum	3				1
soulatrii					
Dillenia serrata	10		$\overline{2}$	$\overline{4}$	3
Enterolobium	$\overline{2}$				1
cyclocarpum					
Vitex coffasus	4	$\overline{2}$			$\overline{2}$
Diospyros celebica	3				$\mathbf{1}$
Maesopsis eminii	3		$\overline{2}$		$\overline{2}$
Weinmania celebica	$\overline{4}$				1
Shizigium sp.	26			17	$\overline{2}$
Rhizosfer wood type	12	6	6	5	
Number of spores*	156	140	17	40	

Table 2. A Number of AVM spores in the various tree rhizosphere

Note: \*Number of spores per 20 g of soil

Based on the soil analysis results in Table 3, the soil pH in the rhizosphere where AVM was obtained ranged from acidic (pH 5.14) to slightly acidic (6.22). Thus, four mycorrhizal genera that react somewhat acidic in soil conditions are still quite good. This can be seen in *Acaulospora* sp1, with a number of spores 57 at pH 5.58 (slightly acidic), and Gigaspora sp4, with a number of spores 34 (pH 5.72), classified as slightly acidic. According to Selvaraj et al. (2001), Gigaspora, Scutellospora, and Acaulospora are generally only found in soils with acidic pH, while Glomus can be found in soils with acidic to neutral pH.

	Soil pH		% Organic C			$\Sigma$ spore/20
No	H <sub>2</sub> O	Criteria*	Walkley & <b>Black</b>	Criteria**	Morpho Species	g
$\mathbf{1}$	5.58	<b>SA</b>	1.36	L	Acaulospora sp 1	57
$\overline{2}$			1.79	L	Acaulospora sp2	12
	5.45	$\mathbf{A}$			Scutellospora sp1	$\mathbf{1}$
3	5.79	<b>SA</b>	1.65	L	Acaulospora sp3	23
					Gigaspora sp2	$\overline{c}$
					Scutellospora sp2	$\overline{2}$
$\overline{4}$	5.83	<b>SA</b>	1.13	L	Acaulospora sp4	16
5	5.51	$\mathbf{A}$	1.54	L	Gigaspora sp3	12
6	5.72	<b>SA</b>	1.25	L	Gigaspora sp4	34
$\overline{7}$	5.14	$\mathbf{A}$	1.08	L	Gigaspora sp5	3
8	5.11	A	1.42	L	Gigaspora sp6	12
					Gigaspora sp7	10
9	5.57	<b>SA</b>	1.17	L	Scutellospora sp3	$\mathbf{1}$
					Scutellospora sp4	3
					Glomus sp1	$\overline{2}$
10	6.05	<b>SA</b>	1.05	L	Gigaspora sp8	11
11	5.91	<b>SA</b>	1.36	L	Gigaspora sp9	$\overline{2}$
	5.65	<b>SA</b>	1.17	L	Gigaspora sp10	$\overline{4}$
12					Acaulospora sp5	$\overline{2}$
13	5.51	$\mathbf{A}$	1.29	L	Gigaspora sp11	$\overline{3}$
14	6.22	<b>SA</b>	0.74	<b>VL</b>	Gigaspora sp12	3
					Scutellospora sp5	11
	5.95	<b>SA</b>	1.22	L	Glomus sp2	$\overline{2}$
15					Gigaspora sp13	$\overline{2}$
					Gigaspora sp14	$\mathbf{1}$
16	5.28	<b>SA</b>	0.84	<b>VL</b>	Gigaspora sp15	$\overline{\mathcal{A}}$
	5.48	A		L	Gigaspora sp16	$\overline{3}$
17			1.09		Scutellospora sp6	11
18	5.88	<b>SA</b>	0.93	<b>VL</b>	Gigaspora sp17	23
					Scutellospora sp7	6
19	5.43	A	1.44	L	Glomus sp3	1
					Glomus sp4	$\mathbf{1}$
20	6.12	<b>SA</b>	1.26	L	Gigaspora sp18	14
21	6.10	<b>SA</b>	1.33	L	Acaulospora sp6	$\overline{4}$
22	5.44	$\mathbf{A}$	1.23	L	Acaulospora sp7	6
					Gigaspora sp19	$\mathbf{1}$
23	5.88	<b>SA</b>	1.17	L	Glomus sp5	$\mathbf{1}$ $\overline{2}$
24	6.16 6.00	SA <b>SA</b>	1.55 1.50	L ${\bf R}$	Glomus sp6	14
25					Acaulospora sp8 Glomus sp7	1
					Glomus sp8	$\mathbf{1}$

Table 3. Relation between soil pH, organic C, and a number of AVM spores

Description: \*A=Acid; SA= Slight Acid; \*\*L=Low; VL=Very Low

The content of soil organic matter based on the results of C-Organic analysis in various rhizospheres of plants in the study site shows a very low to low category. Although categorized as very low or low, it is not a limitation to the presence of mycorrhizal spores. According to Vedere et al. (2022), Yustika & Muchtar (2016), and Murindangabo et al. (2023), the amount of organic matter affects soil moisture status because one of the roles of organic matter is to increase the soil's ability to retain water. In moist soil conditions, the mycorrhizal sporulation process becomes lower, so the number of spores in the soil is also tiny. According to Zarei et al. (2010), mycorrhizal spore populations tend to decrease with increasing soil moisture. Likewise, according to Proborini et al. (2017), the presence of endomycorrhizal spores in nature tends to decrease in number during the rainy season because some of these spores have germinated to form hyphae in the soil. This proves that the existence of AVM is also influenced by the condition of the environment (Huey et al., 2013; Ortas et al., 2018).

### **4. CONCLUSION**

In the nickel post-mining soil, four mycorrhizal genus were found: Gigaspora sp., Acaulospora sp., Glomus sp., and Scutellospora sp. Morpho species of Gigaspora, Acaulospora, Glomus and Scutellospora were 18, 8, 8, and 7, respectively. The observations of the presence of AVM in the rhizosphere of trees showed that Gigaspora was found in 12 types of tree rhizosphere. In the natural forest area, the genus Gigaspora sp1 was found. Gigaspora occupies the tree rhizosphere more dominantly than other mycorrhizas. Gigaspora is most commonly found in the rhizosphere of *Casuarina rumphiana* and *Shizigium* sp. Acaulospora appears dominant in the rhizosphere of *Macaranga gigantea*, *Casuarina rumphiana*, and *Acacia auriculiformis*. Scutellospora, primarily found in *Eucalyptus urograndis* and *Shizigium* sp., Glomus dominant found in *Casuarina rumphiana, Dillenia serrata* and *Maesopsis eminii.*

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