Isolation of Iindigenous arbuscular mycorrhizal fungi (AMF) to support revegetation on the nickel post-mining land

Isolation of Iindigenous arbuscular mycorrhizal fungi (AMF) to support revegetation on the nickel post-mining land

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Abstract. The revegetation on the nickel post-mining area requires symbiotic associations with mycorrhizal fungi to support the plant growth because it faces major obstacles of harsh soil condition. The study was conducted to identify the AMF species status on the nickel post-mining land and its association with pioneer plant roots. Soil and root sampling werewas collected on 4 type areas of nickel post-mining land i.e TR (top soil + revegetation), TNR (top soil + no revegetation), NTR (no top soil + revegetation) and NTNR (no top soil + no revegetation) to determine spore density, soil chemical properties and AMF colonization. The results showed that the nickel mining activities interfere in soil fertility and soil microbial population. It was denoted by lack available nutrient and low AMF diversity. Population of AMF only 7 – 83 spore per gram soil. Glomus or Acaulospora is dominating genus in each area. However, However, AMF occurrence is a vital to help the pioneer plants growth. It was proven by 163 species of pioneer plants that colonize nickel post-mining land associated with the AMF in their root system. Grass species have highest AMF colonization level. Therefore, inoculation of AMF indigenous isolate is needed as an intervention ion post-mining reclamation effort, so the possibility of symbiosis between plant roots and AMF becomes increasing. Eventually the development of natural plants will be accelerated to catalytic the natural succession process.

1. Introduction

Nickel mine is one of the country's foreign exchange contributors, and Indonesia is ranked 4th of the world as a nickel producer. However, in addition to generating foreign exchange, the nickel mining industries also caused deforestation as they apply open-pit mining methods. Nickel mining leaves the post-mining lands with very low soil properties i.e. no organic matter, low pH, low nutrient availability and high soil temperatures. Moreover, according to [1] found that nickel mining led to heavy metal pollution i.e. Ni, Co, Fe, Mn, Cu, Pb and Zn in the soil. Nickel mining activities have also been shown to decrease the soil microbial communities [2]. The decline in soil microbial communities, especially beneficial soil microbes will further hamper the the development of natural plants.

AMF (Arbuscular Mycorrhizal Fungi) is one of the soil microbial components that have an important long-term role for the development of plant communities [3][4][5]. AMF established mutualism symbiosis with vascular plant. The fungi get in touch with root plants, but the symbiosis is created with the plant, as the photosyntethic assimilates are delivered to roots system. Hence, the plant provides constant and direct access to carbohydrates, such as glucose and sucrose, for the fungus. In reply, the plant gets the benefits for water and mineral nutrients due to the large surface area of fungal

hyphae and the fungi can mobilize soil minerals unavailable to the plants' roots. The effect is thus to improve the plant's mineral absorption capabilities. According to [6] and [7], The arbuscular mycorrhizal association able to increase P absorption and other nutrients, as well as drought tolerance. Moreover [3} [8] [9] [10], stated arbuscular mycorrhizae also improve the soil physical properties by the improvement of soil structure because one of the role of AMF hyphae contributes to the formation of soil aggregates. Therefore, the AMF associations play a role in accelerating plant survival and growth, especially in the marginal land that extremely low nutritional status.

The declining AMF population will cause the development of the vegetation community to be inhibited. According to [11], AMF has great potential in the development and survival of plants in natural conditions and environmental stresses on mined land. The characteristic of the nickel post-mining lands are also suspected causing AMF population and infectivity decline due to low soil quality. Meanwhile AMF is vital for the successful reclamation of post-mining land [12] [13]. Low AMF population and decreased infectivity will hamper mineralization and immobilization of nutrients, thus inhibiting the development of native plants, and further affect the process of natural succession. In this research, isolation of AMF was conducted to analyze species diversity, species richness and spore density of AMF on the nickel post-mining area. In addition, measurements of AMF infection level in the pioneer plants were also undertaken to determine the significance of AMF's role in the successful of vegetation invasion and colonization on the area.

2. Material and Methods

2.1. Study area and sampling method

Location of soil and root sampling was conducted in the mining area of Stargate Pacific Resources (SPR) company in Konawe Utara Regency, Southeast Sulawesi Province, Indonesia. Geographically the location of mining concession rights of PT. The SPR approved by the principle of forest use lies between 03 ° 17 '24 "LS - 03 ° 22' 1.2" LS and 122 ° 14 '45.6 "BT - 122 ° 17' 42" BT. The post-mining nickel area is 3 years old and has been arranged by overburden materials. Some of these areas have also been spread with topsoil and have been revegetated or no revegetated. The location of soil and root samplings in the post-mining area was carried out in 4 type areas TR (top soil + revegetation), TNR (top soil + no revegetation), NTR (no top soil + revegetation) and NTNR (no top soil + no revegetation).

Collecting soil samplings was performed to AMF spore isolation and soil chemical properties analysis. Soil sampling was done by subplot size 20 cm x 20 cm [13] in main plots 10 m x 10 m on dry season (September 2015). Preparation of main plots in each type of area is carried out by systematic with random start. Subplots are placed at 4 points of the main plot angle’s and 1 point from the intersection of the main plot diagonal line. The soil samples were taken at a depth of 0-20 cm. Furthermore, soil samples were kept in a plastic bag and then composted according to its subplot. Root samplings were collected from all pioneer plants in each main plot. Sampling of pioneer plant specimens was also performed for its identification purposes.

2.2. AMF isolation and identification

The collected soil samples were processed for AMF spore isolation and identification. These process were done on October – Desember 2015. The spore extraction was conducted following wet sieving and decanting methods from Gerdemann and Nicolson in [14] and followed by centrifugation techniques from [15] that has been modified. Spores made of preparations are spores that have a complete structure. Preparation of spores using Polyvinyl Lacto Glycerol (PVLG) solution as a preservative and Meltzer’s solution as a dye. The identification was done morphologically based on the spore shape and its reaction to Meltzer's solution. The spore color change in Meltzer's solution is one of the indicators for determining the genus [15].

2.3. Assessment of AMF colonization level
Roots were stored in 50 % (v/v) ethanol then cleared and stained using the method of [16]. The slide method of [17] was used observing the percentage of mycorrhizal colonization.

2.4. Soil chemical analysis
The evaluation of soil chemical properties consisting pH, C-org content, N total, P available (P_2O_5), and CEC (Cation Exchangeable Capacity).

2.5. Data analysis
The AMF community structures were analyzed using several diversity parameters including species richness and important value indexes [[18], [19]], as well as the Shannon-Wiener diversity index. The soil chemical properties, AMF spore density, species diversity and level of AMF colonization in the pioneer root system were analyze by ANOVA in SPSS 25. The one way analysis of variance was carried out to compare the means of different treatments (area). Means separation tests were performed using Duncan Multiple Range Test (DMRT) at the $P<0.05$ level after significant F values were obtained.

3. Result and Discussion

3.1. Soil chemical properties
Statitistical F-tests revealed that among all soil parameters measured in nickel post-mining land, content of soil C-organik showed significant differences the areas. Meanwhile, N content, P available, K content and CEC were non-significant. However, most soil chemical parameters showed very low or low category in the category set by the Indonesian Centre for Agricultural Land Resource Research and Development (ICALRD) (Table 1). This mean that the soil chemical properties of nickel post-mining is harsh for plant growth even though after top soil spreading and revegetation. Available phosphorus concentration increased with top soil spreading and more enhancing with revegetation. Topsoil and vegetation restoration can significantly improve the content of soil available phosphorus [20] [21]. Different trend was showed by N and K content. Top soil amendment and revegetation have no influence to the availability this macronutrient.

Table 1. Soil N, P, and K availability in nickel post mining area. Soil chemical quality categories follow ICALRD's standard. Different letters within columns indicate significant differences (F-test, $p<0.05$).

Areas	N content (%)	Category	P available (ppm)	Category	K content (cmol/kg)	Category
NTNR	0.11 a	Low	2.33 a	Very low	0.38 a	Medium
NTR	0.10 a	Low	4.67 a	Very low	0.53 a	Medium
TNR	0.08 a	Very low	4.33 a	Very low	0.27 a	Medium
TR	0.09 a	Very low	6.50 a	Very low	0.32 a	Medium

The organic C content of soil on nickel post-mining was very low (Table 2), which may account for the lack of improvement in the CEC. The increasing of organic C content was denoted by areas which have been spread with top soil and revegetated. According to [20], top soil spreading significantly increased soil organic matter and indicated increasing of organic C content of soil. Furthermore, trees can potentially improve soils through numerous processes, including - maintenance or increase of soil organic matter [12].

Table 2. Soil C content, pH, and CEC in nickel post mining area. Soil chemical quality categories follow ICALRD's standard. Different letters within columns indicate significant differences (F-test, $p<0.05$).

Areas	C content (%)	Category	pH	Category	CEC (cmol/kg)	Category
NTNR	0.27 a	Very low	5.38 a	Slightly acid	5.99 a	Low
NTR	0.80 a	Very low	5.68 a	Slightly acid	5.85 a	Low
TNR	0.89 b	Very low	5.56 a	Slightly acid	6.86 a	Low
TR	2.97 b	Very low	5.31 a	Slightly acid	6.97 a	Low

3.2. AMF spore abundance

The nickel mining process which is conducted by open-pit mining method thus removing vegetation and topsoil layer proved to have a very significant impact on the soil microbial community decreasing, i.e., AMF. A very noticeable decrease is the density of the spores. In Figure 1. the nickel post-mining area that has been stockpiled with an overburden material but not followed by topsoil spreading and revegetation (NTNR) has the lowest spore density and so does its species diversity and richness. The decrease of spore density of AMF due to mining activity also occurs on the post-mining land of limestone, tin, and gold [5] [13][22][23][24] that use open pit mining method.

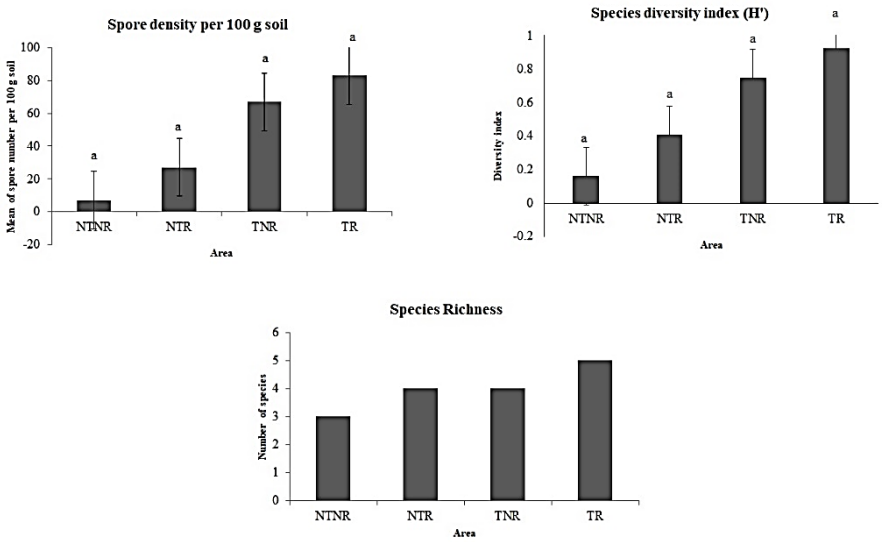

Figure 1. AMF status (mean±SE) in different area of nickel post mining land. NTNR = post-mining area already stockpiled with overburden not following top soil spreading and revegetation, NTR= post-mining area already stockpiled with overburden not following top soil spreading but revegetated;, RT = post-mining area already stockpiled with overburden + topsoil + revegetated. Same letters above bars indicate not significant (Duncan test, $p<0.05$).

The AMF population on the nickel post-mining land increases when the area that has been dumped with the overburden material, then spread with topsoil, moreover when the revegetation was done in the area (Figure 1). The revegetation can increase AMF spore density, species diversity, and species richness. However, when the revegetation was not preceded by top soil spreading, the AMF spores

density, species diversity and species richness were lower than area with top soil but not following by revegetation. It was showed by the comparison of NTR and TNR (see Figure 1.). This result indicate top soil spreading is important in post-mining reclamation. The top soil contain available nutrient that was required for plant growth and appropriate soil microbes for plant survival. According to [25] and [26] topsoil contains mycorrhizal or bacterial symbionts for promoting the establishment and persistence of plant species indigenous to the local environment.

Figure 2. Various AMF species on the nickel post-mining land. A. spore of *Acaulospora* in Meltzer's solution. B. spore of *Acaulospora* in a PVLG solution. C. *Gigaspora* spore in Meltzer's solution. D. *Gigaspora* spore in a PVLG solution. E, G, I. *Glomus* spore in Melzer's solution. F, H, J. *Glomus* spores in a PVLG solution.

A total of 11 morphotypes of AMF spores isolated from nickel post-mining land were identified as 3 genus namely *Acaulospora*, *Gigaspora* and *Glomus* (Figure 2). The presence and density of spores of each genus in each type of area are varied. In addition, not all genus are found in every type of area and the dominating genus is also different (Figure 3).

The genus *Acaulospora*, *Gigaspora*, and *Glomus* were found in the post-mining areas that have been spread by top soil and revegetated (TR). Meanwhile in the other area of nickel post-mining (NTNR, NTR, TNR) only genus *Acaulospora* and *Glomus* were found. The results of this study also indicate that higher density of certain genus spores in an area is also followed by a higher index value importance (IVI). According to [19] the AMF species or genus having INP $\geq$ 20 is the dominant species or genus. *Glomus* is the most dominant AMF genus on the area with top soil and then revegetated, while *Acaulospora* is dominated on the other area. Dominance of *Acaulospora* and *Glomus* are possible by shorter spore production cycles and smaller spore size than Gigasporaceae [27] and *Acaulospora* dominance is also found in limestone spoiled mine areas [13].

The results of this study prove that spreading of top soil can increase the population and the diversity of AMF, especially when followed by revegetation. However, top soil spreading is often constrained by the insufficiency of the top soil itself. Therefore, revegetation supplemented by mycorrhizal inoculum is an effort to catalyse increasing of AMF population and plant establishment. Revegetation of limestone quarry by mycorrhizal inoculated planting increase its indigenous AMF population 2 times higher than revegetation with non-mycorrhizal plants, even 4 times higher than no

revegetated areas [13]. AMF inoculation in revegetation of post-mining land is necessary because it ensures the possibility of arbuscular mycorrhizal associations in plant roots. In addition, the utilization of indigenous AMF isolates in revegetation of post-mining land is recommended as more adaptive to local edaphic conditions.

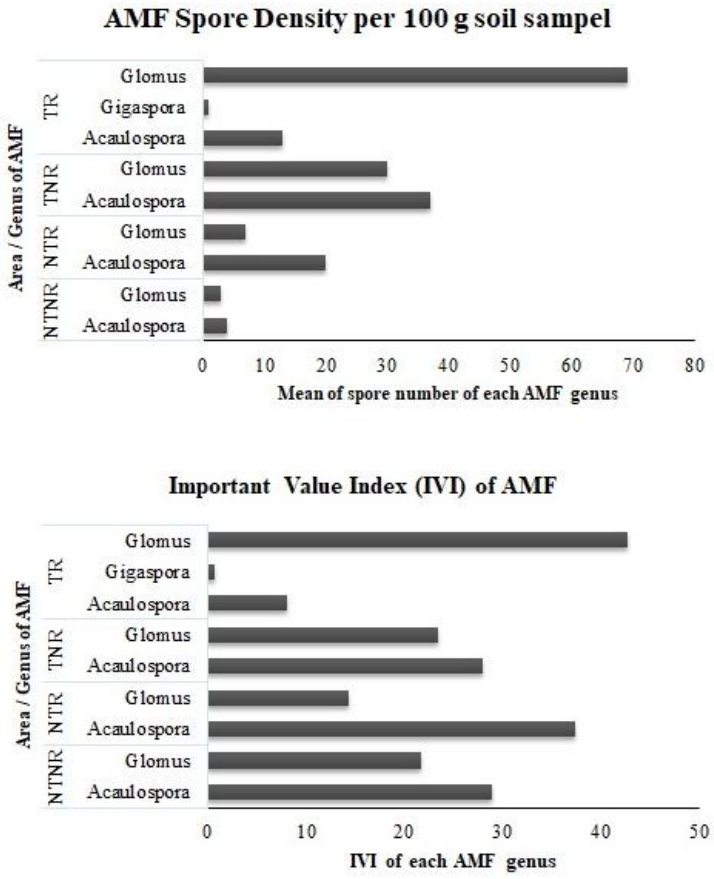

Figure 3. Spore density and important value index (INP) of each genus AMF found in nickel post-mining area. NTNR = post-mining area already stockpiled with overburden not following top soil spreading and revegetation, NTR= post-mining area already stockpiled with overburden not following top soil spreading but revegetated;, RT = post-mining area already stockpiled with overburden + topsoil + revegetated.

3.3. AMF colonization

A total of 16 species of pioneer plants including the species of grasses, herbs, shrubs, and trees that naturally invade and colonize the nickel post-mining land associated with AMF in its root system (Table 2). Similarly, 2 species of trees namely *Paraserienthes falcataria* and *Palaquium luzoniense* Vid that are planted for revegetation of the land. The AMF level colonization of these plants is low because of ≤ 50%.

Table 3. Colonization of AMF in pioneer plant roots that grow spontaneously in each type of area on nickel post-mining land.

Area	Species	Life form	AMF colonization level±SD	AMF Colonization structure
NTNR	*Machaerina glomerata* (Gaudich)	Grass	26.11±12.62	Hypha
	Scleria lithosperma (Linnaeus) Swartz	Grass	10.56±3.47	Hypha, microscleretia
	Trema orientalis	Tree	0.00	-
NTR	*Casuarina sumatrana*	Tree	0.83±0.91	Hypha
	Cynodon dactylon (L.) Pers.	Grass	2.22±2.54	Hypha , spore
	Machaerina glomerata (Gaudich)	Grass	4.00±5.60	Hypha, vesicle
	Palaquiium luzoniense Vid.	Tree	7.78±6.94	Hypha
	Paraserienthes falcataria	Tree	18.89±25.62	Hypha , spore, vesicle
TNR	*Digitaria sanguinalis*	Grass	0.00	
	Dracaena sp	Grass	21.67±28.28	Hypha, vesicle
	Machaerina glomerata (Gaudich)	Grass	5.00	Hypha
	Melastoma malabatrichum	Shurb	8.19±4.58	Hypha , spore, vesicle, microscleretia
	Paspalum schrobiculatum	Grass	21.94±9.03	Hypha , spore, vesicle, microscleretia, arbuscular, Hypha coil
	Sarcotheca celebica	Tree	1.67	Hypha, vesicle
	Scleria lithosperma (Linnaeus) Swartz	Grass	29.44±9.81	Hypha
	Spermacoce sp	Herb	1.11±0.96	Hypha
	Trema orientalis	Tree	10.93±6.93	Hypha , spore, vesicle
	Trichospermum kjelbergii Burret	Tree	8.89±2.54	Hypha , spore
TR	*Callicarpa pachyclada*	Herb	25.00±7.07	Hypha , spore, vesicle
	Centrosema pubescens	Herb	28.33	Hypha, vesicle
	Cynodon dactylon (L.) Pers.	Grass	10.56±7.72	Hypha, vesicle
	Fimbristylis sp	Grass	4.44±4.19	Hypha
	Machaerina glomerata (Gaudich)	Grass	11.67±10.38	Hypha, vesicle
	Melastoma malabatrichum	Shurb	15.33±20.76	Hypha , spore, vesicle
	Paraserienthes falcataria	Tree	26.25±28.01	Hypha , spore, vesicle, microscleretia
	Paspalum schrobiculatum	Grass	30.56±15.44	Hypha , spore, vesicle
	Scleria lithosperma (Linnaeus) Swartz	Grass	11.11±6.12	Hypha
	Scleria sp.	Grass	50.00	Hypha
	Trema orientalis	Tree	22.96±15.92	Hypha , spore, vesicle

Remarks: NTNR = post-mining area already stockpiled with overburden not following top soil spreading and revegetation, NTR= post-mining area already stockpiled with overburden not following top soil spreading but revegetated;, RT = post-mining area already stockpiled with overburden + topsoil + revegetated

The importance of the AMF role to support the successful growth and establishment of plant communities in nickel post-mining land is indicated by the AMF association in the roots of pioneer plants that grow spontaneously on the land. The associations formed between AMF and plant roots will affect the processes in the ecosystem, including determining the biodiversity of plants in their natural communities. Even [28] and [4] suggests that the role of arbuscular mycorrhizae is not limited

only to early colonization (pioneer species) but also to subsequent colonization (early sere species until late sere species).

4. Conclusion

Eventually, it can be concluded that the nickel mining activity proved to harm soil chemical properties and disrupt one of the existence of beneficial soil microbial community, i.e., AMF, which is indicated by the decrease of spore density, diversity, and richness of AMF species. AMF population in nickel post mining area is very low. The AMF spore density is 7 -83 spores per gram soil with species diversity index of 0.17 - 0.92 and species richness of 3-5 species. Furthermore, *Glomus* or *Acaulospora* is dominating genus in each area. The spora density and important value index (IVI) of *Glomus* and *Acaulospora* 69 and 43; 37 and 37 respectively. However, the symbiotic association of arbuscular mycorrhizae is vital for the development of plant communities that naturally colonize the nickel post-mining land. Grass and herbs species included *Scleria sp*., *P. schrobiculatum*, *S. lithosperma*, *C. pubescens* and *M. Glomerata* have higher AMF colonization level than the other pioneer species. The AMF colonization level of these species are 50; 30, 56; 29.44; 28, 33 and 26.25% respectively. Therefore AMF inoculation is required as an intervention on revegetation to restore the site condition of the nickel post-mining area. Although revegetation without AMF inoculation proved to be sufficient to increase the AMF population but with the inoculation of AMF, the facilitative impact on the development of vegetation through natural succession is expected to occur faster.

5. References

[1] Gonzales H and Ramirez M 1995 The effect of nickel mining and metallurgical activities on the distribution of heavy metals *J Geochemi Explor*. **2** 183 - 192.
[2] Herrera A, Héry M, Stach J E M, Jaffré T, Normand P, and Navarro E 2007 Species richness and phylogenetic diversity comparisons of soil microbial communities affected by nickel-mining and revegetation efforts in New Caledonia *Eur J Soil Biol*. **43**(2) 130-139
[3] Smith S E and Read D J 2008 *Mycorrhizal Symbiosis* (New York: Academic Press)
[4] Kikvidze Z, Armas C, Fukuda K, Martínez-García L B, Miyata M, Oda-Tanaka A, Wu B 2010 The role of arbuscular mycorrhizae in primary succession: Differences and similarities across habitats *Web Ecology* **10** (1) 50-57
[5] Singh A K, and Jamaluddin 2011 Status and diversity of arbuscular mycorrhizal fungi and its role in natural regeneration on limestone mined spoils *Biodiversitas* **12** (2) 107-111
[6] Abbaspour H, Saeidi-Sar S, Afshari H, and Abdel-Wahhab M A 2012 Tolerance of Mycorrhiza infected Pistachio (*Pistacia vera* L.) seedling to drought stress under glasshouse conditions. *J Plant Physiol*. **169** (7) 704-709
[7] Cavagnaro T R, Bender S F, Asghari H R, and van der Heijden M G A 2015 The role of arbuscular mycorrhizas in reducing soil nutrient loss *Trends in Plant Science* **20** (5) 283-290
[8] Rillig M C and Mummey D L 2006 Mycorrhizas and soil structure *New Phytol*. **171** (1) 41-53
[9] Wilson G W T, Rice C W, Rillig M C, Springer A and Hartnett D C 2009 Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: Results from long-term field experiments *Ecology Letters* **12** (5) 452-461
[10] Baar J 2010 Development of soil quality metrics using mycorrhizal fungi *Span J Agric Res*. **8** S137-S143
[11] Quoreshi A M 2008 *The Use of Mycorrhizal Biotechnology in Restoration of Disturbed Ecosystem*. In Z Siddiqui, M Akhtar and K Futai (Eds.), Mycorrhizae: Sustainable Agriculture and Forestry (Springer) pp. 1-359
[12] Sheoran V, Sheoran A S, and Poonia P 2010 Soil Reclamation of Abandoned Mine Land by Revegetation : A Review. *Int J of Soil, Sediment and Water* **3**(2) 1-21
[13] Prayudyaningsih R (2017) Akselerasi Suksesi Alami Di Lahan Bekas Tambang Kapur: Efek Katalitik Pertanaman Awal dan Inokulasi Fungi Mikoriza Arbuskula (FMA) [Dissertation]. Universitas Gadjah Mada, Yogyakarta. [Indonesia]

[14] Pacioni G 1992 *Wet Sieving and Decanting Techniques for Extraction of Spores of VA Mycorrhizal Fungi*. In J Norris, D Read and A Varma (Eds.), Methods in Microbiology (San Diego: Academic Press) p. 317 - 322
[15] Brundrett M C, Bougher N L, Dell B, Grove T, and Malajczuk N 1996 *Working with Mycorrhizas in Forestry and Agriculture* (Canbera: Australian Centre for International Agriculture Resarch)
[16] Kormanik P and McGraw A 1982 *Quantification of Vesicular-Arbuscular Mycorrhizae in Plant Roots*. In N. Schenk (Ed.), Methods and Principles of Mycorrhizal Research. (Minnesota: The American Phytopathological Society)
[17] Giovannetti M and Mosse B 1980 An Evaluation of Techniques For Measuring Vesicular-Arbuscular Mycorrhizal Infection In Roots. *New Phytol*. **84** 489-500
[18] Shi Z Y, Chen Y L, Feng G, Liu R J, Christie P and Li X L 2006 Arbuscular mycorrhizal fungi associated with the Meliaceae on Hainan island, China *Mycorrhiza* **16**(2) 81-87
[19] Yang A N, Lu L and Zhang N 2011 The diversity of arbuscular mycorrhizal fungi in the subtropical forest of Huangshan (Yellow Mountain), East-Central China *World J Microbiol Biotechnol.* **27**(10) 2351-2358
[20] Rivera D, Mejias V, Jauregui, B M, Costa-Tenorio, M. Lopez-Archilla A I and Peco B 2014 Spreading Topsoil Encourages Ecological Restoration on Embankments: Soil Fertility, Microbial Activity and Vegetation Cover *PLOS ONE* **9**(7) 1 - 8
[21] Bao Fu, Yanbing Q, and Qiungrui C 2015 Impacts of revegetation managements modes on soil properties and vegetation ecological restoration in degraded sandy grassland in farming-pastoral ecotone *Int. J. Agric. & Biol. Eng.* **5**(1) 26 - 34
[22] Straker C J, Freeman A J., Witkowski E T F and Weiersbye I M 2008 Arbuscular mycorrhiza status of gold and uranium tailings and surrounding soils of South Africa's deep level gold mines II. Infectivity. *S Afr J Bot*. **74**(2) 197-207
[23] Prayudyaningsih R 2008 Keragaman Fungi Mikoriza Arbuskula (FMA) di Lahan Bekas Tambang Kapur, PT. Semen Tonasa dan Efektivitasnya Terhadap Pertumbuhan Semai Kersen (*Muntingia calabura* L.). [Thesis]. Universitas Gadjah Mada, Yogyakarta. [Indonesia]
[24] Nurtjahya E 2008 Revegetasi Lahan Pasca Tambang Timah Dengan Beragam Jenis Pohon Lokal di Pulau Bangka. [Dissertation]. Institut Pertanian Bogor. [Indonesia]
[25] Jasper A D 2007 Benefecial Soil Microorganism of the Jarrah Fores and Their Recovery in Bauxite Mine Restoration in Southwestern Australia *Restor Ecol*. **15**(4) 74 - 84
[26] Munoz-Rojaz M., Erickson T, Martini, D, Dixon, K and Merrit D J 2016 Soil physicochemical and microbiological indicators of short, medium and long term post-fire recovery in semi-arid ecosystem *Ecol Indic.* **63** 14 - 22
[27] Nandakwang P, Elliott S and Lumyong S 2008 Diversity of Arbuscular Mycorrhizal Fungi in Forest Restoration Area of Doi Suthep-Pui National Park *Microsc. Microanal. Res*. **22** 60-64
[28] Wu B, Hogetsu T, Isobe K and Ishii R 2007 Community structure of arbuscular mycorrhizal fungi in a primary successional volcanic desert on the southeast slope of Mount Fuji *Mycorrhiza* **17**(6) 495-506

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