## Phosphorus Availability Affecting the Diversity of Arbuscular Mycorrhizal Fungi (AMF) in the Artisanal Gold Mining Area

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#### Abstract

Arbuscular mycorrhizal fungi (AMF) diversity is influenced by biotic and abiotic factors. Several studies have shown the presence of AMF in ex-gold mining areas with low soil fertility and heavy metal accumulation. The purpose of this study was to analyze the diversity of AMF species in artisanal gold mining areas. The experimental design used is randomized group design with two treatment factors: the interval distance range from tailings disposal point (A) and the vegetation type (B). Interval distance treatment consisted of two levels (A1: 0-10 m and A2: 10-20 m). Vegetation type treatment (B) consists of three levels (B1: Theobroma cacao, B2: Mangifera indica, B3: Artocarpus heterophyllus). Some types of AMF spores found are Acaulospora sp. 1, Acaulospora sp. 2, Acaulospora sp. 3, Acaulospora sp. 4, and Acaulospora sp. 5. The index of species richness and diversity of AMF species in the artisanal gold mining area is low. The existence of AMF symbiosis with plants in artisanal gold mining areas is also indicated by the presence of colonization in the roots of T. cacao, M. indica, and A. heterophyllus with a low to medium category. Pearson correlation test results showed that AMF species diversity index and P availability were not correlated (r = -0.204, p-value = 0.699). Pearson correlation test results also show that AMF colonization and P availability are not correlated (r = -0.756, p-value = 0.082). Although not correlated, based on the graph, it can be seen that the higher the available P element, the smaller the index value of species diversity and AMF colonization.

Keywords: diversity of mycorrhiza, mining land, mycorrhizal colonization, soil properties

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#### Introduction

Arbuscular mycorrhizal fungi (AMF) is a group of fungi that belong to the phylum Glomeromycota, forming symbiosis with the roots of about 80–90% of all types of terrestrial plants (Smith & Read, 2008). The phylum Glomeromycota has four orders, namely Glomerales, Archaeosporales, Diversisporales, and Paraglomerales. This group consists of 11 families and 18 genera (Schüßler & Walker, 2010). AMF has internal hyphae, vesicles, and arbuscular structures in the roots of AMF-infected plants (Brundrett et al., 1996). AMF plays a role in increasing the absorption of nutrients for plants, plant resistance to biotic and abiotic stresses, and heavy metal tolerance in plants (Janeeshma & Puthur, 2020).

AMF diversity is influenced by biotic and abiotic factors (Ma et al., 2023). AMF diversity is influenced by soil chemical properties such as pH and P availability (Bainard et al., 2014; Tuheteru et al., 2020). AMF diversity is also influenced by the type of host plant (Rasmussen et al., 2022).

AMF has the potential to form symbiosis with various types of host plants (Smith & Read, 2008). Several studies found that AMF is able to form symbiosis with several plants in former gold mining areas. Symbiosis of AMF with *Casuarina equisetifolia* and *Ficus adenosperma* plants was

found in mining areas such as PT Freeport Indonesia (Suharno et al., 2014). AMF was found in symbiosis with *Euphorbia* sp. and *Acacia mangium* on ex-gold mining area in Bombana, Southeast Sulawesi (Tuheteru et al., 2023).

Several types of AMF were found in ex-gold mining area despite heavy metal accumulation (Spruyt et al., 2014; Suharno et al., 2014; Tuheteru et al., 2020). AMF species found in gold mine tailings deposition in Timika, Mimika Regency, Central Papua, are Clariodeoglomus etunicatum and C. lamellosum (Suharno et al., 2017). AMF species found in former small-scale gold mining and artisanal gold mining in North Rarowatu District, Bombana Regency, Southeast Sulawesi Province, are Acaulospora scrobiculata, A. tuberculate, Entrophospora colombiana, Glomus coronatum, Sclerocystis sinuosa, Racocetra gregaria, and Scutellospora pellucida (Tuheteru et al., 2020). The types of AMF found in the Vaal Reefs gold mining complex are Diversispora celata, Scutellospora gilmorei, Claroideoglomus lamellosum, and Sclerocystis sinuosa (Spruyt et al., 2014). Previous studies only focused on the types of AMF found in artisanal gold mining areas. However, this study focuses on the effect of phosphorus availability on the types of AMF in artisanal gold mining areas. This study will also analyze the percentage of AMF colonization in plant roots

found in artisanal gold mining areas to determine the level of symbiotic ability of AMF and plants in extreme environments.

Gold mining activities have caused the surrounding soil to have low soil fertility and heavy metal contamination (Ogola et al., 2002; Tuheteru et al., 2023). This is also why research on AMF in the gold mining environment has not been widely carried out because it has environmental conditions that are less supportive of plant and mycorrhizal growth. Gold mining activities have also resulted in heavy metal pollution as a result of gold ore processing and tailings disposal around the mining area (Donkor et al., 2005). One of the artisanal gold mining activities occurred in Bunut, Pesawaran Regency, Lampung Province. The research was conducted here because this area is still actively engaged in gold mining, which has management characteristics that are quite representative of artisanal gold mining areas in other regions. Gold mining in this area is carried out independently (not employed by a mining company) and uses methods that have a negative impact on the environment and public health, which are characteristics of most artisanal gold mining areas. Artisanal gold mining in Pesawaran Regency is estimated to have occurred for more than ten years, hence the soil and river water around the gold mining processing site are estimated to have been contaminated with Hg (Yuwono et al., 2023). The types of heavy metals found in the artisanal gold mining area in Bunut, Pesawaran Regency, Lampung Province, are mercury (Hg) (Kurniawan et al., 2019). Tailings are left unattended and the residual treatment water is discharged into the river, potentially negatively impacting the environment and public health. In this study, we assumed that AMF species could still be found in the artisanal gold mining area, although with a low diversity index. The AMF varieties that can survive marginal land conditions have the potential to be used to support plant growth in the areas. The aim of this study was to analyze the diversity of AMF species in artisanal gold mining areas.

#### Methods

**Study area** Soil and root sampling was conducted at the artisanal gold mining area in Bunut, Pesawaran Regency, Lampung Province. This artisanal gold mining is located at coordinates N5°36'37.5" and E105°05'27.9" as shown in Figure 1. Testing of soil chemical and physical properties was conducted at the Lampung State Polytechnic Analysis Laboratory. Observations of colonization, spore extraction, and morphological identification of AMF were carried out at the Plant Science Laboratory, Faculty of Agriculture, University of Lampung. A single spore culture of AMF was conducted in the greenhouse of the Faculty of Agriculture, University of Lampung.

**AMF exploration at artisanal gold mining area** AMF exploration was carried out by taking soil and root samples. Root samples were taken from plants growing in artisanal gold mining areas. Previously, the types of vegetation around the artisanal gold mining area were identified. Furthermore, the distance between the vegetation and tailings disposal point was measured. Determination of soil and root sampling points was carried out with the treatment of interval distance (A1: 0–10 m; A2: 10–20 m) and vegetation type (B1: *Theobroma cacao*; B2: *Mangifera indica*; B3: *Artocarpus heterophyllus*).

Soil and root samples were taken at a depth of 0–20 cm in a hole of 15 cm diameter using a shovel. The digging point is  $\frac{3}{4}$  from the stem to the outermost crown. Soil and fine roots (Ø < 2 mm) were taken from each vegetation at four replicate points based on the cardinal directions, namely east, south, west, and north. There were 6 soil and root sampling points with four replications. Soil samples from the four points were



Figure 1 Map of the research area (Source: Indonesia Topographic Map 2023).

then composited and taken as much as 1 kg. Each soil and root sample was put into plastic and labeled with the location and date of sampling.

**Testing of initial soil chemical and physical characteristics** Soil samples were taken from the artisanal gold mining area based on the treatment of plant type and distance. Soil sampling points for initial soil chemical and physical characteristics testing corresponded with soil sampling points for AMF exploration. Soil samples were taken using a hoe and then put into plastic and labeled with the name code, location, and date of sampling. The soil was then tested for chemical characteristics in the form of elemental P content using the Olsen method.

AMF colonization in plant roots Observation of AMF colonization was carried out using the root staining method (Clapp et al., 1996). Fine roots that have been collected from the plant are then washed using running water. Roots soaked in 20% KOH solution for 48 hours were then washed using a sieve under running water. Roots were soaked in 0.1 M HCl solution, then soaked in aniline blue dye solution for 48 hours, then soaked in destaining solution for 24 hours. Roots were cut along 1 cm as many as 10, then arranged parallel to the object glass and covered with a cover slip. Prepared root samples were then observed using a compound microscope. Colonization is indicated by the presence of hyphae, vesicles, or arbuscules. Percent colonization is determined based on the category as shown in Equation [1] (O'Connor et al., 2001).

$$\Sigma \text{ colonized roots} = \frac{\Sigma \text{ colonized field of view}}{\Sigma \text{ overall field of view}} \times 100\% \quad [1]$$

AMF spore extraction Spore isolation was carried out using the filter pouring technique (Pacioni, 1992). Fifty grams of soil sample was put into 1,000 ml of water, then stirred, then filtered in a set of sieves (500  $\mu$ m, 250  $\mu$ m, 150  $\mu$ m, and 45  $\mu$ m). The material stored on the sieve (supernatant) measuring 150  $\mu$ m and 45  $\mu$ m was then poured into a Petri dish and then observed under a compound microscope. Observations were made to calculate spore density and separate spores from other materials in the dish. The formula for spore density is shown in Equation *[2]*.

Spore density = 
$$\frac{\sum \text{ spores}}{100 \text{ g soil}}$$
 [2]

**Morphological identification of AMF** PVLG and Melzer solutions were placed on the object glass. Healthy AMF spores were selected and then placed on both solutions and then covered with a glass cover. Observation of morphological identification of AMF is done by observing morphological characteristics such as shape, size, color, carrier hyphae, spore wall, spore adornment, spore mother cell, bulbous suspensor, and germination shield. The naming of AMF spores was carried out by following the naming pattern (Schüßler & Walker, 2010; Redecker et al., 2013).

**Determination of species richness index** (R) and species diversity index (H') Species richness expresses the number of species in a certain area. Determination of the species richness index value (R) uses the Margalef index. The value

of R < 3.5 indicates low species richness, R between 3.5-5.0 is classified as medium species richness, and R>5.0 is classified as high. The value of the species diversity index (H') is expressed based on the Shannon Wiener index. The species diversity index (H') according to Shannon-Wiener is categorized: The value of H'<2.0 is included in the low category, the value of  $2.0 \le H' \le 3.0$  is included in the medium category, and H'>3.0 is included in the high category. Formulas of species richness index (R) and species diversity index (H') are shown in Equation [3] and Equation [4], respectively (Magurran, 2004).

$$R = \frac{(S-1)}{\ln(N)}$$
[3]

note: R = species richness index, S = number of species observed, N = total number of individuals of all species, and ln = natural logarithm.

$$H' = -\sum_{i=1}^{s} \left( \left( \frac{n_i}{N} \right) ln \left( \frac{n_i}{N} \right) \right)$$
<sup>[4]</sup>

note: H' = diversity index value, N = number of individuals of all species,  $n_i$  = number of individuals of the *i*-th species, ln = natural logarithm, and s = number of species in the community.

**Data analysis** Pearson correlation analysis was used to determine the correlation between the diversity index with phosphorus availability and colonization with phosphorus availability. Mychorrizal colonization data were analyzed by using ANOVA and followed by the Duncan Multiple Range Test (DMRT) at the 5% level. Data analysis was done using SPSS software.

## **Results and Discussion**

**Element P availability** The P availability of the six soil sampling points at the artisanal gold mining area is shown in Table 1. The available P content at all points is very high (Eviati & Sulaeman, 2009). The addition of charcoal in the gold extraction process has caused the surrounding soil to have a high available P content through the mechanism of enhancing cation exchange capacity, allowing the soil to retain more phosphorus (Adio et al., 2022). The addition of charcoal will increase the availability of P elements in the soil (Tanzito et al., 2020; Adio et al., 2022).

One of the main sources of P in plants comes from the symbiosis between AMF and plant roots (Balzergue et al., 2013). P elements are available to plants in the form of  $H_2PO^2$  and  $HPO^2$ . The availability of P in the soil is one of the important things for plant growth. The P element has an important role as a store of energy in the form of ATP in the process of photosynthesis and respiration (Silva & Uchida, 2000). Young cells, such as root tips and shoots, require large amounts of P elements.

AMF colonization in plant roots Table 2 shows that AMF colonization found in plant roots in artisanal gold mining areas has a low to medium category. AMF colonization in *T. cacao* and *M. indica* plants at the distance of 10-20 m was lower than that found at the distance of 0-10 m. Conversely, AMF colonization on *A. heterophyllus* plants at the distance of 0-10 m was lower than AMF colonization found at the

distance of 10–20 m. However, the interaction of interval distance and vegetation types treatments were not significant for AMF colonization (F=2.478, p-value=0.126).

AMF colonizes the roots of the host plant, thereby increasing the plant's access to nutrients in the soil (Shi et al., 2023). The percentage of mycorrhizal colonization can be determined by the presence of internal hyphae, vesicles, and arbuscular structures (Montiel-Rozas et al., 2017). The structure of internal hyphae, vesicles, and arbuscules in the roots of plants infected with AMF is shown in Figure 2. The three structures in AMF have their respective functions (Brundrett et al., 1996). Internal hyphae are hyphae that grow inside the root cortex cells that will develop into vesicles and arbuscules. Vesicles originate from the swelling of internal hyphae in terminal and intercalary parts, which has a function to store food reserves containing fatty compounds. Arbuscules are branching internal hyphae with fine structures that function as nutrient transfer contacts between AMF and host plants.

Ĥigh P nutrient content leads to low AMF colonization (Jayani et al., 2018; Ma et al., 2023). Plants control AMF colonization through root exudates, which are influenced by P nutrient availability (Tawaraya, 2022). Table 1 shows that



Figure 2 Structure of AMF. 1). Internal hyphae, 2). Vesicles, and 3). Arbuscule.

Table 1 Pavailability in artisanal gold mining area

Treatment	$P(mg kg^{-1})$	Category*
A1B1	20.91	Very high
A1B2	35.95	Very high
A1B3	51.27	Very high
A2B1	78.24	Very high
A2B2	46.39	Very high
A2B3	25.08	Very high

A1: 0–10 m; A2: 10–20 m; B1: *Theobroma cacao*; B2: *Mangifera indica*; B3: *Artocarpus heterophyllus*. \*(Eviati & Sulaeman, 2009).

the soil in the artisanal gold mining area at the six soil sampling points has a very high available P content so that AMF colonization is in the low to medium category.

The higher the P content in the soil, the lower the AMF colonization. Table 2 shows that AMF colonization on *T. cacao* and *M. indica* plants at the distance of 10-20 m was lower than that found at the distance of 0-10 m. This is because the available P content in the soil of *T. cacao* and *M. indica* plants at the distance of 10-20 m is higher than that of 0-10 m distance, as shown in Table 1. Table 2 shows that AMF colonization on *A. heterophyllus* plants at the distance of 10-20 m. This is probably because the available P content in the soil of 10-20 m. This is probably because the available P content in the soil in *A. heterophyllus* plants at the distance of 0-10 m is higher than that of 0-10 m distance, as shown in Table 1.

AMF diversity Table 3 shows that each sampling point has different spore densities. Spore density is inversely proportional to P content in the soil (Tian et al., 2011; Husna, 2015). The higher the P content, the lower the spore density. Table 3 shows that the density of AMF spores found in the roots of T. cacao and M. indica plants at the distance of 0-10 m is higher than that at the distance of 10-20 m. This could be due to the available P content in the soil. The available P content in the soil of T. cacao and M. indica plants at the distance of 0-10 m is lower than that at the distance of 10-20 m, as shown in Table 1. Table 3 shows that the density of AMF spores found in the roots of A. heterophyllus plants at the distance of 10-20 m is higher than that at 0-10 m distance. This is because the available P content in the soil of A. heterophyllus plants at the distance of 10-20 m is lower than that at the distance of 0-10 m, as shown in Table 1.

Species richness indicates the number of species per soil sample (Magurran, 2004). The species richness index (R) at the six points in the artisanal gold mining area was 0.40–1.54. The value of the species richness index (R) is relatively low. These results indicate that the number of AMF species found in the artisanal gold mining area both at the distances of 0–10 m and 10–20 m in the roots of *T. cacao*, *M. indica*, and *A. heterophyllus* plants are low. This is due to the availability of P in the soil affects the structure of the AMF community (Miao et al., 2023). Long-term P fertilization causes FMA species richness to decrease, especially in the family Acaulosporaceae (Lin et al., 2012).

The species diversity index is used to show the level of

Table 2 Percentage of AMF colonization in roots

Treatment	Colonization percentage (%)	Category*
A1B1	16.67	Medium
A1B2	20.00	Medium
A1B3	13.33	Medium
A2B1	6.67	Low
A2B2	6.67	Low
A2B3	26.67	Medium

A1: 0–10 m; A2: 10–20 m; B1: *Theobroma cacao*; B2: *Mangifera indica*; B3: *Artocarpus heterophyllus*. \*(O'Connor et al., 2001).

species diversity, namely the relationship between the number of species and the number of individuals. The species diversity index (H') at the six points in the artisanal gold mining area is 0.56–1.31. The value of the species diversity index (H') is classified as low. These results indicate that the diversity of AMF species found in the artisanal gold mining areas both at the distances of 0–10 m and 10–20 m in the roots of *T. cacao*, *M. indica*, and *A. heterophyllus* plants are low.

Increasing available P content reduces AMF species diversity (Miao et al., 2023). Table 3 shows that the species richness and diversity of AMF species found in the roots of *T. cacao* and *M. indica* plants at the distance of 0–10 m are higher than those at the distance of 10–20 m. This is because the available P content in the soil of *T. cacao* and *M. indica* plants at the distance, as shown in Table 1. Table 3 shows the species richness and diversity of AMF species found in the roots of *A. heterophyllus* plants at the distance of 0–10 m. This is because the available P content in the soil of T. cacao and *M. indica* plants at the distance of 0–10 m is lower than that at 10–20 m distance, as shown in Table 1. Table 3 shows the species richness and diversity of AMF species found in the roots of *A. heterophyllus* plants at the distance of 10–20 m are higher than those at the distance of 0–10 m. This is because the available P content in the soil in *A. heterophyllus* plants at the distance of 010 m. This shows the species found in the roots of 10–20 m are higher than those at the distance of 0–10 m. This is because the available P content in the soil in *A. heterophyllus* plants at the distance of 010 m. This is because the available P content in the soil in *A. heterophyllus* plants at the distance of 010 m. This is because the distance of 10–20 m is lower than that at the distance of 010 m, as shown in Table 1.

Morphological identification of arbuscular mycorrhizal fungi (AMF) Table 4 shows the results of morphological identification of AMF spores found in the artisanal gold mining area. All AMF spores found in general have almost the same characteristics, namely a single arrangement, round shape, measuring 4–400  $\mu$ m, and the inside reacts with Melzer. The genus Acaulospora has characteristics that distinguish it from other genera (Manoharachary et al., 2002). AMF spores belonging to the genus Acaulospora are round or oval and have a diameter of 40–400  $\mu$ m. The inner wall consists of one or more walls and reacts with Melzer's solution, which produces a pink, red, or purple color.

Figure 3 shows pictures of each AMF spore found, and

Figure 4 shows its reaction with Melzer's solution. All AMF spores found showed that the outer part did not react with Melzer's solution while the inner part did. This reaction is characterized by a darker reddish-brown color compared to the outside. The characteristic that distinguishes the five types of AMF found is the color of the spores. *Acaulospora* sp. 1 has an orange color, *Acaulospora* sp. 2 has a hyaline color, *Acaulospora* sp. 3 has a brownish yellow color, *Acaulospora* sp. 4 has a brownish orange color, and *Acaulospora* sp. 5 has a yellow color.

AMF species of the Acaulospora genus were also found in the gold mining activity area (Prasetyo et al., 2010; Tuheteru et al., 2020; Adyari et al., 2021). The type of AMF found in the community gold mining area in Sekotong, West Lombok, is *Acaulospora scrobiculata*. *A. scrobiculata* and *A. tuberculate* were found in the former small-scale gold mining and artisanal gold mining in North Rarowatu Subdistrict, Bombana Regency, Southeast Sulawesi Province (Tuheteru et al., 2020). AMF species found in the small-scale gold mining area of Pongkor, West Java, are *A. scrobiculata* and *A. cf. delicate* (Adyari et al., 2021).

Relationship of diversity index and AMF colonization with P availability The graph of the relationship between diversity index and AMF colonization with P availability is shown in Figure 5. Pearson correlation test results show that the diversity index of AMF species and P availability are not correlated (r = -0.204, *p*-value = 0.699). Pearson correlation test results also showed that AMF colonization and P availability were not correlated (r = -0.756, *p*-value = 0.082). The value of r<0 indicates that the relationship between the two factors, both AMF diversity index and P availability as well as AMF colonization and P availability is not correlated.

Although not correlated, based on Figure 5, it can be seen that the higher the available P element, the smaller the index value of species diversity and AMF colonization. The

010 5	spore density, species nonness (R), and diversity index (II) of Aivin in the artisanal gold mining area						
	Treatment	Spore density 50 g <sup>-1</sup> soil	Species richness index (R)	Species diversity index (H')			
	A1B1	12	0.40	0.56			
	A1B2	9	1.37	1.31			
	A1B3	7	1.54	1.28			
	A2B1	4	0.72	0.56			
	A2B2	7	1.54	1.28			
	A2B3	14	1.14	1.17			

Table 3 Spore density, species richness (R), and diversity index (H') of AMF in the artisanal gold mining area

A1: 0–10 m; A2: 10–20 m; B1: Theobroma cacao; B2: Mangifera indica; B3: Artocarpus heterophyllus.

Table 4 Identification of AMF spore morphology in artisanal gold mining area

Species	Arrangement	Shape	Color	Reaction with Melzer
Family: Acaulosporaceae				
Genus: Acaulospora				
Acaulospora sp. 1	Single	Round	Orange	The inside reacts with Melzer
Acaulospora sp. 2	Single	Round	Hyaline	The inside reacts with Melzer
Acaulospora sp. 3	Single	Round	Brownish yellow	The inside reacts with Melzer
Acaulospora sp. 4	Single	Round	Brownish orange	The inside reacts with Melzer
Acaulospora sp. 5	Single	Round	Yellow	The inside reacts with Melzer

A1: 0–10 m; A2: 10–20 m; B1: Theobroma cacao; B2: Mangifera indica; B3: Artocarpus heterophyllus.



Figure 3 Images of AMF spores found in the artisanal gold mining area. A). *Acaulospora* sp. 1, B). *Acaulospora* sp. 2, C). *Acaulospora* sp. 3, D). *Acaulospora* sp. 4, and E). *Acaulospora* sp. 5.



Figure 4 Reactions of AMF spores with Melzer's solutions. A). *Acaulospora* sp. 1, B). *Acaulospora* sp. 2, C). *Acaulospora* sp. 3, D). *Acaulospora* sp. 4, and E). *Acaulospora* sp. 5.

availability of P element affects the diversity of AMF. The high availability of P element causes the diversity of AMF species to be smaller (Ma et al., 2023). The result of this study is in accordance with the results of Lin et al. (2012) that the diversity and richness of AMF species decreased due to long-term P fertilization, especially the family Acaulosporaceae.

The availability of P also affects AMF colonization in the roots. The higher the available P element, the smaller the AMF colonization (Figure 5). The result of this study is in

accordance with the results of previous studies that the higher the available P element, the lower or even absent AMF colonization at the root (Vierheilig, 2004; Liu et al., 2016). Highly available P content reduces hyphal and arbuscular colonization. The inverse comparison between P availability and AMF colonization in this study is due to the fact that high P can reduce the ability of the host plant to form symbiosis with AMF (Balzergue et al., 2013). The availability of high amounts of P can alter the composition of root exudates



Figure 5 Relationship graph of phosphor availability with A). AMF species diversity index and B). AMF colonization percentage.

(Vierheilig, 2004). When plants are in a high P availability status, AMF will reduce the stimulation of spore germination and hyphal growth in roots through root exudates. Root exudates do not stimulate root colonization. This is also one of the causes of low AMF colonization in plant roots.

Besides reducing AMF diversity and colonization, high P availability can also cause environmental problems in the form of eutrophication. Eutrophication is the rapid growth of algae and other aquatic plants due to high nutrient content, especially P and N (Shaw et al., 2009). Eutrophication might cause a decrease in oxygen levels in the water, making it unfavorable for the growth of aquatic biota such as fish (Weil & Brady, 2017). Next to the gold mine processing area in Bunut Village, Pesawaran Regency, Lampung, there is a river where waste from gold mine processing is discharged. We think that efforts should be made for gold mining management in this area, especially to reduce the P content in soil and water. Gold processing residual water should not be discharged directly into the river but first accommodated in the wastewater treatment plant to carry out a water treatment before being discharged into the environment. Growing a few crops of grain can reduce excess soil P (Sideman, 2011). However, as the soil in artisanal gold mining areas contains Hg, some grain crops grown could not be consumed by humans or for animal feed. If the few grain crops that contain Hg are consumed by humans or animals, there will be an accumulation of Hg in the food chain. It is also necessary to regularly monitor P levels in soil and water.

#### Conclusion

There are several types of AMF spores found in the artisanal gold mining area, namely *Acaulospora* sp. 1, *Acaulospora* sp. 2, *Acaulospora* sp. 3, *Acaulospora* sp. 4, and *Acaulospora* sp. 5. The index of species richness and diversity among AMF species in the artisanal gold mining area is low. The existence of AMF symbiosis with plants in artisanal gold mining areas is also indicated by the presence of colonization in the roots of *T. cacao*, *M. indica*, and *A. heterophyllus* plants although in the low to medium category. The higher the availability of P, the smaller the index value of species diversity and AMF colonization. 396

#### Recommendation

Action is needed to reduce the high P content in the soil. This is because, besides impacting the low diversity of AMF found, high P also causes other environmental problems such as eutrophication. Reducing P content can be done by growing a few crops of grain and reducing the amount of charcoal added in the gold extraction process. In addition, the types of AMF found in artisanal gold mining areas also need to be cultivated to obtain a mycorrhizal inoculum supporting rehabilitation activities.

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