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# The Influence of Cellulolytic Bacteria on Soil Properties in Mangrove Ecosystems of Banda Aceh and Aceh Besar, Indonesia



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ABSTRACT

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#### Keywords:

Aceh Province, cellulolytic bacteria, mangrove, organic carbon, rehabilitated, soil properties, total population

Mangrove ecosystems harbor diverse bacterial communities that significantly affect soil properties. Among these bacteria are cellulolytic species, which contribute to the breakdown of organic matter. This study aimed to analyze the total population of cellulolytic bacteria and their relationship with soil characteristics in mangrove ecosystems along the coast of Banda Aceh and Aceh Besar, Aceh Province, Indonesia. Six research locations were selected, with soil samples taken at three depth intervals: 0-15 cm (Layer 1), 15-30 cm (Layer 2), and 30-45 cm (Layer 3). The total population of cellulolytic bacteria was found to vary depending on both sampling location and soil depth. The highest total population of bacterial colonies was observed at location 2.3. However, on average, the soil surface (Layer 1) harbored a higher number of cellulolytic bacteria (13.0×10<sup>7</sup> CFU g<sup>-1</sup> dry soil) compared to Layers 2 and 3 (5.0×10<sup>7</sup> and 8.0×10<sup>7</sup> CFU g<sup>-1</sup> dry soil, respectively). A significant correlation was observed between the total bacterial population and organic carbon content (P < 0.05), while no significant correlations were found with soil particle size, pH, salinity, total nitrogen, available phosphorus, or soil moisture (P > 0.05). Unrehabilitated mangrove ecosystems exhibited higher levels of cellulolytic bacterial populations, sand and silt fractions, pH, salinity, organic carbon, total nitrogen, and moisture compared to rehabilitated mangrove ecosystems.

## **1. INTRODUCTION**

Soil, a vital abiotic component, serves as a habitat for a myriad of organisms, including bacteria commonly found in mangrove ecosystems. These bacteria produce extracellular enzymes, such as cellulase, protease, and lipase, which play pivotal roles in these ecosystems [1, 2]. Mangroves are unique tropical ecosystems characterized by high levels of organic matter, nutrient recycling [3], and low-oxygen, muddy soil [4]. Cellulolytic bacteria and other extracellular enzymeproducing bacteria contribute to efficient biodegradation [5] by decomposing cellulose through cellulase synthesis [6]. As the primary structural component of plant cell walls, cellulose is the most abundant biomass on Earth [7]. The presence of cellulolytic bacteria in mangrove soil is facilitated by the decomposition of organic compounds, specifically cellulose from dead plants, which serves as a nutrient source for growth and enzyme production [8, 9].

Bacteria play an integral role in organic matter decomposition and nutrient mineralization for plant use [10-12]. Their population and activities are influenced by seasonal changes and the physical and chemical characteristics of the soil [13, 14]. Furthermore, environmental conditions affect the abundance and activity of cellulolytic bacteria [15, 16]. Several studies have indicated that soil organic matter and pH are among the major factors impacting the number and diversity of microorganisms [17, 18]. According to Fan et al. [19], the total number of bacteria is positively correlated with the organic matter content in an ecosystem. Bacteria directly participate in nutrient cycling and provide insight into soil quality through decomposition processes [20].

Forest ecosystem health assessments often evaluate ecological indicators such as tree growth, species diversity (biodiversity), population, and soil fertility [21]. In mangrove ecosystems, bacterial populations contribute to the biotransformation and biomineralization of minerals [22, 23]. Mangrove soil and leaf litter are abundant in cellulolytic bacteria [24-27], which adapt to salinity changes and low oxygen availability. However, some studies suggest that salinity does not necessarily determine microbial abundance in mangrove ecosystems [28]. Das and Dangar [29] reported average bacterial populations of 1.8-2.1×10<sup>6</sup> and 2.2-6.0×10<sup>6</sup> CFU per gram of mangrove soil with low salinity and without salinity, respectively. Bacterial populations vary in colony number, density, and diversity depending on soil type and depth. Previous studies have found a higher microbial density on the soil surface compared to other layers [30, 31], while others have reported high numbers deep below the soil surface [12, 32, 33]. Microbial biomass populations serve as valuable indicators of soil biology and fertility [34]. However, cellulolytic bacterial populations have not been examined in the mangrove ecosystems of Aceh Province.

Mangrove ecosystems along the coast of Aceh Besar and Banda Aceh districts are divided into rehabilitated and unrehabilitated areas, with different soil characteristics such as sand, silt, and clay percentages, salinity, organic carbon, total nitrogen, available phosphorus, and soil moisture, which affect cellulolytic bacterial species and abundance. The total bacterial population correlates with soil characteristics in the mangrove ecosystems. Therefore, investigating the presence of cellulolytic bacteria within different mangrove soil layers and types is essential. This study aims to analyze the total colonies and population of cellulolytic bacteria and their correlation with soil characteristics in both rehabilitated and unrehabilitated mangrove ecosystems.

## 2. MATERIALS AND METHODS

## 2.1 Description of study site

The study was conducted between July 2020 to February 2021 at two different sites, namely unrehabilitated and rehabilitated mangrove areas located on the coast of Banda Aceh and Aceh Besar districts in Indonesia. The rehabilitated mangrove area was the vegetation planted after the tsunami catastrophe in 2004, while the unrehabilitated was the undestroyed ecosystem. The rehabilitated mangroves were dominated by *Rhizophora* sp., but the three-common species in the unrehabilitated ecosystem included *Rhizophora* sp., *Avicennia marina*, and *Sonneratia alba*. The purposive sampling method was used to select six sampling locations, where numbers 1, 2, and 3 were rehabilitated, while 4, 5, and 6 were unrehabilitated, as shown in Figure 1 and Table 1.



Figure 1. Red dots showed the study location

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NO.	Location	Coordinate
1	Gampong Lambadeuk	05°32'35.8"N 95°14'30.9"E
2	Gampong Dayan	05°33'50.3"N 95°18'13.3"E
	Teungoh	
3	Gampong Pande	05°34'15.3"N 95°18'46.4"E
4	Gampong Ruyung	05°36'08.2"N 95°29'46.8"E
5	Gampong Lamreh	05°36'30.0"N 95°32'18.1"E
6	Gampong Lampanah	05°35'25.1"N 95°40'20.9"E

#### 2.2 Soil sampling

Soil sampling was carried out at six locations which was

further categorized into three sampling points based on soil depth. At each location, samples were selected randomly at three depths, namely 0-15 cm (surface/1st layer), 15-30 cm (2<sup>nd</sup>), and 30-45 cm (3<sup>rd</sup>). There were differences in the level of soil depth sampling applied by several studies. For example, Ambeng et al. [35] collected soil samples from three depths, namely 0-15 cm, 15-30, and 30-45. Pupin and Nahas [36] divided soil depth into 0-5 cm and 5-10 cm, while Akpan and Solomon [37] only obtained samples at the depth of 0-20 cm. Approximately 100 g of soil was collected from each study point using a sterilized spatula, then replicated three times, combined to form a composite sample, and dropped into sterilized plastic bags. The samples were transported to the microbiology laboratory of SKIPM, Aceh for isolation and calculation of the bacteria population. Additionally, they were analyzed for measurements of pH, salinity, organic carbon, Ntotal, P-available, moisture, and soil texture (sand, dust, and clay fractions) at the soil laboratory of the Agriculture Faculty, Universitas Syiah Kuala (USK).

#### 2.3 Preparation of selective media

Selective media for cellulolytic bacteria were prepared by weighing 1 g of CMC, 0.02 g of MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.05 g of KH<sub>2</sub>PO<sub>4</sub>, 0.075 of KNO<sub>3</sub>, 0.002 of FeSO<sub>4</sub>, 0.004 of CaCl<sub>2</sub>, 0.2 g of yeast extract, 1.5 g of Bacto agar, and 0.1 g of glucose. All these materials were put into 100 mL of distilled water and poured inside an Erlenmeyer flask, then covered with aluminum foil and plastic wrap. The media were sterilized in an autoclave at 121°C with a pressure of 1 atm for 15 minutes and subsequently transferred into a sterilized petri dish.

#### 2.4 Estimation of cellulolytic bacterial population

Up to 1 g of the mangrove soil sample was weighed and diluted from  $10^{-1}$  to  $10^{-6}$  with a NaCl physiological solution. About 1 mL of the  $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$  dilutions were spread on media enriched with 1% CMC (*Carboxy Methyl Cellulose*) using a scattering rod in a duplicate manner. This was incubated at 30°C for 48 h to count the number of colonies formed in different samples. The total population of cellulolytic bacteria per unit gram of the media (CFU. g<sup>-1</sup>) was also calculated using the *Total Plate Count* (TPC) method [37]. The number of microorganisms obtained with the TPC method is only an estimate and possibly greater than the actual number [38]. Bacteria are counted only from Petri dishes which have 30-300 colonies [39]. The total population can be calculated using the following formula [39, 40].

Total population (CFU) 
$$g^{-l}$$
 of dried soil= $\frac{(number of colinies)X(df)}{dw soil}$ 

df = dilution factor  $(10^{-4}, 10^{-5}, \text{ and } 10^{-6})$ 

dw = dry weight of soil sample (g) = wet weight x (1-water content)

#### 2.5 Data analysis

Data on the cellulolytic bacterial population were analyzed descriptively, then presented in tables. The soil properties distribution was analyzed descriptive statistical with boxplot and biplot. The correlation between bacterial population and soil properties was determined using the Spearmen test, and the data obtained were analyzed with the R Studio software. Differences between the two environments were assessed using the Mann-Whitney-Wilcoxon Test and visualized using R Studio's Ggplot.

#### 3. RESULTS AND DISCUSSION

Based on the results, the number of cellulolytic bacteria colonies was different in each soil depth and sampling location with three dilutions ( $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$ ). Figure 2 shows bacteria colonies growing on *Carboxy Methyl Cellulose* (CMC) media incubated at 37°C for 48 h.





Generally, it was observed that the total colony decreased with increasing dilution. In the mangrove soil with  $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$  dilution, the bacterial population ranged from 3.9 to  $32.9 \times 10^5$ ,  $3.7 \cdot 32.9 \times 10^6$ , and  $3.1 \cdot 27.2 \times 10^7$  CFU. g<sup>-1</sup> dry soil, respectively. The highest population of cellulolytic bacteria, reaching  $32.9 \times 10^5$ ,  $32.9 \times 10^6$ , and  $27.2 \times 10^7$  CFU. g<sup>-1</sup> dry soil was found at location 2.3 with a soil depth of 30-45 cm (3<sup>rd</sup> layer). Location 3.1 had  $24.7 \times 10^7$  CFU. g<sup>-1</sup>, while 5.1 had

 $22.0 \times 10^7$  CFU. g<sup>-1</sup>. The lowest population was found at locations 1.2, 1.3, and 6.1, namely  $3.1 \times 10^7$  CFU. g<sup>-1</sup>. Complete data on the total and average total population at the six locations and three depths are presented in Tables 2 and 3.

The number of bacterial colonies in mangrove soil varies based on habitat characteristics. The total population of cellulolytic bacteria at the 10<sup>-5</sup> dilution ranged from 3.7 to  $32.9 \times 10^6$  CFU. g<sup>-1</sup>, higher than the  $1.8 - 2.1 \times 10^6$  CFU. g<sup>-1</sup> in soil containing salinity [29]. Moreover, Khotimah et al. [41] stated the population of cellulolytic bacteria in peat soil forests was  $4.9 \times 10^4$  CFU. g<sup>-1</sup>. The range of total bacteria is different at each level of dilution. According to Olsvik et al. [42], the purpose of multilevel dilution is to reduce the number of microbes growing in a media, hence, only fewer microbes are produced at a higher dilution. Cellulolytic bacteria populations differ between regions and environments [43]. This study shows that variations in the cellulolytic bacterial population are influenced by environmental factors and soil characteristics.

The highest total population of cellulolytic bacteria at location 2.3, this is possibly due to a higher soil organic carbon (SOC) content (2.18%) at that location compared to other locations. A high density of the bacteria population correlates to soil physical and chemical characteristics as well as thickness. The higher the sediment thickness, the higher the concentration of bacteria [35]. A high bacterial population indicates an adequate supply of food sources. Bacteria break down organic matter and recycle nutrients, thereby influencing both soil chemical and physical characteristics. Several studies stated soil pH and organic matter content as the main characteristics affecting the number and diversity of microorganisms [17, 18].

Table 2. Number of colonies and the	he total population of	cellulolytic bacteria	in mangrove soil
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Station	Dilution 10 <sup>-4</sup> (colony)	Total Population (CFU, g <sup>-1</sup> )	Dilution 10 <sup>-5</sup> (colony)	Total Population (CFU, g <sup>-1</sup> )	Dilution 10 <sup>-6</sup> (colony)	Total Population (CFU, g <sup>-1</sup> )
1.1	300	30.6×10 <sup>5</sup>	300	30.6×10 <sup>6</sup>	87	8.9×10 <sup>7</sup>
1.2	38	3.9×10 <sup>5</sup>	36	3.7×10 <sup>6</sup>	30	3.1×10 <sup>7</sup>
1.3	250	26.1×10 <sup>5</sup>	52	$5.4 \times 10^{6}$	30	$3.1 \times 10^{7}$
2.1	300	30.8×10 <sup>5</sup>	300	30.8×10 <sup>6</sup>	68	$7.0 \times 10^{7}$
2.2	300	31.0×10 <sup>5</sup>	48	$5.0 \times 10^{6}$	33	$3.4 \times 10^{7}$
2.3	300	32.9×10 <sup>5</sup>	300	32.9×10 <sup>6</sup>	256	$27.2 \times 10^{7}$
3.1	300	31.7×10 <sup>5</sup>	300	31.7×10 <sup>6</sup>	240	$24.7 \times 10^{7}$
3.2	300	31.0×10 <sup>5</sup>	67	$6.9 \times 10^{6}$	32	3.3×10 <sup>7</sup>
3.3	88	9.3×10 <sup>5</sup>	53	5.6×10 <sup>6</sup>	31	3.3×10 <sup>7</sup>
4.1	300	31.8×10 <sup>5</sup>	263	$27.9 \times 10^{6}$	72	$7.6 \times 10^{7}$
4.2	153	16.0×10 <sup>5</sup>	113	$11.8 \times 10^{6}$	91	$9.5 \times 10^{7}$
4.3	53	$6.0 \times 10^5$	42	$4.7 \times 10^{6}$	42	$4.7 \times 10^{7}$
5.1	226	$24.0 \times 10^5$	300	31.9×10 <sup>6</sup>	200	22.0×107
5.2	104	$11.1 \times 10^{5}$	40	$4.3 \times 10^{6}$	39	$4.1 \times 10^{7}$
5.3	230	$24.7 \times 10^5$	220	$23.7 \times 10^{6}$	64	6.9×10 <sup>7</sup>
6.1	250	25.9×10 <sup>5</sup>	106	$11.0 \times 10^{6}$	30	$3.1 \times 10^{7}$
6.2	144	15.2×10 <sup>5</sup>	66	$7.0 \times 10^{6}$	39	$4.1 \times 10^{7}$
6.3	130	$14.7 \times 10^{5}$	77	$8.7 \times 10^{6}$	44	$5.0 \times 10^{7}$

1, 2, 3, 4, 5, 6: sampling location; 1, 2, 3: level of soil depth (layer)

Table 3. The average total bacterial population at different soil depth

Variable	Unit	1st layer (0-15 cm)	2 <sup>nd</sup> layer (15-30 cm)	3 <sup>rd</sup> layer (30-45 cm)
Total bacterial population	CFU×10 <sup>5</sup> . g <sup>-1</sup>	29.1	18.0	19.0
	CFU×106. g-1	27.3	6.5	13.5
	CFU×10 <sup>7</sup> . g <sup>-1</sup>	12.2	4.6	8.4

Table 4. Characteristics of mangrove soil based on depth

Parameters	Unit	1st layer (0-15 cm)	2nd layer (15-30 cm)	3 <sup>rd</sup> layer (30-45 cm)
Sand	%	59.8	53.5	42.8
Silt	%	35.0	32.0	43.2
Clay	%	5.2	14.2	14.0
Texture	-	Sandy loam	Sandy loam	Dusty loam
pН	-	7.6	6.5	7.3
Salinity	µscm <sup>-1</sup>	5.6	6.4	9.6
	ppt	3.02	3.49	5.39
Organic Carbon	%	1.00	0.95	1.27
N total	%	0.08	0.06	0.09
P available	ppm	27.92	26.05	26.19
Moisture	%	3.72	4.47	7.99

According to Fan et al. [19], the total number of bacteria is positively correlated with the organic matter content in an ecosystem. Furthermore, Huang et al. [44] stated that bacterial number and diversity increase proportionally with soil organic matter. Commonly, at the 3<sup>rd</sup> soil layer near wider roots, the mangrove rhizosphere is rich in nutrients, including sugars, amino acids, organic acids, and fatty acids from root exudates, which commonly lead to a dominant bacterial population [45]. Bacteria in the rhizosphere have a symbiotic relationship with plant roots since their exudates serve as the main food source for microorganisms [46].

Otherwise based on soil depth, the average total population of cellulolytic bacteria on the soil surface was higher compared to 2<sup>nd</sup> and 3<sup>rd</sup> layers for all dilution series. The values obtained in the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> layers at 10<sup>-6</sup> dilution were  $12.2 \times 10^7$ ,  $4.6 \times 10^7$ , and  $8.4 \times 10^7$  CFU. g<sup>-1</sup> dry soil, respectively. Meanwhile, at dilutions  $10^{-4}$  and  $10^{-5}$ , each layer had  $29.1 \times 10^{5}$ , 18.0×10<sup>5</sup>, and 19.0×10<sup>5</sup> CFU. g<sup>-1</sup> and 27.3×10<sup>6</sup>, 6.5×10<sup>6</sup>, and 13.5×10<sup>6</sup> CFU. g<sup>-1</sup> dry soil, as presented in Table 2. Kruskal Wallis analysis showed that the relationship between bacterial population and soil depth (layer) had significant relationship where Psig.<0.05. Moreover, the analysis obtained that there was a significant difference the bacterial population at 1<sup>st</sup> layer and 2<sup>nd</sup> layer (Psig. <0.05), whereas, there were no significant difference at 2<sup>nd</sup> and 3<sup>rd</sup> layers, and at 1<sup>st</sup> and 3<sup>rd</sup> layers (Psig.>0.05). The Psig value of 1<sup>st</sup> and 2<sup>nd</sup> layers, 2<sup>nd</sup> and 3<sup>rd</sup> layers, and 1<sup>st</sup> and 3<sup>rd</sup> layers were 0.003, 0.882, and 0.078, respectively.

The highest bacterial population was detected in the 1<sup>st</sup> layer, followed by the 3<sup>rd</sup> and 2<sup>nd</sup>. The soil surface contains a high number of bacterial colonies, which decreases with increasing depth due to less sunlight and organic matter. Furthermore, organic matter is mainly concentrated on the surface due to leaf litter [47]. The lowest bacterial population is found in the 2<sup>nd</sup> layer at a depth of 15-30 cm. Some factors causing a smaller number of microorganisms are compact soil and low organic matter [48], as well as soil strata [31].

The bacterial community in soil sediments depends on depth, porosity, organic matter, and pH [31]. Soil organic carbon is higher in fine soil with small porosity compared to coarse soil with big porosity. Soil texture influences bacterial population, where a high population is possibly due to higher percentages of silt and clay with fine texture, than sand with coarse texture. Carney and Matson [49] mentioned that finetextured soils support more microbial biomass than their coarse-textured counterpart. Moreover, texture as an abiotic factor may also affect mineral distribution, organic matter retention, microbial biomass, and other soil characteristics [50]. The small size of soil pores and pore space distribution greatly influence bacteria and fungi abundance as well as high carbon levels [51]. As in research Hamarashid et al. [52], the highest bacteria population (8.77 and 8.03 log CFU. g<sup>-1</sup> dry weight of soil) in dusty clay soil and silty clay, while sandy loam and silty soil have the lowest (6.07 and 6.42 log CFU. g<sup>-1</sup>), the same result as location 2.3 supported by fine texture and high soil organic carbon.

Table 4 showed the value of pH, salinity, organic carbon, N total, P-available, moisture, soil fraction, and the texture of each soil layer. Soil fractions in the study area consisted of sand, silt, and clay. The sand was higher at the 1<sup>st</sup> layer than at the 2<sup>nd</sup>, and 3<sup>rd</sup>, with values of 59.8%, 53.5%, and 42.8%, respectively. The soil texture of the 1<sup>st</sup> and 2<sup>nd</sup> layers were sandy loam, while the 3<sup>rd</sup> had dusty loam. The soil pH was 6.5 - 7.6 and the salinity ranged from 5.6 - 9.6 ( $\mu$ scm<sup>-1</sup>) which was equal to 3.02 - 5.39 ppt. The highest salinity and soil moisture value were in the 3<sup>rd</sup> layer, the value was 5.39 ppt and 7.99%. There was more soil organic carbon (SOC) and N-total in the 3<sup>rd</sup> layer compared to the other layers (1.27 and 0.09%).

Soil acidity (pH) level affects microorganisms living in the mangrove areas. The pH at the study site low and leads to acid in 2<sup>nd</sup> soil layer, and neutral in the 1<sup>st</sup> and 3<sup>rd</sup> layer, the average value measured at the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> depths were 7.6, 6.5, and 7.3, respectively. The bacterial population observed decreased with decreasing pH, indicating that low soil pH leads to fewer bacteria. These results were consistent with the statement of Zi et al. [53] that soil microbial population and pH decrease proportionally due low pH inhibited the presence of soil microorganism. Furthermore, recent studies showed a relationship between bacteria composition, diversity, and soil pH [54, 55], bacterial diversity and abundance high in soil neutral and low in acidic soil [56]. Soil surface has higher pH than the deeper layers due to high organic matter from decomposed leaf litter, causing soil sediment to become neutral [55]. In soil, pH is an important soil property that can stimulate soil microbial community structures [57]. The pH value at the study site was still in the suitable range that favors bacterial growth.

Furthermore, the soil salinity increased with depth, where a higher bacterial population was found at a soil depth containing lower salinity (1<sup>st</sup> layer) compared to the 2<sup>nd</sup> and 3<sup>rd</sup>. This contradicts the result by Pupin and Nahas [36] who detected higher salinity (11.62 mscm<sup>-1</sup>) on the mangrove soil surface (0-2 cm). The salinity contained in the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> layers were 5.6 mscm<sup>-1</sup> (3.02 ppt), 6.4 mscm<sup>-1</sup> (3.49 ppt), and 9.65 mscm<sup>-1</sup> (5.39 ppt). Several studies reported that salinity is not a determining factor for the presence of microbes associated with the dynamics of mangrove ecosystems [28]. However, soil salinity can affect microorganisms' composition and number [58]. The bacterial community in saline soil changes with salinity [59, 60], where the number and

phylogenetic diversity of bacteria decrease alongside increasing salinity. High salinity was reported to have a significant negative impact on the microbial population [61].

According to Wang et al. [62] and Ramirez et al. [63], soil Nitrogen (N) was associated with bacterial biomass and diversity, and soil bacterial biomass will increase in mangrove soil containing high P element [64]. Nitrogen (N) is an important element that affects soil fertility and environmental quality. The range of N-total contained in soil for each depth was 0.08% (1<sup>st</sup> layer), 0.06% (2<sup>nd</sup>), and 0.09% (3<sup>rd</sup>), while the values of P-available in each depth were 27.92 ppm, 27.05 ppm, and 26.19 ppm, respectively. The highest P-available was in the 1<sup>st</sup> layer which contained the highest bacterial population. The availability of sufficient N and P may elevate plant productivity, leading to the increased release of plant exudates into the soil. Consequently, cellulose-degrading bacteria that utilize the litter from plant in the area will become more abundant [65]. High soil nutrient levels (N, P, K) in the rainy season boost the microbial population [66]. Increasing both P and N has a synergistic effect on microbial population growth [67].

Figure 3 shows the distribution of bacterial population and soil characteristics in study area. A non-normal distribution of

the total population was formed because the boxplot was not symmetrical, with more skewness to the right and three outliers. Up to 50% of the total population was below  $4.92 \times$  $10^7$  CFU. g<sup>-1</sup>. Sand, silt, and N-total were normally distributed since the boxplot was symmetrical with no outliers. Half of these three parameters were below 52%, 39%, and 0.07%, respectively. Furthermore, clay, pH, salinity, organic carbon, P-available, and soil moisture had non-normal distributions. Furthermore, half of them were below 5%, 7.40, 6.25 µscm<sup>-1</sup>, 1.09%, 26.43 ppm, and 5.15%, respectively.

Figure 4 shows that the correlation between the total population and soil characteristics statistically, some variable lacks a parametric pattern, but some produced two curves. Due to the non-normal distribution of the data, the nonparametric Spearmen correlation method was used.

Based on Table 5, there was a strong significant correlation (0.65) between the cellulolytic bacteria population and organic carbon (Psig<0.05). However, the correlation with soil fraction, pH, salinity, N-total, P-available, and soil moisture was insignificant correlation (Psig>0.05). The total population had a negative correlation with sand, clay, pH, and P-available, but it shared a positive correlation with silt, salinity, N-total, P-available, and moisture.



Figure 3. Boxplot of the soil characteristics and cellulolytic bacteria population in each sampling location

Table 5. Correlation between the total population and soil characteristics

No	Variable 1	Variable 2	Correlation	P-sig	Significant/ notsignificant
1	Total population	Sand	-0.36	0.14	Not significant
2	Total population	Silt	0.43	0.08	Not significant
3	Total population	Clay	-0.13	0.61	Not significant
4	Total population	pH	-0.07	0.77	Not significant
5	Total population	Salinity	0.15	0.54	Not significant
6	Total population	Organic Carbon	0.65	0.00*	Significant
7	Total population	N-total	0.35	0.16	Not significant
8	Total population	P-available	-0.20	0.42	Not significant
9	Total population	Moisture	0.17	0.50	Not significant



Figure 4. The distribution of correlation between the total bacterial population and soil characteristics

Soil organic carbon (SOC) significantly influenced the bacteria population and the correlation between both was strong and positive. Commonly, high SOC can stimulate high bacterial population activity. Hua et al. [68] stated that the dynamics of the bacterial population have a high correlation with soil carbon availability. Bacteria as microorganisms can promote the formation of soil aggregates to store carbon. They are also essential decomposers that play a critical role in carbon cycling [69, 70]. The organic carbon in the 1<sup>st</sup> and 3<sup>rd</sup> layers was categorized as low, while the 2<sup>nd</sup> was very low. Organic carbon is classified as very low (provided the content <1.00%), low (1-2.00%), moderate (2.01-3.00%), and high (>5.00%) [71].

The boxplot in Figure 5 explains the differences between

soil parameters measured in the two study areas (rehabilitated and unrehabilitated) based on median values. The data obtained were not normally distributed and included some outlier points.

The unrehabilitated mangroves had a higher total bacterial population  $(5.07 \times 10^7 \text{ CFU}. \text{ g}^{-1})$  than the rehabilitated counterpart  $(3.47 \times 10^7 \text{ CFU}. \text{ g}^{-1})$ . Percentage values of the sand fraction had a nearly similar median between both areas, namely 52% and 53%. Soil organic carbon was higher in the unrehabilitated mangroves than in the rehabilitated with values of 1.23% and 0.90%, respectively. Furthermore, the values of dust, pH, salinity, N-total, and moisture were higher in the unrehabilitated area. In contrast, there was more P content in the rehabilitated area.

Table 6. The differences between total population and soil characteristics based on the s	tudy areas
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No	Parameters	Median		
		Mangrove Rehabilitated	Mangrove Unrehabilitated	
1	Total population (10 <sup>7</sup> CFU. g <sup>-1</sup> )	3.47	5.07	0.666
2	Sand (%)	53	52	0.796
3	Silt (%)	31	43	0.931
4	Clay (%)	5	5	0.863
5	pH	7.23	7.86	0.222
6	Salinity (mscm <sup>-1</sup> )	5.6	7.6	0.077
	(ppt)	3.02	4.19	
7	Organic carbon (%)	0.90	1.23	0.113
8	N-total (%)	0.06	0.07	0.489
9	P-available (ppm)	38.05	7.55	0.001*
10	Moisture (%)	3.09	5.93	0.014*

\* Significantly different at  $\alpha~5\%$ 



Figure 5. Ggplot distribution of bacterial population and soil characteristics in rehabilitated and unrehabilitated mangrove

The unrehabilitated mangroves contained more bacteria and organic carbon than the rehabilitated mangrove ones due to more vegetation. There were 70 individuals.  $100m^{-2}$  in unrehabilitated compared to 39 individuals.  $100m^{-2}$  in their counterparts. Vegetation litter significantly contributes organic carbon to mangrove soil [72], which promotes the abundance and diversity of cellulolytic bacteria. Furthermore, the number of microbes can vary in soils due to distinctions in the amount of litter and vegetation in different ecosystems [73]. Cellulolytic bacteria provide carbon sources to improve soil fertility and mangrove productivity [74].

Furthermore, the Mann-Whitney-Wilcoxon test was used to determine the significance of differences in soil parameters between the rehabilitated and unrehabilitated mangrove areas. Significant differences were found between P-available and soil moisture (Psig<0.05), but the total population, sand, silt, clay, pH, salinity, organic carbon, and N-total had no significant differences (Psig>0.05) between study areas, as presented in Table 6. Kurth et al. [75] found that higher soil P content leads to an increased absolute and relative abundance of bacteria involved in P changes. Baumann et al. [76] concluded that the microbiome directly transfers and immobilizes P, while sandy soil texture reduces its absorption, leading to high P content.

### 4. CONCLUSION

The result of research concluded that the bacterial population in mangrove soil varies based on soil depth and mangrove sites (rehabilitated and unrehabilitated). In the average, the total bacterial population isolated was higher in the surface soil or 1<sup>st</sup> layer with a depth of 0-15 cm, than in the 2<sup>nd</sup> (15-30 cm) and 3<sup>rd</sup> layers (30-45 cm), and followed by 3<sup>rd</sup> layer. Differences in the level of soil depth sampling will lead to differences in bacteria population, and there was the significant relationship between bacteria population and soil depth. Bacteria population was significantly different at 1<sup>st</sup> and

2<sup>nd</sup> soil layers. Specifically, location 2.3 contain more cellulolytic bacteria than other location due to highest soil organic carbon in this location. This study shows that variations in the cellulolytic bacterial population are influenced by environmental factors and soil characteristics, such as pH, salinity, soil fraction, organic carbon, N-total, P-available and moisture. The value obtained had a strong correlation soil organic carbon content with total of bacteria population, and fine soil texture support the presence of bacteria population compare to the coarse soil. The presence of bacterial populations can affect the soil characteristics and vice versa.

Furthermore, more bacterial population and soil organic carbon were recorded in soil of the unrehabilitated mangrove site compared to the rehabilitated counterpart. The number of mangrove vegetation is higher in mangrove unrehabilitated than rehabilitated, so contribute more litter production and stimulate soil fertility in unrehabilitated site. Soil characteristics including P-available and moisture significantly different (Psig<0.05) between both sites, however the total population, sand, silt, clay, pH, salinity, organic carbon, and N-total had no significant differences (Psig>0.05) between sites.

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