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The Influence of Length of Rehabilitation Process for Ex-Nickel Mining Land on Soil pH, Soil Organic Matter, Population and Distribution of Soil Microbes

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ABSTRACT

This study aimed to analyze the influence of rehabilitation length process of the ex-nickel mining land on soil pH, SOM, population and soil microbial distribution in East Halmahera, North Maluku, Indonesia. Purposive sampling method was taken under completely randomized design (CRD) with variation of rehabilitation length process: 0, 2, 4, 6, and 8 years. The parameter included soil pH, SOM, population and soil functional bacteria. Data was analyzed using ANOVA and continued by Duncan Multiple Range Test (DMRT) at 5% error level. The result showed the length of rehabilitation process can reduce soil pH, increasing bacteria and fungi population. Four years of rehabilitation process in 2020-Nancy with special treatment of intensive watering resulted the best soil quality characterized by soil pH 6.48, SOM 4.03%, bacteria population of 85 × 10^1 CFU/g, fungi population of 7 × 10^1 CFU/g, non-symbiotic nitrogen-fixing bacteria of 7.0 × 10^1 CFU/g, phosphate-solubilizing bacteria of 0.5 × 10^1 CFU/g, potassium-solubilizing bacteria of 1.0 × 10^1 CFU/g, and proteolytic bacteria of 3.0 × 10^1 CFU/g. The rehabilitation length of 4 years with intensive watering is recommended to provide nutrient and soil microbial on ex-nickel mining rehabilitation.

1. INTRODUCTION

Nickel mining lands have undergone changes in physical, chemical, and biological conditions due to mining activities. The changes can have a negative impact on the distribution and activity of soil microbes (Mentis, 2020). Mining activities cause damage to the topsoil, loss of vegetation, and soil pollution. Damage to the topsoil can reduce the availability of organic matter, which is a source of energy and nutrients for soil microbes. Loss of vegetation can reduce the supply of organic carbon to the soil. Soil pollution causes the death of soil microbes and disrupt the balance of the soil ecosystem (Bing *et al*., 2016). The result of all this is a decline in soil quality and land carrying capacity.

Soil microbes in ex-mining lands had important role in ecological restoration and bioremediation processes (Neina, 2019). Microorganisms have certain mechanisms to change pollutants into a source of energy in their life mechanisms, so they can be used as biofertilizers in phytoremediation (Joniec *et al*., 2019; Rusnam *et al*., 2024). The diversity of microbial communities in ex-mining land had influenced of the level organic matter and metal contamination. In addition, ecological restoration efforts have been shown to have a positive impact on the structure of microbial communities, the recovery of contaminated land and the improvement of ecosystem services. Microbial communities play a crucial role in ecological restoration by driving essential processes that sustain ecosystem health and resilience. These microorganisms, including bacteria, fungi, and archaea, facilitate nutrient cycling, enhance soil structure, and support plant growth by forming symbiotic relationships with vegetation. In degraded environments, reestablishing a balanced microbial community can accelerate the recovery of soil fertility, promote biodiversity, and improve ecosystem stability (Atuchin *et al*., 2023; Yang *et al*., 2023).

The low distribution of soil microbes in mining land can have a negative impact on soil fertility and crop productivity. Therefore, efforts are needed to restore the distribution and activity of soil microbes in mining land (Herrera *et al*., 2007; Joshi & Garkoti, 2023). The restoration of the distribution and activity of soil microbes in mining land is one of the keys to improving soil fertility and crop productivity in that land (Feng *et al*., 2019). Rehabilitation of ex-mining land is a land restoration activity that is carried out by reconstructing the irregular topography into regular topography, with the aim of restoring, maintaining and improving land functions to maintain carrying capacity, productivity and its role in ecosystem services (Hu *et al*., 2020; Tong *et al*., 2022).

The restoration of soil fertility through the rearrangement of topography will improve micro and macro aggregates, thus providing pore space for microhabitats in regulating the activity and modulation of soil microbial populations and soil functional bacteria. Rehabilitation treatments consisting of planting certain vegetation are not able to increase soil fertility to a level that can support plant growth and soil biota populations. The length of rehabilitation plays a crucial role in the success of rehabilitation efforts. Adequate time allows for the proper establishment of vegetation, stabilization of soil, and restoration of ecological functions. A longer rehabilitation period provides the opportunity for ecosystems to recover more fully, enhancing biodiversity and ensuring that the land can be sustainably used in the future. Insufficient rehabilitation time, on the other hand, may lead to incomplete recovery, increased erosion, and long-term environmental degradation. Therefore, the time allocated for rehabilitation is a key factor in determining the overall effectiveness and sustainability of these efforts (Leomo *et al*., 2021). Based on the statements above, this research aimed to determine the effect of rehabilitation duration on soil pH, soil organic matter (SOM), as well as the population and distribution of microbes, particularly functional bacteria, in the rehabilitated land of ex-nickel mines in East Halmahera, North Maluku Province.

2. MATERIAL AND METHODS

2.1. Time of Research, Location and Sample Collection

A field experimental was conducted from May 2023 to October 2023 at the ex-nickel mining land rehabilitations in East Halmahera, North Maluku, Indonesia. Eight rehabilitation locations were selected and the exact coordinate was determined GPS using a GPS, including Sandoro (0°50'0,07" N, 128°16'44,83" E), North Caleban A (0°49'40,33" N, 128°17'39,48" E), North Caleban B (0°49'40,33" N, 128°17'39,48" E), 2022-Hikari (0°49'58,47" N, 128°16'50,48" E), 2020-Nancy (0°50'26,16" N, 128°17'9,52" E), 2018-Suwota 2A (0°50'26,20" N, 128°17'29,48" E), 2018-Suwota 2B (0°50'26,20" N, 128°17'29,48" E), and 2014-Suwota 1 (0°50'24,69" N, 128°17'39,04" E). The soil was collected from a depth of 0-20 cm as much as 500 g from each of the 8 locations. Wherever possible, soil samples were taken as close as possible to the plant roots.

2.2. Experimental Design and Data Analysis

The research used descriptive explorative and field survey using purposive sampling method. Design of research was Completely Randomized Design (CRD) with length of rehabilitation process, as the treatment, namely: 1. Sandoro (0 year); 2. Caleban Utara A (0 year); 3. Caleban Utara B (0 year); 4. 2022 Hikari (2 year); 5. 2020 Nancy (4 year); 6. 2018 Suwota 2A (6 year); 7. 2018 Suwota 2B (6 year); and 8. 2014 Suwota 1 (8 year). Each location was divided into 3 points as the replications, thus there were 24 experimental sampling units. Data was analyzed using ANOVA and continued by Duncan Multiple Range Test (DMRT) at 5% error level.

2.3. Parameter and Soil Analysis

Soil pH was determined according to (Miller & Kissel, 2010). Soil pH was measured by putting a 10 g of soil and 50 ml aquades in a plastic bottle (soil-to-water ratio of 1:5), shaking it for 60 min, then measure using a pH meter that had been calibrated with buffer solution pH 4 and pH 7. The pH value was displayed on the pH meter.

Soil organic matter (SOM) was determined using Walkley and Black method (de Oliveira Morais *et al*., 2019). A 0.5 g of soil with the mixture $K_2Cr_2O_7$ 1 N 5 ml and H_2SO_4 7.5 ml in 100 ml Erlenmeyer flasks. The sample was let for 30 min and filled with H_2O till 100 ml, and then filtered by Whatman 42 filter paper to determine C-organic by spectrophotometer (Spectonic Genesys 20 Visible). The content of SOM (%) was calculated by multiplying C-organic with a factor of 1.74.

Microbial population and functional bacteria was determined using Total Plate Count (TPC) method (Mailoa *et al*., 2017). Media for bacterial growth was prepared using nutrient agar (NA) composed of 0.3 g beef extract, 0.5 g peptone, 1.8 g bacto agar and 100 ml aquades. To prepare media for fungal growth, potato dextrose agar (PDA) was required (3.9 PDA instant and 100 ml aquades). Media for non-symbiotic nitrogen-fixing bacteria growth used Lowenstein Jensen medium (2 g sucrose, 0.1 g K_2HPO_4, 0.05 $MgSO_4$, 0.05 g NaCl, 0.01 g $FeSO_4$, 0.0005 g N_2MoO_4, 0.2 $CaCO_3$, 1.8 bacto agar and 100 ml aquades). Media for phosphate-solubilizing bacteria growth used Pikovskaya agar (1 g glucose, 0.5 g $Ca_3(PO_4)_2$, 0.05 g $(NH_4)_2SO_4$, 0.02 g KCl, 0.01 g $MgSO_4$, 0.01 g $MnSO_4$, 0.1 g $FeSO_4$, 0.05 g yeast extract, 1.8 g bacto agar and 100 ml aquades). For potassium-solubilizing bacteria growth, Alexandrov agar was prepared (0.5 g glucose, 0.0005 g $MgSO_4$, 0.2 g mineral K, 0.01 $FeCl_3$, 0.2 g $Ca_3(PO_4)_2$, 0.2 g $CaCO_3$, 1.8 bacto agar, and 100 ml aquades). Media for the growth of proteolytic bacteria used Skim Milk Agar (SMA) composed of (1 g skim milk powder, 1.8 bacto agar, and 100 ml aquades). All of the above media were sterilized in an autoclave at 121°C with 1.5 atm for 25 min. The sterilized media (10-15 ml) was poured into a petri dish and was let to harden slightly. Sterilization of equipment was carried out in an autoclave at 121°C with 1 atm for 20 min.

The physiological saline solution (garfis) was prepared by weighing 0.9 g NaCl and dissolving it in 100 ml of aquades. Each soil sample is weighed 10 g and added to 90 ml of garfis in an Erlenmeyer flask and shaking it for 15 to 20 min until homogen. Then, 1 ml of the solution was pipetted and added to a test tube containing 9 ml of garfis (10^{-2} dilution). Next, inoculation was carried out on solid media in a petri dish using the spread plate method. To observe the growth of bacterial populations, inoculation was carried out on NA media, at a dilution of 10^{-6}. To observe the growth of fungi, inoculation was carried out on PDA media, at a dilution of 10^{-4}. To observe the growth of functional bacteria, inoculation was carried out on Lowenstein Jensen media for non-symbiotic nitrogen-fixing bacteria, Pikovskaya media for phosphate-solubilizing bacteria, Alexandrov media for potassium-solubilizing bacteria, and SMA media for proteolytic bacteria, at a dilution of 10^{-7} with each made in duplicate. The samples were then incubated for 5 × 24 h at 35°C. Observations were made every 24 h until no more growth of microbial colonies.

3. RESULT AND DISCUSSION

Ecosystem of ex-nickel mining land in East Halmahera, North Maluku, have different soil pH, SOM and biological properties as summarized in Table 1. One of the causes is the presence of plant growing in the location, both those deliberately planted and plants grow wildly. Vegetation cover can protect the soil from erosion around the mining site. Important to select trees species with fast growing, tolerance of draught, tolerance of soil acidity, and metal contaminant for the success rate of rehabilitation, because rehabilitation needs time and the long-term livelihood following mining (Prematuri *et al*., 2020a). Silvicultural characteristics can be defined as the stabilizer of microclimate to development soil microorganisms and natural plants (Armstrong *et al*., 2014).

Table 1. Soil pH, SOM, and biology properties of ex-mining lands

Rehabilitation Area	Rehabilitation Length (year)	pH H_2O		SOM (%)		Bacteria Population (× 10 CFU/g)		Fungi Population (× 10 CFU/g)	
Sandoro	0	6.84^{ab}	N	4.10	M	3.2	U	0.2^{bc}	U
North Caleban A	0	6.11^{c}	MA	2.60	M	1.2	U	0.0^{c}	U
North Caleban B	0	6.20^{bc}	MA	3.63	M	2.5	U	0.0^{c}	U
2022-Hikari	2	6.40^{abc}	MA	3.75	M	2.3	U	0.2^{bc}	U
2020-Nancy	4	6.48^{abc}	MA	4.03	M	85	U	7.1^{a}	U
2018-Suwota 2A	6	6.06^{c}	MA	3.81	M	4.0	U	0.5^{bc}	U
2018-Suwota 2B	6	6.87^{ab}	N	2.40	M	5.3	U	1.5^{b}	U
2014-Suwota 1	8	7.04^{a}	N	3.88	M	4.3	U	1.2^{bc}	U
Significance		0.033*		0.188^{ns}		0.116^{ns}		0.000**	

* = significant; ** = very significant; ns = not significant. Status based on Ritung *et al*. (2011): N = Neutral, MA = Moderately Acid, M = Medium, U = Under Quality

3.1. Soil pH

Table 1 showed that soil pH was moderately acid to neutral. The soil pH values around 6.06 – 7.04. ANOVA results showed that treatment (rehabilitation length) had a significant effect on soil pH (p<0.05) and DMRT results, showed that 2014-Suwota 1 (0 year) had the highest pH with 7.04. The results showed that length of rehabilitation process can generally increase soil pH, especially in location of 2014-Suwota 2B. The length of rehabilitation process promoted more plants grow. The factor that causes the decrease in soil pH is plant growing in the land. Plants produce root exudates, including organic acids that will lower soil pH. Prematuri *et al*., (2020b) stated that the decomposition process of plant litter also produces organic acids that lower soil pH. Microorganisms present in the land produce secondary metabolites that lower soil pH. The location of 2014-Suwota 1 land had poor plant growth, so the decrease in soil pH was not significant resulted the highest soil pH.

This finding is in line with Cui *et al*., (2012) stated that the overall soil pH of rehabilitation land tends to increase over time. Li *et al*., (2018), further stated that most soil properties had greater changes in the first 10 years after reclamation, compared to the following years, and that soil pH decreased rapidly during the initial reclamation. Soil pH has important to the amount of nutrients available for plants and the level of soil pollution. In the current research, the pH value is within the optimum range of around 6 – 9 (Stefanowicz *et al*., 2020; Wang *et al*., 2023) which will further influence growth of microorganisms in the soil.

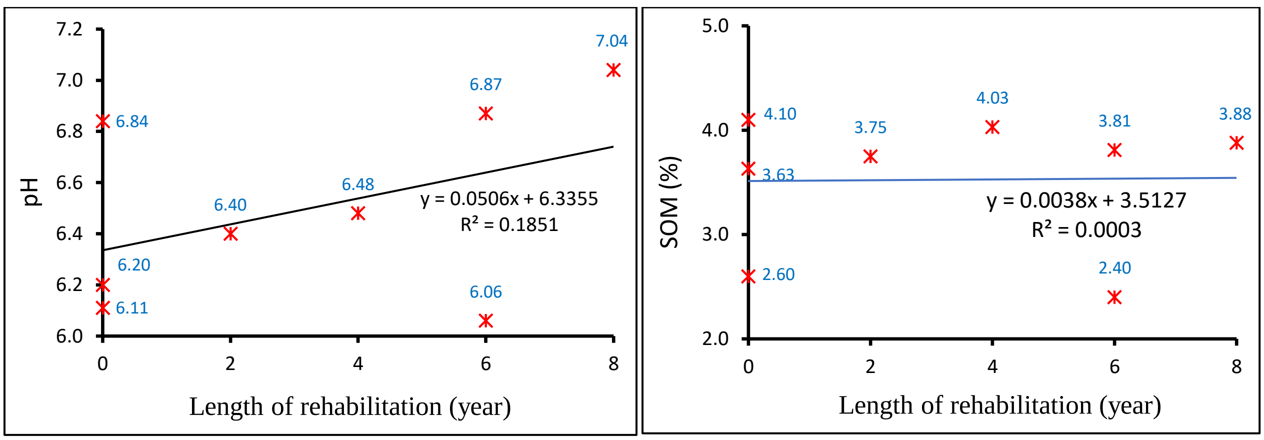

Figure 1. Effect of rehabilitation time on soil pH (left), and soil organic matter (SOM) (right)

3.2. Soil Organic Matter (SOM)

SOM in rehabilitated land were in the medium category. SOM value is around 2.40 – 4.10% (Figure 1) and ANOVA results showed that length of rehabilitation process had no significant effect (p>0.05). The Sandoro (0 year) had the highest SOM of 4.10%. High rainfall in the ex-nickel mining lands in East Halmahera affects the content of SOM. Suleymanov *et al*., (2021) stated that the loss of SOM is through mineralization, erosion, and leaching. The exfoliation of the top soil layer and then transferring it to bank soil also causes the SOM of the ex-nickel mining rehabilitation land to become low. This was supported by research of Meena *et al*., (2020) that the process of stripping topsoil layers from mining sites and storing them as replacement fill for other land will also affect the amount of SOM.

SOM has crucial role of soil physical properties, increase activities of soil microorganisms and supply nutrients for plant and production (Jiang *et al*., 2023). Sandoro has cover by topsoil from bank soil which is mining process before. While, higher SOM in 2020-Nancy (4 year) by 4.03% gets to decompose leaves and branches of the plant. Rehabilitation in 2020 Nancy has good and have natural ecosystem of forest, had a special treatment with intensive watering of land. This causes an effect had to growth of plants and good soil quality. Nickel mining impact was on the ecosystem and soil properties, including reducing SOM and organic carbon, even though SOM has important to soil fertility and water holding capacity to plants. That is why management of topsoil is important role for rehabilitation to carbon losses (Lima *et al*., 2016; Tibbett, 2008).

3.3. Population of Microbial

Soil microbes have important role in the nutrient cycle and the improvement of soil quality (Danapriatna *et al*., 2024; Mbukwa *et al*., 2023). In general, the length of rehabilitation process was highest the population of bacteria and fungi (Figure 2). ANOVA results showed that the length of the rehabilitation process had not a significant effect on population bacteria (p>0.05), but the length of the rehabilitation process had a significant effect on fungi population (p<0.01). DMRT results, showed that 2020-Nancy (4 years) with the highest fungi population of 7.1×10 CFU/g. The length of the rehabilitation process can increase the fungi population. Plants affect the abundance of soil microorganisms. Plants release root exudates in the form of organic compounds that serve as a source of nutrients and energy for microorganisms. This is evident in land that has not been or in the early stages of rehabilitation in Sandoro, North Caleban A, and North Caleban B, where there are no plants growing so the number of fungi is far below the land that has been rehabilitated. In general, soil microbes on former nickel mining rehabilitation land in East Halmahera, North Maluku, are below standard (< 10^6 CFU/g). According to (Raza *et al*., 2023), the growth of microbes is influenced by the environment, both biotic and abiotic factors. One of the environmental factors that influence the growth of microorganisms such as bacteria and fungi is the availability of nutrients that support their lives. If compared between fungi population based on the length of rehabilitation time, fungi population found on the rehabilitation land has higher than the land that is new or just being rehabilitated.

Nickel mining causes land damage and the loss of native microbes in the soil. Soil microbes such as bacteria obtain nutrients and energy from the decomposition of plants and organic matter, while fungi form a symbiosis with plants to obtain carbohydrates and nutrients (Nadalia & Pulunggono, 2020). The symbiosis between fungi and plant roots in providing water and nutrients is assisted with hyphae of fungi that move in the soil.

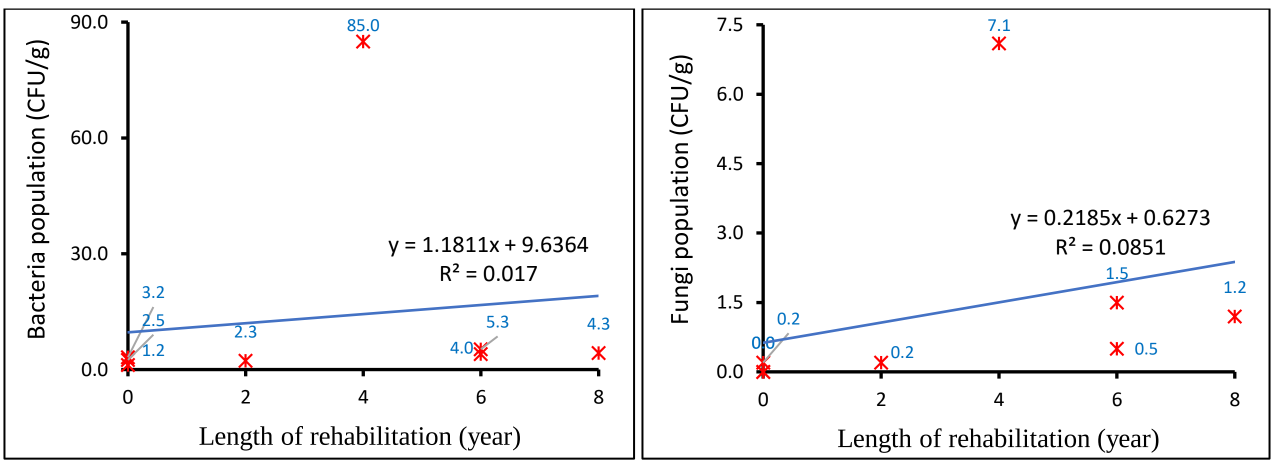

Figure 2. Effect of rehabilitation time on the bacteria population (left) and fungi population (right)

3.3. Soil Microbial Distribution

The presence of functional bacteria in rehabilitation land is influenced by vegetation, plant growth conditions, and plant type. The length of the land rehabilitation process is influenced by plant growth. In this research, the length of rehabilitation process, can affect to more plants grew. Syaf *et al*. (2020), state that soil pH, organic matter, and nutrients affect plant growth. Ex-nickel mining rehabilitation land in East Halmahera, North Maluku Province, has a soil pH in the range of moderately acidic to neutral and medium category of SOM, which can support the growth of microorganisms in the soil. Special treatment with watering intensive on 2020 Nancy caused good plant growth and the discovery of a number of functional bacteria that were different from other the length of rehabilitation process. Table 2, showed that distribution of soil microbes in 2020-Nancy site is as follows: non-symbiotic nitrogen-fixing bacteria of 7.0×10 CFU/g, phosphate-solubilizing bacteria of 0.5×10 CFU/g, potassium-solubilizing bacteria of 1.0×10 CFU/g, and proteolytic bacteria of 3.0×10 CFU/g which distinguishes it from other rehabilitation lands. Overall, the distribution of soil microbes is a complex and dynamic process that is influenced by many factors. Nickel mining has an impact on soil microbes and changes in the activity and diversity of soil microbes. Therefore, it is necessary to consider how to manage ex-nickel mining land for the sustainability of the agroecosystem.

Table 2. Soil microbial distribution of functional bacteria (× 10 CFU/g).

Ex-Nickel Mining Rehabilitation	Non-Symbiotic Nitrogen-Fixing Bacteria	Phosphate-Solubilizing Bacteria	Potassium-Solubilizing Bacteria	Proteolytic Bacteria
Sandoro	3.0	0.0	0.0	0.0
North Caleban A	1.0	0.0	0.5	0.0
North Caleban B	0.0	0.0	0.0	0.0
2022-Hikari	5.0	0.5	0.5	0.0
2020-Nancy	7.0	0.5	1.0	3.0
2018-Suwota 2 A	0.0	0.0	0.0	0.0
2018-Suwota 2 B	1.0	0.5	0.0	0.0
2014-Suwota 1	0.5	0.0	0.0	0.0

4. CONCLUSIONS

Soil chemical characteristics on ex-nickel mining rehabilitation East Halmahera, North Maluku province, Indonesia have moderately acid to neutral pH (6.06 – 7.04); medium SOM (2.60 – 4.10); low bacteria population (1.2×10 – 85×10 CFU/g); low fungi population (0.0×10 – 7×10 CFU/g). The length of rehabilitation process can reduce soil pH, increasing bacteria and fungi population. However, the length of rehabilitation process and the special treatment of intensive watering in the 2020 Nancy (4 year) had effect to growth of plants and good soil quality. The 2020 Nancy had soil pH 6.48, SOM 4.03%, bacteria population 85×10 CFU/g, and fungi population 7×10 CFU/g, it was good and different than other the length of rehabilitation process. The soil microbial distribution in the 2020 Nancy had non-symbiotic nitrogen-fixing bacteria of 7.0×10 CFU/g, phosphate-solubilizing bacteria of 0.5×10 CFU/g, potassium-solubilizing bacteria of 1.0×10 CFU/g, and proteolytic bacteria of 3.0×10 CFU/g, it was good and different than other land. The 2020 Nancy with 4 year of length rehabilitation process with special treatment of intensive watering has recommended to provide nutrient and soil microbial on ex-nickel mining rehabilitation.

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