Correlations between Soil Microbe and Soil Chemical Properties in Limestone Mining Area: Case Study at Southern Jember Indonesia

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***Abstract*—**An investigation at the Southern limestone mountainous area of Jember-East Java Indonesia, was performed to study the importance of soil chemical properties, population of total bacteria and fungi in the rhizosphere of some essential species. The objectives of the research were: (a) to compare the condition between exploited limestone mining area and unexploited area on population of rhizosphere of some plant species soil microbe and some soil chemical properties, and (b) to identify the correlation between soil microbial population and some soil chemical properties. The research was conducted by two steps, namely exploitation in the field and laboratory analysis. Soil samples were taken under the specified dominant plants at four locations of the limestone mountain. The four locations representing the high (>84 m ASL), middle (52 – 78m ASL), and low (< 34m ASL) sites of the mountain topography and one location of rehabilitation area (< 25m ASL). The west site was indicating the exploited limestone mining area, whereas the east site was the unexploited area. It was found that *Jatropha gossypifolia L, Calotropis gigantea,* and *Randia sp,* are the dominant species plants at the two sites. Soil samples of plants rhizosphere were analyzed for total bacteria, total fungi, soil pH, organic carbon content, total nitrogen, and concentration of P-available. The results of this study revealed that the population of total bacteria ranged between 3.70 to 48.23 cfu ($x10^4$ g^{-1} dry soil), whereas total fungi ranged between 3.13 to 33.06 cfu ($x10^2$ g^{-1} dry soil). The correlation between total bacteria and organic carbon and total nitrogen were positively correlated, with r = 0.73 and 0.60, respectively. On the contrary, there were no correlations between total fungi with all chemical properties.

***Keywords*—**limestone mining area, Jatropha gossypifolia L, Calotropis gigantean, Randia sp.

I. Introduction

NATURAL ecosystems are complex and dynamic environments, in which the physical and chemical environments can vary both with respect to time and space. Besides that, soil microbe activities are also to influence soil condition and ecosystems.

Biodiversity of soil microbes has been regarded as human and vegetation life resource, especially the one connected with biological and environmental resources. Soil is a unity of subsistence that includes the varieties of microbes, because microbial community is one of the important components of soil, therefore, the microbial activity and species compositions are generally influenced by the physical characteristic and soil chemical properties, climate and vegetation [1]. Moreover, soil microorganisms and their activities have the importance role in transformation on plant nutrients to available form and also have many metabolisms related to soil fertility improvement. Soil fungi and bacteria play a focal role in nutrient cycling by regulating soil biological activity [2]. Generally, a reduction in any group of species has little effect on overall processes in soil because other microorganisms can take on its function.

Limestone is a sedimentary rock composed largely of the mineral calcite (calcium carbonate: CaCO3). Limestone is pervious, but not porous, water does not pass through the actual rock, but it does pass down the joints. At Southern Jember Indonesia some of land is Limestone Mountain which has mined several years ago. East site area is still natural ecosystem without mining activities, but at west site is post exploitation area that there no activities more than fifteen years ago. Top soil of the area is shallow (< 20 cm). Actually, interaction between activity of soil microbes and plant give positive impact at limestone.

Nevertheless, there is little information on the fluctuations of bacterial community and fungi in response to the conditions of Limestone Mountain. The objectives of the research were: (a) to compare the condition between exploited limestone mining area and unexploited area on population of rhizosphere of some plant species soil microbe and some soil chemical properties, and (b) to identify the correlation between soil microbial population and some soil chemical properties.

II. Materials And Methods

A. Study Sites

The study was conducted during November to December 2011 in limestone mountain at latitude 08°20'20,1" to 08°20'22,4" and longitude 113°28'14,7" to 113°28'22,9" Southern Jember, East Java Indonesia (Fig. 1). Climate is tropical with temperature ranging between 30 to 40°C and annual rainfall of 728 – 1812 mm.

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http://dx.doi.org/10.15242/IICBE.C914048

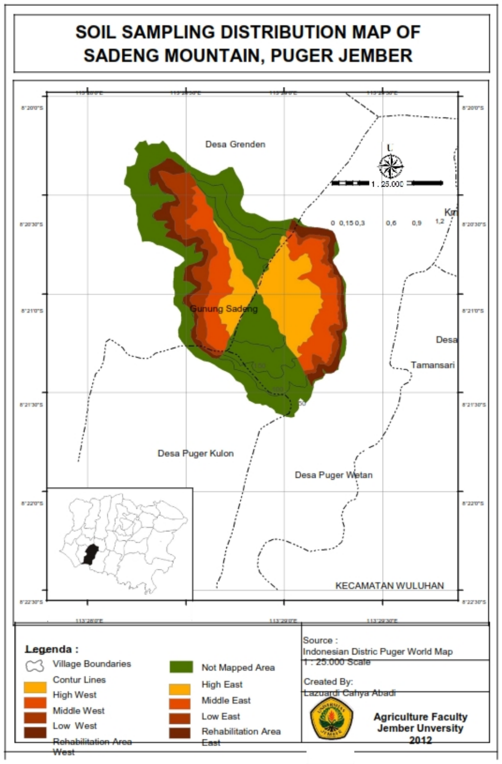

Fig.1 Sampling location of Limestone Mountain area

B. Soil Sampling and Preparing Analysis

Soil from some plant rhizospheres representing dominant plant at four locations; high (>84 m Above Sea Level (ASL)), middle (52 m – 78 m ASL), low (< 34 m ASL) and rehabilitation site (< 25 m ASL) with three replicates were collected and stored in sterilized polythene bags in cold storage box and were used for the isolation of bacteria and fungi. The remaining soil samples were air-dried and used for the determination of soil chemical properties. Soil moisture content was determined gravimetrically by oven drying 10 g of fresh sieved soil for 24 h at 105°C.

C. Isolation of Bacterial and Fungal Population

Soil bacterial population was estimated by using the nutrient agar medium at 10^5 dilutions. While fungal population was estimated by using the potato dextrose agar medium with dilution plate method. The inoculated Petridishes were incubated at room temperature for 3-5 days for bacteria and fungi respectively. To calculate the populations of bacteria and fungi, colonies developed on Petri dishes were counted with colony counter and expressed as number of colony forming units (cfu) g^{-1} dry soil.

D. Soil Chemical Analysis

Dry soil was used to analyze total Nitrogen (Kjeldahl method), C-organic content (Curmis method), concentration of available-P (Olsen method), Cation Exchange Capacity (Ammonium extraction), and pH.

III. Results And Discussion

A. Soil chemical characteristic

Soil characteristics of two sites were determined and some chemical properties were shown in Table 1. It can be seen that soil acidity of all samples are neutral (7.38 – 7.76), while total Nitrogen is medium to very high (0.32% - 0.83%). In addition, organic carbon content is medium to high, concentration of P_2O_5 is low to high (14.41 – 56.48 ppm) and cation exchange capacity is low to high (10.99 - 39.94 $cmol.kg^{-1}$). Generally, soil chemical characteristics on control site are lower than soil covered by vegetation.

Table 1
Soil Properties In Two Sites Of The Area

Site/ slope	altitude (m ASL[a])	Dominant species	pH	N-total %	C-org %	P_2O_5 ppm	CEC cmol. kg^{-1}
West							
High	91	*Control (bare)*	7.41	0.43 (M)	1.86 (L)	14.41 (L)	23.84 (M)
		Jatropha gossypifolia	7.43	0.51 (H)	2.62 (M)	14.41 (L)	37.92 (H)
		Randia sp	7.41	0.79 (H)	4.05 (H)	56.48 (H)	36.70 (H)
Midle	78	*Jatropha gossypifolia*	7.45	0.69 (H)	3.90 (H)	20.01 (M)	38.75 (H)
		Calotropis gigantea	7.42	0.72 (H)	4.60 (H)	23.03 (M)	39.94 (H)
Low	25	*Jatropha gossypifolia*	7.43	0.48 (M)	3.60 (H)	36.64 (M)	10.99 (L)
		Calotropis gigantea	7.38	0.83 (VH)	5.47 (H)	55.58 (H)	17.78 (L)
Base	20	*Tectona grandis*	7.50	0.35 (M)	2.10 (M)	24.72 (M)	26.08 (H)
East							
High	*84*	*Control*	7.52	0.35 (M)	2.02 (M)	20.53 (M)	37.21 (H)
		Jatropha gossypifolia	7.58	0.56 (H)	3.84 (H)	26.73 (M)	24.60 (L)
		Randia sp	7.56	0.65 (H)	3.34 (H)	33.06 (M)	34.96 (H)
Midle	*52*	*Calotropis gigantea*	7.62	0.70 (H)	3.57 (H)	14.48 (L)	30.09 (H)
		Jatropha gossypifolia	7.62	0.45 (M)	2.63 (M)	20.66 (M)	32.73 (H)
Low	*34*	*Jatropha gossypifolia*	7.57	0.59 (H)	2.76 (M)	26.16 (M)	39.55 (H)
		Calotropis gigantea	7.62	0.61 (H)	3.43 (H)	29.76 (M)	39.22 (H)
Base[b]	*25*	*Tectona grandis*	7.76	0.43 (M)	2.25 (M)	28.49 (M)	37.46 (H)

ASL[a] = above sea level; base[b] = rehabilitation area
Categories: L=low; M=medium; H=high; VH=very high

Comparison of soil characteristics were based on the results of west and east sites on four plants rhizosphere. The soil of west sites in the *Randia sp* rhizosphere showed higher on nitrogen total and phosphate concentration, whereas *Calotropis gigantea* has highest on carbon organic content (Table 1). On east site *Calotropis gigantea* showed higher on organic matter content and nitrogen total.

The soil chemical status base on location that represents high (altitude) does not have specific trend at both sites (Table 1). The high location or altitude do not influence on status of that properties. Hence, the improvement of soil chemical status was more affected by rhizosphere rather than altitude.

B. The Number of Soil Bacteria and Soil Fungi

Table 2 shows that, at all plant rhizosphere, the number of soil bacteria is greater than total fungi. Soil reactions at all locations are neutral. Such situation is in line with the result in [3] which shows that neutral to alkaline pH is favorite condition for bacteria. Generally, in neutral pH, bacterial growth has positive correlation with diversity, *richness*, *bacterial community* [4], and composition of soil bacteria [5]. However, in this study, it was found that there is no correlation between pH and soil bacteria. There are many aspect influence soil microorganisms (bacteria and fungi) population and activity.

In contrast to bacterial growth, generally fungal growth was optimal at pH 4.5-5, and then decreases above pH 5.

TABLE II
TOTAL BACTERIA AND TOTAL FUNGI IN TWO SITES OF THE AREA

Site/ slope	altitude (m above sea level)	Dominant species	total bacteria (10^4cfu.g^{-1} dry soil)	total fungi (10^2cfu.g^{-1} dry soil)
West				
High	91	*Control (bare)*	3.70	4.16
		Jatropha gossypifolia	6.78	26.74
		Randia sp	25.69	18.02
Midle	78	*Jatropha gossypifolia*	22.42	5.19
		Calotropis gigantea	48.23	16.66
Low	25	*Jatropha gossypifolia.*	22.80	6.10
		Calotropis gigantea	44.65	15.30
Base	20	*Tectona grandis*	24.78	29.69
East				
High	*84*	*Control (bare)*	14.82	4.22
		Jatropha gossypifolia.	28.80	13.78
		Randia sp	32.62	33.06
Midle	*52*	*Jatropha gossypifolia*	23.83	11.32
		Calotropis gigantea	31.72	3.13
Low	*34*	*Jatropha gossypifolia.*	24.80	9.77
		Calotropis gigantea	37.73	16.68
Base	*25*	*Tectona grandis*	26.44	0.35

The number of soil microbes which is found at plant rhizosphere related to root exudate. Some researched showed that root exudate plays a central role in influencing interactions with neighboring plants and microbes. The quantity and quality of root exudates depends on plant species, cultivar, developmental stage and environmental factors. A small change in root exudates may lead to large alterations in the population of microorganisms in the rhizosphere [6]

TABLE III
CORRELATION COEFFICIENT FOR THE RELATIONSHIPS BETWEEN TOTAL BACTERIA AND TOTAL FUNGI AND SOME SOIL CHEMICAL PROPERTIES

Microbes	Organic C	Total N	pH	P-available	CEC
Bacteria	0.630**	0.550**	0.11	0.203	0.19
Fungi	0,070	0.120	0.10	0.412	0.33

N=25: p<0.05 (0,396); p<0.01 (0,505)

It can be observed from Table 3 that in this limestone mountain, significant positive correlation is only seen between soil bacteria with organic carbon and nitrogen. Actually, microbe activities at rhizosphere affect on soil nutrient cycle. Dominated bacteria improves concentration of total soil nitrogen, furthermore *Azotobacter* which was found on rhizosphere [7] as a *free living microorganisms* can fix of nitrogen from atmosphere. This is shown by the coefficient correlation (r=0.55) between bacteria and nitrogen (Table 3). The presences of soil bacteria are important agent to improve availability of soil nitrogen (Table 2).

Concentration of soil phosphate in the area is medium to high because of the area has limestone parent material with weak laterisation. Usually limestone parent material contain medium to high of total phosphate, also calcium base concentration. Availability of soil phosphate more influenced by other factors than microbial activity, even though their activity able to solubilize insoluble phosphate (r=0.203 and 0.412).

A number of soil bacteria and fungi are capable to dissolve soil phosphate continuously. Their activity increase phosphate availability through several mechanisms, i.e: mineralization of organic phosphate by phosphatase enzyme [8] to an organic phosphate which can be used by the plants, competition of adsorption site, and changes of soil reaction. A number of phosphate solubilization microorganisms on all of plant rhizosphere ranged 0.56 – $36.44.10^4$ cfu.g^{-1} dry soil [7] produce some organic acid.

Most of the phosphate solubilisation bacteria (PSB) genus such as *Bacillus*, *Pseudomonas*, and *Azotobacter* are known to produce several organic acids such as citrate acid, acetate etc. as the result of secondary metabolism [9]. The organic acids as the secondary metabolyte of microbe and the result of root secretion increase phosphate soil availability through several mechanisms: (1) P release which is bound by Fe, Al and Ca so that there is a complex forming of organic metals [10] ; (2) Citric ion or other ions (such as malate, acetate, and oxalic) can be adsorbed by adsorption site which is the same as phosphate anion so that there is P release simultaneously; (3) organic acid anion adsorption (citric acid, etc) changes the surface charge to become negative charge.

The distribution of soil bacteria under *J. gossypifolia L* and *C. gigantea* rhizosphere at east site shows different model, but at west site both of plant have similar trend. The bacteria population increases with a decrease in altitude (Fig 2).

The trends of soil fungi on both plant rhizosphere differ with soil bacteria trend. Soil fungi population decreases as altitude decreases (Fig 3), except at east site of *C.gigantea* rhizosphere.

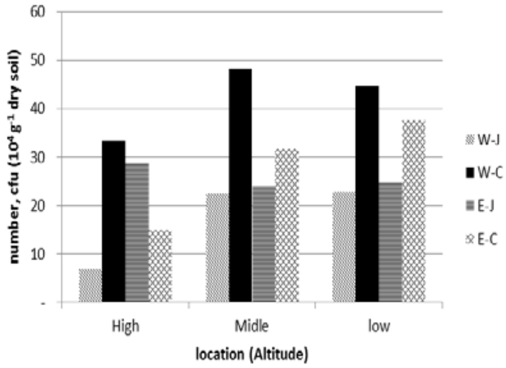

Fig. 2 Population of bacteria at west and east of *J.gossypifolia* and *C. gigantea* rhizosphere at three location (altitude)
W-J=west- *Jathropa gossipyfolia*; W-C= west-*Calotropis gigantea*
E-J=East- *Jathropa gossipyfolia*; E-C= east-*Calotropis gigantea*

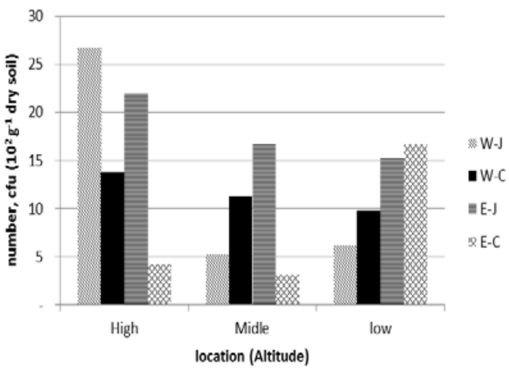

Fig. 3 Population of fungi at west and east of *J.gossypifolia* and *C. gigantea* rhizosphere at three location (altitude)
W-J=west- *Jathropa gossipyfolia*; W-C= west-*Calotropis gigantea*
E-J=East- *Jathropa gossipyfolia*; E-C= east-*Calotropis gigantea*

Generally, populations of soil bacteria and fungi on *C.gigantea* are higher than on *J. gossypyfolia* rhizosphere, (Table 2). The genus *Calotropis* consists of common weeds which occur in arid ecosystems but have become naturalized in warm climates, where they grow commonly in disturbed areas. Genus *Calotropis* like *C.procera* and *C. gigantea* as a medical plant also support the microbial activities, especially at their rhizosphere. Reference [11] concluded that rhizosphere of medicinal plants like *Calotropis procera* harbor bacteria and actinomycetes showing various PGP activities, good NH_3 production and IAA production activities and antifungal activity against *Aspergillus.* So, *C.gigantea* rhizosphere gives refuge to the activitiy and growth of soil microbe.

Reference [12] stated that genus *Jatropha* is capable to grow on marginal land, and helps to reclaim problematic lands and prevent soil erosion. So the cultivation of *Jatropha* leads to the conservation of degraded lands, soil restoration and management by preventing soil erosion, protects plants against wind erosion and the roots also form a protection against water erosion.

The relationship between geology (parent material), climate, soil and vegetation has been very important for restoration ecosystem. Geological processes that interact with climatic elements and living organisms produce the soil in which plants grow. Soil which is nothing more than disintegrated rock caused by lime mining activities modified by plants, water, decomposed organic material and microbial activity, is the medium in which plants grow and obtain their nourishment. Once vegetation established, naturally or man-made, it modifies the soil development processes due to the parent material, topography, climatic conditions etc.

Finally, the exploited area has similar soil fertility status with natural area after approximately fifteen years mining. This condition indicated that improvement of soil fertility and micro environment were suitable for microorganisms and plant. So the genesis or soil development at west site may occur continuously.

ACKNOWLEDGMENT

I thank to Lazuardi, and Fahmi for their valuable helps during data collection in the field. Also thank to Mrs Umiyah, MSc for plant identification.

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