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Microbial diversity on sedimentated rice fields due to coal mining activities in Tenggara Seberang subdistrict of Kutai Kartanegara

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Abstract. The results showed that on upland rice fields with sediment found five genus of fungus with number of colonies 4.0×10^3 cfu/g to 9.3×10^4 cfu/g; three bacterial families with number of colonies 7.1×10^4 cfu/g to 2.8×10^5 cfu/g; and five genera of nematodes with the amount of 2.6×10^2 /kg of soil to 1.1×10^3 /kg of soil. In unpolished upland rice fields were found four genus of fungus with colonies of 2.4×10^3 cfu/g to 8.4×10^4 cfu/g, three bacterial families with number of colonies 1.2×10^5 cfu/g to 2.7×10^5 cfu/g and four genera of nematodes with the amount of 9.6×10^2 /kg of soil to 1.1×10^3 /kg of soil. The most common microbes are *Aspergillus*, *Cladosporium*, *Penicillium*, *Phytium* and *Trichoderma* (fungi), *Achromobacteraceae*, *Brevibacteriaceae*, *Micrococcaceae* (Bacteria), as well as *Dorylaimus*, *Hemicycliophora*, *Mononchus*, *Meloidogyne*, *Paratrichodorus*, *Radopholus*, *Rotylenchulus*, *Rhabditis*, *Seinura* and *Trichodorus* (Nematodes). Fungi, bacteria and nematodes have a good role in the process of soil decomposition. The results of soil chemical analysis showed that soil fertility is lower in upland rice fields with sediments compare to those without sediment.

Keywords: bacterial, fungus, nematodes, soil

1. Introduction

Environmental problems will lead to wider aspects in East Kalimantan, such as the decrease of soil productivity, soil compaction, sedimentation, the movement of soil or avalanche, disturbance to the security and health of the population, disruption of flora and fauna, as well as the microclimate change [1]. Sediment deposition caused by erosion that occurs during mining activities and after mining can lead to disruption of plant growth due to reduced oxygen. Factors that cause erosion by water in the form of rainfall, topography, soil type, land use and cover crop. Sedimentary sediments on land usually consisting of clay, dust, sand and large sand. The presence of sediment deposition usually does not affect soil fertility, it can even increase soil fertility in the land because it can bring nutrients and microbes from eroded land if the land has a good soil fertility rate.

The presence of microbes in the soil plays an important role in biogeochemical cycles and is highly responsive for recycling of organic compounds. Soil microbes affect ecosystem conditions in the soil by their contribution in the provision of plant nutrients, plant growth, soil structure and soil fertility [2-4].



The soil fertility criterion is determined by a combination of three interacting factors, ie physical, biological and chemical factors. Physical characteristics and soil chemistry can be understood more perfectly than their biological characteristics. Therefore, more is known physical status and soil chemistry, and little information about the biological status of the soil. There is little difficulty in determining the biological status of the soil because the substance is alive, dynamic and can change in space and time.

The dynamic nature of the soil biological status provides great opportunities for its management. The soil biological status can provide early warning of soil degradation, making it possible to implement more sustainable land management practices [5]. The biological aspects of the soil are complex and require better understanding because there is not much information about the amount and diversity of soil microbes, and how their activity levels in maintaining fertile and productive soils [6].

Microbes are organisms that have a very narrow niche that is very vulnerable to environmental changes. These vulnerabilities spur mutated microbes to survive in new environmental conditions [7]. The research aims to find out the diversity of microbes as well as the level of soil fertility in upland rice fields sedimentation due to coal mining activities in Tenggara Seberang Subdistrict of Kutai Kartanegara.

2. Material and Methods

2.1. The methods

This research is a qualitative descriptive research. The sampling is aimed to identify microbial and chemical analysis. The soil samples were collected from six different locations in field near the operational activity area of PT MSJ mining company in Bukit Pariaman Village, Tenggara Seberang Subdistrict, Kutai Kartanegara. They were the areas which were three upland rice fields with sediments (*UTM 50 M0522356 9976041*, *UTM 50 M0519390 9971851 (S2)*, *UTM 50 M0519289 9971079 (S3)*), and three upland rice fields without sediment (*UTM 50 M0522371 9976059 (U1)*, *UTM 50 M0519413 9971802 (U2)*, *UTM 50 M0519392 9971058 (U3)*). These samples then were analyzed in the Laboratory of Plant Pests and Diseases Science and Soil Science Laboratory of the Faculty of Agriculture, Mulawarman University, Samarinda, East Kalimantan, Indonesia.

2.2. Soil Microbial Analysis

For microbial analysis, the sample was taken from the rhizosphere area of the sample plants to identify the total population of bacteria, fungi, and nematodes. 1 kg of each soil sample was taken from the depth of 0-30 cm and mixed into one, then from that mixture, 1 kg was taken to represent a sample. Furthermore, the samples are packed in a plastic bag and labeled corresponding to its coordinate. Then, the sample was picked up to the laboratory in cool box, to maintain the existing microbes well preserved. This process then will be followed by the determination of the total population of microbes on the standard procedure for microbial analysis. In relation to be method used to calculate the microbes population, this study applied plate count method with a colony counter. A total of 1 g of soil was added by 9 mL of distilled water and made serial dilution to 10^{-6} . Dilution is used to define the population of each of the different parameters. The total population of bacteria was calculated using the dilution with a ratio of 10^{-5} and 10^{-6} under the media of *nutrient agar* (NA), within 2-days incubation period. While for the total fungi, this study used a ratio of dilution at 10^{-3} and 10^{-4} and using the media of *Potato Dextrose Agar* (PDA) within three days period of incubation. On the other hand, to determine the total population of nematodes, using a stereo microscope, a direct counting from a total of 50 g of soil were taken and extracted according to *Baermannfunnel method* within 2-3 days period of incubation.

2.3. Soil Chemical and Physical Analysis

The soil chemical analysis comprises more aspects such as pH, organic C, C/N ratio, total-N, P-available, K-available, and CEC. The analysis was conducted in the laboratory. The soil pH level was

usually measured in water suspension. The physical analysis was focused on the soil texture such as sand, silt, and clay

3. Results And Discussion

3.1. Microbial Biomass

Research shows that the presence of sedimentation has little effect on microbial development on upland rice fields, compared with land without sediment. Although on the third location without sediment the number of microbes and the population was slightly higher than the third location with sediment (Table 1).

In the first field, the diversity of unsedimentary field fungi has three genera (Aspergillus, Phytium, and Trichoderma) better than sedimentary fields with two genera (Aspergillus and Phytium), but sedimentary field has a slightly better number of fungal colonies. The diversity of nematodes in unsedimentary land has only one genus (Seinura) equal to the sedimentary field but differentiated genus (Rhabditis), but the nematode density on unsedimentary field is better than the sedimentary field. Diversity of bacteria in unsedimentary field is two families (Micrococcaceae and Achromobacteraceae), whereas in the sediment field there is only one bacterial family (Micrococcaceae) but has better colony than unsedimentary field.

In the second field, the diversity of fungi on unsedimentary field has three genera (Aspergillus, Penicillium, and Trichoderma) equal to the field with sediment containing three genera (Phytium, Cladosporium, Penicillium) and there are two different genera, besides the number of fungi colonies is better than sedimentary field. The diversity of nematodes in the unsedimentary field has only two genera (Rotylenchulus, Rhabditis), whereas the sedimentary field has three genera (Dorylaimus, Rhabditis, Hemicycliophora) and more nematodes. The diversity of bacteria and the number of colonies in the unsedimentary field there is one family (Micrococcaceae) is the same as the field with the sediment has one bacterial family (Micrococcaceae).

In the third field, the diversity of fungi on unsedimentary field has three genera (Aspergillus, Penicillium, and Trichoderma) lower than the sedimentary field having four genera (Aspergillus, Penicillium, Trichoderma and Cladosporium) and there is addition of the Cladosporium genus. Diversity nematodes on unsedimentary land have seven genera (Paratrichodorus, Meloidogyne, Trichodorus, Radopholus, Mononchus, Rhabditis, and Seinura) more than the sedimentary field which has two genera (Mononchus and Paratrichodorus). Diversity of bacteria in unsedimentary field there is one family (Micrococcaceae and Achromobacteraceae), whereas in sedimentary field there is only 1 bacterial family.

Sedimentation that occurs due to erosion will bring soil particles together with microbes present in the soil particles. In addition, sediment deposits that occur will cover the pores of the soil so that microbes in sediment-covered soil cannot develop because there is no oxygen intake. So that the microorganisms in the sedimented soil are the microbes carried on the sediment.

Soil fertility can be detected from the number of microbial populations living in it. The high number of microbes is a sign of the high level of soil fertility because microbes require organic materials for growth. Organic matter is subsequently overhauled by microbes to become nutrients available to plants and in the soil. The soil fertility provides nutrients that allow well-adapted species within a community to grow rapidly and dominate [8]. In addition, soil microbial diversity plays a key role in maintaining multifunctional ecosystems because the quality and quantity of soil organic matter is strongly influenced by the structural dynamics and functions of soil microbes that are strongly supportive in the decomposition process of litter and mineralization of organic matter [9-10].

Microbes are involved in activities related to plant growth, by encouraging nutrient cycling and stress reduction, play a beneficial role in nutrient-deprived lands [11]. Soil microbial biomass in soil fractions is responsible for the energy and nutrient cycles, as well as the transformation of organic matter [12,13]. In this regard, Soil microorganisms play a role in the mineralization of nutrients

through the decomposition of organic matter. Microbial biomass is a small but important nutrient reservoir (C, N, P, and S) and many of these nutrient transformations occur in microbial biomass [14]. Soil microbial biomass also contributes to soil structure and soil stabilization [10], microbial as an indicator compared to superior organisms are more sensitive to different ecological factors such as plant diversity, soil organic matter content, humidity, and climate change, therefore microorganisms are widely used as an indicator of soil quality[15].

Table 1. The inventory of microbial diversity in upland rice fields with sediment and unsediments

Sample	Fungi		Nematode		Bacteria	
	cfu/g	Genus	Population /kg of soil	Genus	cfu/ g	Characteristic/family
Location 1						
Sediment	9,3 x 10 ⁴	<i>Aspergillus</i> sp.	2,6 x 10 ²	<i>Rhabditis</i> sp.	2,8 x 10 ⁵	Colonies of beige, thick, serrated, rough, coccus, gram (+) <i>Micrococcaceae</i>
		<i>Phytium</i> sp.				
Un-sediment	1,1 x 10 ⁴	<i>Aspergillus</i> sp.	9,6 x 10 ²	<i>Seinura</i> sp.	1,8 x 10 ⁵	Colonies of beige, thick, serrated, rough, coccus, gram (+) <i>Micrococcaceae</i> Colonies white, smooth, serrated, basil, gram (-) : <i>Achromobacteraceae</i>
		<i>Phytium</i> sp.				
		<i>Trichoderma</i> sp.				
Location 2						
Sediment	4,0 x 10 ³	<i>Phytium</i> sp.	1,1 x 10 ³	<i>Dorylaimus</i> sp.	9,4 x 10 ⁴	Colonies of beige, thick, serrated, rough, coccus, gram (+) <i>Micrococcaceae</i>
		<i>Cladosporium</i> sp.				
		<i>Penicillium</i> sp.				
Un-sediment	8,4 x 10 ⁴	<i>Aspergillus</i> sp.	1,0 x 10 ³	<i>Rotylenchulus</i> sp <i>Rhabditis</i> sp.	9,4 x 10 ⁴	Colonies of beige, thick, serrated, rough, coccus, gram (+): <i>Micrococcaceae</i>
		<i>Penicillium</i> sp.				
		<i>Trichoderma</i> sp.				
Location 3						
Sediment	5,0 x 10 ³	<i>Aspergillus</i> sp.	5,8 x 10 ²	<i>Mononchus</i> sp. <i>Paratrichodorus</i> sp.	7,1 x 10 ⁴	Colonies beige, thick, serrated, smooth, small basil, gram (-): <i>Brevibacteriaceae</i> Colonies White, smooth, serrated, basil, gram (-): <i>Achromobacteraceae</i> Colonies of beige, thick, serrated, rough, coccus, gram (+): <i>Micrococcaceae</i>
		<i>Penicillium</i> sp.				
		<i>Trichoderma</i> sp. <i>Cladosporium</i> sp.				
Un-sediment	2,4 x 10 ³	<i>Aspergillus</i> sp.	1,1 x 10 ³	<i>Paratrichodorus</i> sp. <i>Meloidogyne</i> sp. <i>Trichodorus</i> sp. <i>Radopholus</i> sp. <i>Mononchus</i> sp. <i>Rhabditis</i> sp. <i>Seinura</i> sp.	1,2 x 10 ⁵	Colonies beige, thick, serrated, smooth, small basil, gram (-): <i>Brevibacteriaceae</i> Colonies of beige, thick, serrated, rough, coccus, gram (+): <i>Micrococcaceae</i>
		<i>Penicillium</i> sp.				
		<i>Trichoderma</i> sp.				

Microorganisms form both saprophytic or symbiotic relationship with plants, where the habit of symbionts may be parasitic or mutualistic. Among the favorable saprophytic specific bacterial microbial rhizosphere, i.e. called plant growth that promotes rhizobacteria (PGPR), and fungi, such as pathogen antagonists *Trichoderma*.

Aspergillus, *Cladosporium*, *Penicillium*, *Phytium* and *Trichoderma* in soils play a greater role in soil decomposition [16]. *Micrococcus* and *Achromobacter* are two of the many PGPR found in rooting in the soil [17-18]. The role of PGPR for plants is to act as biofertilization in the soil, stimulate root growth, rhizoremediation, and control stress in plants, while PGPR in biological control mechanisms play a role in reducing the impact of diseases on plants due to plant capability in antibiosis, systemic resistance induction, and competition for nutrition and recesses [19].

Meloidogyne, *Radopholus*, *Rotylenchulus* more acts as parasitic nematodes in plants that are more commonly found in East Kalimantan [20] than other parasitic nematodes such as *Hemicycliophora*, *Paratrichodorus* and *Trichodorus* [21]. *Seinura* in addition to its role as decomposer also plays a role in controlling the number of soil microorganisms as a predator in other organisms. *Rhabditis* plays a role in soil decomposition and is a fungus eater on the ground [22-23]. *Dorylaimus* is an omnivorous nematode capable of feeding on different food sources depending on environmental conditions and food availability [22, 24]. Nematodes play a role in the decay of organic matter so that it has a role such as earthworms, which play a direct role in the decomposition process even in nematodes are often considered saprophagous. In fact, such nematodes feed on the bacteria that abound in decaying organic matter and not on the organic matter itself [22-25].

3.2. Chemical Analysis of Soil Fertility

Based on the analysis of soil chemical properties on the first location, it was found that the soil observed at the sediment site had high cation exchange capacity (CEC), high base saturation (BS), slightly acid pH, high organic carbon (first location of sediment) and very high (first location without sediment), low N total element, low available P (first location is seded) and very high (first location without sediment) and K available very high. Based on the soil fertility criterion [26] that the soil fertility level at the first location with sediment studied was moderate. From the analysis of soil chemical properties tend to decrease soil fertility quality due to sediment, such as very low N content available, resulting in very low soil fertility.

Table 2. Results of Chemical Analysis in upland rice fields with sediment and unsediments

Sample	pH	C Orga- nik	N Total	C/N Ratio	P ₂ O ₅ Bray 1	K ₂ O Mor- gan	KT K	Kej Basa
		%			ppm P	ppm K		%
Location 1								
<i>Sediment</i>	6,95	4,92	0,19	28	4,6	88,8	24,3	94,9
<i>Unsediment</i>	6,6	5,35	0,18	27	29,9	103,8	25,1	77,3
Location 2								
<i>Sediment</i>	7,05	4,77	0,16	30	2,5	77,8	13,5	100,0
<i>Unsediment</i>	6,78	3,89	0,21	18	5,3	90,3	13,5	89,6
Location 3								
<i>Sediment</i>	6,10	4,02	0,24	17	4,5	92,8	17,5	77,7
<i>Unsediment</i>	5,95	3,98	0,22	18	4,6	100,3	18,6	78,0

In the second location, based on the analysis of soil chemical properties, it was found that the soil observed at the sediment site had low cation exchange capacity (CEC), very high alkaline

saturation (KB), slightly acid pH, high organic carbon, low total N element (location second sediment) and medium (second location without sediment), P is very low (second location with sediment) and low (second location without sediment) and K available is very high.

In the third location, the level of soil fertility in the studied site was low. The analysis of soil chemical properties has a tendency to decrease the chemical quality due to sediment, such as low N content available, resulting in very low soil fertility. Based on the analysis of soil chemical properties it is known that the soil observed at the sediment site has medium cation exchange capacity (CEC) moderate, base saturation (BS) is very high, soil pH is slightly acidic, organic carbon is high, element of moderate total N, P is low and K available very high.

The organic chemistry of organic matter affects the decomposition rate of organic matter on the soil, the quality of organic matter is determined by the ratio of C / N, N total, P total, lignin and polyphenols[27-28]. In the decomposition occurs the physical and chemical processes of organic matter into other chemical compounds with the help of microbes [9-13].

CEC is very important to know because able to give criteria of soil fertility, the higher the CEC then the higher also long-term soil fertility [30]. CEC in a high soil indicates the soil has a large enough negative charge so that the land is capable absorbing more potassium, to be subsequently released when the potassium content in the solution is reduced[31].

3.3. Physical Analysis of Soil Fertility

The result of analysis of soil physical characteristic that is texture on upland rice field and un-sediment field (Table 3). Sand is one part of a fraction that has a particle size of 2 μ m-50 μ m and belongs to a class group with a coarse texture [31]. The percentage of fine and coarse sand on the sedimentary ground in the first location of 24.0% and 9.8%, the second sites 26.1% and 4.2% and the third sites 15.1% and 3.2%. On land without sediment in the first location 1.8% and 1.5%, second location 20.8% and 0.7% and third location 11.5% and 1.9%. From the data, it was found that the fine sand content of the sediments on site 2 was higher in fine sand content than the first and third locations, but the first location of the sand content was higher than the second and third locations. In addition, if sediments are compared to land without sediment, it is seen that the sediment area has a finer sand content than roughly sediments. The sediment transport mechanism is based on the depth of the sediment flow carrying the flow of water with a base sedimentary charge in the form of coarse particles such as sand moving and can be rolled or jumped from high to low.

Dust is one of the soil materials that have smaller particle sizes of the sand is 50 μ -2 μ [31]. Dust is included in the form of rinse load fine particles carried by the flood stream down to a calm/stagnant stream or lower ground. The results showed that the dust content of the sampling sites in the sediments field was higher with 44.5%, 55.5%, and 63.1% respectively of the sediments, ie 41.9%, 49, 2% and 59, 4%.

Table 3. Results of physical Analysis in upland rice fields un-sediments

Sample	Particle Distribution				Texture
	Clay	Silt	Sand		
			Fine	coarse	
%					
Location 1					
<i>Sediment</i>	24,3	41,9	24,0	9,8	L

<i>Unsediment</i>	52,2	44,5	1,8	1,5	SiC
Location 2					
<i>Sediment</i>	20,5	49,2	26,1	4,2	L
<i>Unsediment</i>	23,5	55,0	20,8	0,7	SiL
Location 3					
<i>Sediment</i>	22,3	59,4	15,1	3,2	SiL
<i>Unsediment</i>	23,5	63,1	11,5	1,9	SiL

The clay has a particle size of less than 2μ , with this measure making the clay the smallest of all soil textures [31]. The results showed that the clay content at the sampling sites in the land without sediment was higher at 52.5%, 23.5%, and 23.5% respectively of the sediments i.e. 24.3%, 20.5% and 22.3 %.

The many sediments containing clay fractions have a slow rate of water exchange, which is due to the very small size of the clay particles. As a result, the O_2 exchange brought by water entering into the sediment becomes obstructed. However, at the site of seeded in the early days of flooding and puddles then muddy will be more dangerous because of the influence of clay coating and cover the pores of the soil so that no oxygen enters the soil.

Severe quality deterioration is the effect of sediment on soil physical properties. The accumulation of sediment causes oxygen cannot enter the root zone. This effect is similar to a prolonged flood. This means that the existing sediments inhibit the entry of oxygen to the root zone during the sediment is wet and during that time the roots of plants lack oxygen. Oxygen will re-enter the root zone after the sediment is dry and by that time the new flood effect ends.

3.4. Land improvement with sediment

Dedicated land can be repaired and used if channel normalization and sediment deposition ponds are made so that water does not pass through the land and disturb the land. The damaged land can only be used if the mud is dry and fertility improvement is done by using organic fertilizer or addition of soil microorganisms. The mixture of topsoil and fertilizer has successfully increased the number of fungal genus genera, the number of nematode genus and they can improve the soil fertility in post-mining areas, can increase C, N and P, as well as the growth of plant, regarding the pH soil (which is closed to neutral) [1]. The increase in microbial activity depends on the annual increase in C and N in cultivation of plants. Soil microbial activity is not only able to release nutrients for plants but also able to stimulate the process of mineralization and mobilization of pollutants and xenobiotic, so that microbial activity is important in the biogeochemical cycle [10, 12, 32]. Therefore, microbial activity depends on nutritional conditions, temperature and availability of water in the soil.

4. Conclusion

Microbial life in the soil plays an important role in controlling the stability of soil ecosystems. Soil sedimentation and soil management may affect the structure of microbial communities in the soil. By understanding the soil environmental conditions well from the physical, chemical and biological aspects, so the management of the land to maintain the fertility of the land can be done appropriately. Fungi, bacteria, and nematodes have a good role in the process of soil decomposition. The results of soil chemical and physical analysis showed that soil fertility is lower in upland rice fields with sediments compare to those without sediment.

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