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Soil microorganisms numbers in the tailing deposition ModADA areas of Freeport Indonesia, Timika, Papua

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ABSTRACT

Djuuna IAF, Masora M, Puradyatmika P (2011) Soil microorganisms numbers in the tailing deposition ModADA areas of Freeport Indonesia, Timika, Papua. Biodiversitas 12: 198-203. The objective of this study was to examine the number and distribution of bacteria, fungi and actinomycetes in the inactive tailing deposition areas of Freeport Indonesia Mining and Gold Company, Timika. One hundred ninety eight composite samples (0-20 cm) were taken from four location of inactive tailing ModADA (Modification Aijkwa Deposition Areas) namely double levee-bottom (fine texture); double levee-middle (medium texture); double levee-top (coarse texture); Mile 21 and transmigration areas of I to V. The conventional method of dilution and Plate Count Agar were used to examine the population of soil bacteria, fungi and actinomycetes. pH and moisture content were also analyzed. The numbers of bacteria in the tailing deposition areas are in the range from $3.48x10^5$ CFU/g soil to $102.83x10^5$ CFU/g soil, soil fungi from $1.51x10^5$ CFU/g soil to $106.61x10^5$ CFU/g soil and actinomycetes range from $0.32x10^4$ CFU/g soil to $113.74x10^4$ CFU/g soil. While in some transmigration areas, the number of soil bacteria, fungi and actinomycetes were lower than in the tailing areas. The number of soil bacteria and fungi were higher than actinomycetes. However, the coefficient of variation of actinomycetes (107%) was higher than soil fungi (89%) and bacteria (68%). Tailing deposition areas are considered as a good habitat for soil microorganisms. Overall, the number of soil organism in the tailings areas are considered medium to high, however to understand their functioning in each location under different land use system, more research are needed to evaluate their roles especially in the decomposition of soil organic matter.

Key words: tailing deposition, soil bacteria, fungi, actinomycetes.

INTRODUCTION

Tailings are small sized residue of mined material that generated from the separation process of copper, gold and silver by flotation technique of the concentrate rock (Mahler and Sabirin 2008). The production of copper, gold and silver of Freeport Indonesia Company produce a big amount of tailing in average of 230,000 tons/day which are deposited in the lowland area called Modified Ajkwa Deposition Area (ModADA) (PTFI 2003, 2005, 2007).

Tailing deposit has a particle size varies from coarse to fine, with less organic matter and contains very little nutrients. Taberima et al. (2011) has reported that tailing deposited in ModADA areas deficient in some macro nutrients, while base cations and some micro nutrients are abundant. Some of the nutrients from tailing deposit areas are generally not in the available form for plants, therefore its fertility level is very low. The availability of natural organic matter is very low affected by climate factor (rainfall, temperature, sunlight, humidity), organic matter availability, soil reaction, and the variety of decomposer microorganism. Soil microorganism especially decomposer microorganism is very important to the stability of organic matter weathering.

The information about the variation and status of soil microorganism such as bacteria, fungi and actinomycetes is needed to improve the fertility and productivity of tailing. These microorganisms are the largest group of soil microorganism (micro biota living in the natural habitat including tailing area. Bacteria is a dominant group of microorganisms in the soil with population of $>10^8$ CFU per gram soil and 10^4-10^6 units? number of species. Actinomycetes is the second largest group of microorganisms with density of population about 10^6-10^7 CFU per gram soil, while fungi in the third position with population density of 10^4-10^6 CFU per gram soil (Celentis Analytical 2003; Handayanto and Hairiah 2007). These groups of organisms are useful as quality and healthy indicator of soil. They have ecosystem function as one of sensitive biological marker and useful to identify the disturbance and damage of ecosystem (Roper and Ophel-Keller 1997).

Therefore, basic data about soil microorganism biodiversity especially the group of soil decomposer which is beneficial for plant growth in tailing deposition area is very important in the management and reclamation planning of tailing area. This information will be used as an important factor in the evaluation and identification of alterations occurred in the tailing deposition area.

As an effort to identify the availability of bacteria, fungi, and actinomycetes from the tailing deposition areas, the isolation and identification of this micro biota group from the tailing aiming to find out the micro biota population and distribution from several locations in inactive tailing deposit area (ModADA) and transmigration agricultural area as a standard of comparison.

MATERIALS AND METHODS

Soil sampling and analysis

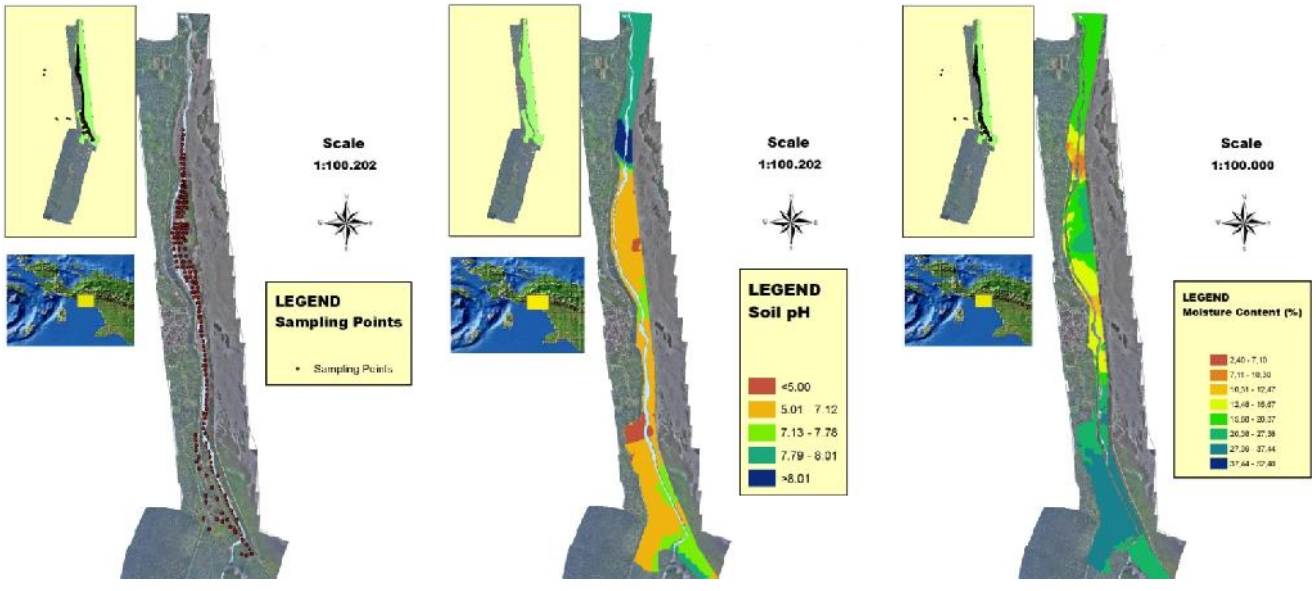
Inactive tailing sample is taken from the depth of 0-20 cm in 8 locations of ModADA areas and Transmigration Area i.e. lower-ADA (fine deposit); middle-ADA (medium deposit); upper-ADA (coarse deposit); old-ADA (inactive deposit area); Double Levee; Mil 21; and transmigration area SP I, II, IV and V. Research site and point of sample taken is presented on Figure 1A. The laboratory analysis was conducted in Timika Environmental Laboratory (TEL) Freeport Indonesia Company, for soil pH and soil moisture content. For the observation of microorganism population, it was conducted in Water Microbiology Laboratory, Public Health and Malaria Control, Freeport Indonesia Company, Kuala Kencana, Timika and continued in Soil Biology Laboratory Faculty of Agriculture and Agricultural Technology) and Microbiology Laboratory of Faculty of Mathematics and Natural Sciences of University of Papua Manokwari.

Soil samples were taken compositely in the depth of 0-20 cm by using a soil auger in each 200 m interval, but in the location which its width was smaller than the distance between points, was 50-100 m. In each point, sample was taken as many as 10 augers with 1 meter distance circularly in order to get composite sample from each point. Coordinate point of each sample point was determined by Global Positioning System (GPS). The number of the whole samples taken was 198 points, or as many as 1980 composed auger samples.

Isolation and identification of bacteria, fungi and Actinomycetes

The isolation of soil bacteria, fungi and actinomycetes was conducted by dilution method using NaCl 0.85% as a solvent with the dilution series of 10^{-1}-10^{-7}. Sample (100 µL) was poured into a petridish contained Nutrient Agar (NA) for bacteria, Potato Dextrose Agar (PDA) media for fungi and Starch-Casein Agar (SCA) media for actinomycetes which was then incubated in the incubator of temperature at 27-30^{0}C. After 2-3 days incubation (bacteria); 3-5 days (fungi); and 5-7 days (actinomycetes), observation was conducted based on 30-300 colony/plate thinning. The amount of colonies was counted by using Plate Count Method (Lay 1994).

All microorganisms were identified by isolating each microorganism by using universal media as a growing media for soil microorganism. Each microorganism could be purified in a special media. Afterward, the microorganism in pure culture was identified microscopically by Wet mounts and Gram stain, and biochemical test. Morphological observation of the microorganism found by microscope included cell size, form of mycelia and other characteristics, and then it was identified based on the characteristics of microorganism found and followed by Bergey's Manual Handbook of identification for bacteria; identification of fungi using the method of Cappuccino and Sherman (2001) and Atlas (1995), and for actinomycetes using the method from Sembiring (2000), Sembiring et al. (2000) and Prescott et al. (1999).



A **B** **C**

Figure 1. Research site map and point of sample taken in tailing deposition area of PT. Freeport Indonesia, Timika (A); and distribution map of soil pH (B) and soil moisture content (C).

Microbial Biomass Carbon (MBC) with Fummigation-Extraction method (Vance et al. 1987; Sparling 1990).

Data analysis

All data were then analyzed by using statistic analysis which consists of two stages: (i) data distribution was done by using conventional statistic (mean, minimum, maximum, median, standard deviation, skewness, kurtosis and coefficient of variation, and histogram), which was assumed implicitly that observation conducted was independent to every sample point; and (ii) Geostatistical Analysis was used to analyze the distribution of soil microorganisms, based on the location the samples was taken. Krigging interpolation was used to estimate data on one or more points not taken as a sample.

Microorganism distribution map was created by using GIS software ArcView/ArcMap ® (version 9.2 ESRI), with Spatial Analyst and Geostatistical Analyst extensions.

RESULTS AND DISCUSSION

Soil characteristics (pH and moisture content)

Data on soil pH and moisture content in the tailing areas is presented in Table 1. In general, the range of soil pH in the tailing areas was from 4.61 to 8.67 with the mean of 7.00. While the moisture content ranged from 2.40 to 62.00% with the mean of 20.13%. Soil pH and moisture content were factors that affected the number and distribution of soil microorganism in the soil.

The distribution of soil pH and moisture content in the tailing areas is presented in Figure 1B and 1C. It showed that most of the tailing areas have high value of soil pH, however only few locations have had lower soil pH (4.6-4.9). In contrast, only few areas in Mile 21 and 23 have >45.7 % of soil moisture content. This might be caused by high level of rain and the areas were located nearby river.

Population and distribution of soil microorganisms

Isolation result showed that bacteria, fungi and actinomycetes population in all study location were varied and ranged between $3.48x10^5$-$102.83x10^5$ CFU/g (bacteria), $1.51x10^5$-$106.61x10^5$ CFU/g (fungi) and $0.32x10^4$-$113.74x10^4$ CFU/g (actinomycetes). If it is compared between these three microorganisms, it can be seen that bacteria and fungi population were higher than actinomycetes. However, the Coefficient of Variation (CV) of actinomycetes was higher (107%) than fungi (89%) and bacteria (68%) (Table 2). In other words, the higher the coefficient of variation values the more the number of variation in the soil.

The comparison of soil microorganism population in tailing area and transmigration area (SP I, II, IV and V) was presented in Table 2. The total number of soil microorganism in tailing area tended to be higher than the average population of soil microorganism in transmigration area. The average population of microorganism in tailing area was $16.84x10^5$ CFU/g (bacteria), $11.83x10^5$ CFU/g (fungi), and $9.94x10^5$ CFU/g (actinomycetes). While the

Table 1. Mean of soil pH and moisture content in the tailing and transmigration areas

Variable	Mean ($x10^5$CFU/g dried soil)	Median ($x10^5$ CFU/g dried soil	SD	Kurtosis	Skewness	Min ($x10^5$ CFU/g dried soil)	Max ($x10^5$ FU/g dried soil)	CV (%)
Soil moisture content (%)	20.13	17.95	11.05	0.40	0.81	2.40	62.00	54.92
pH H_2O (1:2)	7.00	7.42	1.13	-0.93	-0.64	4.61	8.67	16.09

Table 2. The average population of bacteria, fungi and actinomycetes (0-20cm) in PTFI tailing area and transmigration area SP I, II, IV and V, Mimika District

Variable	Mean ($x10^5$CFU/g dried soil)	Median ($x10^5$ CFU/g dried soil	SD	Kurtosis	Skewness	Min ($x10^5$ CFU/g dried soil)	Max ($x10^5$ FU/g dried soil)	CV (%)
PTFI tailing area (n=190)								
Bacteria	16.84	12.82	11.34	16.19	2.73	3.48	102.83	67.36
Fungi	11.83	8.44	10.45	34.95	4.39	1.51	106.61	88.35
Actinomycetes	9.94	7.01	10.53	49.57	5.55	0.32	113.74	105.91
Transmigration areas (n=8)								
Bacteria	7.37	7.04	2.37	0.76	0.56	3.88	11.70	32.14
Fungi	5.47	4.83	1.90	-0.92	0.50	2.92	8.31	34.67
Actinomycetes	5.63	5.80	2.41	-1.73	-0.26	2.47	8.52	42.90
PTFI tailing area and transmigration area (n=198)								
Bacteria	16.46	12.38	11.28	16.23	2.74	3.48	102.83	67.99
Fungi	11.57	8.12	10.32	35.71	4.44	1.51	106.61	89.06
Actinomycetes	9.77	6.95	10.36	51.17	5.64	0.32	113.74	106.83

Note: SD= Standard Deviation, CV= Coefficient of Variation

average population of microorganism in transmigration area was $7.37x10^5$ CFU/g (bacteria), $5.47x10^5$ CFU/g (fungi), and $5.63x10^5$ CFU/g (actinomycetes).

Kriging interpolation result showed that the distribution of bacteria, fungi and actinomycetes in tailing area tended to be the same that was following the distribution of vegetation and soil characteristics such as pH and soil moisture content. Generally, soil pH and soil moisture content were the factors which could affect the total number, activity and distribution of microorganism in soil (Figure 2A, 2B, and 2C). The population of bacteria, fungi and actinomycetes was higher on secondary forest vegetations and natural succession area compared with the location planted by some agricultural crops.

Microbial Biomass Carbon (MBC)

The MBC in tailing deposition areas of ModADA ranged from 26.25 to 4957.29 ppm with the mean of 1774.61 ppm. Microbial Biomass C is one parameter that has been used to determine the amount of C in the soil microbe, therefore the MBC value has been indirectly correlated to soil C. The MBC in the tailing areas was tend to follow the distribution of soil texture, which is on the fine texture areas, the MBC was higher compare to coarse texture areas. This pattern can be affected the number of microorganisms in the tailing areas.

Based on the isolation result of the amount of soil microorganism found, therefore identification result of soil microorganism in tailing area and its surrounding was found 10 species of bacteria i.e. *Nitrosomonas* sp., *Clostridium* sp., *Bacillus cereus, Bacillus subtilis, Thiobacillus* sp., *Arthrobacter* sp., *Desulfovibrio* sp., *Serratia marcescens, Chromobacterium violaceum,* and *Pseudomonas* sp.; four species of fungi i.e. *Aspergillus fumigatus, Aspergillus* sp., *Aspergillus niveus,* and *Penicillium chrysogenum*; and also three species of actinomycetes i.e. *Micrococcus, Mycobacterium,* and *Arthrobacter*.

Results of soil microorganism population showed that the high number of soil microorganism in tailing area and its surrounding was not followed by range of this soil microorganism species in all of sample taken points.

Population and distribution of soil organisms are highly varied in the soil depended on soil types and characteristics, land cultivation and vegetation growing on it. Differences of land use resulted in different population of bacteria, fungi and actinomycetes (Gofar et al. 2007). There were three main factors which influenced population and biodiversity of soil microorganisms i.e. (i) weather, especially precipitation and humidity; (ii) soil condition/characteristic, particularly the acidity, humidity, temperature and the availability of soil nutrients; and (iii) type of vegetation such as forests, bushes and grass field (Hanafiah et al. 2005). In general the number of bacteria in the soil was higher compared to the number of other microfloras such as fungi, actinomycetes and algae, however individually the number was lower (Alexander 1977). This also could be seen toward comparison of the number of bacteria, fungi, and actinomycetes in tailing area and its surroundings. Bacteria were a group of microorganism in the soil which was the most dominant and included half of microbe biomass in the soil (Subba Rao 1944). Number of bacteria in the soil usually ranged between 10^8-10^9 CFU/g soil, while number of fungi and actinomycetes respectively ranged between 10^7-10^8 and 10^5-10^6 CFU/g soil.

Number and activity of soil microorganism was influenced by climate, vegetation and habitat of its surroundings including the soil characteristics and land use pattern. Vegetation difference and land use pattern in tailing area and transmigration area resulted the difference

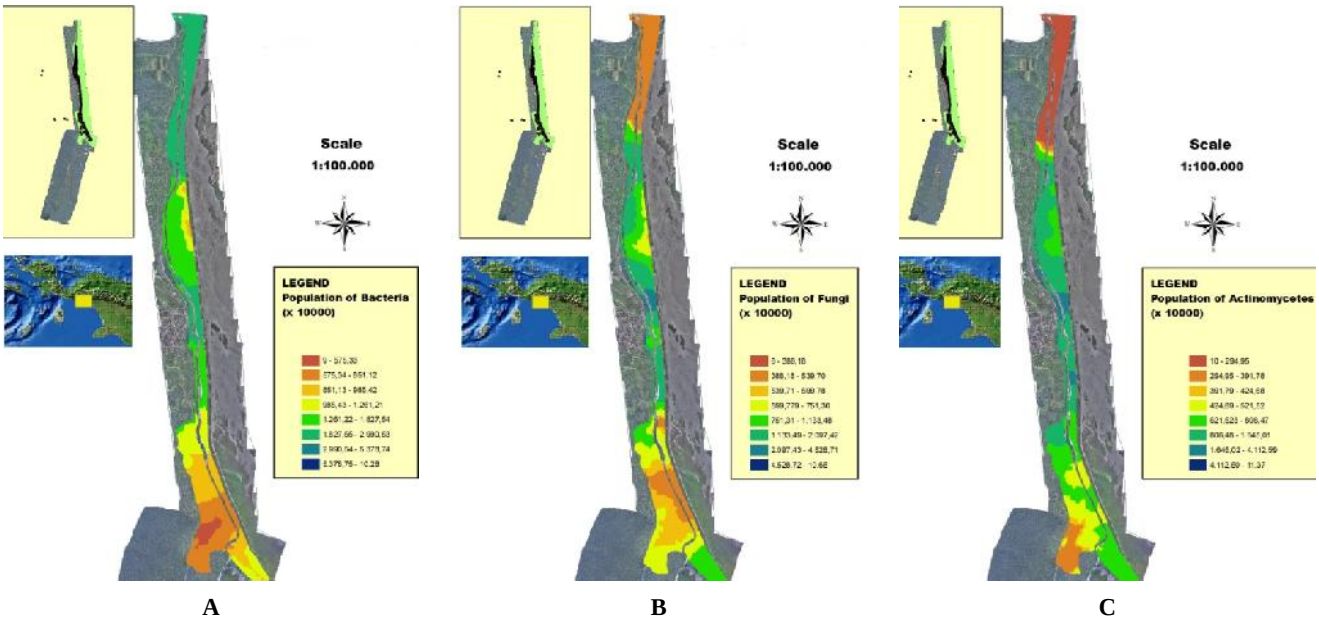


Figure 2. Distribution map of soil (A) bacteria, (B) fungi and (C) Actinomycetes in tailing area PT Freeport Indonesia, Timika.

on number of soil microorganism, because generally lands used for agricultural crops conservation, some treatments such as the continual application of chemical fertilizer and pesticide could influence the number and distribution of soil microorganism. In the contrary, in some location in tailing area, the number and distribution of soil microorganism was low. It could be happened due to the organic matter content of the soil was low. Generally soil with highly sand content has low organic matter content.

The species of soil microorganism found in all points of sample taken generally could exist and grow mostly in several species of soil such as *Pseudomonas* sp and *Bacillus* sp; however, their normal population in the soil was categorized as low. These bacteria were also known as zymogene and fermentative microorganism that needed energy from outside the soil (Subba Rao 1994). Included in this bacteria group was cellulose decomposed bacteria, nitrogen consumption bacteria and bacteria which could break ammonium into nitrate. Besides, among the bacteria species found in the study location, it was also found several colonies of bacteria resembling *Chromobacterium violaceum* (natural antibiotics producer bacteria "*violacein*"), and *Thiobacillus* and *Desulfovibrio* which are also species of bacteria which could oxidize and reduce sulfur so they were called the group of sulfur bacteria.

Majority, these bacteria exist and grow well in waterlog or anaerobe areas, but *Thiobacillus* bacteria group could also exist in aerobe condition. *Thiobacillus* is a type of bacteria that could oxidize inorganic sulfur compound so it was called special bacterium. This bacterium could produce sulfate acid if sulfur element was added in the soil to decrease soil pH as low as 2.0 long after it was incubated with the bacteria (Subba Rao 1994). In addition to this, *Thiobacillus* commercially played very important role in mining industry in the process of acid waste (Horan 1999). Another type of sulfur bacteria like *Desulfovibrio,* was reducing inorganic sulfate into sulfide hydrogen so the existence of this bacterium could reduce sulfur content for plant's nutrient and because of that it could influence agriculture production. This bacterium mostly live and grow well in anaerobe condition and could produce sulfide hydrogen. Some tailing areas which covered by waterlog condition could potentially be occupied by this type of bacteria, therefore regular monitoring in this areas can reduce the population of this bacteria.

Generally, the existence of other soil microorganisms such as fungi and actinomycetes was influenced by quality and quantity of organic substance in the soil. Fungi lived dominantly in the acid soil and monopolize the use of natural substrate in the soil. Group of soil fungi and actinomycetes found in the study location was the group of soil fungi and actinomycetes which was generally found in the soil such as *Aspergillus* sp. and *Streptomyces* sp. *Aspergillus* sp. was one of soil fungi type which produced substance which was similar to humic substance in soil and because of that it was somewhat important in maintaining soil organic substance (Subba Rao 1994).

Different with fungi, actinomycetes was not tolerant to acid and their number would decrease at pH 5.0. Usually, actinomycetes would grow well at pH between 6.5 and 8.0. So was with the level of moisture quite high, the number of actinomycetes was decreased.

Soil pH was one of soil chemical characteristics which very influenced to the number and distribution of microorganism in the soil. Population and distribution of this microorganism in tailing area and its surroundings were influenced by soil pH; however the relationship between pH and the soil microorganism was significantly negative. In general, number of soil microorganism would increase at soil pH nearly neutral. But several soil microorganism species could be also tolerant to the soil which was so acid or alkaline. Soil pH which was a little bit low (4.6-4.9) in several locations of sample taken, could impact in the increasing of metal solubility so that observation was needed if location of low pH was found.

Besides soil pH, soil moisture was one of environmental factors that influenced the number and activity of microorganism in the soil. Generally soil microorganism preferred the environment which was nearly moist, but several organisms were tolerant to dry condition and stagnation. This was the same with the observation result that soil moisture level influenced significantly negative to the population and distribution of soil microorganism in tailing area and its surroundings. The higher the level of soil moisture content in tailing area, the number of the distribution of the soil microorganism was low.

Soil organic matter is an important fraction that can support population of microorganism in the soil especially for those microorganisms in the organic matter. Microbial biomass is a living component in the organic matter which contains 2-7% from C organic in the soil (Gupta and Roget 2003). The total Microbial Biomass C in the tailing areas was in the range of 26.25-4957.29 ppm (very low to very high). Generally, the total number of MBC on the soil surface is 250 mg C/kg in the sandy soils and 1100 mg C/kg in the clay soils and high of organic matter. Although the MBC is only small part of organic matter (2-7%), its live and dynamic properties has been made sensitivity to most of land management compared to the total organic matter (Gupta and Roget 2003). Microbial biomass C in the soil can be used as an indicator to predict the soil fertility especially the status of organic matter in the soil (Sparling 1992).

Soil microorganism variation comprised number, distribution and species of existed microorganism in tailing area and its surroundings showed that the tailing area was one of good habitats for soil microorganism that was not different with other habitats. Because generally the number and distribution of soil microorganism in this area was categorized as middle-high from the average number of most organisms found in other types of soil. By the existence of soil microorganism population and distribution in the tailing area also showed that tailing areas was not the toxic habitat for this microorganism group. But the number of organism found in the tailing area was not followed by high variety of the species. This was caused by the characteristic of each organism that was number and distribution in the soil which was highly influenced by several soil characteristics such as physical, chemical and biological characteristic and other ecologycal factors. Soil

organic matter was one of the factors that highly determined the survival of soil microorganism, so that the increasing of soil organic matter in tailing area was really needed to maintain, to keep and to increase the number and the type of soil organism. In addition, planting some plants which was easily decomposed in tailing area was one of the alternatives that could increase the number and the species of soil microorganism and also to keep its balance and stability.

CONCLUSION

Number of soil organism in tailing area and its surroundings was categorized as middle-high. The average number and population of soil microorganism in tailing area and its surroundings were $16.46x10^5$ (bacteria), $11.57x10^5$ (fungi) and $9.78x10^4$ (actinomycetes) CFU/g soil. There were 10 species of bacteria i.e. *Nitrosomonas* sp, *Clostridium* sp, *Bacillus cereus, Bacillus subtilis, Thiobacillus* sp., *Arthrobacter* sp., *Desulfovibrio* sp., *Serratia marcescens, Chromobacterium violaceum,* and *Pseudomonas* sp.; four fungi species i.e. *Aspergillus fumigatus, Aspergillus* sp., *Aspergillus niveus,* and *Penicillium chrysogenum,* and also three species of actinomycetes i.e. *Micrococcus, Mycobacterium,* and *Arthrobacter.* Majority of bacteria and fungi found was decomposer microorganism of organic matter particularly cellulose decomposers and phosphate solubilizers. The biodiversity of soil microorganism comprised number, distribution and species of organisms in tailing area and its surroundings showed that tailing area was also one of a good habitat and was not toxic for soil microorganism which was not different with other natural habitats.

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