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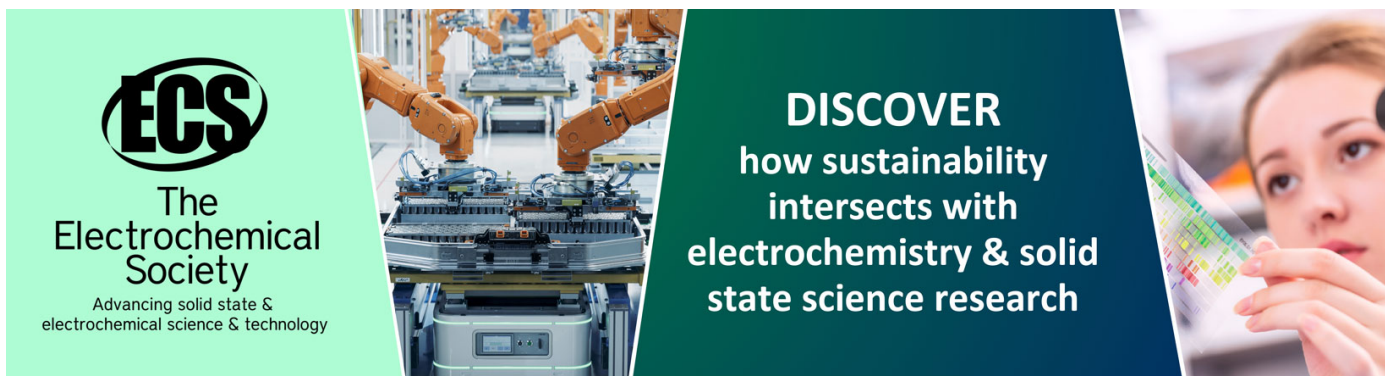
Application of *Aspergillus fumigatus* from *Rhizophora mucronata* leaves that have decomposition in various salinity levels in Belawan

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Application of *Aspergillus fumigatus* from *Rhizophora mucronata* leaves that have decomposition in various salinity levels in Belawan

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Abstract. *Rhizophora mucronata* leaf litter is a source of organic material for several organisms found in mangrove forest ecosystems. Leaf litter that falls on the forest floor will undergo a process of decomposition by biological agents within a certain time period. One effort to accelerate decomposition process is the use of fungi *Aspergillus fumigatus*. The purpose of this study was to measure the rate of decomposition and to measure the levels of carbohydrates and proteins contained in *R. mucronata* litter with different levels of salinity in the mangrove area of Hamparan Perak District. Carbohydrate and protein analysis is carried out at the Medan Industrial Research and Standardization Center. The average decomposition rate (k) of *R. mucronata* leaf litter at the 0-10 ppt salinity level is 10.89, the 10-20 ppt salinity is 7.74 and the 20-30 ppt salinity is 7.81. The Leaf Litter of *R. mucronata* experienced a decrease in carbohydrate levels. The highest carbohydrate content was found in Observation I station 3 which was 12.2% while the protein content in *R. mucronata* litter had increased. The highest protein content was found in observation VI of station 3 which was 6.78%. The decomposition rate in Belawan waters shows that productivity is good.

1. Introduction

Mangroves are ecosystems that are rarely found because they are only 2% of the earth's surface. Indonesia is a country that has the largest mangrove ecosystem in the world among several other countries. Mangrove ecosystems have several ecological, economic, social, and cultural benefits. *Rhizophora mucronata* is a true mangrove type. These species can reach a height of 27 m, and rarely exceed 30 m. The stem has a diameter of up to 70 cm with dark to black bark. The distribution of *R. mucronata* is East Africa, Madagascar, Mauritania, Southeast Asia, throughout Malaysia and Indonesia, Melanesia, and Micronesia. The ecology where *R. mucronata* grows in the same area as *Rhizophora apiculata* but is more tolerant of harder substrates and sand [1]

Mangrove litter in the forest floor will undergo a decomposition process that can be organic material for the surrounding mangrove ecosystem. Production and decomposition factors have a big influence in determining the amount of organic matter. Decomposition is the process of decomposing dead organic matter carried out by organisms/microorganisms into mineral materials and humus [2]. The process of decomposition of mangrove litter starts from the destruction carried out by macrozoobentos and the litter will be cut into smaller sizes. Decomposition is followed by biological processes carried out by bacteria and fungi as decomposers to decompose organic particles by



releasing enzymes so that they can decompose organic matter into proteins. Decomposition products are not only used as a nutrient source for plants but also as an important food source for fish and invertebrates [3].

Aspergillus is a fungus that will be able to live in media with a high degree of acidity and sugar content. This fungus can cause spoilage in fruits or vegetables. *Aspergillus* is parasitic, some saprophytic. *Aspergillus fumigatus* fungi can be found in decomposed soil, water, and plants, especially in manure and humus [4].

Fungi are the main decomposers in the decomposition of mangrove leaves because they capability to decompose cellulose and lignin. The process of decomposition by fungi in mangroves is strongly influenced by the environment such as salinity. The level of salinity will affect the many types of fungi in the mangrove ecosystem. Decomposed litter will be tested to determine the levels of carbohydrates and protein contained. Therefore, the present study aimed to measure the rate of litter decomposition after administration of *Aspergillus fumigatus* fungi at various levels of salinity and to find out the carbohydrate and protein contained in a litter.

2. Materials and Method

2.1. Study sites

The research was conducted in December 2019 until March 2020. This research was carried out in the mangrove area of Paluh Kurau, Kecamatan Hamparan Perak, Kabupaten Deli Serdang, North Sumatra. dry weight assessment was carried out at the Forest Cultivation Laboratory, Faculty of Forestry, University of North Sumatra, while Carbohydrate and Protein analyzes were carried out at the field industry standardization and research center Medan. (Research location can be seen in the Figure 1, Figure 2 and Figure 3).



Figure 1. Station 1 (0-10 ppt salinity)



Figure 2. Station 2 (11-20 ppt salinity)



Figure 3. Station 3 (21-30 ppt salinity)

2.2. Tools

The tools used in this research are refractometer, Global Positioning System (GPS), litter bag with size 30×40 cm made of nylon, needle, Erlenmeyer flask, Beaker glass, Autoclave, oven, analytical balance, digital camera, Petri dishes, 100 ml Kjeldahl flasks, distilleries, and accessories, electric heaters or burners. The materials used in this study are *Rhizophora mucronata* leaf litter, PDA media, seawater, markers (stationery), raffia, yarn, cotton, mask, cling wrap, aluminum foil, label paper, sample envelope, Selen mixture, Conway indicator, boric acid solution, hydrochloric acid solution, sodium hydroxide solution.

2.3. Sampling sites

Data collection techniques using Purposive Sampling (data retrieval through consideration) is to determine the 3 points of observation stations based on differences in salinity. The determination of the station point is done by measuring the level of salinity using a refractometer. Station 1 with salinity 0-10 ppt, station 2 with salinity 11-20 ppt, station 3 with salinity 21-30 ppt.

2.4. Collection of *Rhizophora mucronata* leaf litters

The leaf litter of *R. mucronata* as much 50 g was put in a litter bag measuring 40×30 cm and made of nylon with a 1×1 mm mesh. The number of bags containing litter prepared was 54 bags (18 bags $\times$ 3 levels of salinity).

2.5. Fungal application

The fungus isolate that will be used is *Aspergillus fumigatus*. Furthermore, isolates were taken by cutting in order of $5 \times 5 \times 2$ mm. These pieces of agar that have been overgrown with fungi are then put into test tubes filled with 2.5 ml of sterile water to be used as suspensions. As much as 2.5 ml of the suspension are spread evenly on the leaf litter found in the litter bag.

2.6. Estimation of *R. mucronata* leaf litter decomposition rate

Estimation of *R. mucronata* Leaf Litter Decomposition Rate obtained to using formula [5]:

$$X_t = X_0 \cdot e^{-kt}$$

$$\ln\left(\frac{X_t}{X_0}\right) = -kt \quad (1)$$

Where:

X_t = litter dry weight after the observation time (g)

X_0 = initial litter weight (g)

e = natural logarithm number (2.72)

k = value of litter decomposition rate

t = observation time (days)

2.7. Carbohydrate content analysis

Hydrolysis of carbohydrates into monosaccharides which can reduce Cu^{2+} to Cu^{+} . The excess of Cu^{2+} can be controlled by Yodometry. The level of carbohydrate in the decomposition of *R. mucronata* leaves can be determined by first calculating the ash content in the following way: Weigh carefully about 5 g of the sample into a 500 ml erlenmeyer, then add 200 ml of 3% HCl solution, simmer for 3 hours with an upright cooler. Cool and neutralize with 30% NaOH solution (with litmus and phenolphthalein) and add a little CH_3COOH 3% so that the atmosphere of the solution is slightly acidic, then transfer the contents into a 500 ml measurement and squeeze until the line mark and then strain. Pipette 10 ml filter into a 500 ml erlenmeyer, add 25 ml Luff Schoorl solution (with a pipette) and some boiling stones and 15 ml distilled water. Heat the mixture with a steady flame, try so that the solution can boil within 3 minutes (use a stop watch), continue to simmer for exactly 10 minutes (counted when starting to boil) then quickly cool in a tub of ice. After chilling add 15 ml of 20% KI solution and 25 ml of 25% H_2SO_4 slowly, then pull the turret immediately with a 0.1 N tio solution (use a 0.5% starch solution pointer) also do the blank. Calculation: (Blank titers) $\times$ N tio $\times$ 10, equivalent to reduced irrigation. Then look in the list of Luff-Schoorl how many mg of sugar is contained for ml of tio used.

$$\text{Glucose content} = \frac{W_1 \times fp}{W} \times 100\%$$

$$\text{Carbohydrate content} = 0.9 \times \text{glucose content} \quad (2)$$

Where:

W_1 = sample weight

W = glucose contained for ml used (mg)

F_p = dilution factor

2.8. Protein content analysis

Protein analysis of the Kjeldahl method can basically be divided into three stages: (1) the destruction stage, (2) the distillation stage and (3) the titration stage. This method is suitable for semimicrobial use, because it only requires a small number of samples and reagents and a short analysis time. The detailed analysis method is described as follows: weigh 2 g of the sample, put it in a 100 ml Kjeldahl flask, then add 1 g of the selen mixture and 25 ml concentrated H_2SO_4 , then heat it on an electric heater or an incendiary flame until it boils and the solution becomes clear greenish (about 1-2 hours). Allow to cool, then dilute and put into 100 ml measuring cup, right up to the line mark. Pipette 25 ml of solution and put it into a distiller, add 5 ml of 40% NaOH and a few drops of PP indicator. Flute for about 9 minutes, as a container to use 25 ml of 4% boric acid which has been mixed with the Conway indicator. Rinse the cooling end with distilled water. Then titer with 0.1N HCl solution. Work on blanking. Determination of protein content can be done by the formula:

$$\text{Protein content} = \frac{(V_1 - V_2) \times N \times 0.14 \times f_k \times f_p}{W} \quad (3)$$

Where:

W = sample weight

V_1 = HCl volume of 0.1 N used in the sample spin

V_2 = volume of HCl used in blanking

N = normality of HCl

F_k = protein from food in general 6.25, milk and processed products 6.38, peanut oil 5.46

F_p = dilution factor

3. Results and Discussion

3.1. Decomposition rate

Litter *R. mucronata* leaves experienced a weight loss for 90 days. from the 15th day until the k-90 day continued to decline. This happens due to the decomposition process. The decomposition process from station 1 to station 3 is different. Decomposed leaves undergo physical changes ranging from whole to leaf fragments in Figure 4. The average residual litter decomposition can be seen from the graph above where the largest decrease in dry weight / litter residual or weight reduction is very drastic at the beginning of the observation on the 15th day seen at station II which is 17.33 g with a shrinkage of 32.67 g and the percentage of decomposition rate of 65%. Whereas the weight depreciation at stations I and III was 29.2 g and 26.91 and the percentage decomposition rate was 58% and 54%. Based on the weight loss or residual litter of *R. mucronata* leaves, it can be known that the average decomposition rate of *R. mucronata* leaf litter can be seen periodically in Table 1.

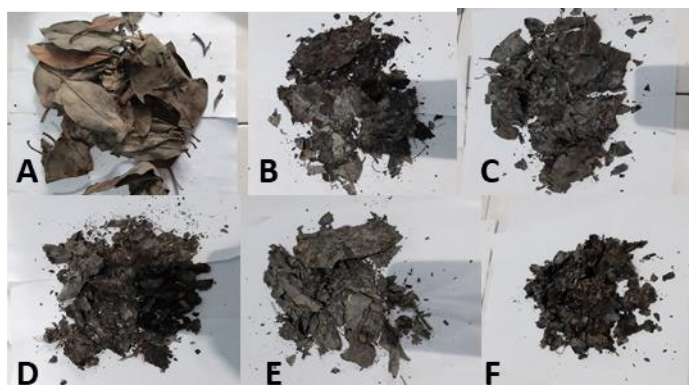


Figure 4. Physical appearance of *R. mucronata* leaf litters from the 15th day until the 90th day. (A) 15th day (B) 30th day (C) 45th day (D) 60th day (E) 75th day (F) 90th day.

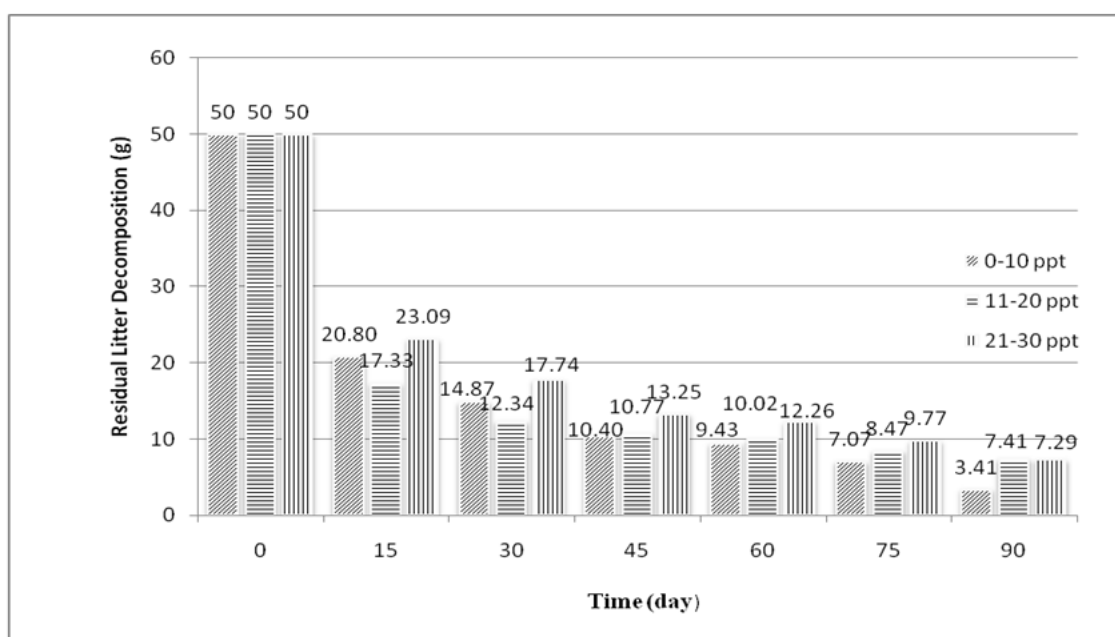
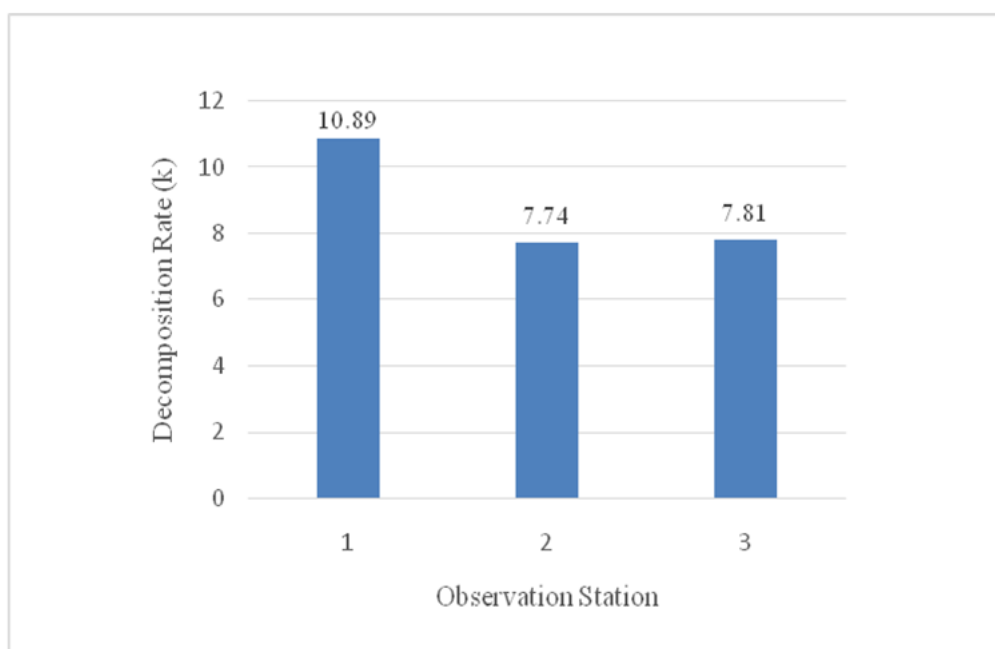


Figure 5. Average residual litter of *R. mucronata* leaves over the observation period of 90 days.

Table 1. The average time of leaf litter decomposition of *R. mucronata*

Station	Time (Days)							Average
	0	15	30	45	60	75	90	
1	0	1.95	1.17	0.88	0.68	0.57	0.52	0.96
2	0	2.18	1.26	0.87	0.67	0.55	0.47	1.00
3	0	1.79	1.08	0.82	0.63	0.54	0.57	0.90
Average	0	1.97	1.17	0.86	0.66	0.55	0.52	

From the table above, it can be seen that the highest decomposition rate of *R. mucronata* leaf litter occurs at the beginning of the 15th-day short composition and then decreases slowly with increasing observation time until the end of the 90th-day short composition. The highest decomposition rate at the beginning of the 15th-day observation was 1.97 g / day and the lowest decomposition rate occurred at the 90th observation of 0.52 g / day. The graph below shows the average constant value of the decomposition rate of the *R. mucronata* leaf from each station.

**Figure 6.** The average constant value of the decomposition rate of the *R. mucronata*.

The measurement results of the average value of the decomposition rate constant (k) litter at each station respectively amounted to 10.89, 7.74, and 7.81 from the initial weight of the 15th observation to the observation period of the 90th day. the highest value of the decomposition rate constant was found the station I at 10.89 and the lowest at station II at 7.74.

The largest decrease in dry weight/litter residual or very drastic weight reduction occurred at the beginning of the observation on the 15th day seen at Station II which was 17.33 g with a shrinkage of 32.67 g and the percentage decomposition rate of 65%. Whereas the weight depreciation at stations I and III was 29.2 g and 26.91 and the percentage decomposition rate was 58% and 54%. The influence of chemical physics plays a major role in the decomposition process. In the first 15 days, the litter quickly contracted due to macrozoobenthos activity which acts as the main enumerator for eating leaves. In the leaves that are still new, many elements that are food for macrozoobenthos so that the litter is cut into pieces faster.

Accordance with the statement of [4] which states that the litter produced by mangroves will later be decomposed. The process of decomposition of mangrove litter starts from the destruction carried out by macrozoobenthos and the litter will be cut into smaller sizes. Decomposition is followed by biological processes carried out by bacteria and fungi as decomposers to decompose organic particles by releasing enzymes so that they can decompose organic matter into proteins.

The value of the decomposition rate (k) on observations was 10.89, 7.74, and 7.81 from the initial weight of the 15th observation to the observation period of the 90th day. The highest decomposition rate coefficient value was found the station I at 10.89 and the lowest at station II was 7.74. The high salinity value of station II and station III can inhibit the decomposition process. Besides, there are differences in the number of microorganisms the station II and station III so that the constant value at station III is higher than station II. Accordance with [6] states that the increase in salinity can cause inhibition of soil microorganism activity which is reflected in the form of changes in CO₂ content, cellulase activity, and humification of plant residues. The speed of decomposition is influenced by leaf type, microorganism activity, water velocity, and length of submergence under the water surface.

3.2. Macrozoobenthos

Macrozoobenthos is one of the early decomposers who squeezed or chopped the remnants of leaves which were then re-released as dirt then continued by bacteria and fungi to break down organic matter into proteins and carbohydrates. The most number is the station I.

Table 2. Macrozoobenthos species found in *R. mucronata* litter bags

Class	Order	Genus
Bivalvia	Venorida	<i>Polymesoda</i>
Crustaceae	Decapoda	<i>Chiromantes</i>
Gastropoda	Mesogastropoda	<i>Telescopium</i>
Malacostraca	Decapoda	<i>Litopenaeus</i>
Turbellaria	Macrostomida	<i>Microstonum</i>

The number of macrozoobenthos is also influenced by the level of salinity. macrozoobenthos which have a shell is the most suitable in the area of maternity. So many crabs, clams, and snails are found in litter bags. This is consistent with the statement of [7] which states that the development of salinity affects the development of macrozoobenthos types. The presence of river or rainwater input will reduce salinity levels, which will result in the death of several types of macrozoobenthos. The life of some macrozoobenthos depends on the low salinity, but there is also the opposite. Species of makrozoobenthos can be seen in Figure 7.



Figure 7. Makrozoobenthos found in leaf *R. mucronata* leaf litter (A) snail (B) crab (C) worm (D) mussel (E) shrimp.

In addition to bacteria and fungi macrozoobenthos also plays an important role in the decomposition process. Giving fungi *A. fumigatus* to accelerate decompose organic particles by releasing enzymes so that it can break down organic matter into proteins. This is consistent with the statement of [3] which states that the fungus *A. fumigatus* can cause spoilage in fruits or vegetables. *Aspergillus* is parasitic, some saprophytic. fungi *A. fumigatus* can be found in decomposed soil, water, and plants, especially in manure and humus.

3.3. Carbohydrate and protein content

The decomposition process of *R. mucronata* leaf litter occurs from the 15th day until the 90th day. Searasah of *R. mucronata* leaves experienced a decrease in carbohydrate levels. Based on the results of the Medan Industrial Standardization Research Laboratory Laboratory the highest carbohydrate content was found in observation I station 3 which was 12.2%. The carbohydrate content of *R. mucronata* leaf litter can be seen in the table 3. The decomposition process of *R. mucronata* leaf litter occurs from the 15th day until the 90th day. Leaves of *R. mucronata* have increased levels of protein. Based on the results of the Medan Industrial Standardization Research Laboratory Laboratory, the highest protein content was found in observation VI of station 3, which was 6.78%. The protein content of *R. mucronata* leaf litter can be seen in the Table 4.

Table 3. Test results of carbohydrate content in *R. mucronata* leaf litter.

Observation	Station	Results (%)
I	1	10.0
	2	8.07
	3	12.2
IV	1	7.16
	2	7.50
	3	6.02
VI	1	2.64
	2	3.06
	3	5.46

Table 4. Test results of protein content in *R. mucronata* leaf litter

Observation	Station	Results (%)
I	1	4.88
	2	4.36
	3	5.76
IV	1	5.35
	2	6.07
	3	5.08
VI	1	6.09
	2	6.77
	3	6.78

The testing of carbohydrate and protein levels is done by the sampling method. The test results showed a decrease in carbohydrate levels from the 15th observation to the 90th observation. The higher the level of salinity and the length of time of decomposition, the carbon nutrients tend to decrease. The nutrient element of carbon is one of the nutrients that play a role in the formation of carbohydrates, namely Carbon (C), hydrogen (H), and oxygen (O). Accordance with the statement of [8] which states that the content of carbon nutrients tends to decrease with the addition of decomposition time and reduction of litter particle size.

Microorganisms as decomposers to decompose organic particles by releasing enzymes so that they can decompose organic matter into proteins. The test results showed an increase in protein levels from the 15th observation to the 90th observation. Accordance with the statement of [9] states that in the mangrove ecosystem, the food chain that occurs is the detritus food chain. Detritus food chain (detritus food chain) starts from the decomposition process of leaves and mangrove twigs (dead organic material) broken down by microorganisms (bacteria and fungi) to produce detritus. Detritus is then eaten by detritus-eating animals, then eaten by predators. During the decomposition process, mangrove litter gradually increases in protein content.

4. Conclusion

The average decomposition rate (k) of *R. mucronata* leaf litter at 0-10 ppt salinity is 10.89, 10-20 ppt salinity is 7.74 and 20-30 ppt salinity is 7.81. The Leaf Litter of *R. mucronata* have decreased carbohydrate content. the highest carbohydrate content was found in observation I station 3 which was 12.2% and the lowest was in observation VI station 1 which was 2.64%. While the protein content in *R. mucronata* litter increased. The highest protein content was in observation VI of station 3 which was 6.78% and the lowest was in observation I of station 2 which was 4.36%.

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