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Distribution of Arbuscular Mycorrhiza Fungi in different plants within tailings deposition areas of Freeport, Timika, Central Papua, Indonesia

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Abstract. *Djuuna IAF, May NL, Kubangun SH, Massora M, Aibini SW. 2023. Distribution of Arbuscular Mycorrhiza Fungi in different plants within tailings deposition areas of Freeport, Timika, Central Papua, Indonesia. Biodiversitas 24: 4515-4522.* This study investigated the presence of Arbuscular Mycorrhiza Fungi (AM fungi) in various vegetation types within tailings deposition areas in Freeport Timika, Papua. Soil and root samples were collected from 41 sampling locations, representing 28 types of vegetation growing in these areas. AM fungi spores were extracted using the wet sieving technique, while root samples were cleaned, stained, and analyzed for the percentage of root infection using the gridlines method. Some soil characteristics were also analyzed, including pH (H_2O), moisture content, organic carbon (C), Total nitrogen (N) and phosphorus (P), and soil texture. The study showed low total soil N and P levels, with organic C ranging from low to high and a pH range from 6 to 6.7. The spore number ranged from 2 to 20 spores/10 g soil, and the percentage of root colonization varied from 17 to 89.5%. The highest number of spores (20 spores/10 g soil) was found in the rhizosphere of *Homalanthus* sp. and *Polyalthia glauca*, while the highest percentage of root colonization (89.5%) was observed under *Pandanus* sp. at Upper ADA. Notably, the highest number of spores did not correspond to the highest percentage of infected roots. Additionally, four genera of AM fungi, namely *Glomus*, *Acaulospora*, *Scuttelospora* and *Gigaspora*, were found to thrive in tailings areas and have the potential to be developed as biofertilizers for tailings rehabilitation programs.

Keywords: Arbuscular mycorrhiza fungi, gold tailings, vegetation

INTRODUCTION

Freeport Indonesia is one of the largest mining companies in Indonesia, involved in the mining, processing, and exploration of copper and gold ores in the highlands of Mimika, Papua. The mining process generates tailings or leftover sand, resulting from fine rock after copper and gold are obtained through the flotation process at the ore processing operations. These tailings contain the highest mineral content with physical characteristics ranging from coarse to medium and very fine textures, affecting soil formation (PTFI 2007). The soil derived from these tailings has very low organic matter and a limited amount of nutrients. Taberima et al. (2010) reported that tailings contain only 0.02% of total N (very low), ≤20 me/100g (low to medium) of cation exchange capacity (CEC), 0.1-2% of organic C, and a pH value from 7 to 8. Moreover, the nutrients in tailings soil are not in a form that is readily absorbed by the plants, resulting in a very low soil fertility level. Therefore, the natural revegetation process in Freeport Indonesia work areas is significantly affected in both lowland and highland areas. Therefore, to support the revegetation in these areas, it is necessary to implement various strategies using existing technologies. One method is the application of Arbuscular Mycorrhiza (AM) Fungi as a biofertilizer source.

Arbuscular Mycorrhiza Fungi (AM fungi) are ubiquitous in soils and establish symbioses with the roots of about 85% of all terrestrial plants. These Fungi are important in enhancing the plants' uptake of soil nutrients, particularly phosphorus (Smith and Read 2008). This symbiotic association is thus generally considered beneficial for the host plant's growth and development under limited phosphate availability. AM fungi are essential components that promote plant growth, especially in post-mining sites (Gardner and Malajczuk 1988; Wang 2017; Husna et al. 2019; 2021). Furthermore, it is important in the agricultural growth, productivity, and quality (Chen et al. 2018) of plantation and forestry crops, especially those cultivated on less fertile soils. It has an essential biological role and contributes to improving nutrition and plant growth (Diagne et al. 2020), biological protection (Azcón-Aguilar and Barea 1996; Rouphael et al. 2015; Lin et al. 2021), plant resistance to drought (Abdel-Salam et al. 2018; Begum et al. 2019; Li et al. 2019; Zhang et al. 2019), synergy with other microorganisms particularly bacteria (Christensen and Jacobsen 1993; Artursson et al. 2005), as well to maintain plant diversity in different ecosystems (Heijden et al. 1998; Klironomos et al. 2000; Bagyaraj and Revanna 2017; Horn et al. 2017; Zhaoyong et al. 2017), and to improve soil quality through its structure, texture, and plant health (Zou et al. 2016; Thirkell et al. 2017). These fungi also assist the revegetation process by increasing mineral solubility,

nutrient uptake, binding soil particles into stable aggregates, and increasing tolerance to drought and heavy metal toxicity (Hildebrandt 2007; Amir et al. 2013; Asmelash et al. 2016; Chen et al. 2018; Husna et al. 2019). In addition, they enhance plant growth on critical and degraded lands (Al-Karaki 2013), including those affected by mining activities. Several studies have previously been conducted on AM fungi in Freeport Indonesia's Modified Ajkwa Deposition Areas (Mod ADA). These studies have focused on various aspects, such as the examination of the spatial distribution of AM fungi (Djuuna et al. 2010), Fungi associated with dominant plant growth in Mod ADA (Suharno et al. 2014), the association of AM fungi with Fern (Suharno et al. 2016), and the association of AM fungi with *Brachiaria precumbens* (Poaceae) (Suharno et al. 2017). This study examined the highest number of plants (28 types) at three different areas of Mod ADA, including Upper, Middle, Lower Mod ADA, and Mile Point 21 (MP 21), characterized by different soil textures and plant types. The aim of this study was to assess the distribution and variability of AM fungi in the soils and roots of plants growing under varying soil textures in the tailings sites of Mod ADA and MP 21.

MATERIALS AND METHODS

Soil and root sampling

Soil and plant root samples (0-20 cm) were collected from multiple locations within the Mod ADA areas, particularly at the Upper, middle, and lower ADA and MP 21. Therefore, 41 sampling points were established, encompassing 28 distributions of existing plant species, as presented in Figure 1. Subsequently, soil and root samples were taken to the laboratory for further preparation and analysis.

Soil analysis

Soil samples obtained from all sampling locations were analyzed to determine various chemical and physical properties, including soil pH (H_2O), organic C (%) (Walkley and Black), Total N (%) (Kjeidahl), and P (ppm) (Bray I), soil moisture content (%) through the gravimetric method, and soil texture using a hydrometer.

Mycorrhiza bioassay method

Mycorrhiza bioassays measure the infectivity in the soil at a point in time, indicating the potential of AM Fungal propagules present to colonize plant roots. The 41 composite soil samples collected from Mod ADA and MP 21 areas were used as growth media for *Zea mays* (corn) in plastic pots containing 500 grams of soil. The soil was consistently watered to maintain field capacity for the bioassay duration. Each pot was initially sown with five seeds of corn and then thinned to two plants per pot. The pots were then randomized in blocks and placed in a glasshouse for maintenance. After six weeks from seedling emergence, plants were harvested, and the roots were further assessed for AM Fungal colonization.

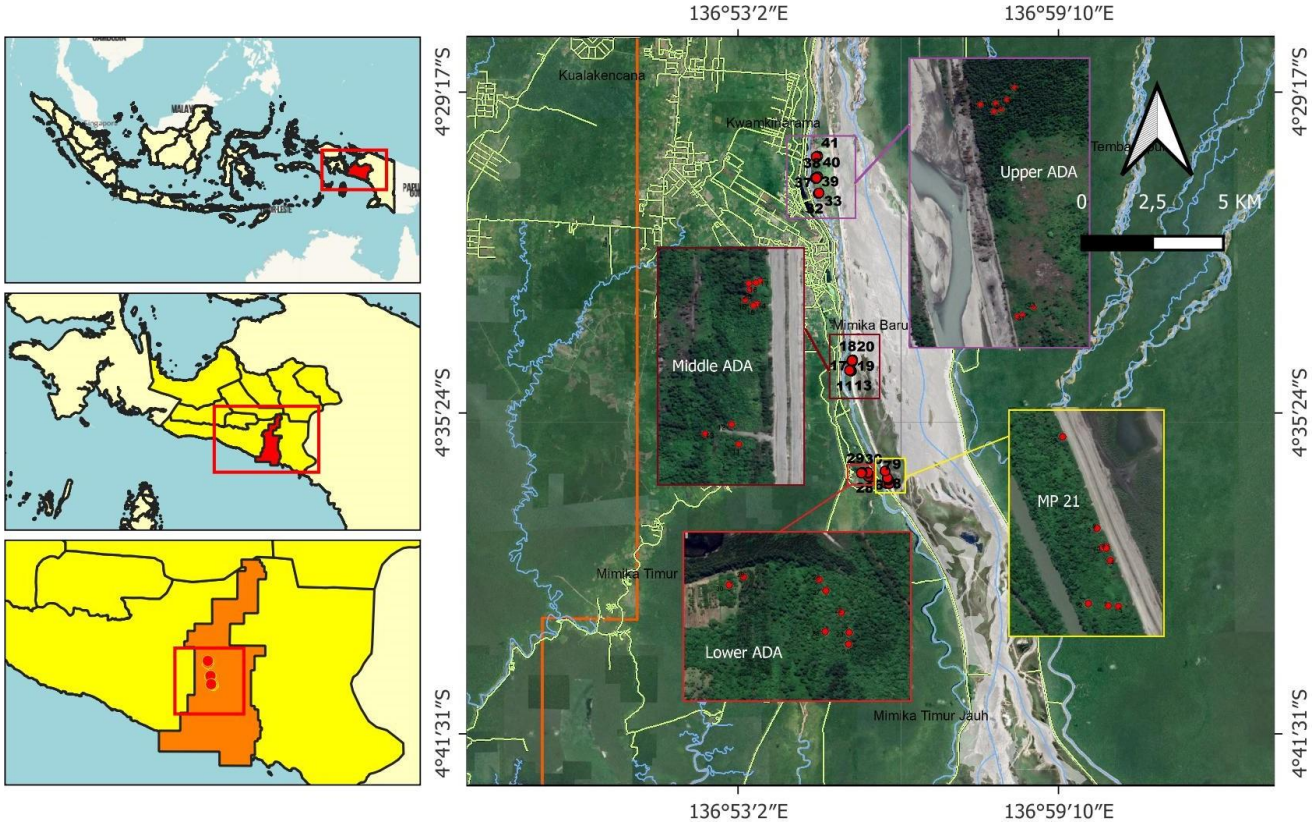

Figure 1. Sampling location of soils and roots at Mod ADA areas and MP 21 of Freeport Indonesia of Timika District, Central Papua Province, Indonesia

Isolation and extraction of AM fungal spores

AM fungal spores were isolated and extracted from soil samples using the wet Sieving and decanting technique (Gederman and Nicholson 1963). For this procedure, 10 g of soil was added to 100 mL of water and mixed vigorously with a glass rod for about 30 seconds to free AM fungi spores from the soil. The suspension was then allowed to settle for 10-15 seconds before gradually decanting it through standard sieves with mesh sizes of 300 um, 125 um, 106 um, and 63 um, respectively. The contents retained on each sieve were decanted into separate Petri dishes using a wash bottle for further processing. Subsequently, the number of spores found in each sample was calculated as the total number per 10 grams of soil.

Morphological observation of spores for identification

AM fungi was identified by determining the spores' morphology, which involved placing the spores in a Petri dish. Spores were carefully selected using a dissecting microscope with tweezers and prepared as AM fungi specimens using the Polyvinyl-lacto-glycerol (PVLG) reagents (Koske and Tessier 1983). The specimens were then examined under a compound microscope for further identification. AM fungi spore was identified based on the Gedermann and Trappe identification guide (1974). Furthermore, morphotypes of AM fungi present in samples were identified up to the genus level (Abbott 1982).

Assessment of AM fungi colonization

Plant roots collected from the field and bioassay plant, particularly fine roots, were washed thoroughly until no soil was attached. The roots were then cleared and stained with Trypan Blue, following the method by Abbott and Robson (1981). The stained roots were examined under a dissecting microscope to assess the percentage of root length infected. The presence or absence of AM fungi that intersected the grid in the field of view was recorded for 100 intercepts, and the root length was estimated (Newman 1966). The percentage of roots infected by AM fungi was calculated using the formula by Brundrett et al. 1996 as follows:

(Total number of fields of view colonized/Total observed field of view) x 100%

RESULTS AND DISCUSSION

Number of spores and percentage of roots infected by AM fungi

Table 1 shows that the average number of AM Fungal spores in plants' root areas (rhizosphere) across all sampling locations were classified as low and ranged from 2 to 20 spores/10 g of soil. The highest number of spores was found in the Upper ADA (8.7 spores/10 g of soil), followed by the Lower ADA (7.6 spores), Middle ADA (7.2 spores), and the lowest in the MP 21 areas (7.2 spores). Moreover, based on plant types, a high number of AM fungi spores was observed in the rhizosphere of *Homalanthus sp* (Middle ADA) and *Polyalthia glauca* (MP 21), compared to other types of plants. The lowest spore numbers were found in *Casuarina litorea, Rhus taitensis, Campnosperma brevipetiolatum* (Middle ADA) and *Ficus benjamina* (MP 21), with the low number attributed to the soil properties of tailings, which lack organic matter and some soil nutrients.

AM fungi colonization

Based on the examination of the field root samples, the percentage of root colonization by AM fungi ranged from low to high, with values of 17.2 to 89.5%. On average, the Middle ADA had the highest percentage of root infection (72.78%) compared to the other sampling areas, while the lowest was in the Lower ADA areas (42.1%). Based on the type of plant, the highest percentage of root infection by AM fungi (>80%) was found in the rhizosphere of *Ficus elastica, Maccaranga aleuritoides, Glochidion macrocarpus,* and *Ficus armitii* in the Middle ADA, *Euodia elleryana* and *Pandanus* sp. in the Upper ADA, and *Decaspermum fruticosum* and *Polyalthia glauca* in the MP 21. Although the percentage of infection varied among different plants, all plants examined were infected by AM fungi, indicating a well-developed association between AM fungi and plant roots growing in the tailings soil of Mod ADA and MP 21 areas. Similar trends were observed in the root infection of AM fungi using the bioassay method. The percentage of root infection in the bioassay plant was slightly higher compared to the field sample plant, which ranged from 22.22% to 94.52%. However, based on the sampling location, the average percentage of infection was highest in the Middle ADA areas (75.77%), followed by the Upper ADA (68.14%) and MP 21 (67.13%), while the lowest was in the Lower ADA (47.02%). Most of the soil samples in the bioassay experiment exhibited high levels of AM Fungal root infection.

AM fungi spore morphotypes

The identification process revealed the presence of several spore types in all observation locations, with the majority belonging to the genus Glomus, along with some from the Acaulospora, Scutellospora, and Gigaspora genera (Table 1). Glomus was found in all sampling locations and plant rhizospheres (Figure 2), and the spore morphotypes were more diverse in Mod ADA areas than in MP 21.

The presence of spores and the percentage of root infection by AM fungi at all sampling locations indicate that the Mod ADA and MP 21 tailings areas are suitable habitats for the growth and development of AM fungi.

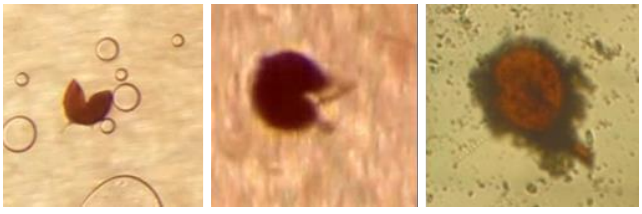

Figure 2. Representation of Glomus Spores Morphotypes in study areas of Mod ADA

Table 1. Number of spores and percentage of roots infected by AM fungi at Mod ADA and MP 21 areas

Sampling location/ plant	Spore numbers/ 10 g of soil	% AM fungi	% root infected (bioassay)	AM fungi spores morphotypes
Lower ADA				
Pandanus sp.	10	53.8	58.82	Glomus
Casuarina litorea	6	48.3	53.33	Glomus
Octomeles sumatrana	12	36.3	41.28	Glomus
Rhus taitensis	6	17.2	22.22	Glomus, Scutellospora
Campnosperma brevipetiolatum	6	61.9	65.96	Glomus, Scutellospora
Ficus elastica	4	46.6	51.58	Glomus, Scutellospora, Acaulospora
Maccaranga aleuritoides	8	55.5	60.53	Glomus, Scutellospora
Glochidion macrocarpus	16	41.2	46.24	Glomus, Scutellospora, Acaulospora
Homalanthus sp	12	32.2	37.23	Glomus
Ficus armitii	6	28.0	32.99	Glomus, Scutellospora, Acaulospora
Mean	7.6	42.1	47.02	
Middle ADA				
Pandanus sp	6	55.9	70.37	Glomus
Casuarina litorea	2	59.0	72.41	Glomus
Campnosperma brevipetiolatum	2	67.3	72.31	Glomus, Scutellospora
Paraserianthes falcataria	12	67.4	60.87	Glomus
Ficus armitii	12	85.9	90.91	Glomus, Scutellospora, Acaulospora
Alstonia scholaris	2	65.4	64.04	Glomus, Scutellospora
Terminalia catappa	4	86.6	91.58	Glomus, Scutellospora, Acaulospora
Artocarpus communis	8	81.0	86.05	Glomus, Scutellospora
Premna corymbose	4	81.3	86.27	Glomus, Scutellospora, Acaulospora
Malotus philppinensis	20	73.0	78.02	Glomus
Mean	7.2	72.78	75.77	
Upper ADA				
Maccaranga aleus	8	74.5	94.52	Glomus, Acaulospora
Euodia elleryana	4	88.9	48.00	Glomus, Gigaspora
Alstonia scholaris	14	73.9	70.51	Glomus
Piper aduncum	8	43.1	82.08	Glomus, Scutellospora
Rhus taitensis	10	77.1	70.18	Glomus
Campnosperma brevipetiolatum	6	65.2	79.47	Glomus
Pandanus sp	12	89.5	93.94	Glomus
Octomeles sumatrana	8	65.5	78.87	Glomus, Acaulospora
Cassuarina litorea	6	43.0	48.10	Glomus, Scutellospora
Pometia pinnata	12	42.0	47.01	Glomus, Acaulospora
Leucaena glauca	8	31.8	36.84	Glomus, Gigaspora
Mean	8.7	63.14	68.14	
Mile Point (MP) 21				
Pandanus sp	4	31.2	92.20	Glomus, Acaulospora
Campnosperma brevipetiolatum	8	67.1	83.33	Glomus
Timonius timon	4	74.4	36.21	Glomus, Scutellospora
Schefflera sp	16	52.9	72.09	Glomus
Decaspermum fruticosum	4	80.1	79.38	Glomus
Ficus benjamina	2	78.3	57.89	Glomus
Polyalthia glauca	20	87.2	85.14	Glomus
Maccaranga aleus	6	35.3	40.30	Glomus
Metroxylon sagu	4	68.1	73.08	Glomus
Elaesis guineensis	6	46.7	51.72	Glomus
Mean	7.2	62.13	67.13	

Soil properties

The physical and chemical properties of the soil from all sampling locations in the Upper, Middle, Lower Mod ADA, and MP 21 areas are presented in Table 2. It was observed that the pH ranged from 5.2 to 6.7 (acid to neutral), with an average soil pH of 6.0 (slightly acidic). The organic C content ranged from 0.40 to 7.10% (very low to very high), with the Lower ADA observation site exhibiting a very high organic C content, followed by Upper ADA (low), Middle ADA, and MP 21 (very low). The total N at all observation locations ranged from very low to high, with an average of very low to low. Soil P content in all locations was generally low, ranging from 3.20 to 17.20 ppm, with the Lower ADA showing the highest average soil P value compared to other locations. Soil water content at all sampling locations ranged from 37.3 to 86.2%, and most of the areas comprised coarse particle sizes classified as loamy to loamy sand in terms of texture. Generally, the soils in tailings areas are classified as poor in nutrients and organic matter.

Table 2. Soil properties in the Mod ADA areas of Upper, Middle, Lower, and MP 21

Sampling location/ plant types	pH (H_2O)	C-org (%)	N-Total (%)	P (ppm)	Moisture content (%)	Texture class
Lower ADA						
Pandanus sp	5.8	6.94	0.47	16.50	85.9	Loamy
Casuarina litorea	5.8	0.80	0.08	5.70	86.2	Loamy
Octomeles sumatrana	5.2	6.14	0.43	14.50	86.1	Sandy clay loam
Rhus taitensis	5.2	3.75	0.34	10.50	85.9	Sandy clay loam
Campnosperma brevipetiolatum	5.6	5.98	0.45	14.80	84.9	Sandy clay loam
Ficus elastica	5.5	7.10	0.53	17.20	86	Sandy clay
Maccaranga aleuritoides	5.9	6.86	0.48	15.60	86.2	Sandy clay loam
Glochidion macrocarpus	5.8	6.62	0.48	15.10	86	Sandy loam
Homalanthus sp	6.0	0.88	0.09	6.50	86.2	Sandy clay
Ficus armitii	6.3	5.58	0.49	15.40	41	Sandy clay loam
Mean	5.71	5.06	0.38	13.18	62	
Middle ADA						
Pandanus sp	5.8	0.71	0.06	4.10	38.2	Sandy loam
Cassuarina litorea	6.4	0.48	0.05	3.60	41.1	Loamy sand
Campnosperma brevipetiolatum	5.8	0.40	0.06	3.20	44.1	Sandy loam
Paraserianthes falcataria	5.8	0.88	0.08	4.50	41.2	Sandy loam
Ficus armitii	5.6	0.48	0.05	3.40	37.9	Sandy loam
Alstonia scholaris	6.2	0.64	0.06	4.40	39.3	Sandy loam
Terminalia catappa	6.5	0.55	0.06	3.70	45.2	Sandy loam
Artocarpus communis	6.5	0.72	0.07	4.50	38.2	Sandy loam
Premna corymbose	5.3	0.80	0.08	4.70	37.8	Sandy loam
Malotus philppinensis	6.1	1.51	0.13	5.20	41.7	Sandy loam
Mean	6.0	0.71	0.07	4.13	40.47	
Upper ADA						
Maccaranga aleus	6.3	1.20	0.11	6.00	86.1	Sandy clay loam
Euodia elleryana	6.2	1.51	0.14	6.50	86.2	Sandy clay loam
Alstonia scholaris	5.8	6.06	0.57	15.90	86.1	Sandy clay loam
Piper aduncum	5.9	3.03	0.29	13.80	40.8	Loamy sand
Rhus taitensis	5.8	1.75	0.18	6.30	41.7	Sandy loam
Campnosperma brevipetiolatum	6.3	3.27	0.29	7.50	44	Loamy sand
Pandanus sp	6.3	2.63	0.27	6.50	40.1	Sandy loam
Octomeles sumatrana	6.1	0.95	0.08	5.20	37.5	Loamy sand
Cassuarina litorea	6.7	1.75	0.16	5.80	37.3	Loamy sand
Pometia pinnata	6.0	1.11	0.12	7.00	40.2	Loamy sand
Leucaena glauca	6.2	2.95	0.27	13.0	45	Loamy sand
Mean	6.1	2.32	0.25	9.41	53.18	
MP 21						
Pandanus sp	6.1	0.71	0.07	4.70	86	Loamy
Campnosperma brevipetiolatum	6.6	0.55	0.06	3.90	85	Loamy
Timonius timon	6.4	0.64	0.06	4.40	40.2	Sandy loam
Schefflera sp	6.4	0.95	0.09	5.30	39	Sandy loam
Decaspermum fruticosum	6.5	1.12	0.10	5.80	37.4	Sandy loam
Ficus benjamina	6.2	1.03	0.10	4.50	39.8	Sandy loam
Polyalthia glauca	6.3	0.95	0.10	4.10	86.1	Sandy clay loam
Maccaranga aleus	6.5	1.20	0.10	6.00	85.9	Sandy clay loam
Metroxylon sagu	6.2	1.27	0.13	5.70	86	Sandy clay loam
Elaesis guineensis	6.1	0.64	0.06	5.30	86	Loamy
Mean	6.3	0.93	0.09	4.97	65.0	

Discussion

The results indicate that certain soil chemical properties across the study areas were low. This is consistent with the study by Taberima et al. 2010 reported that low total N, Organic-C, CEC, K, and Na in tailings, low to medium Ca and Mg, high levels of available P and Base Saturation, and a pH range from acid to neutral. Suryanto and Susetyo 1997 stated that tailings soil has a coarse texture, low water retention, low soil chemical properties, low CEC, and lacks both inorganic and organic colloids while containing heavy metal elements (Setyaningsih 2017). Low soil chemical properties contribute to lower soil fertility levels on tailings land. In addition, the low soil pH in tailings areas should affect the number and population of soil microbes, including AM fungi. Essentially soil microbes tolerant to soil pH would be present, as most soil microbes prefer a pH level close to neutral; at this pH level, plants grow well and produce more root exudates as a carbon source for survival and multiplication of microbes. Soil characteristics have been found to influence the activity of AM fungi in the soil

(Alguacil 2016), including tailings soil. The abundance of AM fungi is related to soil phosphorus level (Jacobsen et al. 2001; Yang et al. 2014; Higo et al. 2020) and can be influenced by soil moisture (Deepika and Kothamasi 2014; Bhardwaj and Chandra 2018), soil texture, and aggregation (Bearden and Peterson 2000; Rillig and Mummey 2006; Leifheit 2013). The low number of AM fungi spores in the study areas is also attributed to the soil properties of tailings and the type of plant. Zhu et al. 2020 pointed out that environmental factors, such as soil organic C, N, P, and pH, influence AM fungi's quantity and variety. The low number of spores and diversity may also be due to the inhibition of AM fungi developmental processes like spore germination, sporulation, colonization, hyphae extension, and arbuscular formation. Notably, all plant samples grown in Mod ADA and MP 21 areas were colonized by AM fungi, and these 28 plant types exhibited robust growth and adaptability in tailings conditions. The highest number of AM fungi spores were found in the rhizosphere of *Homalanthus sp* and *Polyalthia glauca*. The presence of different plant types also affected the abundance of AM fungi (Govindan et al. 2019).

Bagyaraj and Revanna (2017) stated that plant biodiversity and ecosystem productivity increase with the increasing number of AM fungi. That indicates that the diversity of AM fungi in the soil is important in maintaining plant biodiversity and ecosystem functioning. In the bioassay plants, the root infection percentage was slightly higher than in the field sample plant. Mycorrhizal bioassays measure the infectivity of arbuscular mycorrhizal (AM) fungal propagules in the soil at one point or over time if sequential harvests are included. However, the bioassay environment may differ from the field conditions. Djuuna et al. (2009) explained that Bioassays have the potential to assist in predicting how roots might become colonized in field soils, but calibrations are necessary. The number of spores in this study (2-20 spores/10 gram of soil) are slightly higher than those reported by Suharno et al. (2016), which ranged from 8 to 12 spores/10 gram of soil. The lower number of AM fungi spores in the MP 21 areas may be attributed to the conversion of some areas into agroecosystem crops that significantly affect AM spore numbers and their diversity. Furthermore, AM fungi diversity and spore numbers are relatively higher on newly developed plant communities (natural succession) than on agroecosystems crops (Hatfindo 1998).

AM fungi have potential roles in restoring and improving degraded soils, including tailings, and promoting plant growth and survival under mining conditions (Jasper et al. 1988; Juge et al. 2021). However, mining activities reduce AM fungi diversity in mining-impacted sites (Husna et al. 2015; Yang et al. 2015; Wang 2017).

The dominant AM fungi morphotypes across the study were from the genus of *Glomus*, followed by *Scuttelospora*, *Acaulospora*, and *Gigaspora*. This finding is consistent with the study by Suharno et al. (2017) in tailings areas of ModADA, except for the genus Gigaspora. Husna et al. (2015) reported that Glomus is a genus of AM fungi that is tolerant and adaptive to different soils and environmental conditions, indicating that it survives in varying pH conditions. Samal et al. (2023) also found that the genus Glomus had the highest spore abundance on post-mining land with *Arenga pinnata*.

In conclusion, the number of AM fungi spores across the study areas was classified as low, which may be attributed to the low soil nutrients and organic matter of tailings. The percentage of root colonized by AM fungi was high for most plant species, including the bioassay plant. AM fungi colonized all plants grown in Mod ADA and MP 21 tailings areas, indicating that the Mod ADA and MP 21 tailings areas are suitable habitats for the growth and development of AM fungi. However, it is essential to implement more tailings management that involves the application of organic matter to increase AM fungi diversity. Four morphotypes of AM fungi, namely *Glomus* sp., *Scuttelospora* sp., *Acaulospora* sp., and *Gigaspora* sp., were identified, which have shown promising growth in tailings areas and have the potential to be developed as biofertilizers for tailings rehabilitation program.

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