International Journal of Life Science and Agriculture Research

ISSN (Print): 2833-2091, ISSN (Online): 2833-2105

Volume 02 Issue 07 July 2023

DOI: https://doi.org/10.55677/ijlsar/V02I07Y2023-07

Page No : 200-205

Total Bacteria and Fungi in Gold-Mine Tailing in Buru of Indonesia after Three-Year Natural Revegetation

Reginawanti Hindersah[1], Agustinus Marthin Kalay[2], Pujawati Suryatmana[1], Triyani Dewi[3]

[1]Faculty of Agriculture Universitas Padjadjaran, Jl. Ir. Soekarno Km. 21, Sumedang 45363, Indonesia

[2]Faculty of Agriculture Universitas Pattimura, Jalan Ir. Putuhena, Kampus Poka Ambon 97233, Indonesia

[3]Research Center for Horticultural and Estate Crops, National Research and Innovation Agency, Jl. Raya Bogor-Jakarta, Cibinong, Bogor, West Java 16915, Indonesia

ABSTRACT: The concern of agricultural environment damage due to amalgamation process of gold mining in Indonesia is increased. Gold mine tailing deposition in agricultural land pose a serious problem about the mercury (Hg) accumulation in soil. The objective of this study was to verify the population of heterotroph-aerobic bacteria and filamentous fungi in the tailing deposit grown with pioneer plants in comparison to both population in bare tailing after three-year revegetation. Moreover, the research was conducted to analyze the different of acidity (pH) and electrical conductivity (EC) of both sites. The study area was performed in artisanal and small-scale gold mining in Waelo village of Buru Regency, Maluku Province. The bacterial and fungal population in tailing that is naturally grown by sedges and grasses were lower than that in bare tailing without vegetation. In general, the bacterial and fungal count of tailing with vegetation was 9.3 and 4.3. while in bare tailing were 8.49 and 3.79. The acidity of both locations was not different but the pH was strongly acid ranged from 3.52-4.29. The EC was lower (21.61) in the tailing with vegetation compared to bare tailing without vegetation (58.94). This study suggested that the fungal and bacterial population as well as pH were increased after revegetation that benefit plant growth.

KEYWORDS: Acidity, Electrical conductivity, filamentous fungi, heterotroph bacteria, vegetation.

Published Online: 31 July 2023

Corresponding Author: Reginawanti Hindersah

INTRODUCTION

Severe environmental damage by anthropogenic activity is happen in mining area. In Indonesia, artisanal and small-scale gold mining (ASGM) is usually conducted in the area near the National Mining Company. However, ASGMs utilizes amalgamation method to extract the gold. Mercury (Hg) gold-recovery process was mixed with grounded mining materials recovered from the earth. In mineral mining, separating valuable mineral produced tailing as a by-product with less economic value.

In gold mining area of Buru Island, tailings were disposed on the agricultural area and hence covered the fertile soil. Tailings are characterized by low essential macronutrients Nitrogen and Phosphor; organic matter and cation exchange capacity (Yuningsih et al., 2021). Moreover, the composition of tailing particle is dominated by sand or clay; while the acidity of tailing usually acid or alkaline (Yuningsih et al., 2021; Wangi et al., 2023). Low content of essential nutrient in tailing may bring about nutrient deficiency in plants.

In the context of soil genesis, the tailing over the soil become a parent material of Entisols soil that do not yet have pedogenic horizon development other than an A-horizon (Poppiel et al., 2018). Since Entisols develop from newly deposit material, water availability is prominent to accelerate horizon development which is induce for plant growth. Due to global climate change, minimum, maximum, and average temperature in Buru is slightly higher. Therefore, increasing evapotranspiration and lowering water availability may be evidenced. Actually, the annual rainfall in Buru was > 3,000 mm; at 2020, the rainfall (3.655 mm) was higher than that in 2018 and 2019. However, in Buru Island the water scarcity is occurred in certain months so the growth of pioneer plant/vegetation on tailings were retarded.

Tailing revegetation of tailings through natural method that relates to natural succession is important in post-mining landscapes (Hendrychová, 2008). In general, degraded mine may undergo natural revegetation through spontaneous plant succession. Pioneer plants grown in disturbed land contribute to soil characteristics improvement that support growth of subsequent plants. Revegetation

by biological has been performed in 2017 in limited area of tailing in agricultural area (Hindersah et al., 2019). After three years, pioneer plants were naturally grown but, in some location, they failed to grow (Figure 1).

Pioneer plant grown in tailing may change the microbial population. Roots of pioneer plants provide water and exudates that induce microbial proliferation; and accumulate soil organic matter (Arocena et al., 2010). Microbes colonized the rhizosphere have prominent role in maintaining nutrient cycling in soil, providing nutrients and growth factors for plant growth and improving soil structure for root growth (Jacoby et al., 2017; Gupta, 2011). The bacteria and fungi are dominant beneficial microbes in the plant rhizosphere. the population of both heterotroph microbes largely depend on organic-C, pH and EC.

The soil acidity determines the microbial growth. Fungus is dominant in soil with lower pH but bacteria prefer neutral acidity (Rousk et al., 2010). Bacillus, Lysinibacillus, Staphylococcus, Brevundimonas, Alcaligenes, Enterobacter, Klebsiella, Escherichia, and Aeromonas were tolerant to acidic soil environment (Goswami et al., 2017), while. Firmicutes, Proteobacteria and Actinobacteria are alkali-tolerant (Namirimu et al. 2019) The electrical conductivity represents the ion content soil and have strong relation with salinity (Ismayilov et al., 2021). In general, higher EC restricted nutrient uptake due to osmotic pressure enhancement while lower EC may affect due to limited amount of nutrient in soil solution (USDA, 2011). The objective of the research was to determine the population of heterotroph-aerobic bacteria and filamentous fungi in the tailing deposit grown with pioneer plants in comparison to both population in bare tailing. Moreover, the research was conducted to analyze the acidity and electrical conductivity of both sites.

MATERIALS AND METHODS

The study was conducted in Waelo village in Waelata district of Buru Regency, Maluku Province, Indonesia (Figure 1). The tailings of ASGM were deposited on the 15-m deep storage pool that used to be the paddy field.

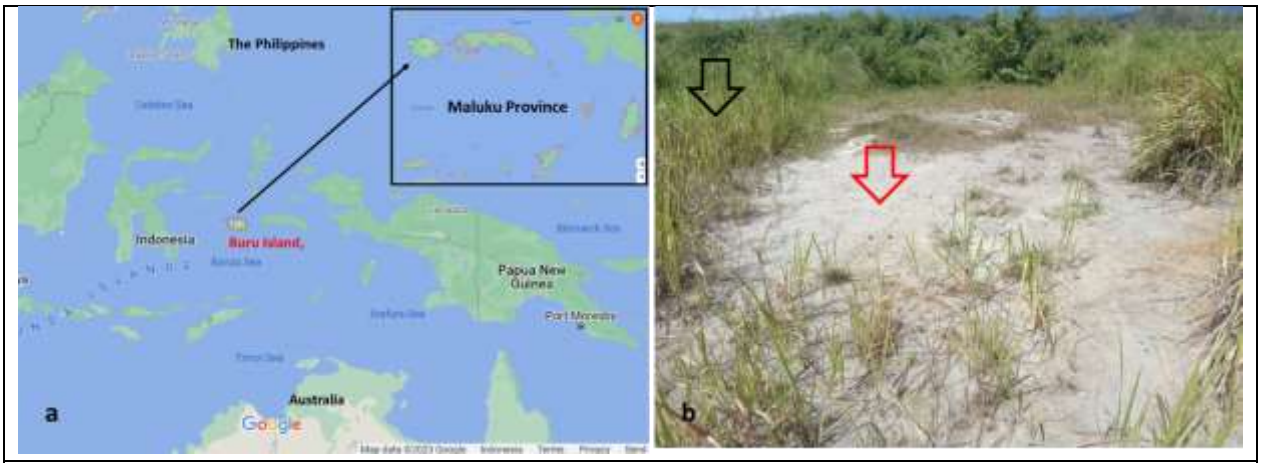

Figure 1. a. Study location in Buru Island of Maluku Province, Indonesia; b. gold-mine tailing with vegetation (black arrow) and without vegetation (red arrow) three-year vegetation.

The study location is in tropical region with the altitude of 9 m above sea level and geographical position is 3◦28'26"S-126◦59'19"E. The annual temperature is approximately 27 ^{o}C, with the minimum and maximum temperature of 24 ^{0}C and 31 ^{0}C at 2020. Naturally, pioneer plants included grass and sedges grow naturally on tailing. The grass species was *Digitaria ischaemun, Cynodon dactylon, Digitaria* sp. and *Cyperus compressus*; while the grass was dominated by *Fimbristylis* spp., *Imperata cylindrica* and *Cyperus* spp.

The research was carried out by descriptive quantitative method with the primary data taken up from the tailing piles. Samples were collected by purposive method from two sites included tailing piles which is grown by pioneer plants for two years and bare tailings where pioneer plant had failed to grow (Figure 1). The samples were taken from 5 sampling point of tailing that already grown by pioneer plants and another 5 samples of bare tailing. The tailing was collected by using auger at the depth of 20 cm by composite method. The soil was cleaned from the shoot of pioneer plant; then a total of five subsamples on the distance of 10 cm from sampling point were collected and then mixed, put in the plastic bag.

For bare tailing sampling, a total of five subsamples were taken at the distance of 20 cm; the weight is subsamples was 200 g. The five subsamples were mixed thoroughly and put in the plastic bag. All samples were subjected to bacterial and fungal count as well as pH and EC measurement. The density of microbes was determined by serial dilution plate method (Ben-David & Davidson, 2014) with nutrient agar and potato dextrose agar for bacterial and fungal count respectively. Bacterial plate agar was stored at 30^{o}C for 48 hours while fungal plate agar for 72 hours before counting. The pH was determined by potentiometer with pH meter and the EC was measured by EC meter. All measurement were performed in duplo. The average and standard deviation of all data were

calculated from two replications. The Student-t test with $p \leq 0.05$ was performed to compare the means of parameters of two different populations.

RESULTS

Based on serial dilution plate method, the population of total bacteria in tailing with pioneer plants and bare tailing were approximately 10^9 and 10^8 CFU/g respectively, which is equal to 9 and 8 of $\log_{10}$ (Table 1). Meanwhile the fungal population was approximately 4 of $\log_{10}$ in tailing that naturally grown with pioneer plants and 3 of $\log_{10}$ in bare tailing where the pioneer plants did not grow. Before experiment, the bacterial and fungal population was about 5 of $\log_{10}$ and below 2 of log10 respectively. The study showed that total bacterial count of both tailings was higher even though the colony characteristics did not vary. Nonetheless, the fungal population in each tailing condition was quite low. In general, the population of bacteria and fungi in mineral soil is 10^7 and 10^5 CFU/g respectively.

Table 1. Population of total bacteria and fungi in tailing after three-year revegetation

Tailing Condition and sample points	Microbial population ($\log_{10}$ of CFU/g)	
	Total bacteria	Total fungi
With pioneer plants		
A-1	9.31 ± 0.47	4.53 ± 0.03
A-2	9.09 ± 1.00	4.25 ± 0.1
A-3	9.63 ± 0.09	4.29 ± 0.12
A-4	9.58 ± 0.26	4.16 ± 0.31
A-5	9.35 ± 0.58	4.35 ± 0.33
Average	9.392 ± 0.22	4.31 ± 0.14
Without pioneer plants		
B-1	8.71 ± 0.12	3.52 ± 0.22
B-2	9.26 ± 0.63	4.10 ± 0.32
B-3	6.77 ± 0.09	3.48 ± 0.11
B-4	9.31 ± 0.69	3.99 ± 0.07
B-5	8.40 ± 0.42	3.87 ± 0.24
Average	8.49 ± 1.03	3.792 ± 0.28

Average data and standard deviation were calculated form two replications

The acidity of tailing irrespective of the presence of pioneer plant were strongly acid (Figure 2). In both soil condition the pH was less than 5. The average pH of tailing with and without the presence of vegetation were 4.06 ± 0.02 and 3.8 ± 0.08.

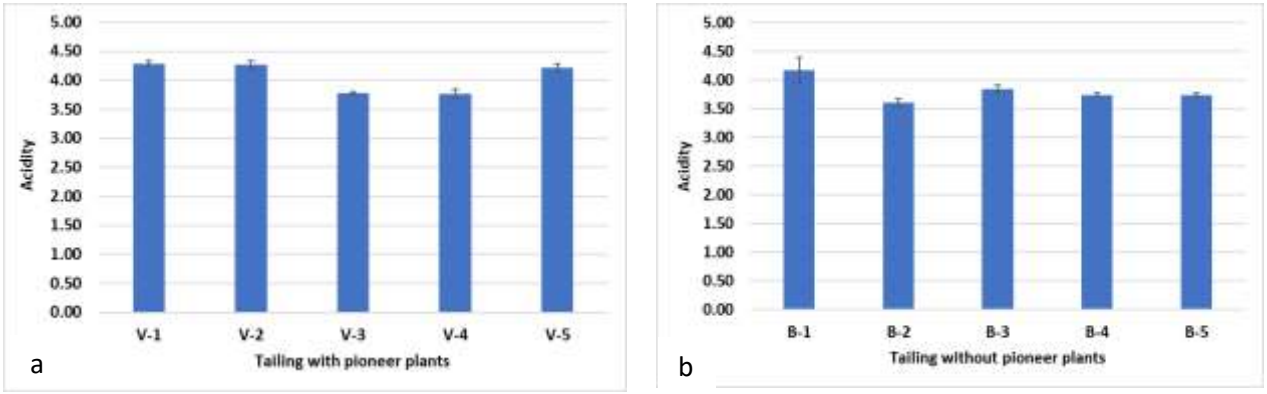

Figure 2. Acidity (pH) of gold-mine tailing grown with pioneer plants (a) and without plants (b) after 2-year revegetation. Average data and standard deviation were calculated form two replications. V1-V5 and B1-B5 are the sampling points.

The EC of all tailing condition were very high that exceed the EC of normal soil. According to USDA (2011),

the EC (ds/m) < 2 showed that soil is not saline, but EC > 16 is strongly saline. This study demonstrated that the lowest EC in tailing with pioneer plant was 7.82 ds/m and the highest was 35.60 s/m (Figure 3), but the average was still very high, 21.61 ds/m. The bare tailing has the EC of 58.94 ds/m; the highest and lowest EC was 109 ds/m and 37.66 ds/m.

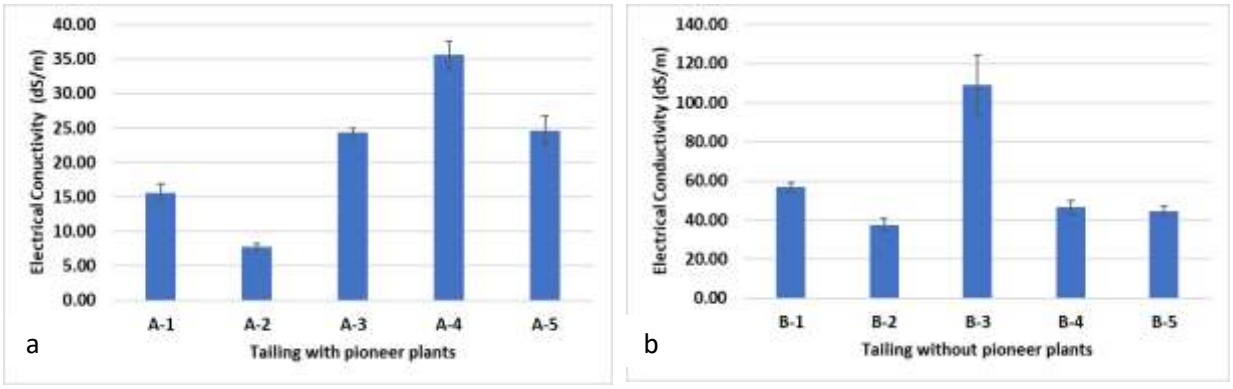

Figure 3. Electrical conductivity (EC) of gold-mine tailing grown with pioneer plants (a) and without plants (b) after 2-year revegetation. Average data and standard deviation were calculated form two replications. V1-V5 and B1-B5 are the sampling points.

Based on Student-t test with $p \leq 0.05$, the total bacterial and fungal population of tailing with pioneer were significantly higher than bare tailing; but the EC of bare tailing was clearly lower (Table 2). Two years after revegetation, the pH of both tailing condition was not different.

Table 2. The p-value of Student-t test for total bacteria, total fungi, acidity and electrical conductivity of tailing with and without vegetation

Tailing with and without overburden	Total Bacteria	Total Fungi	pH	EC
different	0.848	0.522	0.244	37.326
t-test value	1.91	3.75	-1.6	-2.72
p value	0.129*	0.013*	0.153	0.042*

*Significant at $p \leq 0.05$

DISCUSSION

Heterotroph bacteria and fungi benefits the exudates for carbon and nutrient source. Therefore, the population of both fungi and bacteria in tailing grown by pioneer plants were higher based on the Student-t test (Table 2). The adaptability of these plants indicates that these plants can produce root exudate, which can act as a supplier of carbon and nutrient sources for indigenous bacteria and fungi in the tailings area. Plant roots exert the exudates composed of Low and high molecular weight compound (Ma et al., 2022). Increasing the population of bacteria and fungi indicates that the condition of root area is improved for microbial and pioneer plants growth, which will interact positively with each other. Plants and soil microbes engage in biochemical interactions and exchange signal molecules (Pinton, 2007). Root exudates provide microorganisms with a source of carbon, while microorganisms are the most active biological factor affecting the formation and stability of aggregates (Ma et al., 2022). Concluded decades ago that the exudates determined the microbial diversity in the rhizosphere (Nye, 1981) This increase of microbes in tailing with vegetation showed the potency of pioneer plants to begin successful tailings revegetation at the Buru gold mine.

On the other hand, Bare tailing, which is not grown by any vegetation, did not received additional organic matter from vegetation. Since the temperature and humidity in tropic is high, decomposition rate will be intensified. It is supposed that the organic matter content of bare tailing is reduced that hence reduce the growth of heterotroph bacteria and fungi. The other reason, microbes include bacteria and fungi have possibly exclusive characteristic and are unculturable. According to Raaijmakers & Weller (2001) 98% of the soil microbes cannot be cultured.

The pH of both locations was increased compared to the initial pH at 2017; before the revegetation, the acidity of tailing at 2017 was 2.7 which is strongly acid. Acidity increment was possibly caused by the leaching of organic acid out of the root zone since in the rainy season flood is occured. It is likely that plant adsorb NO_3^- and root release the OH^- and HCO_3^- (Hinsinger et al., 2003). On the other hand, the oxidation of Fe II leads to a decrease in soil pH. Even though the soil was strongly acid, the bacteria survive in this harsh environment. These results agree with the research of Goswami et al. (2017) that verified the presence of certain bacterial genus in soil with the pH of 3.8-5.5.

This current research showed that pioneer plants enabled to grow in soil with high EC; and lower the EC after three years revegetation. Soil electrical conductivity (EC) measures the ability of soil water to carry electrical current. Therefore, the concentration Cations Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, and NH_4^{+} and anions SO_4^{2-}, Cl^{-}, NO_3^{-}, and HCO_3^{-} dissolved in soil water regulates the soil EC (do Carmo et al., 2016). Material used in gold mining possibly contained cations and anions described above as well as various metallic cations such as zinc and copper. The presence of vegetation reduced the EC since roots in soil play a significant role in adsorbing the ions and reduce the EC.

CONCLUSION

After three-year revegetation, the population of bacterium and fungus in tailing that was naturally grown by sedges and grasses were lower than microbial count in bare tailing without vegetation. In general, the bacterial and fungal count of tailing with vegetation was 9.3 and 4.3. while in bare tailing were 8.49 and 3.79. The acidity of both locations was strongly acid 3.52-4.29; which is higher than the pH before vegetation (2.7). However, there was no difference pH in both locations. Pioneer vegetation reduced the EC of soil; whereas the EC of bare tailing was 58.94 ds/m; more than twice of EC in tailing with vegetation (21.61 ds/m). The study indicated that pioneer plants have the role not only in the succession process of revegetating gold mine tailings, but also in improving the tailing quality during natural revegetation.

Acknowledgement

Authors thank to the Agricultural Office of Buru Regency for the consent to implement this study.

References

Arocena, J.M., van Mourik. J.M., van Mourik, J.M., Schilder, M.L.M., Cano, A.F. (2010). Initial Soil Development Under Pioneer Plant Species in Metal Mine Waste Deposits. *Restoration Ecology* 18(s2), 244 – 252.DOI: 10.1111/j.1526-100X.2009.00582.x

Ben-David, A., & Davidson, C.E. (2014). Estimation Method for Serial Dilution Experiments. *Journal of Microbiological Methods,* 107, 214-221. https://doi.org/10.1016/j.mimet.2014.08.023

do Carmo, D.L., Silva, C.A., de Lima, J.M., & Pinheiro, G.L. (2016). Electrical Conductivity and Chemical Composition of Soil Solution: Comparison of Solution Samplers in Tropical Soils. *Revista Brasileira Ciencia do Solo,* 40, e0140795. https://doi.org/10.1590/18069657rbcs20140795

Goswami, G., Deka, P., Das, P., Bora, S.S., Samanta, R., Boro, R.C., & Barooah, M. (2017). Diversity and Functional Properties of Acid-Tolerant Bacteria Isolated from Tea Plantation Soil of Assam. *3 Biotech*, 7(3), 229. doi: 10.1007/s13205-017-0864-9

Gupta, V. (2011). Microbes and Soil Structure. In: Gliński, J., Horabik, J., Lipiec, J. (eds) Encyclopedia of Agrophysics. Encyclopedia of Earth Sciences Series. Springer, Dordrecht. https://doi.org/10.1007/978-90-481-3585-1_91

Hindersah., R., Kalay, A.M., Komarya, A., & Kamaluddin, N.N. (2019). Natural Revegetation of Tailing Deposited on Agricultural Area in Buru, Maluku. *IOP Conf. Series: Earth and Environmental Science*, 308, 012054. doi:10.1088/1755-1315/308/1/012054

Hinsinger, P. & Tang, C. (2003). Origins of Root-Mediated pH Changes in The Rhizosphere and Their Responses to Environmental Constraints: A review. *Plant and Soil*, 248**,** 43–59. https://doi.org/10.1023/A:1022371130939

Ismayilov, A.I., Mamedov, A.I., Fujimaki, H., Tsunekawa, A., & Levy. G.I. (2021). Soil Salinity Type Effects on the Relationship between the Electrical Conductivity and Salt Content for 1:5 Soil-to-Water Extract. *Sustainability,* 2021, 13(6), 3395; https://doi.org/10.3390/su13063395

Jacoby R, Peukert, M., Succurro, A, Koprivova, A., & Kopriva, S. (2017). The Role of Soil Microorganisms in Plant Mineral Nutrition—Current Knowledge and Future Directions. *Frontier in Plant Science*, 8:1617. doi: 10.3389/fpls.2017.01617.

Namirimu, T., Kim, J., & Zo, Y-G. (2019). Isolation and Identification of Alkali-tolerant Bacteria from Near-Shore Soils in Dokdo Island. *Microbiology & Biotechnology Letters*, 47(1):105-115 https://doi.org/10.4014/mbl.1807.07018

Ma, W., Tang, W., Dengzeng, S., D., Zhang, D., Zhang, T., & Ma, X. (2022). Root exudates contribute to belowground ecosystem hotspots: A review. Frontier in Microbiology, 2022, 13. https://doi.org/10.3389/fmicb.2022.937940

Nye, PH. 1981. Changes of pH Across the Rhizosphere Induced by Roots. *Plant and Soil*, 61, 7–26. https://doi.org/10.1007/BF02277359

Pinton R., Veranini Z. & Nannipieri P. (2007). The rhizosphere. Biochemistry and organic substances at the soil-plant interface. New York, USA: Taylor & Francis Group, LLC.

Poppiel, R.B., Lacerda, M.P.C., de Oliveira Junior, M.P., Demattê, J.A.M., Romero, D.J., Sato, M.V., de Almeida Júnior, L.R., & Casso, L.F.M. (2018). Surface Spectroscopy of Oxisols, Entisols and Inceptisol and Relationships with Selected Soil Properties. *Revista Brasileira Ciencia de Solo*, 42, e0160519. https://doi.org/10.1590/18069657rbcs20160519

Raaijmakers J.M. & Weller D.M. (2001). Exploiting Genotypic Diversity of 2,4-diacetylphloroglucinol- Producing *Pseudomonas* spp.: Characterization of Superior Root-Colonizing *P. fluorescens* Strain Q8r1-96. *Applied & Environmental Microbiology*, 67, 2545-2554.

Rousk, J., Bååth, E., Brookes,P ., Lauber, L.L., Fierer, N., Lozupone, C., Caporaso, J.G. & Knight, R. (2010). Soil Bacterial and Fungal Communities Across a pH Gradient in an Arable Soil. *ISME Journal*, 4, 1340–1351 (2010). https://doi.org/10.1038/ismej.2010.58.

USDA. 2011. Soil Quality Indicator. United States Department of Agriculture. Natural Resources Conservation Service.

Wangi, A.P., Hindersah, R., Sule, M.I.S. (2023). Effect of Cow Manure on Soil Organic Carbon, Total Nitrogen, and Growth of Choy Sum in Gold Mine Tailings. *International Journal of Life Science and Agriculture Research*, 2(6), 127-133. DOI: https://doi.org/10.55677/ijlsar/V02I06Y2023-06 Ismayilov

Yuningsih, L., Hermansyah, Ibrahim, E., & Marsi, (2021). Analysis on The Characteristics of Ex-Mining Soil After 5 Years and 10 Years of Revegetation, *Media Konservasi*, 26(3), 239-247. DOI: 10.29244/medkon.26.3.239-247 E-ISSN: 2502-6313