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The Fungal Development During The Leaf Litter Process Decomposition of *Avicennia marina*

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ABSTRACT

The existence of mangrove ecosystems decreases from land conversion into residential areas, industries, plantations, road facilities and the construction of ponds. This research was conducted in the mangrove forest area of Secanang Belawan Village, Medan, at the Biotechnology Laboratory of the Department of Forestry, Faculty of Agriculture, USU and at the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, USU Medan. The study's objective was to identify the different fungal species that were present at 10 ppt salinity levels, 10–20 ppt, and 20–30 ppt as well as the pace at which *Avicennia marina's* leaf litter. The research method used litter bags filled with leaf litter of *Avicennia marina* (50 g) with 7 treatments, 3 replications and 3 levels of salinity. The *A. marina's* leaf litter that had a salt level of 10 ppt had the highest level of decomposition. The dry weight of leaf litter left in the litter bag, which is 4.92 g on average. The largest dry weight of the remaining litter was found in an environment with a salinity of 20 – 30 ppt, which is an average of 10.42 g. The rate of decomposition of *A. marina's* leaf litter in an environment with a salt level greater than 30 ppt is 6.53/yr (or almost equal to residence period of = 0.15 yr). The number of fungal species discovered in the leaf litter of *A. marina*, which goes through a breakdown process at salinity levels between 0 and 10 ppt and contains 9 species, is another way to observe the impact of salt level. In comparison to the large at salinity levels of 10 to 20 ppt and 20 to 30 ppt, respectively, there were populations of fungus that underwent the process of decomposition in *A. marina's* leaf litter, with an average of 5.99 x 102 cfu/ml and 5.5 x 102 cfu/ml, the fungal population that was the highest concentration was 10.72 x 102 cfu/ml.

Keyword: *Avicennia marina*, Decomposition, Fungi, Leaf Litter, Microorganisms

1. Introduction

Salinity, along with other animal and microbial components including nails, worms, and bacteria and fungi, as well as environmental components like soil type and water pH, can influence how rapidly litter decomposes [1]. Other elements that influence the rate at which litter decomposes include energy release, tidal frequency, soil temperature, humidity, and the presence of organisms that break down rubbish in mangrove forests [2,3]. The majority of biodiversity and 91% of the biomass in the ecosystem of mangroves are made up of microbes, which include bacteria and fungi. [4] Fungi that create various enzymes can speed up the decomposition of litter. For microorganisms to thrive and aid in the decomposition of litter, several parameters must be met. These include the presence of nutrition, the accessibility of extractable materials, the preferred temperature, pH, O_2, CO_2, and sufficient water.

Carbohydrate breakdown provides energy for the metabolism of bacteria and fungi [5]. The previous research [6] discovered that variations in salinity also resulted in variations in the quantity of fungus participating in the breakdown of *A. marina's* leaf litter. Because decomposers and plant symbionts play a significant role in ecological and biogeochemical processes, microbes, which make up 91% of the total biomass of mangrove

ecosystems, represent a significant component of this biodiversity [7]. They are a significant mineral in mangrove sediments and a crucial source of food for benthic organisms, both of which greatly aid in the decomposition of organic matter derived from mangrove materials [8,10]. Microorganisms, in particular those produced from the detritus of coastal ecosystems like mangroves, perform a crucial ecological function in the breakdown of organic waste and produce detritus that is protein-rich and used as fish food [11]. *Pestalotiopsis agallochae* and *Cladosporium marinum*, two parasitic fungi, appeared to be on the leaves of *Accoecaria marina* and *Excoecaria agallocha* [12]. Mangrove leaf breakdown can be broken down into three parts [13]. Significant nitrogen and carbon losses happen within a few days of the first stage of decomposition, which is mostly brought on by microbial breakdown of biomass from organic materials. The total amount of material, which includes both organic and inorganic components, is substantial [14]. The following two stages a process of leaf litter decay involve the breakdown of stable structural elements and biological matter, which are then the litter will next decompose physically and biologically, in that order [1].

In comparison to *A. marina, R. mucronata's* leaf litter decomposed more quickly (p 0.001). It took *A. marina* 49.55 days and *R. mucronata* 44.43 days (t1/2) to reduce the initial dry mass by 50%. The results of the investigation show [15]. The breakdown rate of *A. marina* (k = 0.83) was higher than that of *R. apiculata* (k = 0.41). Although leaves originally have a high organic content, as they deteriorate, they gradually decline. The nutrients released during decomposition are not lost to the system and are still accessible to the plant for primary productivity following release [1,16]. The study's objective was to identify the different fungal species that were present at 10 ppt salinity levels, 10–20 ppt, and 20–30 ppt as well as the pace at which *A. marina's* leaf litter.

2. Method

2.1 Material and Tools

Litter decomposition experiments were carried out in the mangrove forest area of Secanang Belawan Village, Medan, at the Biotechnology Laboratory in the Faculty of Agriculture, USU, and the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, USU. This research activity lasted for six months (April to November 2022). Leaf litter from *A. marina* that was gathered was used as the materials with a net and picked leaves that have undergone senescence from the tree, Potato Dextrose Agar, Malt Extract Agar, 96% Alcohol, Sterile Water, Aluminum foil, spirits, plastic bags. The field equipment materials used were litter bags made of nylon with a mesh size of 2 mm, nylon ropes for fastening the litter bags, wooden stakes made of bamboo, permanent markers, stationery, and others. The tools used are a hand refractometer to measure salinity, a light microscope, an autoclave, a Petri dish, a test tube, a slide glass, a cover slip, tweezers, a culture storage box, slide box, Erlenmeyer flask, digital camera measuring cup and others.

2.2 Data Collection Techniques

To ascertain the impact of seawater salinity level, duration of decomposition, population, species diversity, and how often each one is colonized species fungi, data on these parameters were gathered. The following salinity levels are used to deposit the leaf litter of *A. marina* such as (a) salinity below 10 ppt, (b) salinity between 10 and 20 ppt, and salinity between 20 and 30 ppt.

Following the placement of the litter with varying salt levels in the field, data collecting was done. The observations were made in three replications for 90 days, each with a bag containing 50 g of litter collected over the subsequent 90 days such as (a) no littering (control), (b) day 15th, (c) day 30th, (d) day 45th, (e) day 60th, (f) day 75th, and (g) day 90th.

2.3 Method of Research

2.3.1 A clump of A. marina's leaf litter

A 30 × 40 cm piece of gauze or nylon, tied above the highest tide line, between two trees, was used to gather leaf litter. A total of 3150 g of *A. marina's* leaf litter were collected (50 grams of litter times 7 treatments times 3 replications times 3 salinity levels).

2.3.2 Placing leaf litter on the field of A. marina

Nylon litter bags (mesh size of 1x1 mm and 40x30 cm) were filled with 50 g of *A. marina's* leaf litter. The total number of bags was 54 (six samplings, three repetitions, and three salinity levels). Salinity was calculated using a hand refractometer. Three plots measuring 430 cm x 50 cm were created at locations with predetermined salinity levels. One plot yielded about 21 bags containing 50 g of leaf litter from *A. marina*.

Bags containing waste are collected until the 90th day (7 times). A total of three bags were collected every fifteen days at each salinity level.

2.3.3 Fungi isolation of leaves layer of A. marina

Dilution is used to determine the fungal population by making a series of sample suspensions. The procedure was carried out to dilute *A. marina's* leaf litter and isolate the fungus on media in a petri dish as follows: (a) A total of 10 grams of *A. marina's* leaf litter sample that had been mashed in a mortar was put into a 250 ml Erlenmeyer pumpkin. Furthermore, the sterilized water is put into the litter environment until the volume reaches 100 ml. After the dilution of *A. marina* leaves reaches an optimal level of 0.1 ml, the suspension of the dilution results is taken from each level of dilution. Furthermore, the suspension was cultured on a petri dish with PDA media that had been treated with the antibiotic Kemicetine at a dose of 0.1 g/l and placed at room temperature. Observation of emerging colonies was carried out 1–12 days later during the incubation period. To get the number of fungal populations per ml, the number of colonies per ml is calculated and then multiplied by the dilution factor.

2.3.4 Fungus identification

Pure rejuvenated fungal cultures are cultured at room temperature for 5-7 days in PDA media. The macroscopic characteristics of the developed fungal isolates were then examined, especially the characteristics of the colonies, including the color of the spore mass or conidia, colony size, and colony color. Shear culture, involving the cultivation of fungal isolates on 4 x 4 x 2 mm agar plates, is another method used to grow mushrooms. A soaked plastic box (30 x 20 x 6 cm) was used to contain the isolates on this slide. Permanent cultures are also prepared by adding one drop of lactophenol solution to sliced agar. After that, the lactophenol solution on the slide was covered with a cover slip. A light microscope is used to examine this glass culture to identify small characteristics of the fungus, including characteristics of the type of hyphal branching, the presence or absence of septations in the hyphae, conidiophores, conidiogenesis, characteristics of the conidia or spores (shape and order), and size of spores. Then properties are compared with the identification keys of fungi [17-23].

2.3.5 Estimation of litter decomposition rate

The rate of litter decomposition is determined by employing the formula [24]:

$$Xt = Xo \, . \, e^{-k\,t} \quad (1)$$

$$\ln (Xt/X0) = -k\,t \quad (2)$$

information :

x_t = dry weight of litter after the t watching period (g)
x_0 = starting weight of the litter (g)
e = number of natural logarithms (2.72)
k = rate of decay of litter
t = days spent observing

2.3.6 The Index of Fungal Diversity

The index of fungi species diversity was derived using [25] formula:

$$H' = -\sum_{i=1}^{s} (Pi \ln Pi) \qquad Pi = \frac{ni}{N} \quad (3)$$

Note :

H' = Diversity
Ni = Number of species to i
s = Number of species
N = Total number of all types
Pi = Proportion of total test sample

3. Result and Discussion

3.1 Decomposition Rate of A. marina's Leaf Litter

Figure 1 shows the dry weight of *A. marina's* leaf litter remains after several long decomposition periods at various salinity levels. The highest concentration of *A. marina's* leaf litter was at a salinity of 20-30 ppt, which was around 10.42 g dry weight. The dry weight of *A. marina's* leaf litter was the smallest (the largest loss), namely 4.92 g. This amount was found in leaf litter that underwent a process of decomposition due to salinity < 10 ppt.

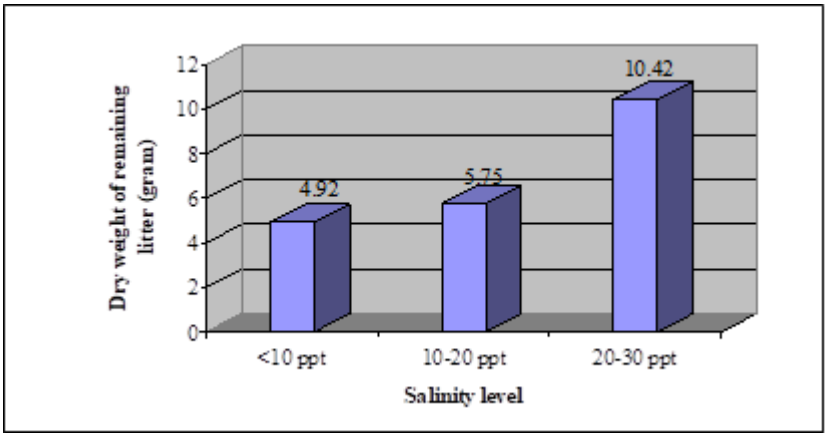

Figure 1. The remaining dry weight of *A. marina* leaves decomposed for 90 days in an environment with varying salinity

The average time and rate of decomposition for *A. marina's* leaf litter discovered in environments with varying salt levels are shown in Table 1. It is conceivable that the leaf litter of *A. marina*, which with a salinity of 10 ppt underwent a breakdown process with a value of k of 9.67/year, experienced the highest decomposition rate, is what this observation means. It is also demonstrated by how long the litter remains in an environment with a salt level of less than 10 ppt (0.10 years).

Table 1. The average decomposition rate and tonnage of litter discovered in varied salinity

No.	Salinity level	k (year^{-1})	The length of time there is litter (year)
1	< 10 ppt	9.67	0.10
2	10 – 20 ppt	9.01	0.11
3	20 – 30 ppt	6.53	0.15

As the particle sizes of the litter or plant material decrease, the dry weight loss is faster because of fungal attack [26]. The wider the tidal environment, the greater, the heterogeneity of variables including light (canopy gap), salinity, and sediment (space and nutrients), all of which will affect the existence and survival of organisms [27]. The effects of increased salinity on soil microbial activity can be seen in changes to the CO2 concentration, cellulase activity, and humification of plant residues [28]. The type of leaf, the activity of the microorganisms, the water's velocity, and how long the object has been submerged in the water all affect how quickly something decomposes [29]. The rate of leaf litter degradation is related to the tidal frequency and the chemical quality of the leaf litter [30].

3.2 Various Fungus Species Found in The A. marina's Leaf Litter that Degrade at Different Salinities

3.2.1 The types of fungus present in A. marina's leaf litter that have not gone through the process of decomposition (control)

As the particle sizes of the litter or plant material decrease, the dry weight loss is faster because of fungal attack [26]. The wider the tidal environment, the greater, the heterogeneity of variables including light (canopy gap), salinity, and sediment (space and nutrients), all of which will affect the existence and survival of organisms [27]. The effects of increased salinity on soil microbial activity can be seen in changes to the CO_2 concentration, cellulase activity, and humification of plant residues [28]. The type of leaf, the activity of the microorganisms, the water's velocity, and how long the object has been submerged in the water all affect how quickly something decomposes [29]. The rate of leaf litter degradation is related to the tidal frequency and the chemical quality of the leaf litter [30].

3.2.2 Fungi species of the A. marina's leaf litter that decompose at a salinity level of less than 10 ppt

Nine different species of fungi were discovered in *A. marina's* leaf litter that was decomposing at a salinity level of less than 10 ppt. *Aspergillus* sp. 1, *Penicillium* sp. 3, *Aspergillus* sp. 2, *Fusarium* sp. 1, *Aspergillus* sp. 4, *Penicillium* sp. 4, *Curvularia lunata* (Figure 2), and *Fusarium* sp. 2 are the different forms of fungi.

3.2.3 Fungi species that decompose at salt levels can be within A. marina's leaf litter. 10 - 20 ppt

Aspergillus sp. 1, *Penicillium* sp. 3, *Aspergillus* sp. 2, *Aspergillus* sp. 4, *Aspergillus* sp. 3, *Fusarium* sp. 2, and *Penicillium* sp. 1 were the species of fungus discovered in the leaf litter of *A. marina* that degraded at a salinity level of 10 to 20 ppt (Figure 2).

3.2.4 Fungi species from the A. marina plant were discovered in its leaf litter after it had begun to decompose at a salinity level of 20 to 30 ppt

A total of 8 different fungal isolates from *A. marina's* leaf litter that had decomposed at a salinity range of 20 to 30 ppt. According to Figure 2, there are six different species of fungi: *Aspergillus sp. 1, Aspergillus sp. 2, Aspergillus sp. 3, Trichoderma sp., Aspergillus sp. 4, Penicillium sp. 1, Aspergillus sp. 6, and Penicillium sp. 4.*

Below are photos of each mushroom taken at different salt levels. *Fusarium* sp. 1 was different from the litter of the control species, *A. marina* (Figure 2a). *Curvularia lunata* from *A. marina* litter degraded at a salinity of less than 10 ppt (Figure 2b). Figure 2c depicts the appearance of the *Penicillium* sp.3 fungus that was isolated from *A. marina's* leaf litter that had 10 to 20 ppt of salinity. The emergence of the fungus *Penicillium* sp. 4 in leaf litter that has decomposed at a salinity of 20 to 30 ppt is shown in Figure 2d.

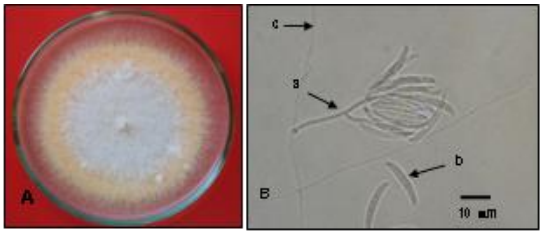

Figure 2a. Fusarium sp. 1. Colonies aged 14 days on PDA media (A) and microscopic forms (B), Conidiophores (a), Conidia (b) and Hyphae (c)

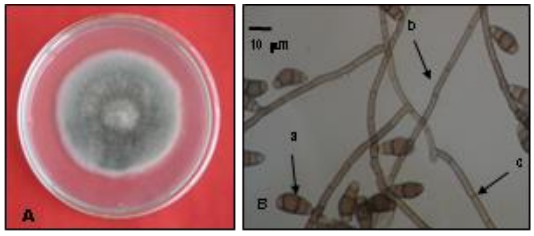

Figure 2b. Curvularia lunata. Colonies aged 14 days on PDA media (A) and microscopic forms (B), conidia (a), hyphae (b) and septa (c).

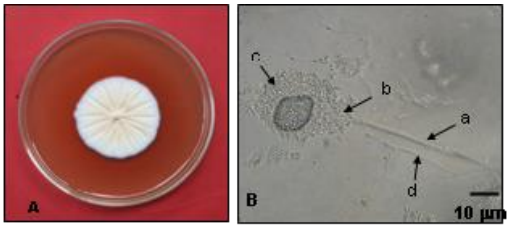

Figure 2c. Penicillium sp. 3. Colonies aged 14 days on PDA media (A) and Microscopic forms (B), Conidiophores (a), Fialid (b), Conidia (c) and Hyphae (d)

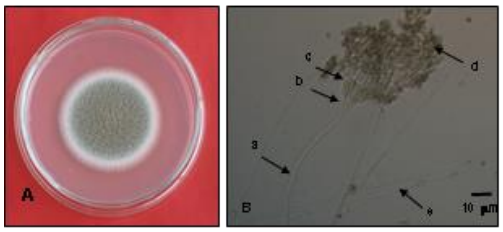

Figure 2d. Penicillium sp. 4. Colonies aged 14 days on PDA media (A) and Microscopic forms (B), Conidiophores (a), Metula (b), Fialid (c), Conidia (d) and Hyphae (e)

Figure 2. Various fungus species found in the leaf litter of *A. marina*

The number of fungal species found in leaf litter of *A. marina* that decomposed at salinity levels of 10 ppt, 10 – 20 ppt, 20 – 30 ppt, and control is in Figure 3. *A. marina's* leaf litter underwent a decomposition process at salinity levels between 10 and 20 ppt. It is where the greatest diversity of fungus species is found. It happens because the conditions are identical to fresh (brackish) conditions. These conditions are more favorable for the development and growth of various forms of fungi than conditions with a higher salt content. These species number is eight at a salinity of 20 to 30 ppt. Their number decreases as the salinity increases.

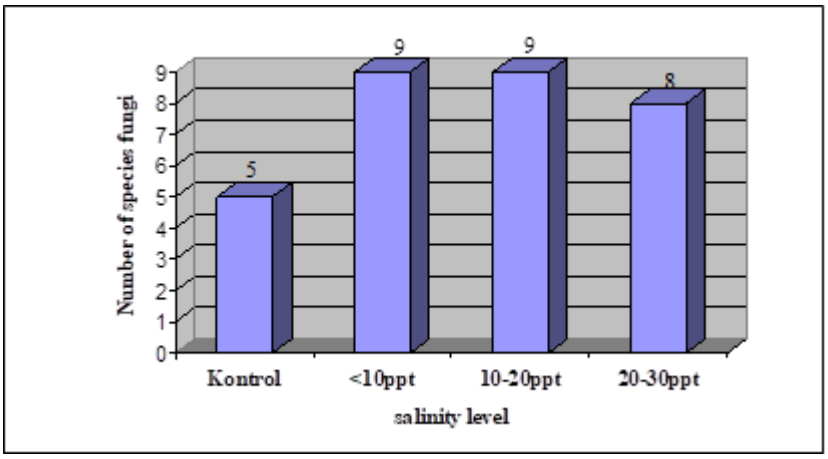

Figure 3. The variety of fungus species that have decomposed in the leaf litter of *A. marina* after being exposed to different salinity levels

This result differs from previous research [6], in which the number of fungal species was higher at salinities > 30 ppt than at salinities between 10 and 20 ppt and 20 and 30 ppt when the litter was decomposing. It is thought to occur due to the influence of river flows near the study site. It is estimated that fungal propagules originating from washed land in the form of spores, hyphae, conidia, and others are carried away by river flows so that when high tides occur, many propagules may be carried to the study site and attached to the leaf litter of *A. marina* and are involved in the decomposition of the trash came from areas where salt levels were greater than 30 ppt. It is also possible that this fungal propagule was in a dormant condition in the leaf litter of *A. marina*. Furthermore, the dormant propagules when isolated in the laboratory can grow and develop. The research [31] found that the propagules viability of 30 fungi species was preserved in the sand submerged in seawater for five weeks.

3.3 Comparison of The Populations of Fungus at Varying Salinities

The leaves of *A. marina* contained the highest fungal population, which has an average of 14.66 x 102 cfu/ml and had not yet completed field decomposition (Figure 4). The number of fungi in *A. marina's* leaf litter that decomposed at salinity levels of 10 ppt or less, or 10.72 x 102 cfu/ml, was higher than the amounts of fungi that decomposed at, or an average of 5.99 x 102 cfu/ml and 5.5 x 102 cfu/ml, respectively. The leaf litter of *A. marina*, which had through a decomposition process at a saline level of 10 ppt, contained the highest number of fungus species at all salinity levels investigated. The quantity of fungi, which makes up the largest group in rubbish has decomposed at a salinity level of less than ten ppt, follows a pattern comparable to this one.

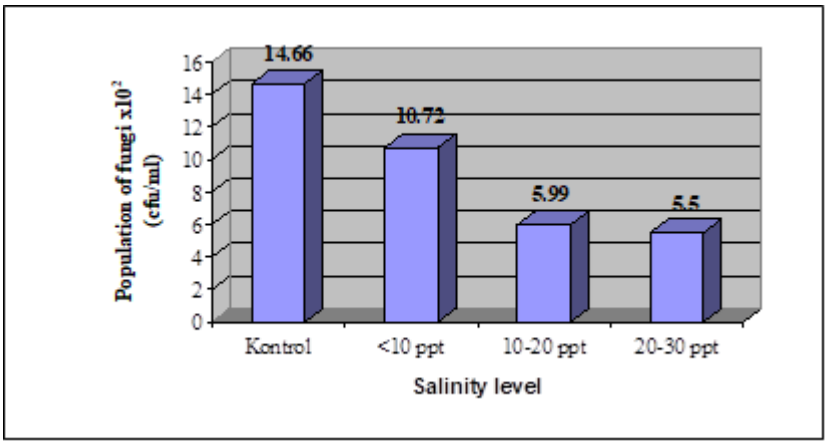

Figure 4. The fungus is found in leaf litter of *A. marina* that has not decomposed in environments with varying levels of salinity.

Fungal populations decrease as environmental salt increases. It occurs in *A. marina's* leaf litter which undergoes a decomposition process at a salinity level that is approximately equivalent to litter, namely 10 ppt. In this condition, the elements needed for the development and repair of various forms of fungi are more abundant. In contrast to salinities of 10–20 ppt and 20–30 ppt, some fungi can develop and mature more effectively at less than 10 ppt.

3.4 Fungi Diversity Index

The leaf litter of *A. marina* that had been decomposing at various salinity levels ranged from low to moderate in terms Shannon Index for fungal species diversity. *A. marina's* leaf litter degraded in environments with salinities of 10 ppt, 10 - 20 ppt, and 20 - 30 ppt had indices of fungal species diversity of 2.59, 1.23, and 0.18, respectively. The fungal diversity index, which was 0.49 and was detected in the leaf litter of *A. marina* but hadn't gone through the decomposition process, was lower than the species diversity index. There was a significant variation in the variety of fungus species in the leaf litter of *A. marina* that degraded at each of the tested salinity levels. Here, it is evident that the population of fungi and the variety of fungi species are at their peak at salinities of less than 10 ppt. According to [32], oxygen availability, soil temperature, humidity, nutrient concentration, and soil organic matter content are the elements that influence species diversity and population size variety of soil organisms.

4. Conclusion

Decomposing control litter at locations with salinity less than 10 ppt contained the highest number of fungal populations. The maximum decomposition rate was found in *A. marina's* leaf litter at a salinity of 10 ppt. The dry weight of the remaining waste left in the trash bag is an average of 4.92 g. The dry litter weight that survived was highest in an environment with a salt content of more than 30 ppt, which was an average of 13.25 g. The value of litter decomposition rate was placed at the salinity level <10 ppt, 10 – 20 ppt, and 20 – 30 ppt (respectively 9.67, 9.01, and 6.53) has residence times of 0.10 years, 0.11 years, and 0.15 years. The process of decomposing litter occurs more slowly at a salinity level of 20 - 30 ppt compared to other salinity levels.

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