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Fungal diversity associated with the decomposition of *Avicennia marina* leaf litter at various salinity levels

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Abstract. *Yunasfi, Susetya IE, Utomo B, Dalimunthe A, Samsuri, Zaitunah A. 2024. Fungal diversity associated with the decomposition of* Avicennia marina *leaf litter at various salinity levels. Biodiversitas 25: 792-800.* The process of litter decomposition in mangrove ecosystem is influenced by both biological and environmental factors. Biological factors include organisms, such as worms, snails, bacteria, and fungi, while environmental factors are soil, water pH, salinity, and others. Therefore, this study aimed to determine the effect of salinity level and length of decomposition period, dominant fungi, as well as carbohydrate and protein levels in *Avicennia marina* leaf litter. In the experiment, litter bags containing 50 g of *A. marina* leaf litter were used and placed at salinity levels of <10 ppt, 11-20 ppt, and 21-30 ppt. The results showed that the highest decomposition rate i.e., 6.90/year, with length of time = 0.15 years was found in *A. marina* leaf litter at a salinity level of 21 ppt-30 ppt. At salinity levels of <10 and 10-20 ppt, the decomposition rate was 4.55 and 5.91, respectively, while the litter period in soil was 0.22 and 0.17 years. Furthermore, the residual leaf litter decomposed at salinity levels of <10 ppt, 11-20 ppt, and 21-30 ppt was 6.67 g, 4.57 g, and 4.36 g, respectively. The average value of the Shannon diversity Index for fungal species in *A. marina* leaf litter, which had experienced decomposition ranged from low to moderate. At salinity levels of <10 ppt, 10-20 ppt, and 20-30 ppt, the values obtained were 1.96, 1.86, and 1.95 respectively. The fungal species diversity index was greater than the control, 1.26. Based on the results, *A. marina* leaf litter placed at salinity level of <10 ppt had the highest carbohydrate and protein content of 12.10 and 9.12%, respectively, while the lowest protein content of 8.28% was recorded at salinity level of 20-30 ppt. This study showed that the longer the decomposition period at various levels of salinity, the higher the protein content in absolute terms.

Keywords: *Avicennia marina*, carbohydrates, decomposition, leaf litter, mangrove, protein, salinity

INTRODUCTION

Mangrove forest in South and Southeast Asia was reported to cover 41.4% of the global total area (Singh et al. 2014). This unique ecosystem thrives in tidal swamp areas, estuaries, as well as tropical and subtropical coastlines (Raghukumar 2017). Mangrove vegetation adapts morphologically and physiologically to conditions that fluctuate salinity, temperature, sediment, and low oxygen concentrations (de Souza et al. 2013; Raghukumar 2017), contributing specifically to primary productivity in the form of litter production as well as decomposition and release of nutrients (Kumar et al. 2014; Lovelock et al. 2015; Carugati et al. 2018; Kamaruzzaman et al. 2019; Ribeiro et al. 2019; Mahmudi et al. 2021). Mangrove serves as an important coastal ecosystem that provides nutrients to river estuaries and the surrounding environment, through litter decomposition. An important aspect lies in the export of organic material which is important for coastal areas (Yuwono 2015; Taketani et al. 2018; Das et al. 2019). The release of nutrients from mangrove leaf litter plays an important role in biogeochemical cycles in the aquatic environment, directly or indirectly affecting water quality and food availability for fish and shrimp (Alam et al. 2021).

Mangrove ecosystem offers an environment where fungi can grow, due to the large amount of litter produced by the vegetation. Meanwhile, fungi play an important role in the decomposition of lignocellulosic material (de Souza et al. 2013). During this process, fungi are considered to be one of the most active microorganisms, even compared to bacteria, specifically in the early stages of decomposition (Raghukumar 2017). Studies on fungi decomposition show that this process of decomposition occurs frequently in marine ecosystems (Tennakoon et al. 2022). Leaf litter decomposed by fungal communities generally occurs in three different phases, namely early, intermediate, and late (Kamel et al. 2020; Moitinho et al. 2022). However, leaf degradation in mangrove ecosystem is dynamic and generally occurs in a few weeks which can change other processes.

In a specific case study at Pichavaram mangrove on the southeastern coast of India, 15 fungi were obtained from wet wood, roots, and leaf litter of *Avicennia marina* and *Rhizophora mucronata*. The most abundant fungi (9 spp.) were found associated with wood litter of both plant species followed by root litter of *R. mucronata*, with *Aniptodera chesapeakensis*, *Halorosellinia oceanica*, *Halosarpheia marina*, *Periconia prolifica*, and *Phoma* sp. being dominant (5-6.3%). *A. marina* wood litter was

mostly occupied by *Halosarpheia marina* (28%), while *R. mucronata* was occupied by *Phoma* sp. (24%). The time in days required to lose 50% of the initial dry mass (t1/2) was 49.55 days for *A. marina* and 44.43 days for *R. mucronata*. The organic content in leaf was initially high but decreased gradually during decomposition, negatively correlating with the inorganic content (Farooqui et al. 2014). Mangrove forest in Nguling, Pasuruan resulting from reforestation with an area of approximately 57.1 ha demonstrated the potential to contribute Nitrogen of 129.58 to 184.69 kg/ha/year or 7.40 to 10.55 tons/year, while phosphorus was estimated at 6.57 to 9.13 kg/ha/year or 0.38 to 0.52 tons/year (Mahmudi et al. 2021). Total mangrove litter production (dry weight) ranged from 1.78 to 2.53 g/m^2/day, with the largest contribution stemming from leaf litter at 76.02 to 79.57% of the total. The decomposition rate of litter ranged between 3.84% to 4.18%, and the half-life or t50 to lose 50% of the initial weight was 19 to 27 days. Therefore, this study aimed to determine the effect of salinity level and length of decomposition period on the rate, dominant fungi, as well as carbohydrate and protein levels in *A. marina* leaf litter.

MATERIALS AND METHODS

Place and time

This study was conducted in Deli Belawan River mangrove forest area and at the Forest Cultivation Laboratory, Faculty of Forestry, University of North Sumatra. The identification of fungal species was confirmed at the Laboratory of Gunung Batu Bogor Forestry Research Institute. The experiment was carried out from June 2023 to September 2023 and a map of the field study locations was presented in Figure 1.

Collection of *A. marina* leaf litter

Leaf litter was collected using 5 to 10 gauze/nylon cloths measuring 3 x 4 m, which were placed by tying between two trees at a height above the highest tide line. The samples obtained weighed approx 3750 g and leaf litter of *A. marina* was also collected by gathering senescent leaf, attached to trees or those on mangrove soil.

Placement of *A. marina* leaf litter in the field

A total of three locations were selected for litter placement in the field, namely salinity level of <10 ppt (down part of river), with the distance of 750 m from river mouth, salinity level of 11-20 ppt (middle part of river) with the distance of 350 m from river mouth, and salinity level of 21-30 ppt (upper part of river) with the distance of 50 m from river mouth of Deli. About 50 g *A. marina* leaf litter was placed in 40 x 30 cm litter bags made of nylon with 1 x 1 mm mesh. The number of litter bags required was 75 including 8 observations x 3 repetitions x 3 levels of salinity plus control. The placement in the field was carried out based on the level of salinity measured with a hand refractometer. At the locations with salinity levels described above, one plot measuring 440 x 40 cm was made, with a total of three plots, and then litter bags were placed randomly. A total of three bags containing litter were taken from each level of salinity once every fifteen days until the 120th day (8 times).

Isolation of fungi from *A. marina* leaf litter

The isolation of fungal population was performed out using the dilution method by making a series of sample suspensions (Kiraly et al. 1974).

A total of 10 g of *A. marina* leaf litter samples were crushed in a mortar were transffered into a 250 mL Erlenmeyer flask, then water was added from the litter environment previously sterilized until the volume reached 100 mL. 0.1 mL suspension of the desired dilution (10^{-3}) was taken from each level. The suspension was poured onto Petri dishes containing PDA media, the antibiotic Kemicetine was used at a dose of 0.1 g/L to prevent bacterial growth, and then the plates were incubated at room temperature. For each dilution level, experiment was repeated two times, and observations of the colonies formed were carried out 1 to 12 days after the incubation period.

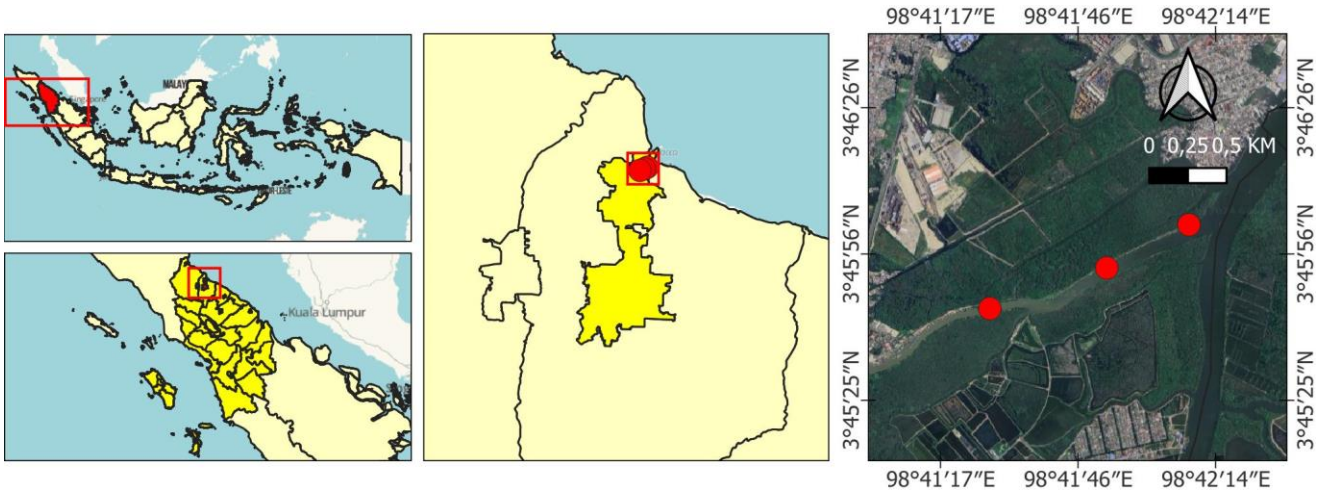

Figure 1. Map of study locations on Deli River in Medan, Belawan, North Sumatra, Indonesia

Estimation of litter decomposition rate

The litter decomposition rate was obtained using Formula (1) (Olson 1963):

$$X_t = X_{o.}\, e^{-k\,t} \quad (1)$$
$$\ln (X_t/X_0) = -k\,t$$

For the determination of the length of time that litter was presented (residence time) on the forest floor, Formula (2) was used:

$$1/k \quad (2)$$

Where: $X_{t:}$ Litter dry weight after observation time t (g); $X_{0:}$ Initial litter weight (g); e: Natural logarithmic number (2.72); k: Litter decomposition rate; t: Observation time (days)

Determination of species diversity index of fungi found in *A. marina* leaf litter

The fungi species diversity index was calculated using the Shannon Index (Shannon and Weaver 1949 referred to Ludwig and Reynolds 1988) with Formula (3), as follows:

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

Where: H': Diversity of species; ni: Number of species to I; s: Number of species; N: The total number of all species; Pi: The proportion of the total test sample

Determination of carbohydrate content

Carbohydrate content in decomposed *A. marina* leaf litter was determined by first calculating ash content as follows:

The porcelain cup was dried in the oven at 105°C for between 2 and 3 hours, followed by cooling in a desiccator and weighed (X). A test sample of 5 g dry weight (Y) was put into a porcelain cup, placed over a Bunsen flame until it released smoke and then placed in an electric furnace at a temperature of 400-600°C. The sample was removed when it turned to white ash and cooled in a desiccator. After 1 hour, the ash was weighed (Z) and the content was determined using the Formula (4).

$$\text{Ash content} = \frac{(Z - X)}{Y} \text{ x } 100\% \quad (4)$$

Where: X: Porcelain cup weight; Y: Initial test sample weight; Z: The weight of the test sample after it turns to ash

Determination of carbohydrate levels was carried out using Formula (5):

$$\text{Carbohydrate levels} = \{\text{Dry weight-Ash}\}\% \quad (5)$$

Determination of protein content

Protein analysis was carried out in three stages, such as: (1) destruction, (2) distillation, and (3) titration. During the analysis, 0.3 g dry weight of the test sample (X) was put into the digestion flask, then 3 small tablespoons of the Selen mixture catalyst and 20 mL of concentrated H_2SO_4 were added homogeneously. The mixture was heated with a digestion device for 10 minutes at a low position and for 5 minutes at a high position until the solution became clear and yellowish-green in color. This process was carried out in the acid chamber (Destruction Stage).

After the digestion flask was cooled, the test sample solution was placed into a distillation flask and diluted with 300 mL of water without Nitrogen. Several boiling stones were added along with 100 mL of 33% NaOH, then the distilling flask was quickly installed on top of the still. This distillation process was carried out until all N was captured by H_2SO_4 in the Erlenmeyer flask or when 2/3 of the liquid in the flask had evaporated (Distillation Stage).

The remaining H_2SO_4 contained in the Erlenmeyer flask was titrated again using 0.3 N NaOH solution. The titration process ended when the color changed to blue-green. The volume of NaOH was recorded as Z mL, then compared with the blank Y mL (Titration Stage). Protein content was determined using the following Formula (6):

$$\text{Protein content} = \frac{(Y - Z) \text{ x } NaOH \text{ x } 14 \text{ x } 6.25}{X} \text{ x } 100\% \quad (6)$$

Where: X: Test sample weight; Y: Blank; Z: Titrated volume of NaOH

RESULTS AND DISCUSSION

Decomposition rate of *A. marina* leaf litter at various salinity levels

The dry weight of *A. marina* leaf litter that was subjected to a long period of decomposition at various levels of salinity is presented in Figure 2.

The smallest residual dry weight was 2.26 g, obtained at a salinity level of 21-30 ppt. The percentage of remaining *A. marina* leaf litter that had experienced decomposition for 15 to 120 days at various levels of salinity is presented in Figure 3.

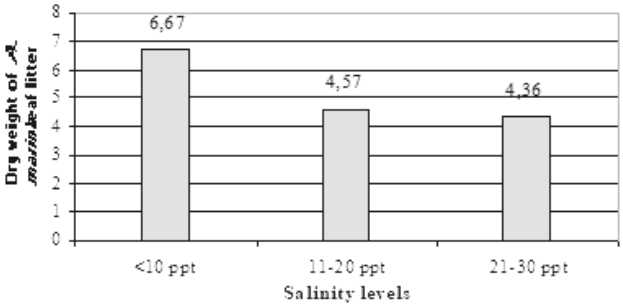

Figure 2. Dry weight of *A. marina* leaf litter decomposed for 120 days in environments with various salinity levels

Figure 3 shows that the large loss of dry weight occurred after litter experienced decomposition for 15, 30, 45, and 60 days. The loss of dry litter weight was greater than those that experienced decomposition for 105 and 120 days. In general, dry weight loss of *A. marina* leaf litter decomposed at all salinity levels showed the same pattern. The average decomposition rate and duration of leaf litter found in environments with various levels of salinity are presented in Table 1. Based on the results, the highest decomposition rate occurred at a salinity level of 21-30 ppt with k value of 6.8/year and length of litter period at 0.15 years.

The litter decomposition rate was assessed based on the reduction in dry weight for 15 to 120 days at all salinity levels, attributed to physical processes in the form of large litter destruction. Besides, the large reduction in dry weight was also caused by other species of organisms living in the location. The organism contributing to the decomposition process of *A. marina* leaf litter included worms (*Nereis* sp.) (Figure 3), found after 45 days at all locations with various salinity levels. At salinity levels of <10 ppt, 10-20 ppt, and 20-30 ppt, the average number of worms found was 13, 4, and 15, respectively. These worms can exist for 15-30 days after decomposition, this may be due to their relatively large size (5 to 6 cm long with a body diameter of 3 to 4 mm). *Nereis* sp worms are found more abundant at a salinity level of 21-30 ppt, and the lower the salinity, the smaller the number of *Nereis* sp worms (Yunasfi et al. 2023a)

In *A. marina* leaf litter which had experienced decomposition for 60 days at all salinity levels, no worms were found. According to a previous report, the process of decomposing litter can be accelerated by adjusting the particle size (Goodman et al. 2019). The distribution and size of litter particles also influence the growth area for decomposing microorganisms and maintaining sufficient porosity for the aeration process (Du et al. 2020). The larger the size of litter particles, the more difficult for microorganisms to reach the center, causing slow decomposition. As stated by Cristiano and Sabatino (2023), the speed of decomposition is influenced by the rate at which litter is fragmented. This breakdown is mostly carried out by many soil animals such as snails, worms, insect larvae, and others. Furthermore, litter quality also plays a crucial role in the decomposition process (García-Palacios et al. 2013; Zhou et al. 2015; Maeda 2016). The litter decomposition rate in river flows is positively related to water temperature (Tiegs et al. 2019; Amani et al. 2019) because the temperature often stimulates fungi activity and is driven by environmental factors, including water chemistry and the quality of litter organic matter, season, and tidal zone (Fernandes 2014; Kristensen et al. 2017). Decomposition continues with a biological process carried out by bacteria and fungi as decomposers to break down organic particles by releasing enzymes to form protein. The products are not only used as a source of nutrients for plants but are also an important source of food for fish and invertebrates (Sari et al. 2014).

Apart from worms, another type of organism found was snails, detected only in litter that had decomposed for 75 to 120 days at a salinity level of 21-30 ppt (Figure 3). These snails also play a role in the decomposition process of *A. marina* leaf litter. The results showed that the remaining leaf litter at a salinity level of 21-30 ppt was less than those at <10 ppt and 11-20 ppt. At salinity level of 21-30 ppt, large snails were found in leaf litter that had experienced decomposition for 75, 90, 105, and 120 days, respectively, with an average number of 7, 29, 52, and 92 snails. In leaf litter that experienced decomposition at salinity levels of <10 ppt and 11-20 ppt, snails with smaller bodies were also found (Figure 4).

Banerjee et al. (2016) reported that C nutrient content decreased with increasing length of decomposition period. In *A. marina* leaf litter decomposed at a salinity level of 0-10 ppt in the control, C nutrient content ranged from 48.68 to 32.23% on day 120. N nutrient content increased with the length of the decomposition period, rising from 0.96% to 1.69 in the control on day 120. According to Siska et al. (2016), factors that influence decomposition rate include leaf morphology and anatomy, N elements, the condition of the substrate where plants live, and the physical environment. *A. marina* has thin leaf morphology and litter possesses a higher nitrogen content compared to *R. apiculata*.

Fungal species found in undecomposed *A. marina* leaf litter

In the control, litter was not decomposed in the field, five species of fungi were isolated (Table 2). Based on the results, three species of *Aspergillus* were isolated, namely *Aspergillus* sp. 1 with an average of 2 x 10^2 cfu/mL, *Aspergillus* sp. 2 with 6.33 x 10^2 cfu/mL, and *Aspergillus* sp. 3 with an average of 1.33 x 10^2 cfu/mL. *Curvularia lunata and Fusarium* sp. 1 were found in litter with an average colony number of 1.67 x 10^2 cfu/mL, and 5.67 x 10^2 cfu/mL, respectively.

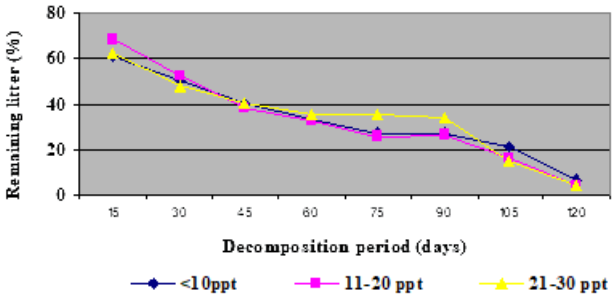

Figure 3. The percentage of remaining *A. marina* leaf litter decomposed for 15 to 120 days in environments with various salinity levels

Table 1. Average decomposition rate and litter length with various levels of salinity

Salinity level	k (year^{-1})	Length of litter period (year)
<10 ppt	4.55	0.22
11-20 ppt	5.91	0.17
21-30 ppt	6.90	0.15

Fungal species found in decomposed *A. marina* leaf litter at a salinity level of <10 ppt

In *A. marina* leaf litter decomposed at a salinity level of <10 ppt, a total of 15 fungi species were found. The amount of each species present varied from one time to the next, with only two species found in almost every decomposition period, namely *Aspergillus* sp. 2 except for day 15 and *Aspergillus* sp. 1, except for day 30, 45, 75, and 90 days. The most frequently found fungi was *Aspergillus* sp. 2, at 5.79 x 10^2 cfu/mL.

Aspergillus sp. 4 was the second most numerous fungi, with an average number of 2.91 x 10^2 cfu/mL. Litter colonization by this species occurred after decomposition for 30, 75, and 90 days, while the third most common fungi colony found was *Aspergillus* sp. 1 with an average amount of 2.64 x 10^2 cfu/mL. This species colonized litter that had decomposed for 15, 60, 105, and 120 days. *Trichoderma* sp. 1 colonized litter that decomposition for 105 days and the number of colonies successfully isolated was 2.03 x 10^2 cfu/mL.

Fusarium sp. 2 also colonized *A. marina* leaf litter which experienced decomposition in an environment with a salinity level of <10 ppt, while the number of colonies averaged 0.76 x 10^2 cfu/mL. *Penicillium* sp. 1 was successfully isolated on average 0.46 x 10^2 cfu/mL in litter decomposited for 60 days. *Trichoderma* sp. 2 and *Aspergillus* sp. 3 were isolated with an average colony number of 0.27 x 10^2 cfu/mL. Furthermore, *Trichoderma* sp. 2 was obtained in leaf litter subjected to decomposition for 45 and 120 days. Other species of fungi, found once out of eleven observations were as follows: (i) *Penicillium* sp. 2, (ii) *Aspergillus* sp. 5, (iii) *Fusarium* sp. 3, (iv) *Penicillium* sp. 3, (v) *Penicillium* sp. 4, (vi) *Curvularia lunata*, and (vii) *Trichoderma* sp.3. According to Yunasfi et al. (2021), the fungal species found in salinity levels 0-10 ppt were similar to those in Desa Nelayan Belawan, namely *Aspergillus* sp. 1, *Aspergillus* sp. 2, *Aspergillus* sp. 1, and *Penicillium* sp. The details of average number of colonies for each species can be seen in Table 3.

Fungal species found in decomposed *A. marina* leaf litter at a salinity level of 11-20 ppt

The fungal species that mostly found in decomposed *A. marina* leaf litter was *Aspergillus* sp. 2 with an average colony number of 2.0 x 10^2 cfu/mL. *Aspergillus* sp. 2 was found in leaf litter that had decomposed for 15, 45, 60, 105, and 120 days but not on days 30, 75, and 90. The highest number of colonies successfully isolated was recorded in litter that was decomposed for 15 days with average number of 8 x 10^2 cfu/mL.

Table 2. Average number of colonies for each fungal species in *A. marina* leaf litter (control)

Fungal species	Average number of colonies x 10^2 (cfu/mL)
Aspergillus sp. 1	2
Aspergillus sp. 2	6.33
Aspergillus sp. 3	1.33
Curvularia lunata	1.67
Fusarium sp. 1	5.67
Number of colonies	17.33

Table 3. Average number of colonies for each fungal species in *A. marina* leaf litter

Fungal species	Average number of colonies x 10^2 (cfu/mL)
Aspergillus sp. 1	2.64
Aspergillus sp. 2	5.79
Aspergillus sp. 3	0.27
Aspergillus sp. 4	2.91
Aspergillus sp. 5	0.27
Penicillium sp. 1	0.46
Penicillium sp. 2	0.27
Penicillium sp. 3	0.06
Fusarium sp. 1	0.06
Fusarium sp. 2	0.76
Fusarium sp. 3	0.12
Trichoderma sp. 1	2.03
Trichoderma sp. 2	0.27
Trichoderma sp. 3	0.03
Curvularia lunata	0.03
Number of colonies	15.97

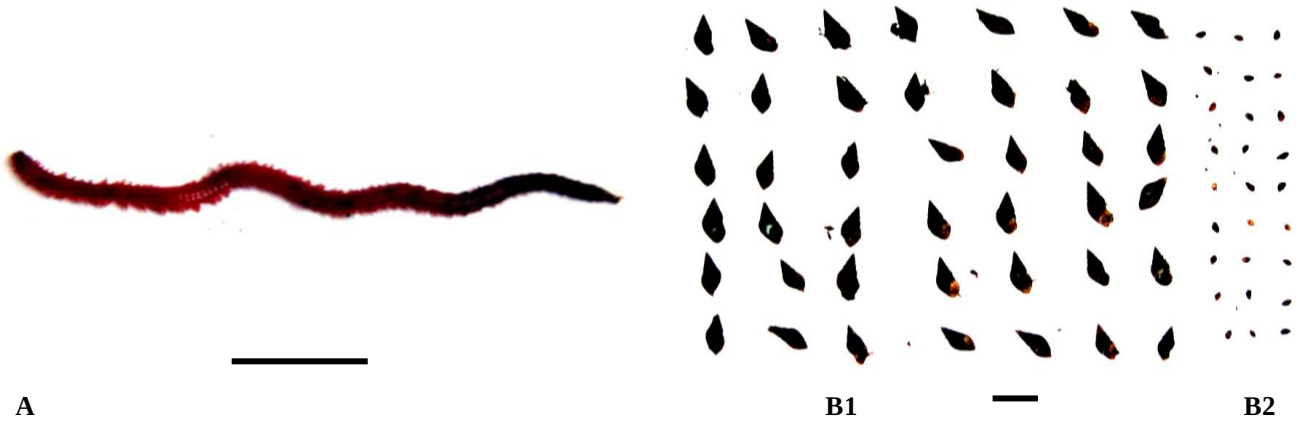

Figure 4. Worms (*Nereis* sp.) found in *A. marina* leaf litter decomposed for 45 days at salinity levels of <10 ppt, 10-20 ppt, and 20-30 ppt (A), and (B1) large snails found in the litter decomposed leaf of *A. marina (*B2) small snails. Bar = 1 cm

Aspergillus sp. 4 was found in litter that was decomposed for 30, 75, 90, and 120 days with average colony number of 1.76 x 10^2 cfu/mL, ranking as the second largest after *Aspergillus* sp. 2. *Aspergillus* sp. 3 and *Penicillium* sp. 1 occupied the third position with average colony number of 0.46 x 10^2 cfu/mL for each species, while *Penicillium* sp. 1 was successfully isolated from litter that had decomposed for 60 days. Furthermore, the fungal species obtained from litter decomposed at a salinity level of 11-20 ppt, was *Aspergillus* sp. 1 which was isolated for 15, 60, 90, and 105 days with a colony count of 0.39 x 10^2 cfu/mL.

Aspergillus sp. 5 was also obtained from leaf litter with an average number of 0.27 x 10^2 cfu/mL colonies for 45, 105, and 120 days of decomposition. Other species of fungi that were isolated at salinity level of 11-20 ppt were as follows: i) *Trichoderma* sp. 1 with an average number of colonies 0.18 x 10^2 cfu/mL, ii) *Trichoderma* sp. 4 with an average of 0.12 x 10^2 cfu/mL, iii) *Fusarium* sp. 2 with colonies averaging 0.09 x 10^2 cfu/mL isolated after decomposition for 75 days, v) *Penicillium* sp. 3 and vi) *Penicillium* sp. 2. The fungal species found at salinity levels of 11-20 ppt were *Aspergillus* sp. 1, *Penicillium* sp. 3, *Aspergillus* sp. 2, *Aspergillus* sp. 4, *Aspergillus* sp. 3, and *Fusarium* sp. 2 (Yunasfi et al. 2023c). The details of average number of colonies for each species can be seen in Table 4.

Fungal species found in decomposed *A. marina* leaf litter at a salinity level of 21-30 ppt

From *A. marina* leaf litter decomposed at a salinity level of 21-30 ppt, a total of 12 fungal species were isolated. *Aspergillus* sp. 4 was found in leaf litter that was decomposed for 30, 75, 105, 135, and 120 days with an average colony number of 1.15 x 10^2 cfu/mL. *Trichoderma* sp. 4 ranked third with an average colony number of 0.73 x 10^2 cfu/mL and the species was successfully isolated after decomposition for 30, 45, and 105 days. *Aspergillus* sp. 3 was also obtained averaging a colony of 0.64 x 10^2 cfu/mL, with a constant presence in decomposition, except after 60 and 120 days. At salinity level of 21-30 ppt, *Aspergillus* sp. 1 was found with the number of colonies being 0.46 x 10^2 cfu/mL, and this species was discovered 15 days after the decomposition period. In addition, *Penicillium* sp. 1 was successfully isolated from litter after 60 and 90 days of decomposition with an average colony number of 0.36 x 10^2 cfu/mL.

A total of 12 fungal species, namely *Aspergillus* sp. 5, *Penicillium* sp. 3, *Trichoderma* sp. 1, *Fusarium* sp. 3, *Aspergillus* sp. 7, and *Aspergillus* sp. 6 were found in leaf litter decomposed at salinity level of 21-30 ppt. *Aspergillus* sp. 5 was isolated from litter that had experienced decomposition for 120 days with an average colony number of 0.27 x 10^2 cfu/mL. The colony of *Penicillium* sp. 3 was successfully isolated reaching 0.06 x 10^2 cfu/mL, while *Trichoderma* sp. 1 was obtained with an average colony of 0.06 x 10^2/mL. *Fusarium* sp. 3 with 0.03 x 10^2/mL was isolated after the litter was decomposed for 120 days. *Aspergillus* sp. 7 with 0.03 x 10^2/mL colony was isolated from litter after 75 days of decomposition, while *Aspergillus* sp. 6 was successfully obtained after 60 days. According to Yunasfi et al. (2019; 2023b), 6 different species of fungi were decomposed at a salinity level of 21-30 ppt, namely *Aspergillus* sp. 1, *Aspergillus* sp. 2, *Aspergillus* sp. 3, *Trichoderma sp.*, *Aspergillus* sp. 4, *Penicillium* sp. 1, *Aspergillus* sp. 6, and *Penicillium* sp. 4. The details of average number of colonies for each species can be seen in Table 5.

Fungal species diversity index in *A. marina* leaf litter

The average value of the Shannon Index for the diversity of fungal species found in decomposed *A. marina* leaf litter at various salinity levels ranged from low to medium. Diversity index at salinity levels of < 10 ppt, 11-20 ppt, and 21-30 ppt were 1.96, 1.86 and 1.95, respectively. These values were greater compared to the species diversity index of the control, namely 1.26.

The largest number of fungal species found at a salinity level of < 10 ppt was because the condition was almost the same as the fresh (brackish) environment which was quite good for the growth and development of various types of fungi compared to higher salinity levels. This was attributed to the influence of river flows near the study location.

Table 4. Average number of colonies for each fungal species in *A. marina* leaf litter

Fungal species	Average number of colonies x 10^2 (cfu/mL)
Aspergillus sp. 1	0.39
Aspergillus sp. 2	2.0
Aspergillus sp. 3	0.46
Aspergillus sp. 4	1.76
Aspergillus sp. 5	0.27
Penicillium sp. 1	0.46
Penicillium sp. 2	0.03
Penicillium sp. 3	0.03
Fusarium sp. 2	0.09
Trichoderma sp. 1	0.18
Trichoderma sp. 4	0.12
Number of colonies	5.79

Table 5. Average number of colonies for each fungal species in *A. marina* leaf litter

Fungal species	Average number of colonies x 10^2 (cfu/mL)
Aspergillus sp. 1	0.46
Aspergillus sp. 2	1.52
Aspergillus sp. 3	0.64
Aspergillus sp. 4	1.15
Aspergillus sp. 5	0.27
Aspergillus sp. 6	0.03
Aspergillus sp. 7	0.03
Penicillium sp. 1	0.36
Penicillium sp. 3	0.06
Fusarium sp. 3	0.03
Trichoderma sp. 1	0.06
Trichoderma sp. 4	0.73
Number of colonies	5.34

Fungal propagules originating from leached land in the form of spores, hyphae, conidia, and others are carried by river flows, hence, during high tide, many propagules may be carried to the location and stick to *A. marina* leaf litter. Different soil conditions, tillage, river water depth, plant species, and environmental stress, have a significant impact on fungal diversity and biological functions (Giard-Laliberté et al. 2019; Vanegas et al. 2019; Zhang et al. 2019).

The diversity of fungal species in decomposed *A. marina* leaf litter at all salinity levels showed significant differences. Although the largest number of fungal species and populations was found at a salinity level of <10 ppt, this did not apply to species diversity. According to Begum et al. (2013); Lange et al. 2015; Peralta et al. 2018; Lazarova et al. 2021), factors influencing population density and species diversity of soil organisms include oxygen supply, humidity, soil temperature, nutrient content, and the amount of soil organic matter. For fungal communities, the decomposition process of leaf litter generally occurs in three different phases namely initial, intermediate, and decomposition (Baldrian 2012; Farooqui et al. 2014; Adrianto et al. 2015; Moitinho 2018).

Total carbohydrate content of *A. marina* leaf litter

The average total carbohydrate content found in decomposed *A. marina* leaf litter at various levels of salinity is presented in Figure 4. There were differences between carbohydrate content at a salinity level of <10 ppt, 11-20 ppt, and 21-30 ppt. At a salinity level of <10 ppt, the highest carbohydrate content in leaf litter was 12.10%. For leaf litter decomposed at 11-20 ppt and 21-30 ppt, carbohydrate content was lower with an average of 11.32% and 10.33%, respectively. The details of carbohydrate content is presented in Figure 5.

Figure 6 shows the pattern of changes in carbohydrate content, starting from litter decomposition for 15, 45, 75, and 105 days. Changes in the total carbohydrate content of leaf litter at the three levels of salinity showed the same pattern. In this case, there was an increase in the total carbohydrate content after decomposition at 15 and 45 days, while content after decomposition at 45, 75, and 105 days showed a decreasing pattern. Despite the increased percentage of total carbohydrate content in leaf litter decomposed for 15 to 105 days, the absolute value in litter decreased.

Protein content of *A. marina* leaf litter

The average protein content of decomposed *A. marina* leaf litter at various salinity levels is presented in Figure 6. The highest (9.12%) protein content, was found after decomposition at a salinity level of <10 ppt, while the lowest (8.28%) was found at 20-30 ppt. Based on the results, the longer the decomposition period at various salinity levels, the greater the protein level. The details of protein content is presented in Figure 7.

The pattern of changes in protein content at various lengths of decomposition is presented in Figure 8. Based on the results, protein content of *A. marina* leaf litter that experienced decomposition in the field for 75 days increased compared to the control. Although an increase was observed in the percentage of protein content in leaf litter decomposed for 15 to 105 days, the absolute value showed a decrease. The high carbohydrate content at a salinity level of <10 ppt was attributed to the C nutrient which was also higher at this level compared to others. C nutrient plays a crucial role in the formation of carbohydrates together with H and O.

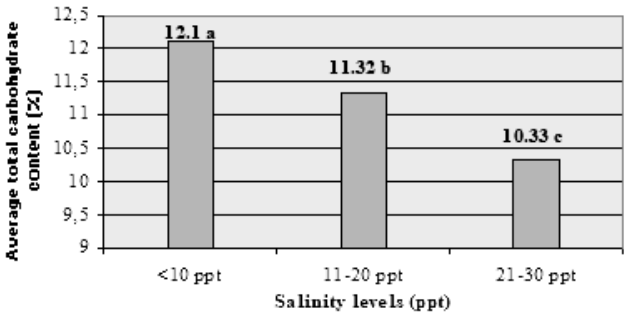

Figure 5. Average of total carbohydrate content of decomposed *A. marina* leaf litter at various salinity levels

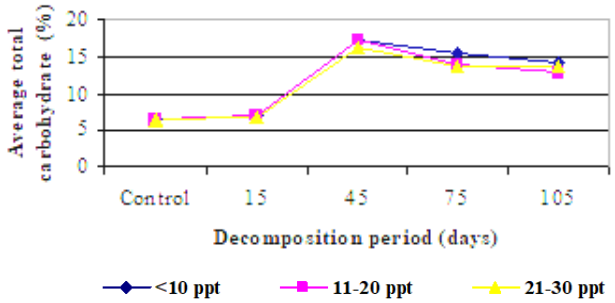

Figure 6. Average carbohydrate content of decomposed *A. marina* leaf litter at various salinity levels

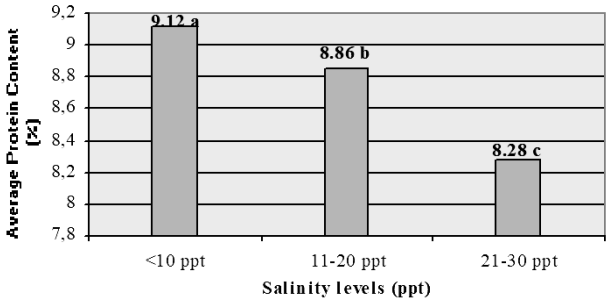

Figure 7. Average protein content of decomposed *A.marina* leaf litter at various salinity levels (after decomposition)

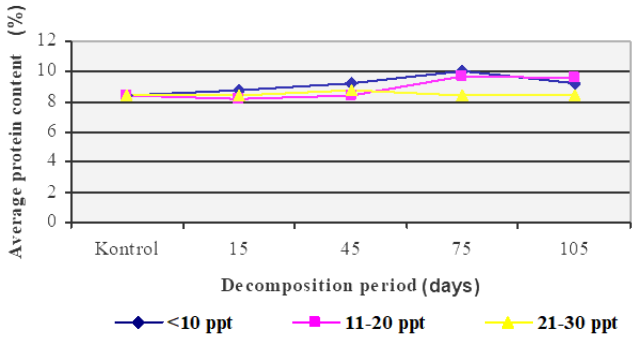

Figure 8. Average protein content of *A. marina* leaf litter over several decomposition periods at various salinity levels

Based on the length of the decomposition period for *A. marina* leaf litter, carbohydrate content increased until the 45th day and then decreased on the 75th and 105th days. Theoretically, the levels of carbohydrates in decomposed litter decrease with a longer decomposition period. In this study, carbohydrate content found in *A. marina* leaf litter was greater than the control. This was attributed to the shorter observation period, reaching up to the 105th day. However, the absolute value of carbohydrate content in leaf litter decomposed at various levels of salinity and length of decomposition showed a decrease.

The highest protein content was found in decomposed *A. marina* leaf litter at a salinity level of <10 ppt. This was attributed to N nutrient levels which increased with a longer litter decomposition period. According to previous studies, N is a good predictor of litter decomposition rate in many ecosystems (Liu et al. 2016; Suseela and Tharayil 2017; Wowor et al. 2019). Microbes influence the formation of organic matter through the transformation of plant residues by extracellular enzymes and the use of organic substrates through cell absorption. Insoluble structural carbohydrates and other complex biopolymers can be attacked by microbial extracellular enzymes (Zimmer 2019). Microbes convert detrital organic matter into biomass in a process called immobilization, increasing amino acids and proteins (Pradisty 2021).

In conclusion, the highest decomposition rate was found at a salinity level of 21-30 ppt, as evidenced by the dry weight of the remaining leaf litter left in the bag, namely 4.36 g. This decomposition rate was smaller compared to the value for litter placed at salinity levels of < 10 ppt and 10-20 ppt, yielding 4.55 and 5.91, respectively, with residence times of 0.22 yr, 0.17 yr, and 0.15. In the control, five fungal species were found, while in leaf litter placed at salinities <10 ppt, 11-20 ppt, and 21-30 ppt, 15, 11, and 12 species were found. The dominant fungal species was *Aspergillus* sp. with colony numbers of 61.67 x 10^2, 28.99 x 10,^{2} and 20.67 x 10^2 at salinity levels of <10 ppt, 11-20 ppt, and 21-30 ppt, respectively.

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