Volume 17, Issue 2, December 2024

p-ISSN 2086-3586
e-ISSN 2460-8483

Biota

Biologi dan Pendidikan Biologi

UNIVERSITAS ISLAM NEGERI MATARAM

Volume 17, Issue 2, December 2024

p-ISSN 2086-3586
e-ISSN 2460-8483

Biota
Biologi dan Pendidikan Biologi

Front cover: Betta Fish (Betta sp.)
(Photo: Thang Cao)
www.pexels.com

Published biannually

June and December

UNIVERSITAS ISLAM NEGERI MATARAM

Volume 17, Issue 2, December 2024

p-ISSN 2086-3586
e-ISSN 2460-8483

Biota
Biologi dan Pendidikan Biologi

Biota: Biologi dan Pendidikan Biologi, a peer reviewed and open access journal published by Universitas Islam Negeri Mataram. It is published half-yearly (June and December) with the objectives to explore and develop the knowledge and technology in bioscience and biology education. Biota receives research articles from all area of science and education in biological fields in order to keep researchers and practitioners informed on current issues as well as to provide information sharing and ideas of research and development in fields of biology.

Volume 17, Issue 2, December 2024
p-ISSN 2086-3586
e-ISSN 2460-8483

Biota
Biologi dan Pendidikan Biologi

Table of Contents

Biota
Biologi dan Pendidikan Biologi

http://biota.ac.id/index.php/jb
DOI: http://dx.doi.org/10.20414/jb.v17i2.508

Research Article

Histopathological Effects of Mangosteen (*Garcinia mangostana* L.) Peel Decoction on Betta Fish (*Betta* sp.) Liver

Wiwin Ariesti[1], Siti Aeniah[1], Shuha Ma'muriyah Halim[1], Fajar Sofyantoro[1], Nastiti Wijayanti[1], Bambang Retnoaji[1], Ardaning Nuriliani[1], Hendry T.S.S.G. Saragih[1], Zuliyati Rohmah[1], Slamet Widiyanto[1], Nur Ainun Oktavia Pusparini[1], Desi Eka Putri Empra[1], Nur Indah Septriani[1]*

[1]*Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia*

**Corresponding author: nurindahseptriani@ugm.ac.id*

Abstract

Mangosteen (*Garcinia mangostana* L.) peel contains bioactive compounds known for their health benefits, yet potential toxicity at certain doses remains a concern. This study evaluates the histopathological effects of mangosteen peel decoction on the liver of Betta fish (*Betta* sp.), a sensitive model organism. Mangosteen peel decoction was prepared and administered to Betta fish at concentrations of 5, 25, and 50 ppm, with a control group receiving no treatment. Fish were observed for changes in swimming activity and appetite over five days. Liver tissues were collected, processed, and analyzed histologically to assess tissue damage including vacuolization, pyknosis, hemorrhage, and necrosis. Data were analyzed using the Kruskal-Wallis and Mann-Whitney tests. Behavioral analysis indicated a dose-dependent reduction in swimming activity and appetite in treated groups. Histopathological examination revealed significant liver damage across all treatment groups, with higher concentrations of decoction correlating with increased hemorrhage, pyknosis, and necrosis. Vacuolization was highest in the control group and lowest in the 50-ppm group. The overall hepatic damage was categorized as moderate, with the control group showing the least damage. Mangosteen peel decoction induced significant hepatic damage in Betta fish, highlighting the cytotoxic effects at higher doses. The observed behavioral and histopathological changes underscore the need for careful consideration of decoction concentrations to avoid adverse effects in aquatic organisms. This study provides crucial insights into the toxicological impacts of mangosteen peel decoction on fish liver health, emphasizing the importance of dose regulation in practical applications. Further research is recommended to explore protective measures and alternative treatments to mitigate liver damage.

Keywords: Betta Fish, Hepatotoxicity, Mangosteen Peel, Necrosis, Pyknosis, Vacuolization

1. Introduction

Mangosteen (*Garcinia mangostana* L.) peel is renowned for its rich content of bioactive compounds, including xanthones, tannins, polyphenols, and flavonoids, which exhibit various health benefits such as antioxidant, anti-inflammatory, and antimicrobial activities (Ibrahim et al., 2016; Obolskiy et al., 2009; Saraswathy et al., 2022; Yuvanatemiya et al., 2022). While these compounds have been incorporated into numerous health products, it is crucial to assess their potential toxicity to ensure their long-term safety. The active compounds in mangosteen peel may elicit side effects at certain doses, necessitating thorough toxicity evaluations (Ibrahim et al., 2016; Saraswathy et al., 2022).

Toxicity testing is a fundamental scientific method employed to ascertain the potential hazards of compounds to living organisms (Aware et al., 2022; Capela et al., 2020; Krewski et al., 2020). In this study, we utilized Betta fish (*Betta* sp.) as an animal model to investigate the toxicity of mangosteen peel extract. Betta fish were chosen due

to their high sensitivity and ease of acclimatization to laboratory conditions (Palmiotti et al., 2023; Yue et al., 2022; Zhang et al., 2022). Similar to zebrafish, which is widely used as a model organism for toxin studies (Sofyantoro et al., 2024), Betta fish offer comparable sensitivity, making them suitable for assessing the impacts of various compounds, including herbal extracts.

Betta fish (*Betta* sp.) are freshwater species native to several Southeast Asian countries, including Indonesia, Thailand, Malaysia, Brunei Darussalam, Singapore, and Vietnam (Yue et al., 2022). These fish are characterized by their distinctive morphology and aggressive territorial behavior (Lichak et al., 2022; Oliveira et al., 2022). Betta fish typically reach sexual maturity and are ready to reproduce between 4 to 12 months of age. Natural spawning occurs primarily during the dry season due to gonadal maturity levels, particularly in males (Lichak et al., 2022; Yue et al., 2022). Optimal spawning temperatures range from 26-30°C, with warmer temperatures promoting spawning activity. Additionally, spawning can result in female mortality due to male aggression if the males are not adequately prepared for reproduction (Lichak et al., 2022; Oliveira et al., 2022).

The liver is a critical organ involved in drug and toxin metabolism, making it highly susceptible to toxic compounds (Hosack et al., 2023; Michalopoulos & Bhushan, 2021). The liver metabolic processes often produce reactive metabolites that can inflict cellular damage (Leung et al., 2012; Simeonova et al., 2014), emphasizing the importance of assessing the effects of mangosteen peel extract on this organ. The objective of this study is to examine the impact of mangosteen peel decoction on the histological structure of the liver in Betta fish, focusing on the extent of damage and histopathological alterations. The findings from this study will provide valuable insights into the safety of long-term mangosteen peel extract usage.

2. Material and Method

Preparation of Mangosteen (*G. mangostana* L.) Peel Decoction

Mangosteen peel symplisia (10 g) was combined with 1000 mL of distilled water and heated to 90°C. After cooling, the decoction was transferred to a container and subsequently diluted to concentrations of 5, 25, and 50 ppm as administered to *Betta* sp.

Animal Acclimatization

Male *Betta* sp., approximately 4-5 months old and weighing approximately 1.51 g of Blitar origin, were acclimatized for 3 days in an aquarium. The fish were divided into four experimental groups: control, 5 ppm, 25 ppm, and 50 ppm. *Betta* sp. were exposed to a light/dark cycle of 12 hours each and were fed once daily. Behavioral observations, including swimming activity and appetite, were categorized into four levels: very active, active, less active, and inactive.

Histological Preparation of Liver

Betta sp. specimens were captured using a net, transferred to plastic containers, and then anesthetized through cold shock by placing them in a freezer at -20°C for 10-15 minutes. The heads and tails of *Betta* sp. were removed, and the remaining body parts were prepared for histological analysis. The hepatic tissues were excised, washed with physiological saline (0.9% NaCl), and fixed in neutral buffered formalin (NBF) for 24 hours. After fixation, the tissues were washed with 70% ethanol until the yellow discoloration was removed, with solution changes every 30 minutes. Dehydration was achieved using a graded series of alcohol solutions: 70, 80, 90, 96%, and absolute. Clearing was performed with toluene, followed by infiltration in paraffin at 65°C, embedding, trimming, and sectioning. Liver histological sections were prepared at a thickness of 5 µm and stained using the Harris Hematoxylin-Eosin method.

Histopathological Examination

Histopathological sections were examined using a Leica light microscope with a 40x10 magnification. Observations were made in four different fields of view per treatment group. The observed pathological changes included hemorrhage, pyknosis, necrosis, and vacuolization. Hepatocyte cell counts were conducted using Image Raster version 3.0. The extent of liver damage was assessed according to the criteria outlined in Table 1.

Table 1. Scoring values of liver damage for histopathological observations (Gibson-Corley, Olivier, & Meyerholz, 2013)

Level of Damage	Description	Score
Normal	normal, clear cell nuclei, round shape	0
Mild	hemorrhage+, pyknosis+, necrosis+, vacuolization+	1
Moderate	hemorrhage ++, pyknosis ++, necrosis ++, vacuolization++	2
Severe	hemorrhage +++, pyknosis +++, necrosis +++, vacuolization +++	3

Description:

"–" : Normal

"+" : Cell damage reaches 25% in five fields of view

"++" : Cell damage reaches 50% in five fields of view

"+++" : Cell damage reaches 75% in five fields of view

Data Analysis

Statistical analysis was performed using the Kruskal-Wallis's test. In cases where significant differences were identified ($p < 0.05$), post hoc analysis was conducted using the Mann-Whitney test to determine significant differences between treatment groups.

3. Results and Discussion

3.1. Results

Behavior of *Betta* sp.

A reduction in both swimming activity and appetite in Betta fish following treatment with mangosteen peel decoction was observed (Table 2). The behavior of Betta fish was noticeably altered following treatment with mangosteen peel decoction. In the control group, Betta fish maintained high swimming activity and appetite throughout the observation period. Conversely, Betta fish treated with mangosteen peel decoction exhibited a dose-dependent decline in both swimming activity and appetite**.**

Table 2. Swimming and feeding behavior of betta fish during treatment

Treatment	Swimming activity (day)					Appetite (day)				
	1	2	3	4	5	1	2	3	4	5
Control	active	very active	active	active	very active	high	high	high	high	high
5 ppm	active	normal	active	less active	less active	high	high	high	moderate	moderate
25 ppm	active	less active	less active	less active	less active	high	moderate	moderate	low	low
50 ppm	active	less active	less active	inactive	inactive	high	moderate	moderate	low	low

Specifically, swimming activity in the control group was consistently high, with fish remaining very active or active throughout the five-day observation period. In the 5-ppm group, the fish were active initially but became less active on days 4 and 5. In the 25-ppm group, there was a reduction in activity from day 2 onwards, with fish becoming progressively less active. The 50-ppm group exhibited decreased activity from day 2, with inactivity observed on days 4 and 5. Similarly, the appetite of Betta fish followed a declining trend with increasing concentrations of the decoction. The control group consistently displayed a high appetite. The 5-ppm group maintained a high appetite initially but showed a moderate reduction on days 4 and 5. The 25-ppm group had a high appetite on day 1, which declined to medium on days 2 and 3 and further reduced to low on days 4 and 5. The 50-ppm group exhibited a high appetite on day 1, which dropped to medium on days 2 and 3 and further to low on days 4 and 5.

Histopathological Analysis of *Betta* sp. Liver

Histological examination of Betta fish liver tissue revealed varying degrees of damage across all treatment groups, including the control, 5 ppm, 25 ppm, and 50 ppm. Notable damage observed included vacuolization, pyknosis, hemorrhage, and necrosis, as detailed in Table 3 and illustrated in Figure 1.

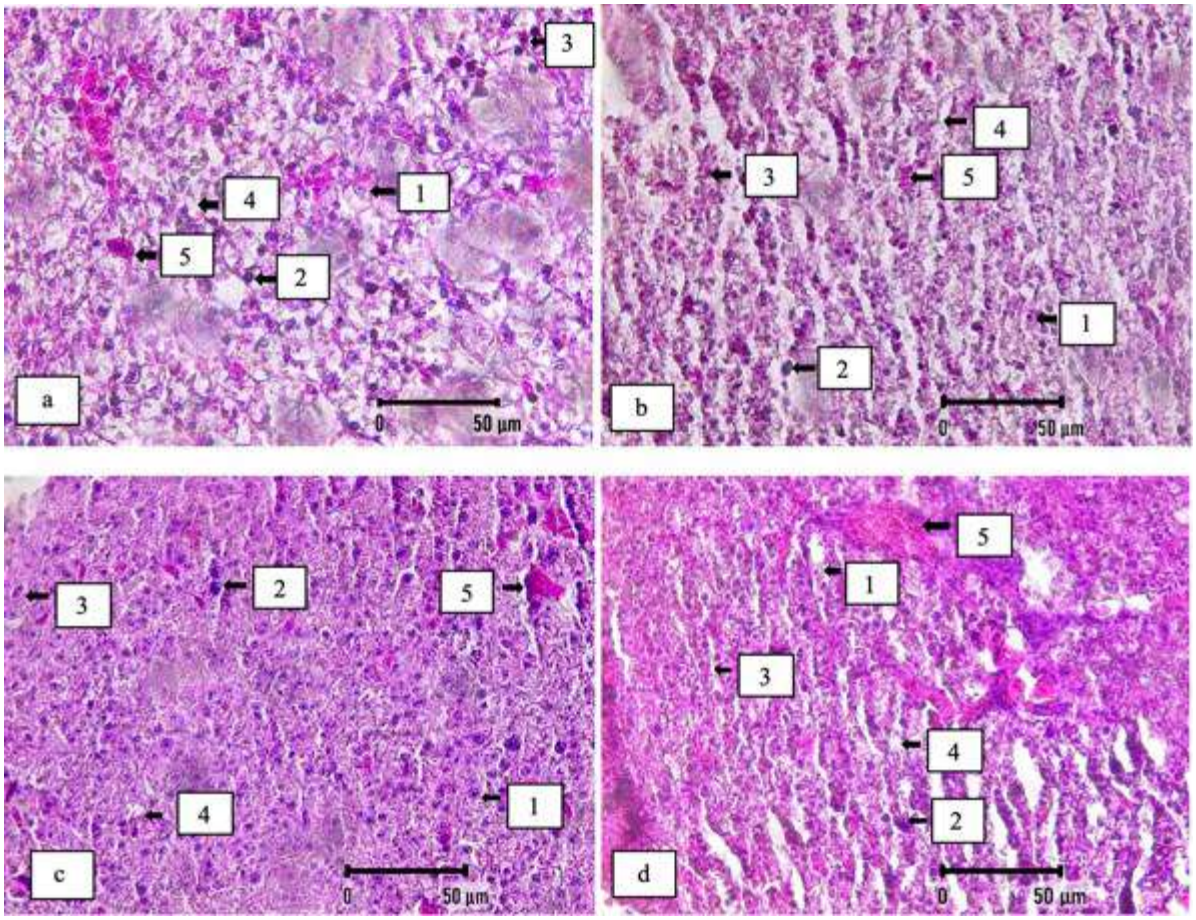


Figure 1. Histological structure of Betta fish liver of control group (a), 5 ppm (b), 25 ppm (c), 50 ppm (d). Normal hepatocytes (1), pyknosis (2), necrosis (3), vacuolization (4), and hemorrhage (5). Hematoxylin-Eosin staining. Scale bar = 50µm.

Figure 1 shows the histological structure of Betta fish liver tissue stained with Hematoxylin-Eosin. In the control group (Figure 1a), the liver tissue exhibited mostly normal hepatocytes with minimal signs of damage. Some vacuolization (4) and pyknosis (2) were observed, but the overall tissue structure remained relatively intact. In the 5-ppm treatment group (Figure 1b), the liver tissue showed increased vacuolization and moderate pyknosis, with some areas of necrosis (3) beginning to appear. Hemorrhage (5) was present but not extensive. The 25-ppm treatment group (Figure 1c) exhibited more pronounced damage. There was a noticeable increase in necrosis and pyknosis, with significant vacuolization and hemorrhage. The liver tissue structure was more disrupted compared to the control and 5 ppm groups. In the 50-ppm treatment group (Figure 1d), the liver tissue showed the most severe damage. Extensive areas of necrosis and pyknosis were observed, with considerable vacuolization and hemorrhage. The hepatocytes were significantly damaged, and the overall tissue architecture was highly compromised.

Table 3. Liver damage in Betta fish based on scoring value

Treatment	Hemorrhage	Pyknosis	Necrosis	Vacuolization	Damage level
Control	0.42 ± 0.15^{a}	1.33 ± 0.14^{a}	1.00 ± 0.00^{a}	2.42 ± 0.15^{a}	Moderate
5 ppm	0.50 ± 0.19^{a}	1.50 ± 0.19^{a}	1.00 ± 0.00^{a}	2.12 ± 0.30^{ab}	Moderate
25 ppm	0.92 ± 0.08^{b}	1.50 ± 0.29^{a}	1.37 ± 0.19^{a}	1.67 ± 0.22^{b}	Moderate
50 ppm	0.83 ± 0.11^{b}	3.00 ± 0.00^{b}	2.80 ± 0.08^{b}	1.00 ± 0.00^{c}	Moderate

Note: Identical letters indicate no significant difference between treatments.

Table 3 presents the scoring values of liver damage in Betta fish across different treatment groups. Hemorrhage, pyknosis, necrosis, and vacuolization were quantified to assess the level of hepatic damage. The analysis revealed that the mangosteen peel decoction exhibited a damaging effect on Betta fish hepatocytes. The Kruskal-Wallis's test revealed significant differences in hepatic damage across treatments, particularly in hemorrhage, pyknosis, necrosis, and vacuolization. Hemorrhage was significantly lower in the control group compared to 25-ppm and 50-ppm groups. Pyknosis was significantly higher in the 50-ppm group compared to other groups. Although no significant differences were observed in necrosis levels among control, 5-ppm, and 25-ppm groups, the 50-ppm group exhibited a higher level of necrosis. The extent of vacuolization was highest in the control group and lowest in 50-ppm group, with 5-ppm and 25-ppm not differing significantly from each other. Hepatic damage across all treatments was categorized as moderate, with the control group showing the least average hepatic damage.

3.2. Discussion

The application of mangosteen (*G. mangostana* L.) peel decoction in aquaculture, particularly in fish species, has attracted significant scientific attention due to its bioactive compounds and associated health benefits. Mangosteen peel is a rich source of xanthones and flavonoids, which possess anti-inflammatory, antibacterial, and antifungal properties (Setiawan et al., 2023; Widyarman et al., 2019). Studies on the embryological development of wader pari fish (*Rasbora lateristriata*) revealed that exposure to higher concentrations of mangosteen peel decoction negatively impacts hatching rates, survival, and heart morphology, with notable cardiac abnormalities such as edema and bending observed at 25 µg/mL (Khasanah et al., 2024). Research on *R. lateristriata* has highlighted its utility as a model organism for embryological and toxicity studies, further validating the relevance of fish models in assessing environmental and compound-related impacts (Retnoaji, Nurhidayat, et al., 2023). Further research on the influence of mangosteen peel extract on bone structure and behavior in wader fish embryos demonstrated that concentrations up to 5 µg/mL are generally safe, showing no significant morphological or skeletal abnormalities. However, higher concentrations caused developmental malformations such as yolk sac edema and collapsed swim bladder, highlighting a dose-dependent toxicity (Retnoaji, Paramita, et al., 2023). The antibacterial efficacy of mangosteen peel extract has also been evaluated against *Aeromonas hydrophila* in African catfish (*Clarias gariepinus*). Concentrations between 6.25% and 25% were found effective in inhibiting

bacterial growth, establishing the extract's potential as a natural antimicrobial agent in aquaculture (Cahya et al., 2023). Similarly, in Nile tilapia (*Oreochromis niloticus*), the incorporation of mangosteen peel extract in nanoemulsion form at a concentration of 6.25 mg/g in the diet significantly improved growth performance, immune response, and resistance to *Aeromonas veronii*. The nanoemulsion formulation enhanced the bioavailability and efficacy of the extract, underscoring its potential as a sustainable aquaculture supplement (Yostawonkul et al., 2023). Additionally, dietary supplementation with mangosteen extracts in African catfish fingerlings at 0.5% inclusion improved hematological parameters such as red and white blood cell counts without negatively impacting growth. These results support the extract's role in enhancing fish health and resilience under aquaculture conditions (Soosean et al., 2010).

In this study, the behavior of Betta fish (*Betta* sp.) was significantly influenced by treatment with mangosteen peel decoction, as evidenced by observed changes in swimming activity and appetite over a five-day period (Table 2). Swimming activity and appetite are fundamental behavioral parameters commonly used to assess the physiological and health status of fish (Svendsen et al., 2021; Zhao et al., 2017). Changes in these parameters can indicate stress, toxicity, or overall well-being in response to environmental factors or treatments. Swimming activity reflects the energy level, muscular coordination, and nervous system functionality, while appetite serves as an indicator of metabolic and digestive health (Gerry & Ellerby, 2014; Nie et al., 2017). In the control group, the fish exhibited consistent and high levels of swimming activity, maintaining an active status throughout the five days, with particularly vigorous activity on days 2 and 5. This stable and active swimming behavior indicates the normal physiological condition of the Betta fish in the absence of any treatment. In contrast, the swimming activity of the treated groups (5, 25, and 50 ppm) progressively declined with increasing concentrations of mangosteen peel decoction. In 5-ppmgroup, treated with 5 ppm, the fish maintained normal swimming activity for the first three days but showed a marked decrease in activity, becoming less active on days 4 and 5. This indicates an initial resilience to the lower concentration of the decoction, with a delayed onset of activity reduction. The fish in group 25-ppm, exhibited a more pronounced decline in activity, becoming less active from day 2 onwards. By day 5, the fish remained less active, suggesting that this intermediate concentration had a quicker and more sustained impact on reducing swimming activity. The 50-ppm group, treated with the highest concentration of 50 ppm, showed the most significant reduction in activity. The fish became less active by day 2 and were completely inactive by days 4 and 5. This rapid and severe decrease in activity at the highest concentration indicates a strong dose-dependent effect of the mangosteen peel decoction on the swimming behavior of Betta fish.

Appetite levels also showed a decreasing trend across the treated groups in comparison to the control group. The control group maintained a high appetite throughout the five-day period, reflecting healthy feeding behavior under normal conditions. In 5-ppm group, the fish maintained a high appetite for the first three days, but

their appetite decreased to medium levels on days 4 and 5. This suggests a gradual reduction in feeding behavior in response to the 5-ppm concentration of the decoction. The 25-ppm group exhibited a more rapid decline in appetite, with high levels on day 1, decreasing to medium by days 2 and 3, and further reducing to low by days 4 and 5. The intermediate concentration of 25 ppm clearly had a more immediate and substantial impact on feeding behavior compared to the lower concentration. The 50-ppm group showed the most drastic reduction in appetite, with high levels on day 1, dropping to medium on days 2 and 3, and reaching low levels on days 4 and 5. The 50-ppm concentration of the decoction had the strongest effect, mirroring the severe reduction in swimming activity observed in this group.

These findings suggest that mangosteen peel decoction exerts a dose-dependent impact on both the swimming activity and appetite of Betta fish. The consistent reduction in these behaviors at higher concentrations indicates potential stress or toxicity effects induced by the decoction. Further research is necessary to elucidate the underlying mechanisms of this impact, including potential physiological and biochemical changes in the fish. In addition, exploring protective measures, such as combining mangosteen peel decoction with protective agents or antioxidants, could mitigate its adverse effects. Studies focusing on identifying specific compounds responsible for the observed effects could also guide safer applications in aquaculture. Understanding these effects is crucial for assessing the safe and effective use of mangosteen peel extracts in aquaculture and other applications involving Betta fish. Overall, the observed behavioral changes underscore the importance of carefully monitoring and regulating the concentration of herbal treatments like mangosteen peel decoction to avoid adverse effects on aquatic organisms.

The histopathological analysis of *Betta* sp. liver tissue reveals significant insights into the effects of mangosteen peel decoction on hepatic health. Across all treatment groups, including the control, 5-ppm, 25-ppm, and 50-ppm, notable liver damage was observed, encompassing vacuolization, pyknosis, hemorrhage, and necrosis. These parameters are commonly used in histopathological studies to assess cellular and tissue health. Vacuolization reflects changes in intracellular organelle dynamics, often indicating cellular stress or metabolic dysfunction (Pham et al., 2016). Similar histopathological parameters, including necrosis and pyknosis, have been utilized in zebrafish exposed to paracetamol to assess hepatotoxicity and provide comparative insights into compound-specific toxicities (Dewanti et al., 2023). Pyknosis, characterized by the condensation of chromatin in the nucleus, is a hallmark of irreversible cellular injury and an early stage of apoptosis (Hou et al., 2016). Hemorrhage, which involves the extravasation of blood, signals damage to vascular integrity within the tissue (Kottke-Marchant, 1994), while necrosis is an end-stage cellular event involving uncontrolled cell death and the breakdown of tissue architecture (Golstein & Kroemer, 2007). These findings are detailed in Table 3 and visually represented in Figure 1. In terms of specific damage metrics, the Kruskal-Wallis's test confirmed significant differences among the treatment groups for all

parameters of hepatic damage. Hemorrhage was significantly more pronounced in the 25-ppm and 50-ppm groups compared to the control, indicating that higher concentrations of the decoction correlate with increased hemorrhagic damage. This suggests a dose-dependent relationship where higher exposure to the decoction exacerbates vascular injury within the liver. Comparable histopathological alterations, including hemorrhage and necrosis, have been observed in *Rasbora lateristriata* exposed to paracetamol, supporting the significance of these markers in evaluating hepatic toxicity (Septriani et al., 2023).

Pyknosis, characterized by the condensation of chromatin in the nucleus, was markedly elevated in the 50-ppm group treated with the highest concentration of mangosteen peel extract. This significant increase in pyknosis in 50-ppm groups compared to other groups underscores the cytotoxic effects of the mangosteen peel decoction at higher doses. These findings suggest that hepatocyte nuclei are particularly susceptible to damage at elevated decoction concentrations, leading to impaired cellular function. Necrosis, another critical indicator of cellular damage, showed a stark increase in the 50-ppm group. While the levels of necrosis did not differ significantly between control, 5-ppm, and 25-ppm groups, the elevated necrosis in 50-ppm group highlights severe cellular injury and death at higher concentration of mangosteen peel extract. This points to the potential lethality of high concentrations of mangosteen peel decoction on Betta fish hepatocytes. Interestingly, vacuolization, the formation of vacuoles within the cytoplasm, was highest in the control group and lowest in the 50-ppm group. The reduction of vacuolization in 50-ppm compared to control, with 5-ppm and 25-ppm groups showing intermediate levels, suggests a complex interplay between decoction concentration and the cellular response. The initial hypothesis that higher decoction levels might increase vacuolization was not supported; instead, a possible explanation could be the severe cellular damage at high concentrations, leading to reduced capacity for vacuole formation or maintenance.

Overall, hepatic damage across all treatments was categorized as moderate, with the control group exhibiting the least average damage. This categorization is crucial for understanding the relative impact of the mangosteen peel decoction. Despite all groups showing medium damage, the gradation of specific types of damage indicates that higher decoction concentrations lead to more pronounced and varied forms of hepatic injury. These findings have significant implications for the use of mangosteen peel decoction in aquatic environments and its potential toxicological impacts on fish liver health. The dose-dependent increase in hepatic damage parameters, particularly hemorrhage, pyknosis, and necrosis, calls for careful consideration of the decoction concentration in practical applications. Further research is necessary to elucidate the mechanisms driving these histopathological changes and to explore potential protective measures or alternative treatments to mitigate liver damage in Betta fish and other aquatic organisms.

Comparing concentrations across studies reveals important insights into the effective and safe use of mangosteen peel extracts. In this study, the 5 ppm concentration

maintained normal Betta fish swimming activity and appetite for three days, indicating a relatively safe threshold for short-term exposure. This aligns with findings in wader fish (*R. lateristriata*), where 5 µg/mL was similarly deemed safe for embryonic development (Retnoaji, Paramita, et al., 2023). However, higher concentrations (25 ppm and above) in Betta fish led to significant reductions in activity and appetite, paralleling the toxic effects reported in wader fish at 25 µg/mL and above (Khasanah et al., 2024). In Nile tilapia, a higher dietary concentration of 6.25 mg/g in nanoemulsion form yielded beneficial effects, likely due to the enhanced bioavailability offered by the nanoemulsion delivery system (Yostawonkul et al., 2023). These comparisons highlight the importance of tailoring concentrations to species-specific tolerances and delivery methods to optimize both safety and efficacy.

Conclusion

This study demonstrates that mangosteen peel decoction has a dose-dependent toxic effect on Betta fish, significantly impacting their behavior and liver health. Higher concentrations of the decoction resulted in decreased swimming activity and appetite, indicating stress or toxicity. Histopathological analysis revealed significant liver damage, including vacuolization, pyknosis, hemorrhage, and necrosis, with severity increasing at higher concentrations. The highest concentration (50 ppm) caused the most pronounced cytotoxic effects, notably in pyknosis and necrosis. These findings highlight the need for careful regulation of mangosteen peel decoction to avoid adverse effects on aquatic organisms. Further research is necessary to understand the mechanisms of these effects and to explore protective measures. This study provides important insights into the safety of mangosteen peel extracts and underscores the importance of comprehensive toxicity assessments.

Acknowledgement

The authors extend their sincere gratitude to Sefi Nur Anggaeni and Suwardi for their invaluable assistance in preparing the tools and materials used in the laboratory.

References

Aware, C. B., Patil, D. N., Suryawanshi, S. S., Mali, P. R., Rane, M. R., Gurav, R. G., & Jadhav, J. P. (2022). Natural bioactive products as promising therapeutics: A review of natural product-based drug development. *South African Journal of Botany*, *151*, 512–528. https://doi.org/10.1016/j.sajb.2022.05.028

Cahya, B., Nirmala, D., & Triantoro, B. (2023). Testing the antibacterial effectiveness of mangosteen peel extract (Garcinia mangostana L) against Aeromonas hydrophila Bacterial infection in dumbo catfish (Clarias gariepinus). *IOP Conference Series: Earth and Environmental Science*, *1273*(1), 012024. https://doi.org/10.1088/1755-1315/1273/1/012024

Capela, R., Garric, J., Castro, L. F. C., & Santos, M. M. (2020). Embryo bioassays with aquatic animals for toxicity testing and hazard assessment of emerging pollutants: A review. *Science of The Total Environment*, *705*, 135740. https://doi.org/10.1016/j.scitotenv.2019.135740

Dewanti, A. I. A., Kunjirika, T. P., Putri, R. R. R. A. D., Adaninggar, A., Raharjeng, A. R. P., Retnoaji, B., Nuriliani, A., Sofyantoro, F., Septriani, N. I., & Saragih, H. T. S. S. G. (2023). Effects of Paracetamol on the Development of Zebrafish (Danio rerio). *Pertanika Journal of Tropical Agricultural Science*, *46*(4), 1173–1188. https://doi.org/10.47836/pjtas.46.4.06

Gerry, S. P., & Ellerby, D. J. (2014). Resolving Shifting Patterns of Muscle Energy Use in Swimming Fish. *PLoS ONE*, *9*(8), e106030. https://doi.org/10.1371/journal.pone.0106030

Gibson-Corley, K. N., Olivier, A. K., & Meyerholz, D. K. (2013). Principles for Valid Histopathologic Scoring in Research. *Veterinary Pathology*, *50*(6), 1007–1015. https://doi.org/10.1177/0300985813485099

Golstein, P., & Kroemer, G. (2007). Cell death by necrosis: Towards a molecular definition. *Trends in Biochemical Sciences*, *32*(1), 37–43. https://doi.org/10.1016/j.tibs.2006.11.001

Hosack, T., Damry, D., & Biswas, S. (2023). Drug-induced liver injury: A comprehensive review. *Therapeutic Advances in Gastroenterology*, *16*, 175628482311634. https://doi.org/10.1177/17562848231163410

Hou, L., Liu, K., Li, Y., Ma, S., Ji, X., & Liu, L. (2016). Necrotic pyknosis is a morphologically and biochemically distinct event from apoptotic pyknosis. *Journal of Cell Science*, jcs.184374. https://doi.org/10.1242/jcs.184374

Ibrahim, M. Y., Hashim, N. M., Mariod, A. A., Mohan, S., Abdulla, M. A., Abdelwahab, S. I., & Arbab, I. A. (2016). α-Mangostin from Garcinia mangostana Linn: An updated review of its pharmacological properties. *Arabian Journal of Chemistry*, *9*(3), 317–329. https://doi.org/10.1016/j.arabjc.2014.02.011

Khasanah, L. U., Paramita, P., & Retnoaji, B. (2024). Mangosteen (*Garcinia mangostana* L.) Peel Decoction Effect on Embryological Development of Wader Pari Fish *Rasbora lateristriata* (Bleeker, 1854). *Journal of Tropical Biodiversity and Biotechnology*, *9*(2), 80645. https://doi.org/10.22146/jtbb.80645

Krewski, D., Andersen, M. E., Tyshenko, M. G., Krishnan, K., Hartung, T., Boekelheide, K., Wambaugh, J. F., Jones, D., Whelan, M., Thomas, R., Yauk, C., Barton-Maclaren, T., & Cote, I. (2020). Toxicity testing in the 21st century: Progress in the past decade and future perspectives. *Archives of Toxicology*, *94*(1), 1–58. https://doi.org/10.1007/s00204-019-02613-4

Leung, L., Kalgutkar, A. S., & Obach, R. S. (2012). Metabolic activation in drug-induced liver injury. *Drug Metabolism Reviews*, *44*(1), 18–33. https://doi.org/10.3109/03602532.2011.605791

Lichak, M. R., Barber, J. R., Kwon, Y. M., Francis, K. X., & Bendesky, A. (2022). Care and Use of Siamese Fighting Fish (*Betta Splendens*) for Research. *Comparative Medicine*, *72*(3), 169–180. https://doi.org/10.30802/AALAS-CM-22-000051

Michalopoulos, G. K., & Bhushan, B. (2021). Liver regeneration: Biological and pathological mechanisms and implications. *Nature Reviews Gastroenterology & Hepatology*, *18*(1), 40–55. https://doi.org/10.1038/s41575-020-0342-4

Nie, L.-J., Cao, Z.-D., & Fu, S.-J. (2017). Digesting or swimming? Integration of the postprandial metabolism, behavior and locomotion in a frequently foraging fish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *204*, 205–210. https://doi.org/10.1016/j.cbpa.2016.12.007

Obolskiy, D., Pischel, I., Siriwatanametanon, N., & Heinrich, M. (2009). *Garcinia mangostana* L.: A phytochemical and pharmacological review. *Phytotherapy Research*, *23*(8), 1047–1065. https://doi.org/10.1002/ptr.2730

Oliveira, J. A., Da Silva Souza, J. G., De Jesus Paula, D. A., Carmo Rodrigues Virote, B. D., & Murgas, L. D. S. (2022). Oxytocin reduces the frequency of aggressive behaviours in male betta fish (Betta splendens). *Behavioural Processes*, *200*, 104689. https://doi.org/10.1016/j.beproc.2022.104689

Palmiotti, A., Lichak, M. R., Shih, P.-Y., Kwon, Y. M., & Bendesky, A. (2023). Genetic manipulation of betta fish. *Frontiers in Genome Editing*, *5*, 1167093. https://doi.org/10.3389/fgeed.2023.1167093

Pham, P. H., Babujee, A., Papazotos, F., & Bols, N. C. (2016). Evaluating the potential role of vacuolization in enhancing or interfering with fish virus detection and replication. *Fish & Shellfish Immunology*, *53*, 107. https://doi.org/10.1016/j.fsi.2016.04.071

Retnoaji, B., Nurhidayat, L., Pratama, S. F., Anshori, K., Hananya, A., Sofyantoro, F., & Bessho, Y. (2023). Embryonic development of Indonesian native fish yellow rasbora (Rasbora lateristriata). *Journal of King Saud University - Science*, *35*(7), 102810. https://doi.org/10.1016/j.jksus.2023.102810

Retnoaji, B., Paramita, P., & Khasanah, L. U. (2023). Mangosteen Garcinia mangostana L. simplicia effect on bone structure and behaviour of Wader Fish Rasbora lateristriata (Bleeker, 1854) Embryo. *Indonesian Journal of Pharmacy*. https://doi.org/10.22146/ijp.6348

Saraswathy, S. U. P., Lalitha, L. C. P., Rahim, S., Gopinath, C., Haleema, S., SarojiniAmma, S., & Aboul-Enein, H. Y. (2022). A Review on Synthetic and Pharmacological Potential of Compounds Isolated from Garcinia mangostana Linn. *Phytomedicine Plus*, *2*(2), 100253. https://doi.org/10.1016/j.phyplu.2022.100253

Septriani, N. I., Saribu, R. L. C. D., Apriliyani, T., Karlina, I., Pusparini, N. A. O., Zusrina, L. M., Sari, R. V. S., Allimi, H. S., Supraitno, M. E., Saeed, F., Simanungkalit, E. E., Paramita, P., Retnoaji, B., Sofyantoro, F., & Wijayanti, N. (2023). Histopathological evaluation of hepatic tissue of yellow Rasbora (Rasbora lateristriata) exposed to paracetamol. *Biological Environment and Pollution*, *3*(1), 8–14. https://doi.org/10.31763/bioenvipo.v3i1.595

Setiawan, A. A., Budiman, J., & Prasetyo, A. (2023). Anti-Inflammatory Potency of Mangosteen (Garcinia mangostana L.): A Systematic Review. *Open Access Macedonian Journal of Medical Sciences*, *11*(F), 58–66. https://doi.org/10.3889/oamjms.2023.8746

Simeonova, R., Kondeva-Burdina, M., Vitcheva, V., & Mitcheva, M. (2014). Some *In Vitro/In Vivo* Chemically-Induced Experimental Models of Liver Oxidative Stress in Rats. *BioMed Research International*, *2014*, 1–6. https://doi.org/10.1155/2014/706302

Sofyantoro, F., Septriani, N. I., Yudha, D. S., Wicaksono, E. A., Priyono, D. S., Putri, W. A., Primahesa, A., Raharjeng, A. R. P., Purwestri, Y. A., & Nuringtyas, T. R. (2024). Zebrafish as Versatile Model for Assessing Animal Venoms and Toxins: Current Applications and Future Prospects. *Zebrafish*, zeb.2023.0088. https://doi.org/10.1089/zeb.2023.0088

Soosean, C., Marimuthu, K., Sudhakaran, S., & Xavier, R. (2010). Effect of mangosteen (Garcinia mangostana L.) extracts as a feed additive on growth and hematological parameters of African catfish (Clarias gariepinus) fingerlings. *European Review for Medical and Pharmacological Sciences*, *14*(7), 605–611.

Svendsen, E., Føre, M., Økland, F., Gräns, A., Hedger, R. D., Alfredsen, J. A., Uglem, I., Rosten, C. M., Frank, K., Erikson, U., & Finstad, B. (2021). Heart rate and swimming activity as stress indicators for Atlantic salmon (Salmo salar). *Aquaculture*, *531*, 735804. https://doi.org/10.1016/j.aquaculture.2020.735804

Widyarman, A., Lay, S., Wendhita, I., Tjakra, E., Murdono, F., & Binartha, C. O. (2019). Indonesian mangosteen fruit (Garcinia mangostana L.) peel extract inhibits Streptococcus mutans and Porphyromonas gingivalis in Biofilms In vitro. *Contemporary Clinical Dentistry*, *10*(1), 123. https://doi.org/10.4103/ccd.ccd_758_18

Yostawonkul, J., Kamble, M. T., Sakuna, K., Madyod, S., Sukkarun, P., Medhe, S. V., Rodkhum, C., Pirarat, N., & Sewaka, M. (2023). Effects of Mangosteen (Garcinia mangostana) Peel Extract Loaded in Nanoemulsion on Growth Performance, Immune Response, and Disease Resistance of Nile Tilapia (Oreochromis niloticus) against Aeromonas veronii Infection. *Animals*, *13*(11), 1798. https://doi.org/10.3390/ani13111798

Yue, G. H., Wang, L., Sun, F., Yang, Z., Shen, Y., Meng, Z., & Alfiko, Y. (2022). The ornamental fighting fish is the next model organism for genetic studies. *Reviews in Aquaculture*, *14*(4), 1966–1977. https://doi.org/10.1111/raq.12681

Yuvanatemiya, V., Srean, P., Klangbud, W. K., Venkatachalam, K., Wongsa, J., Parametthanuwat, T., & Charoenphun, N. (2022). A Review of the Influence of Various Extraction Techniques and the Biological Effects of the Xanthones from Mangosteen (Garcinia mangostana L.) Pericarps. *Molecules*, *27*(24), 8775. https://doi.org/10.3390/molecules27248775

Zhang, W., Wang, H., Brandt, D. Y. C., Hu, B., Sheng, J., Wang, M., Luo, H., Li, Y., Guo, S., Sheng, B., Zeng, Q., Peng, K., Zhao, D., Jian, S., Wu, D., Wang, J., Zhao, G., Ren, J., Shi, W., … Hong, Y. (2022). The genetic architecture of phenotypic diversity in the Betta fish (*Betta splendens*). *Science Advances*, *8*(38), eabm4955. https://doi.org/10.1126/sciadv.abm4955

Zhao, J., Bao, W. J., Zhang, F. D., Ye, Z. Y., Liu, Y., Shen, M. W., & Zhu, S. M. (2017). Assessing appetite of the swimming fish based on spontaneous collective behaviors in a recirculating aquaculture system. *Aquacultural Engineering*, *78*, 196–204. https://doi.org/10.1016/j.aquaeng.2017.07.008

Biota
Biologi dan Pendidikan Biologi
http://biota.ac.id/index.php/jb
DOI: http://dx.doi.org/10.20414/jb.v17i2.506

Research Article

Phenology of Flowering and Fruiting in Rukam Growth (*Flacourtia rukam* Zoll. & Moritzi)

Agustara Dwi Juwita[1*], Robika[1], Dian Akbarini[2]
[1]*Department of Biology, Faculty of Agriculture, Fisheries and Marine Affairs, University of Bangka Belitung*
[2]*Agriculture and Food Security Office, Central Bangka*

**Corresponding author: agustaradwijwt@gmail.com*

Abstract

Rukam is one of the fruiting plants that grow wild in the forest or are planted. Excessive utilization without cultivation efforts triggers a decrease in the population of a species in nature until a species disappears from nature. *Flacourtia rukam* has most recently been assessed for The IUCN Red List of Threatened Species in 2023. Flacourtia rukam is listed as Least Concern. Therefore, knowledge of flower and fruit development is needed to determine the effectiveness of production on rukam plants through phenological studies. This study was conducted to obtain information through morphological changes that occur and the length of time required during the flowering process to fruit formation on rukam. This research was conducted by directly observing and measuring the parts of the inflorescence from the process of fruit formation until the fruit ripenning. The results showed that the morphological structure of rukam flowers is incomplete. The phenology of rukam flowering and fruiting takes 56 - 61 days starting with the flower initiation phase and ending with the mature fruit phase. The stages of morphological changes in flower development can be divided into initiation, small flower buds, large flower buds, and blooming flowers (anthesis). While the stages of morphological changes in fruit, color and size can be divided into young fruit, mature fruit, ripe fruit, and deciduous fruit. All stages of color change occur during the fruit ripening process.

Keywords: Phenology, fertilization flowering, rukam, morphology, local plants.

1. Introduction

Indonesia is one of the tropical countries that has various types of fruiting plants, ranging from planted fruiting plants to fruiting plants that grow wild in the forest. One of the fruiting plants that grow wild in the forest or planted is rukam (Solikin and Budiharta, 2010). Rukam is an edible wild fruit that can be consumed (Lim 2013; Nurtjahya et al., 2012). Rukam is a plant species that belongs to the Magnoliopsida class and the Flacourtiaceae family (Hesthiati et al., 2019). Rukam has short flowers, located in the leaf axils, yellow-green in color (Verheij & Coronel, 1991). Rukam fruit is round, hairless, with a diameter of 2-2.5 cm, green in color when the fruit is young and becomes purple or dark red when the fruit is ripe (Solikin and Budiharta, 2010).

According to (Deshmukh et al. 2011), the consumption of wild fruits is gradually decreasing due to the introduction of exotic fruits. Rukam is one of the local plant species found on Bangka Island. The habitat of rukam in the forest is found in the lowlands up to 2,100 m above sea level (Lim, 2013). Rukam has been used as a natural medicine to treat various diseases. For example, rukam leaves can be applied to treat inflamed eyelids, while a decoction of rukam roots is used by women after childbirth (Rana et al., 2018). Rukam is also used for diarrhea and dysentery (Coronel & Verheij, 1991). The part of rukam that

can be used as a diarrhea medicine is the leaf. The leaves used are young, brightly colored leaves. Rukam leaves contain alkaloid, flavonoid, phenolic and saponin compounds (Fitri *et al.,* 2016). Rukam also has the potential to treat eye worm disease (*thelaziasis*) in cattle (Supriadi and Janah, 2016).

(Rugayah et al., 2017) stated that many plant species are becoming rare due to the fact that their place of growth has been converted into industrial areas, plantations, agriculture, road facilities, transportation, and settlements. (Kusumo et al*.,* 2002) stated that among the many types of fruiting plants are now starting to not be found or have begun to be rare. This is caused by changes in the condition of biological resources, land, and habitat due to uncontrolled utilization. Excessive utilization without cultivation efforts will also increasingly trigger a decline in the population of a species in nature until a species disappears from nature. Therefore, knowledge of flower and fruit development is needed to determine the effectiveness of production on rukam plants through phenological studies. The phenology of flowering and fruiting in rukam plants needs to be studied further in order to obtain information on changes that occur during flowering and fruiting. Population declines can be attributed to a mismatch between flowering time and the availability of pollinators or environmental conditions required for reproduction. Changes in environmental conditions due to climate change cause disruption in plant growth and development, leading to a decrease in fruit and flower production and quality (Adhya et al., 2024). In addition, climate change can also trigger shifts in response, morphology and phenology of plants (Pulatov et al., 2015; Sarvina, 2019).

Phenology is known to be widely used to calendar the growth and development of a plant, especially for plants that are already rare or have low viability (Barlian et al., 1998). The phases of flowering and fertilization that occur naturally in plants can be known from the plant's life cycle. According to (Sitompul & Guritno, 1995) observations of plant phenology are often done is the change of vegetative period to generative and generative period length of the plant. This is usually done through the approach of observing the age of flowers, seed formation, and harvest.

Research related to the phenological phase of rukam has never been reported and is still very limited. Information on the phenology of flowering and fertilization of rukam is important for the preservation of these plants because they have a rare status in their natural habitat. Therefore, research on the phenology of fertilization and flowering of rukam needs to be carried out in order to obtain information through morphological changes that occur in these plant parts.

2. Material and Method

2.1 Material

Time and Place

This study was conducted from November 2022 to January 2023, in the yards of residents in Kuday Village, Sungailiat District, Bangka Regency, Bangka Belitung Islands Province. The research was conducted every day in the morning at 08.00 WIB and in the

afternoon at 16.00 WIB. This is because temperature and humidity tend to be more stable in the morning and evening compared to the heat of the day. This minimizes stress on the plants and allows for more accurate observation of their physiological condition.

Tools and Materials

The tools that will be used in this study are stationery, labels, ruler, vernier, cellphone camera, GPS (*Global Positioning System), lux meter*, *soil tester,* and *thermohygrometer*. Materials that will be used are samples of rukam flowers and fruits in the research location.

2.2 Method

Preliminary Survey

The preliminary survey was conducted directly in the yard of a resident's house in Kuday Village, Sungailiat District, Bangka Regency, Bangka Belitung Islands Province. The preliminary survey was carried out with the aim of knowing the condition of the location at the time of the research, as well as seeing the growth of rukam that allows for observation and data collection. The data used in this observation is primary data generated by researchers from direct measurement and observation at the research site.

Sample Selection Conditions

Flowering and fruiting phenology was observed directly on 1 rukam tree. With one sample, environmental variables and growth conditions can be more easily controlled and measured, so one sample is considered representative for the study. The number of flowers to be observed is 20 flower samples, the observed flower samples are marked (labeled). Observations of flowering phenology were made every day to see the changes. Each number of flowers was counted at the beginning of the observation until the last observation.

Observations and Measurements of Flowering and Fertilization Phases

Observations on flowering phenology can be seen in the limitation of flower stadia. Flower development stadia are based on the criteria used by (Dafni, 1993) with some modifications, namely: initiation stadia, small bud stadia, large bud stadia, open flower stadia, and fruit development stadia. It is necessary to limit each phase of the flower to be observed to facilitate observation. The limitations of each stadia are described in table 1 and table 2. Each stadia is observed for changes in color and shape as well as flower morphology. Observations were made every day by directly observing the development of rukam flowers and fruits. The process of flower and fruit development was then photographed and analyzed to determine the stages of development.

Qualitative Observations

Qualitative data collection observed stages and color changes in flowers and fruits, fruit color and shape, petal shape, and flower and fruit morphology.

Quantitative Observations

Quantitative data collection observed included measures related to the development of rukam flowers and fruits, as well as the duration of vegetative and generative stadia.

Table 1. Fruit Development Phase Observation Results.

Phase	Symbol	Description
Young Fruit	F_4	Small light green fruits start to appear, the flesh is firm, and the flavor is astringent.
Mature Fruit	*F5*	The increase in size and change in fruit color from light green to yellowish red, the texture of the fruit flesh begins to soften, and still has a strong astringent sour taste.
Ripe Fruit	*F6*	There is an obvious color change to dark red or purplish red, the texture of the pulp is soft and contains a lot of water, and has a sweet taste.
Fall Fruit	*F7*	The skin surface is slightly rough and slightly wrinkled, purple-black or brown-black in color, and has a astringent and bland taste.

Measurement of Environmental Factors

Measurement of environmental factors is carried out at the observation point where the rukam grows. environmental factors that support the process of flower development, the aim is to know the weather conditions during the flower development period. Determination of the coordinate location point of the study was determined using GPS (*Global Positioning System*). Other abiotic data to be measured are soil temperature, soil pH, light intensity, air temperature and humidity. Abiotic data measurements are carried out every day in the morning at 08.00 WIB and in the afternoon at 16.00 WIB.

Data Analysis

Observation data obtained were presented in tabulated tables and analyzed. Data analysis was carried out descriptively. In addition, research data using quantitative methods, to explore the results that have been obtained through measurements and then analyzed using descriptive statistics, including vegetative and generative morphological characters (flowers) of rukam plants. The data analyzed aims to describe in detail and systematically starting from the flower initiation phase to the fertilization phase.

3. Results and Discussion

3.1 Results

Based on the research conducted, the following results were obtained.

Figure 1. Flowering process of Rukam; flower initiation phase (1), small bud phase (2), large bud phase (3), flower bloom phase (4), flower fall phase (5).

Figure 2. Flowering process of Rukam; young fruit (a), mature fruit (b), ripe fruit (c).

3.2 Discussion

Stages of Rukam Flower and Fruit Development

The observation period of rukam starts from the appearance of flower buds (initiation stage) to fruit maturity. The stages of development of rukam flowers and fruits starting from the initiation stage until the fruit falls off require a total of 56 - 61 days. The development of rukam flowers goes through 4 stages including initiation (*F0*), small buds (*F1),* large buds (*F3)*, and open flowers (*F3)*.

The initiation phase (*F0*) is the initial stage of observation of flowering on rukam which is characterized by the appearance of clusters in the form of flower bud protrusions that are so small that it is not yet clearly visible part of the flower stalk with other flowers, greenish white in color found on the stem branches and in the leaf axils. The shortest size of this phase is 0.01 mm, the end of this phase is characterized by an increase in the length of the bud stalk of 0.1 cm, and requires a period of 7 days. The next stage enters the small bud phase (*F1*) which is a continuation of the initiation phase (*F0*).

Flower development after the initiation phase then continues with small flower buds that continue to enlarge with a stalk length of 0.1 cm - 0.3 cm. The increase in bud size indicates that the flower buds are showing growth and development (Damaiyani & Metusala, 2011). This phase is also characterized by the appearance of the tip of the bud as it begins to split. The small flower bud phase (*F1*) has a period of 5 days to go to the large flower bud phase (*F2*). A decrease in the success of rukam fruit production can occur due to flower and fruit loss from flower formation to fruit development. Both phases (*F0*) and (*F1*) are prone to shedding. According to (Nurtjahjaningsih et al., 2012), basically the flowering process is an interaction of the influence of external factors, namely temperature, light, humidity, rainfall, and nutrients as well as internal factors, namely genetics and hormones. Changes in the environment can change the flowering response in plants (Darjanto & Satifah, 1990).

The large bud phase (*F2*) is a stage that is almost similar to the previous phase (*F1*). The large flower bud phase (*F2*) is characterized by changes in the bud slightly enlarged like swelling, and the length of the bud stalk also increases from the previous phase (*F1*), the petals have also begun to show their growth. The petals in this phase have begun to form and look whitish light green in color which is not too bright. This phase also experienced an increase in stalk length of 0.3 cm - 0.4 cm and bud length of 0.2 cm - 0.3 cm which lasted for 6 days. Based on observations, rukam flowers do not have a crown, this is in accordance with the statement of (Desitarani et al., 2014) which says that flowers on rukam do not have a crown. However, the pistil which is an important part of the flower is still protected by petals that have not fully bloomed. Each plant species has a different response to the environment for flowering (Thomas, 1993).

The next phase is the flower blossom phase (anthesis). This phase is the stage when flower expansion occurs (flower blooms completely). The anthesis phase (*F3*) occurs after the big bud phase ends until there is no more flower growth and continues until entering the fruit phase until the fruit ripens. The anthesis phase on rukam lasts for 9 days with an increase in stalk length of 0.4 cm - 0.6 cm. Macroscopically the parts of the flower are clearly visible. The anthesis phase (*F3*) occurs simultaneously with the maturity of reproductive organs in rukam flowers.

(Sedgley & Griffin, 1989) stated that in general, the length of time between the stages of flower initiation and blooming varies because it is influenced by the growth pattern, temperature range and humidity where a plant species grows. The process of fruit development begins on the 28th day. The visible characteristics of this phase are the appearance of small light green fruits, hard fruit flesh and no aroma, and a astringent taste. The stage of fruit emergence measures 0.3 cm - 1.1 cm in length and 0.7 cm - 1.3 cm in diameter. The young fruit phase lasts for 10 days and in this phase the fruit will continue to increase in size.

The development of mature fruit (*F5*) takes 5 days which is also characterized by an increase in fruit length of 1.1 cm - 1.6 cm with a fruit diameter of 1.3 cm - 2.3 cm. Mature

rukam fruits show changes in characteristics such as the fruit changing color from green to yellowish red or sometimes pink with a smooth surface of the fruit skin and the texture of the fruit flesh which begins to soften and is slightly watery. (Sedgley & Griffin, 1989) stated that water loss and drying occur at the end of the fruit development process which indicates the destruction of chlorophyll. The flavor of the rukam fruit in this phase also undergoes changes such as a tart taste that is still very concentrated with very little sweetness. (Giovannoni, 2001) states that ethylene plays a role in physiological and biochemical changes that occur during fruit ripening. The moisture content of the fruit is an indicator of fruit maturity.

Ripe fruit development (*F6*) lasts for 14 days after passing the mature fruit phase. The formed rukam fruit will slowly continue to grow until it reaches a size of 2.5 cm in diameter with a purplish red color. Rukam fruits that have the color and size previously experienced changes from day to day ranging from light green, pink, red, until finally turning into a dark red or purplish red color. An increase in temperature can affect the rapid maturity of the fruit. In general, the decrease in water content in fruit is influenced by physiological activity (respiration) and environmental conditions (transpiration) (Agusta & Ahmad, 2016). In addition to visible changes in color and size, the surface of the rind of ripe rukam fruit is shinier and smoother, the water content is more and the acid taste contained is also not too significant from the previous phase. This fruit can be enjoyed and said to be sweet if before consumption first by massaging the fruit because bruising the fruit flesh can eliminate the astringent taste contained therein.

The process of fruit drop lasts for 5 days, in this phase the length of the fruit is < 2 cm - fruit drop and the diameter is < 2.5 cm - fruit drop, has a astringent and tasteless taste, and is purple-black in color. Environmental factors also influence fruit drop. In the natural process of fruit drop, fruits fall as part of the plant's efforts to maintain harmony, resilience or physical strength in its growth. This kind of abortion is often found in various types or varieties of fruits in the tropics and subtropics.

Conclusion

Based on the results of research on the phenology of flowering and fertilization of rukam, it can be concluded that in the morphological structure of rukam flowers are incomplete flowers. The stages of morphological changes in flower development can be divided into initiation, small flower buds, large flower buds, and blooming flowers (anthesis). Based on changes in fruit morphology, color and size can be divided into young fruit, mature fruit, ripe fruit, and deciduous fruit. All stages undergo color changes during the fruit ripening process.

References

Adhya, I., Supartono T., Hendrayana Y., Ismail, A. Y., Herlina, N., & Syahban, F. S. 2024. Fenologi Pembungaan dan Pembuahan Jenis *Goniothalamus macrophyllus* di Hutan Dataran Rendah Kabupaten Kuningan Jawa Barat. *Jurnal ilmu-ilmu hayati*, 23(3), 371-379.

Agusta, W., & Ahmad, U. 2016. Mempelajari Tingkat Kematangan Buah Melon Golden Apollo Menggunakan Parameter Sinyal Suara. *Jurnal Keteknikan Pertanian,* 4(2), 195-202.

Barlian, J. H. Yeni, Masano. 1998. Study of Phenology and Fruit Position Effect and Seed Size on Gmelina Seed Viability. *Bulletin Agronomy,* 26(2), 8–12.

Dafni, A. 1993. *Pollination Biology: a Practical Approach*. Oxford: University Press.

Darjanto, & Satifah, S. 1990. *Pengetahuan Dasar Biologi Bunga dan Teknik Penyerbukan Silang Buatan*. Jakarta: PT Gramedia.

Deshmukh, B. S., Waghmode, A., & Arts, A. 2011. Role of wild edible fruits as a food resource: Traditional knowledge. *International Journal of Pharmacy & Life Sciences*, 2(7), 919–924.

Desitarani, Wiriadinata, H., Miyakawa, H., Rachman, I., Rugayah, Sulistyono, & Partomihardjo, T. 2014. *Buku Panduan Lapangan Jenis-jenis Tumbuhan Restorasi*. Jakarta: LIPI.

Fitri, N., Ulandari, S. A., Waldani, O. I., Noviyani, S., & Allwar. 2016. Uji Aktivitas Antioksidan Ekstrak Daun Rukem (*Flacourtia rukam*) Dengan Metode DPPH (1,1-Difenil-2-Pkrilhidrazil), *HKI: Prosiding Seminar Nasional Kimia-Lombok*.

Giovannoni, J. 2001. Molecular Biology of Fruit Maturation and Ripening. Annu. Rev. *Plant Physiol,* (52), 725-749.

Hesthiati, E., Priatmodjo, D., Wisnubudi, G., & Sukartono, I. G. S. 2019. *Keanekaragaman Hayati Tanaman Buah Langka Indonesia*. Jakarta: Lembaga Penerbit Unas.

Kusumo, S. M., Hasanah, S., Moeljopawiro, M., Thohari, Subandriyo, A., Hardjamulia, A., Nurhadi, & Kasim, H. 2002. *Pedoman Pembentukan Komisi Daerah dan Pengelolaan Plasma Nutfah*. Badan Penelitian dan Pengembangan Pertanian. Bogor: Komisi Nasional Plasma Nutfah.

Lim, T. K. 2013. Edible Medicinal and non Medicinal Plants: *Flacourtia rukam*. Springer, The Netherland.

Nurtjahya, E., Inonu, I., Kartika, & Franto. 2012. *Rona Lingkungan Kawasan Calon Tapak PLTN Bangka Selatan 2012*. Pangkalpinang: UBB Press.

Pulatov, B., Linderson, M. L., Hall, K., Jonsson, A. M. 2015. Modeling climate change impact on potato crop phenology and the risk of frost damage in northern Europe. *Agric For Meteorol*, 214, pp.281-292.

Rana, Ghina, S. E., Lestario, L. N., & Martono, Y. 2018. Effect of Various Concentration Sugar Addition on the Color Stability of Rukem Fruit Anthocyanin Extract (*Flacourtia rukam* Zoll. & Mor.). *Jurnal Aplikasi Teknologi Pangan,* 7(4), 173–179.

Rugayah, Kusumadewi, S., Yulita, Arifiani, D., Rustiami, H., & Girmansyah, D. 2017. *Tumbuhan Langka Indonesia: 50 Jenis Tumbuhan Terancam Punah*. Jakarta: LIPI Press.

Sedgley, M., & Griffin, A. R. 1989. *Sexual Reproduction of Tree Crops*. San Diego: Academic Press Inc.

Sitompul, S. M., & Guritno, B. 1995. *Analisis Pertumbuhan Tanaman*. Yogyakarta: UGM Press.

Solikin & Budiharta. 2010. *Potensi dan Konservasi Buah-Buahan Lokal Jawa Timur*. Jawa Timur: Lembaga Ilmu Pengetahuan Indonesia.

Supriadi, & Janah, M. 2016. Aplikasi Ekstrak Daun Rukam (*Flacourtia* Sp) sebagai Anti Telaziasis pada Ternak Sapi Di Kabupaten Sumbawa. *Lumbung Inovasi: Jurnal Pengabdian Kepada Masyarakat,* 1(1), 41-50.

Thomas, B. 1993. Internal and External Control of Flowering. In: Jordan BR (Ed) *Molecular Biology of Flowering*. Sussex: CAB International.

Verheij, E. W. M., & Coronel, R. E. 1991. *Plant Resources of South-East Asia No. 2: Edible Fruits and Nuts*. Bogor: Prosea Foundation.

Biota
Biologi dan Pendidikan Biologi
http://biota.ac.id/index.php/jb
DOI: http://dx.doi.org/10.20414/jb.v17i2.513

Research Article

Macroscopic Fungi in Grassland and Rubber Plantation Habitat Types in Special Purpose Forest Areas of Universitas Lambung Mangkurat, Indonesia

Elsa Lenia Lefi[1], Mochamad Arief Soendjoto[1, 2, *]. Adistina Fitriani[1], Maulana Khalid Riefani[3]

[1]*Faculty of Forestry, Universitas Lambung Mangkurat, Banjarbaru, Indonesia*
[2]*Master of Biology Education, Postgraduate Program, Universitas Lambung Mangkurat, Banjarmasin, Indonesia*
[3]*Faculty of Teacher Training and Education, Universitas Lambung Mangkurat, Banjarmasin, Indonesia*

**Corresponding author: masoendjoto@ulm.ac.id*

Abstract

In addition to secondary natural forests, other habitat types that also exist in the Special Purpose Forest Area of Universitas Lambung Mangkurat (KHDTK ULM) are grasslands and rubber plantations. While previous studies have documented macroscopic fungal species in secondary natural forests, there has been no documentation on fungi in the last two habitat types. This study aimed to analyze macroscopic fungal species present in these two habitat types and the substrates they inhabit. Fungal species and their substrates were recorded on a 250 m x 8 m track between May and September 2024. Each track was placed at threelocations representing grasslands and three locations representing rubber plantations. In total, ten fungal species were found in grasslands and 7 species in rubber plantations. The similarity index of the fungal communities in the two habitats was categorized as very low (23.53%). Additionally, more fungal species were found on substrates such as dead trunks (rotten wood) compared to those found in the soil.

Keywords: fungi, grasslands, rubber plantations, species richness, substrate.

1. Introduction

The Special Purpose Forest Area (Kawasan Hutan Dengan Tujuan Khusus) of Universitas Lambung Mangkurat, hereinafter referred to as KHDTK ULM is a forest area whose management was handed over to Universitas Lambung Mangkurat by the Ministry of Environment and Forestry (now called the Ministry of Forestry). The 1,617-hectare area located in Karang Intan District, Banjar Regency, South Kalimantan is managed for education and training purposes. Its determination is based on the Decree of the Minister of Forestry and Environment No. 900/Menlhk/Setjen/PLA.0/2016 dated December 6, 2016.

Various activities have been carried out in the area. The Faculty of Forestry ULM carries out fieldwork practices (Praktek Kerja lapang) for its students every year. Numerous research are carried out by both educators and ULM students. The results are published, not only to demonstate ULM's responsibility as the manager of KHDTK ULM, but also to contribute to scientific discussion, enriching insight and facilitating the discovery of new things in science. The publications cover a range of topics, including biodiversity (Purbaya et al., 2020; Saputra et al., 2021, Susilawati et al., 2023; Syaifuddin et al., 2023), ecology (Alfiannoor et al., 2023; Nurhidayati et al., 2021), medicinal plants (Nugroho et al., 2022, 2023), physical, mechanical, and chemical properties of wood

(Fadhil et al., 2020; Pujowati et al., 2022), and phytochemistry (Muhammad et al., 2021; Wibisono et al., 2020).

Recent research results, macroscopic fungi are interesting topic to further study. Macroscopic fungi have visible fruiting bodies (Christita et al., 2017) which generally consist of parts such as blades, caps, stalks, rings, and volva, although some species do not have rings (Fauzan et al., 2023). Fungi, also known as mushrooms, can be observed with the naked eye, without using a microscope (Christita et al., 2017; Fauzan et al. 2023). They play an ecological role, including as organisms involved in the decaying of wood and organic matter (Fukasawa & Matsukura, 2021), nutrient cycles (Salillih, 2023). Economically, they serve important functions, such as a source of food (Pouris et al., 2024) and medicine (Hobbs, 2023; Valverde et al., 2015).

Nearly 36 species of macroscopic fungi have been identified in the KHDTK ULM arboretum, although some of the species' names have not been confirmed (Ayunisa et al., 2020). This number is estimated to be smaller than the number of fungi that can actually be found in the area. KHDTK ULM is not only an arboretum which according to its forest type is classified as a secondary forest. Within this education and training forest area, there are two other types of forests, namely grasslands and rubber plantations.

The purpose of this study was to analyze the presence of macroscopic fungal species in various habitats, specifically in grasslands and rubber gardens. Each species found was documented through photographs. These photographs are not only for identification and publication, but also prepared for field guide materials that will be compiled later.

2. Material and Method

Research location

This study using the exploration method, was conducted in two types of habitats in KHDTK ULM: grasslands and rubber plantations. Each type of habitat is represented by three locations (Table 1 and Figure 1). These locations were determined randomly based on their accessibility by 4-wheeled vehicles and the visibility of the plants directly in the field.

Table 1. Coordinates of research locations and habitat types

Habitat Types	Location 1	Location 2	Location 3
Rubber plantations	S 3^0 30' 49", E 114^0 56' 14"	S 3^0 30' 52.62", E 114^0 55' 54.82"	S 3^0 32' 9.74", E 114^0 56' 28.33"
Grasslands	S 3^0 30' 23.82", E 114^0 57' 16.53"	S 30 30' 54.25", E 1140 56' 48.64"	S 3^0 32' 37.40", E 114^0 54' 57.79"

A grassland is an area dominated by wild grass known as ilalang (*Imperata cylindrica*). This area initially was a forest that was then cut down illegally and abandoned or burned, due to deliberate burning or natural causes. At certain times this area is replanted through reforestation program or critical land rehabilitation, although the outcomes of the program not as expected. Some plants fail to thrive, while others manage to survive even though they only lived a little or up to the level of saplings. In addition to areas formerly

covered by forests, grasslands can also be formed from a thin layer of topsoil that covers or is underneath rocks.

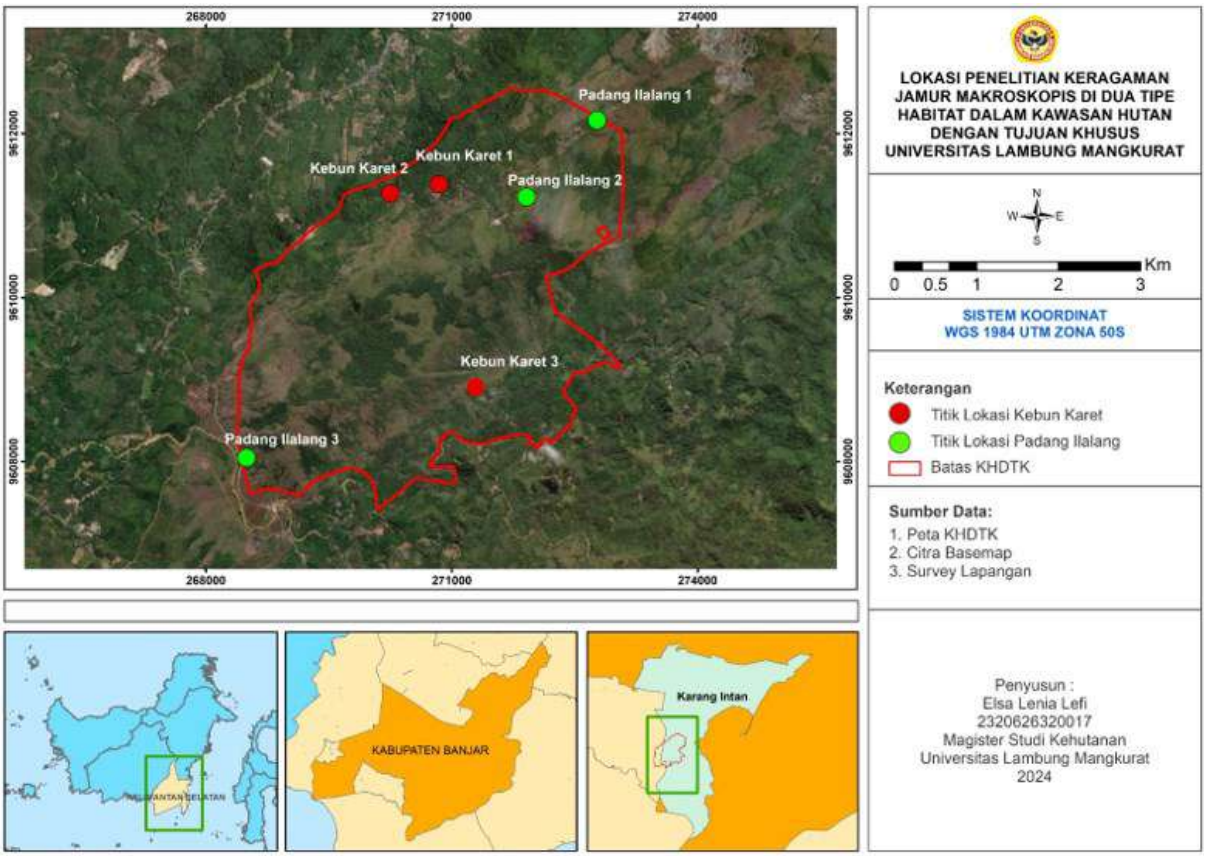


Figure 1. Map of research locations (grasslands and rubber plantations)

Rubber plantations are areas dominated by cultivation of rubber trees (*Hevea brasiliensis*). This area is managed by people who live in nearby villages close to the forest. Their daily activities includes tapping rubber sap, mixing sap, and clearing land from undergrowth or other plants that could hinder sap production.

Data collection and analysis

At each location, an observation path measuring 250 m x 8 m was placed. Macroscopic fungi observed on the path were recorded, their dimensions were measuredand documented through photographs taken from May to September 2024. Photos were taken from various angles, including above, below, and to the side of the object. Fungi were identified by comparing documented photos with those from published articles, such as from Christita et al. (2017), Putra (2021), Putra et al. (2018, 2022), Putri et al. (2024), and Wibowo et al. (2021).

Other objects that are recorded are the substrates where the mushrooms grow. These substrates are categorized into dead trunks, dead branches, or rotten wood (DT), both fallen and still standing; leaf litter (LL), twig litter (TL), and soil (S).

The data were then analyzed in greater detail. Species diversity is identified with species richness (S), which refers to the number of species found or present based on their substrate or present based on their habitat type. The similarity index (Sorensen) is calculated based on Formula 1. The substrate utilization ratio is calculated based on Formula 2.

Species diversity = species richness = **S**

Sorensen similarity index $\mathbf{IS} = \frac{\mathbf{2\,C}}{\mathbf{A + B + 2\,C}}$ ..... Formula 1

Substrate utilization ratio $\mathbf{SUR} = \frac{\mathbf{FPS}}{\mathbf{FAS}}$ ..... Formula 2

In this case:

1) IS = Sorensen similarity index; A = number of fungal species found only in habitat type A; B = number of fungal species found only in habitat type B; C = number of fungal species found in both habitat types; in this case A and also B.
2) SUR = substrate utilization ratio; FPS = number of fungal species living on a particular substrate; FAS = number of fungal species on all substrates.

3. Results and Discussion

3.1. Results

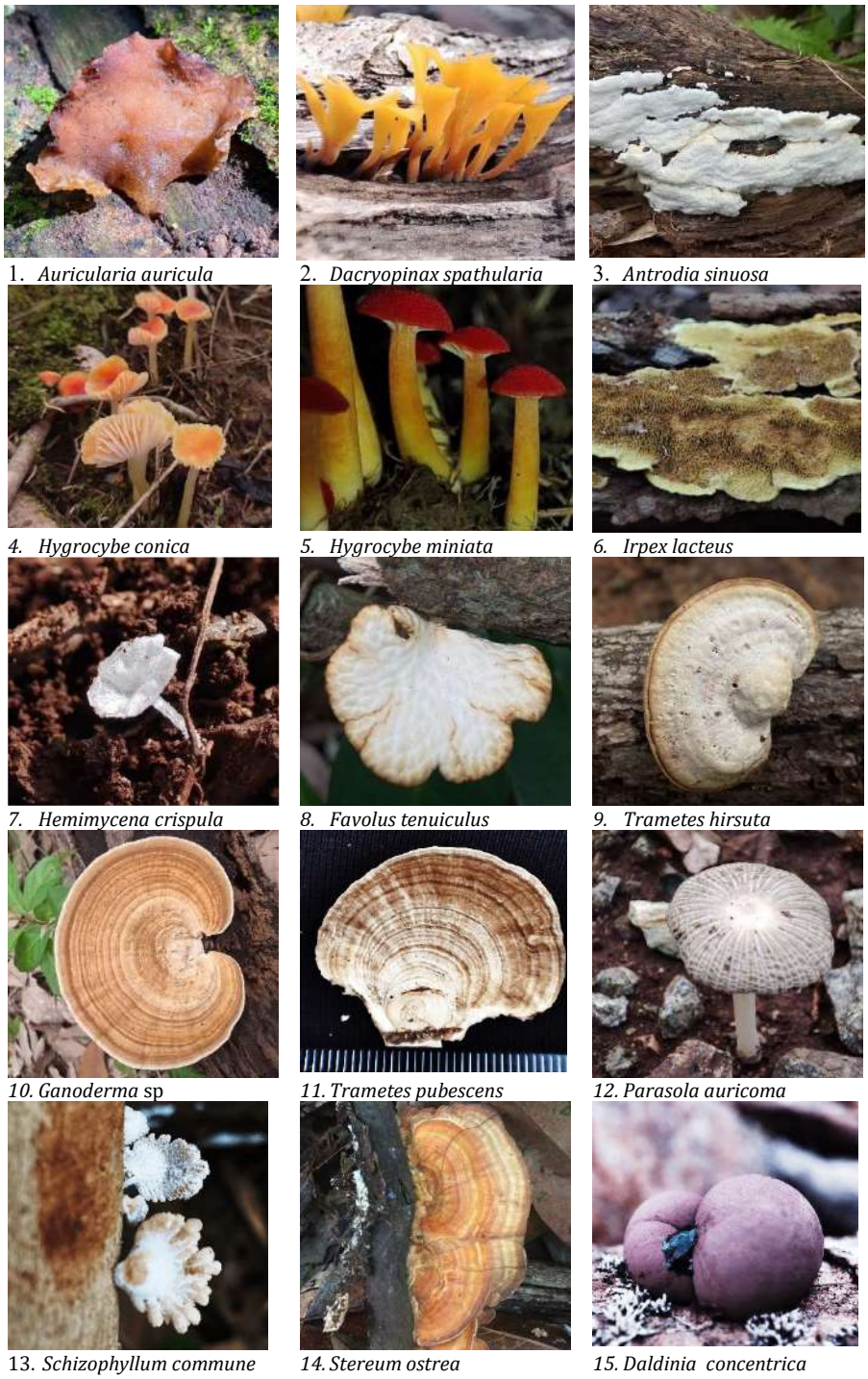
A total of fifteen species (11 families, 2 divisions) of macroscopic fungi were found in the grasslands and rubber gardens (Table 2, Figure 2).

Table 2. Macroscopic fungal species and their substrates in KHDTK ULM

No.	Divisions, families, and species of macroscopic fungi	Vernacular name	Substrate in the grassland		Substrate in rubber plantation	
			DT	S	DT	S
I.	**Basidiomycota**					
	A. Auriculariaceae					
	1. *Auricularia auricula*	Jamur kuping	P	-	-	-
	B. Dacrymycetaceae					
	2. *Dacryopinax spathularia*	Jamur *jelly*	P	-	-	-
	C. Fomitopsidaceae					
	3. *Antrodia sinuosa*	-	-	-	P	-
	D. Hygrophoraceae					
	4. *Hygrocybe conica*	Kulat tiung	-	-	-	P
	5. *Hygrocybe miniata*	Kulat jala	-	-	-	P
	E. Meruliaceae					
	6. *Irpex lacteus*	Jamur-pelapuk putih	P	-	-	-
	F. Mycenaceae					
	7. *Hemimycena crispula*	-	-	P	-	-
	G. Polyporaceae					
	8. *Favolus tenuiculus*	Jamur mekar	P	-	-	-
	9. *Ganoderma* sp.	-	-	-	P	-
	10. *Trametes hirsuta*	Kulat tadung	P	-	-	-
	11. *Trametes pubescens*	Kulat gadong	P	-	P	-
	H. Psathyrellaceae					
	12. *Parasola auricoma*	-	-	P	-	-
	I. Schizophyllaceae					
	13. *Schizophyllum commune*	Kulat taun, jamur gerigit	P	-	P	-

J. Stereaceae					
14. *Stereum ostrea*		-	-	P	-
II. Ascomycota					
K. Xylariaceae					
15. *Daldinia concentrica*	Kulat kancing	P	-	-	-
Richness of fungal species according to their substrate		**8**	**2**	**5**	**2**
Richness of fungal species according to their habitat type		**10**		**7**	

Note: P = present; DT = dead trunks, dead branches, or rotten wood; S = soil



1. *Auricularia auricula* 2. *Dacryopinax spathularia* 3. *Antrodia sinuosa*
4. *Hygrocybe conica* 5. *Hygrocybe miniata* 6. *Irpex lacteus*
7. *Hemimycena crispula* 8. *Favolus tenuiculus* 9. *Trametes hirsuta*
10. *Ganoderma* sp 11. *Trametes pubescens* 12. *Parasola auricoma*
13. *Schizophyllum commune* 14. *Stereum ostrea* 15. *Daldinia concentrica*

Figure 2. Macroscopic fungi species in KHDTK ULM

Fungi were observed in two different types of habitats. The number of species found in the grassland was greater than that in the rubber plantation. Based on the presence of fungi in the two types of habitats, the fungal community similarity index in this study was categorized as very low (23.53%). Only two fungal species were found in both habitat types but on the same substrate. They are *Trametes pubescens* dan *Schizophyllum commune.* In terms of substrate, more fungal species were found in the dead stems than in the soil, in both the grassland habitat and the rubber plantation (Figure 3). Additionally, no fungi were found that utilized leaf litter and twig litter as substrates.

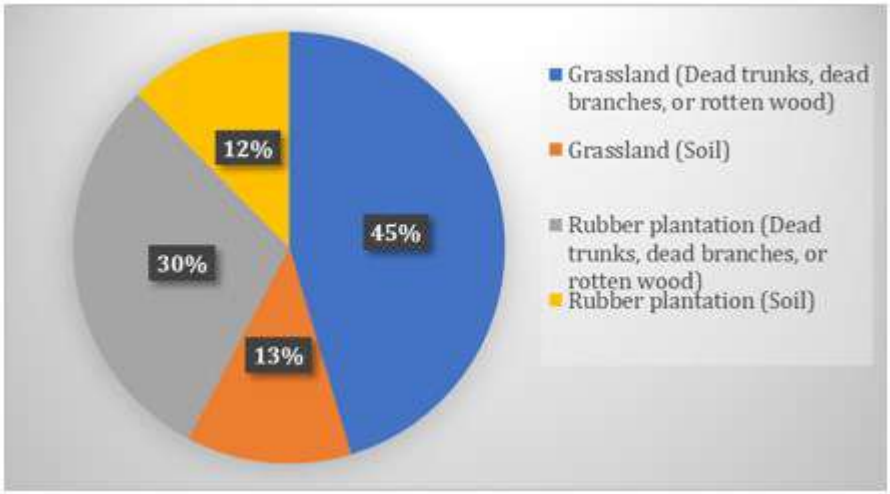


Figure 3. Ratio of fungi utilizing substrate

3.2. Discussion

Macroscopic fungal species

Researchers only observed the existence of species of fungi in grassland and rubber plantation ecosystems, which accounts for the variance in numbers. This number is lower than the number reported by Ayunisa et al. (2020) in secondary forests within the KHDTK ULM, which found 36 species (17 families). While the identification of several fungal species found by Ayunisa et al. (2020) remains unclear, the presence of 15 fungal species in the grasslands and rubber gardens not only contribute to the total number of species, but also show the richness of fungal species in the KHDTK ULM.

Fungi diversity is low in grassland habitats when there are not numerous trees or shrubs. Though the temperature and humidity in grasslands are typically higher, the fungal diversity in these ecosystems is relatively low due to the limited forms of organic materials (such as fallen leaves or plant waste) that are available to fungus. The absence of natural vegetation structure, intense crop management, and pesticide use frequently ends in a decrease of fungi diversity in rubber plantation locations.

Naturally, there are additionally specific times during the year when mushrooms will grow. This happens as consequence of their dependence on specific humidity and temperatures. The humidity in the closed secondary forest area will be higher than that of

the grassland. The forest floor receives very little sunlight because of the shade provided by the many, dense trees. The roots and canopy of plants hold on water when it rains. The vapor of water will be absorbed during this process, leading to lower temperatures, lower humidity, and cooler environments. High intensity light is not needed for mushrooms to survive and grow. Primordia initiation in mushrooms is dependent on light, and it additionally influences the growth of stalks, caps, spores, and hymenium development. Each area has a variable light intensity, which may be caused by uneven canopy cover.

For the record, the following research findings outlines the number of fungal species found in KHDTK and research forests managed by other universities. In KHDTK Tanjungpura University, 17 out of the 24 macroscopic fungal species were found in open-crown peat swamps and 13 species were found in closed-crown peat swamps (Utama et al., 2019). Putra et al. (2019) reported 11 species (7 families) of fungi found in the IPB University Campus Forest. In a subsequent study, Putra et al. (2020) reported 18 species comprising 13 families of fungi.

Several species of mushrooms found in KHDTK ULM are considered beneficial for human health and well-being. *A. auricula* (ear mushroom) contains antioxidant and antibacterial substances (Elfirta & Saskiawan, 2020; Sukmawati et al., 2019). It has medicinal substances that slow down aging, control the digestive system, overcome cardiovascular disorders (Yu et al., 2023), and serve as food ingredients (Arko et al., 2017). *H. conica* (tiung mushroom) is a source of natural antioxidants (Chong et al., 2014; Chun et al., 2021) and can be used as functional food (Chong et al., 2014). *Ganoderma* spp. is widely recognized as a medicinal mushroom (Jong & Birmingham, 1992). *S. commune* has antioxidant and antitumor properties and can also be used as a food ingredient (Arko et al., 2017). Despite these benefits, these mushrooms have not been widely utilized by either ULM or the local community. Usually, community knowledge about edible mushrooms is conveyed orally or by word of mouth (Dewi et al., 2022), a common communication method in traditional communities.

Similarity index and substrate

Environmental factors have a significant impact on an organism's spread and growth (mycelium and mushroom fruit bodies); each species must survive in abiotic environments that are within its tolerance limits (Roosheroe, 2006; Tapwal et al., 2013). The abiotic variables environment (temperature, pH, and humidity) has a significant impact on fungal growth. Different substrates will usually cause different types of mushrooms to grow, as will differences in environmental conditions, such as air humidity, soil humidity, temperature, soil acidity (pH), light intensity. Fungi that grow on dead or living tree trunk substrates, particularly from the Basidiomycota Division, are the primary decomposers of lignin and lignocellulose in wood or roots (Tampubolon, 2012). Mushrooms become parasites, mutualistic symbionts, and decomposers in order to receive nutrition (Wati et al., 2019).

The higher presence of fungal species in dead trunks compared to soil is believed to be due to the several factors. Trunks or branches (also known as wood) are natural materials primarily composed of cellulose, hemicellulose, and lignin along with extractive substances (Augustina et al., 2021; Shobib et al., 2023). In dead wood, these components provide a rich source of nutrients that are abundant and suitable for fungal growth. Nutrients are part of the trunk that is basically organic material, they remain intact even when the trunk is exposed to heavy water or flooding.

The opposite happens in soil substrates. Nutrients resulting from wood decay or the breakdown of organic matter do not remain in the soil for long. Nutrients are not part of the soil and are not bound to it, making them susceptible to loss. Nutrients are easily washed away by infiltrating water, which flows through the pores of the soil to deeper layer. Nutrients are also easily lost by floods. Flooding is a process when water flows rapidly from a certain area to a further area where the ground surface is lower. In such soil substrate, there may be a complete absence of nutrients and fungi.

Conclusion

Out of 15 macroscopic fungal species, 10 species (*Auricularia auricula*, *Dacryopinax spathularia*, *Irpex lacteus*, *Hemimycena crispula*, *Favolus tenuiculus, Trametes hirsuta, Trametes pubescens, Parasola auricoma, Schizophyllum commune, Daldinia concentrica*) were found in the grassland and 7 species (*Antrodia sinuosa*, *Hygrocybe conica*, *Hygrocybe miniata*, *Ganoderma* sp., *Trametes pubescens*, *Schizophyllum commune*, *Stereum ostrea*) in the rubber plantation. Most of the fungi utilized dead stems as their substrate rather than the soil. The similarity index of the fungal communities in both habitat types was classified as very low.

Acknowledgement

This research was funded by the ULM Research and Community Service Institute (Kemendikbudristek) through the Master's Thesis Research scheme under the Implementation Contract for the State University Operational Assistance Program for the 2024 Fiscal Year Research Program Number 1018/UN8.2/PG/2024, dated June 12, 2024. We would like to thank our colleagues from the ULM Faculty of Teacher Training and Education (Muhammad Farhan Azhari, Muhammad Yusuf, Pipin Widyastuti) and also from the ULM Faculty of Forestry (Ahmad Radianoor, Nur Syifa) who helped collect field data and also identify species.

References

Alfiannoor, Nugroho, Y. & Susilawati. (2023). Analisis kesehatan hutan tingkat tiang pada berbagai ketinggian tempat di Bukit Pandamaran KHDTK Mandiangin ULM Kalimantan Selatan. *Jurnal Sylva Scienteae*, 6(5), 713–722. DOI: 10.20527/jss.v6i5. 10650.

Arko, P.F., Marzuki, B.M. & Kusmoro, J. (2017). The inventory of edible mushroom in Kamojang Nature Reserve and Nature Park, West Java, Indonesia. *Biodiversitas,* 18(2), 530–540 DOI: 10.13057/biodiv/d180213.

Augustina, S., Wahyudi, I., , Darmawan, I.W., Malik, J., Kojima, Y., Okada, T., et al. (2021). Pengaruh karakteristik kimia terhadap sifat mekanis dan keawetan alami tiga jenis kayu kurang digunakan. *Jurnal Sylva Lestari*, 9(1), 161–178. DOI: 10.23960/jsl 19161-178.

Ayunisa, S., Naemah, D. & Payung, D. (2020). Inventarisasi jamur makroskopis di KHDTK (Kawasan Hutan Dengan Tujuan Khusus) Universitas Lambung Mangkurat. *Jurnal Sylva Scienteae*, 3(5), 945–953. DOI: 10.20527/jss.v3i5.2564.

Chong. E.L., Sia, C.M., Khoo, H.E., Chang, S.K. & Yim, H.S. (2014). Antioxidative properties of an extract of *Hygrocybe conica*, a wild edible mushroom. *Malaysian Journal of Nutrition*, 20(1), 101-111.

Christita, M., Arini, D.I.D., Kinho, J., Halawane, J.E., Kafiar, J. & Diwi, M.S. (2017). Keragaman dan potensi makrofungi di obyek ekowisata Kaki Dian, Gunung Klabat-Minahasa Utara. *Jurnal Mikologi Indonesia*, 1(2), 82–95. DOI: 10.46638/jmi.v1i2.22.

Chun, S., Gopal, J. & Muthu, M. (2021). Antioxidant activity of mushroom extracts/ polysaccharides—Their antiviral properties and plausible antiCOVID-19 properties. *Antioxidants (Basel)*, 10(12), 1899. DOI: 10.3390/antiox10121899

Dewi, R.F., Ummah, N.R. & Hidayat, N. (2022). Ethnomycology studies of edible and medicinalmacroscopic fungi in Jember Indonesia. *Jurnal Biotik*, 10(2), 218–229. DOI: 10.22373/biotik.v10i2.12513.

Elfirta, R.R. & Saskiawan, I. (2020). The functional character of *Auricularia auricula* crude polysaccharides: Antioxidant and antibacterial activity. *Berita Biologi,* 19(3B), 433–440. DOI: 10.14203/beritabiologi.v19i3 B.3988.

Fadhil, M., Thamrin, G.A.R. & Istikowati, W.T. (2020). Studi pada sifat fisik, mekanik, dan karakteristik anatomi kayu kacang (*Strombosia javanica*) Di Mandiangin, Kabupaten Banjar, Kalimantan Selatan. *Jurnal Sylva Scienteae,* 3(3), 560–567. DOI: 10.20527/jss.v3i3.2190.

*Fahrina, N., Rudy, G.S. & Peran, S.B.. (*2021). Manfaat vegetasi di bawah tegakan karet (*Hevea brasiliensis*) di KHDTK ULM Mandiangin. *Jurnal Sylva Scienteae*, 4(3), 492–500. DOI: 10.20527/jss.v4i3.3750.

Fauzan, F., Taribuka, J. & Patty, J. (2023). Eksplorasi jamur makroskopis di Kecamatan Leihitu Barat Pulau Ambon. *Jurnal Agrosilvopasture-Tech,* 2(1), 78–84. DOI: 10.30598/j.agrosilvopasture-tech.2023.2.1.78.

Fukasawa, Y. & Matsukura, K. (2021). Decay stages of wood and associated fungal communities characterise diversity–decomposition relationships. *Sci. Rep.,* 11, 8972. DOI: 10.1038/s41598-021-88580-2.

Hobbs, C. (2023). The health and clinical benefits of medicinal fungi. *Adv. Biochem. Eng. Biotechnol.*, 184, 285-356. DOI: 10.1007/10_2023_230.

Jong, S.C. & Birmingham, J.M. (1992). Medicinal benefits of the mushroom *Ganoderma. Advances in Applied Microbiology*, 37, 101-134. DOI: 10.1016/S0065-2164(08) 70253_3.

Muhammad, M., Sutiya, B., & Yuniarti, Y. (2021). Uji fitokimia tumbuhan cemara gunung (*Casuarina junghuniana*), Merambung (*Vernonia arborea)*, dan limpasu (*Baccaurea lanceolata*) di Kawasan Hutan Dengan Tujuan Khusus ULM. *Jurnal Sylva Scienteae*, 4(3), 469–475. DOI: 10.20527/jss.v4i3.3747.

Nugroho, Y., Budianto, W.Y. Siahaan, S.C., Agung, P.P., Thalib, I. & Suhartono, E. (2023). Phytochemical analysis, anti-inflammatory, and antioxidant activity of selected medicinal plants in Mandiangin Rainforest in South Kalimantan, Indonesia. *Journal of Tropical Life Science*, 13(1), 137–146. DOI: 10.11594/jtls.13.01.14

Nugroho, Y., Soendjoto, M.A., Suyanto, S., Matatula, J., Alam, S. & Wirabuana, P.Y.A.P. (2022). Traditional medicinal plants and their utilization by local communities around Lambung Mangkurat Education Forests, South Kalimantan, Indonesia. *Biodiversitas*, 23(1), 306–314. DOI: 10.13057/biodiv/d230137.

Pouris, J., Kolyva, F., Bratakou, S., Vogiatzi, C.A., Chaniotis, D. & Beloukas, A. (2024). The role of fungi in food production and processing. *Appl. Sci., 14*(12), 5046. DOI: 10.3390/app14125046

Pujowati, P.S., Sari, N.M. & Rahmadi, A. (2022). Sifat fisik arang alaban, arang mampat dan arang bati-bati dari Kawasan Hutan Dengan Tujuan Khusus Universitas Lambung Mangkurat Gunung Babaris dan Gunung Waringin. *Jurnal Sylva Scienteae*, 5(2), 275–284. DOI: 10.20527/jss.v5i2.5364.

Purbaya, S., Soendjoto, M. A., & Suyanto. (2020). Keragaman dan kemiripan spesies burung pada tiga tipe habitat di Kawasan Hutan Dengan Tujuan Khusus Universitas Lambung Mangkurat (KHDTK ULM). *Jurnal Sylva Scienteae*, 3(4), 741–746. DOI: 10.20527/jss.v3i4.2362.

Putra, I.P. (2021). Panduan karakterisasi jamur makroskopik di Indonesia: Bagian 1 – Deskripsi ciri makroskopis. *Jurnal Penelitian Kehutanan Wallacea,* 10(1), 25–37. DOI: 10.18330/jwallacea.2021.vol10iss1pp25-37.

Putra, I.P., Amelya, M.P., Nugraha, N.H. & Zamia, H.Z. (2019). Notes of some macroscopic fungi at IPB University Campus Forest: Diversity and potency. Biota, 12(2), 57–71. DOI: 10.20414/jb.v12i2.192.

Putra, I.P., Amelya, M.G, Veronica, S. & Kurnianto, M.S. (2020). Fantastic fungi around us: A case study of IPB University Campus Forest. *Jurnal Pena Sains*, 7(2), 68–82. DOI: 1921107/jps.v7i2.6753.

Putra, I.P., Sitompul, R. & Chalisya (2018). Ragam dan potensi jamur makro asal Taman Wisata Mekarsari Jawa Barat. *Al-Kauniyah; Journal of Biology*, 11(2), 133–150. DOI: 10.15408/kauniyah.v11i2.6729.

Putra, Y.G.S., Putra, I.P., Yudistyana, R. & Lukito, E. (2022). *Keanekaragaman Jamur di Kawasan PT Badak NGL*. Bontang: Badak NGL.

Putri, I.A., Kusprianti, E.N., Ardhani, N., Agra, M.A. & Putra, I.P. (2024). Deskripsi morfologi beberapa jamur di Laboratorium Lapang Biologi, IPB University. *Sentri: Jurnal Riset Ilmiah*, 3(1), 69-77. DOI: 10.55681/sentri.v3i1.2120.

Roosheroe, I. G. Sjamsuridzal, W. & Oetari, A. (2006). *Mikologi Dasar dan Terapan*. Jakarta: Yayasan Obor Indonesia.

Salillih, M. (2023). Exploring the ecological role of fungi: A comprehensive study of their function in ecosystems. *Fungal Genom Biol*., 13, 219.

Saputra, F., Fithria, A., & Badaruddin, B. (2021). Keragaman Jenis burung di Kawasan Hutan Dengan Tujuan Khusus Universitas Lambung Mangkurat (KHDTK ULM) Kalimantan Selatan. *Jurnal Sylva Scienteae*, 4(3), 416–427. DOI: 10.20527/jss.v4i3.3742.

Shobib, A., Silva, T.D., Pramudono, B., Rokhati, N. & Kasmiyatun, M. (2023). Analisis komposisi selulosa, hemiselulosa, dan lignin dalam berbagai jenis kayu: Metode Chesson-Datta. *Inovasi Teknik Kimia*, 8(4), 309–314. DOI: 10.31942/inteka.v8i4.9841.

Sukmawati, I.K., Susilawati, E. & Putri, S.D. (2019). Antibacterial activity of extracts and fractions of wood ear mushroom (*Auricularia auricula*). *Pharmaciana*, 9(1), 157–166. DOI:10.12928/pharmaciana.v9i1.11879.

Susilawati, Pujawati, E.D., Hafizianor, Satriadi, T., Novialita, D.R. & Busthami, Y. (2023). Floristic diversity and the structure of the pole level vegetation at Bukit Mandiangin, South Kalimantan. *RJOAS: Russian Journal of Agricultural and Socio-Economic Sciences*, 8(140), 148–155. DOI: 10.18551/rjoas.2023-08.15

Syaifuddin, S., Kissinger, K. & Jauhari, A. (2023). Komposisi dan struktur tumbuhan anggrek di Gunung Bebaris Kawasan Hutan Dengan Tujuan Khusus Universitas Lambung Mangkurat. *Jurnal Hutan Tropis*, 11(2), 240–252. DOI: 10.20527/jht.v11i2.16774.

Tampubolon, S. (2012). Keanekaragaman Jamur Makroskopis di Hutan Pendidikan Universitas Sumatera Utara Desa Tongkoh Kabupaten Karo Sumatera Utara. http://repository.usu.ac.id/bitstream/handle/123456789/52852/Cover.pdf?sequence=7

Tapwal A. Kumar, R. & Pandey, S. (2013). Diversity and Frequency of Macrofungi Associated With Wet Ever Green Tropical Forest in Assam, India. *Biodiversitas*, 14(2): 73-78.

Utama, S., Astiani, D. & Ekyastuti, W. (2019). Keanekaragaman jenis jamur makroskopis pada berbagai kondisi tempat tumbuh hutan rawa gambut di Kawasan Hutan Dengan Tujuan Khusus Universitas Tanjungpura. *Jurnal Hutan Lestari,* 7(3), 1198–1212. DOI: 10.26418/jhl.v7i3.37277.

Valverde, M.E., Hernández-Pérez, T. & Paredes-López, O. (2015). Edible mushrooms: Improving human health and promoting quality life. *Int. J. Microbiol.*, 2015, 376387. DOI: 10.1155/2015/376387.

Wati, R. Noverita, & Setia, T.M. (2019). Keanekaragaman Jamur Makroskopis Di Beberapa Habitat Kawasan Taman Nasional Baluran. *AL-KAUNIYAH: Jurnal Biologi,* 12(2): 171-180.

Wibisono, A., Sunardi, S. & Radam, R.R. (2020). Fitokimia 5 jenis pohon di KHDTK Universitas Lambung Mangkurat Mandiangin Kalimantan Selatan. *Jurnal Sylva Scienteae*, 3(3), 422–431. DOI:10.20527/jss.v3i3.2175.

Wibowo, S.G., Mardina, V. & Fadhliani. (2021). Eksplorasi dan identifikasi jenis jamur tingkat tinggi di Kawasan Hutan Lindung Kota Langsa. *Jurnal Biologica Samudra*, 3(1), 1–13. DOI: 10.33059/jbs.v2i1.3197.

Yu, T., Wu, Q., Liang, B., Wang, J., Wu, D. & Shang, X. (2023). The current state and future prospects of *Auricularia auricula's* polysaccharide processing technology portfolio. Molecules, 28(2):582. doi: 10.3390/molecules28020582.

Biota
Biologi dan Pendidikan Biologi
http://biota.ac.id/index.php/jb
DOI: http://dx.doi.org/10.20414/jb.v17i2.507

Research Article

Analysis of Biomass Organic and Inorganic Carbon Stocks in Silokek Karst Geopark Area, Sijunjung Regency

Chairul[1*], Vera Pertiwi[1], Syamsuardi[1]
[1]Department of Biology, Faculty of Mathematics and Sciences, Universitas Andalas, Padang, West Sumatera, Indonesia

[*]Corresponding author: chairul57@sci.unand.ac.id

Abstract

Climate change caused by increasing greenhouse gas emissions, especially CO2, and deforestation, is an urgent global issue. The REDD+ program initiated by the UN aims to reduce carbon emissions and increase carbon storage in forests. This research aims to measure and analyze biomass and organic and inorganic carbon reserves in the Silokek Geopark area, Sijunjung Regency. The method used in this research is non-destructive sampling and using a destructive sampling method. Transects were carried out using the transect method using purposive sampling and were made in 10 plots 100 m long with square plots measuring 10x10 m for trees, 5x5 m for saplings, and 2x2 m for undergrowth. Next, the limestone samples used the chip sampling method and XRF analysis. The research results showed that the total organic biomass was 326,748 tons/ha, carbon reserves were 153,571 tons/ha, and carbon absorption was 563,607 tons/ha, which is categorized as high. The inorganic carbon content in the Silokek Geopark karst area is 4,908.07 tons/ha and inorganic carbon absorption is 18,012.60 tons/ha.

Keywords: Biomass, carbon stock, CO_2 absorption, Geopark Silokek, karst.

1. Introduction

Climate change caused by increasing greenhouse gas emissions, especially CO_2, and deforestation is an urgent global issue and one of the threats to human populations and organisms on earth (Van de Perre *et al.,* 2018). The REDD+ program initiated by the UN aims to maintain forest carbon storage, by slowing and inhibiting the increase in carbon emissions and providing forest protection, and reducing carbon caused by deforestation (Keohane and Georgia, 2016). In general, tropical forest ecosystems convert more atmospheric carbon into biomass than any other terrestrial ecosystem on earth in any given year (Marvin et al. 2014). Trees in the forest have the potential to store carbon through the process of photosynthesis, where trees sequester carbon in the air and store it in tree body parts such as trunks, branches, and leaves in the form of biomass. According to Danardono et al. (2018) stated that karst is a natural landscape with caves and has the ability to absorb carbon dioxide (CaO et al., 2018; Danardono et al., 2019). Carbon absorbed in karst areas is known as inorganic carbon which is absorbed through the process of forming karst landscapes called karstification (Cao et al., 2018).

Biomass is defined as the total mass or weight of all living creatures in an area at a given time. Carbon reserves are the total quantity of carbon or biomass stored in a component, such as plant, soil, or inorganic biomass in a certain area. Carbon absorption refers to the process by which ecosystems absorb CO_2 from the atmosphere. Carbon

dioxide (CO_2) is absorbed by plants during photosynthesis and converted into organic carbon, or carbohydrates, which are then stored in the plant body's biomass (Karim *et al.,* 2019). The karst ecosystem is one type of ecosystem that can absorb a substantial amount of carbon. The Silokek Karst Geopark is a karst forest that can produce both organic and inorganic carbon in the ecosystem (Danardono *et al.,* 2018). Karst is a natural terrain containing caves that can absorb carbon dioxide (Cao *et al.,* 2018; Danardono *et al.,* 2019).

Based on research conducted in Bantimurung Bulusaraung National Park (Central Sulawesi) indicates that karst forests have a biomass potential ranging from 11.35 to 56.26 tons/ha (Syahrir *et al.,* 2019). Furthermore, based on the West Papua karst area, the predicted biomass is 26.4 tons/ha (Rozak *et al.,* 2021). Moreover, studies conducted in the Biduk-Biduk Karst area of East Kalimantan revealed that the potential for organic carbon stock was 7773.358 tons/ha, whereas inorganic carbon intake was 9026.54 tons/year (Danardono *et al.,* 2022). This shows that karst areas an important role in the carbon cycle in Indonesia, both organic and inorganic.

Based on previous research in the Silokek Geopark area by Pertiwi and Chairul (2024) regarding the analysis of tree strata plant vegetation in the Silokek Karst area, there is a diversity strata (H') of 2.85 which is categorized as medium. This research can be continued by analyzing the biomass content and carbon reserves of above-ground plants and inorganic carbon in the Silokek karst area. This study aims to quantify and assess biomass and organic and inorganic carbon stocks in the Silokek Geopark area of Sijunjung Regency. This research to analyze biomass, and carbon reserves organic and inorganic carbon in the Silokek Geopark Area, Sijunjung Regency. It is hoped that the findings of this study will provide information on biomass estimates, carbon reserves, and organic and inorganic carbon absorption in the Silokek Geopark area, which can be used to improve area management and the potential for long-term biological resource development.

2. Material and Method

This study was conducted from February to May 2024 in the Silokek Geopark Area, Sijunjung Regency, West Sumatra. The research location is at coordinates 00°37'23.4"S / 100°59'22.8"E. The research plot area is 1000 m^2 and has an altitude of 354 mdpl.

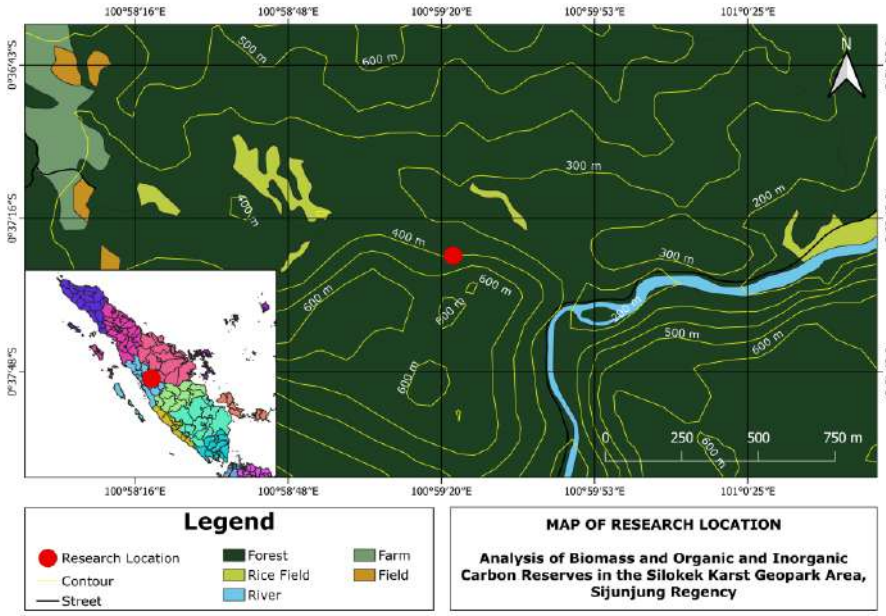


Figure 1. Location of the research area

The following tools were utilized in this study: GPS Garmin, field data sheet, thermohygrometer, soil test, lux meter, measuring tape, DBH meter, hammer and chisel, hand shovel, plant scissors, digital camera, digital scales, and calculator. The following resources were used: a herbarium kit, raffia rope, 5-kilogram plastic packing, permanent marker, writing instruments, and 70% alcohol.

This research uses a non-destructive sampling method by measuring tree diameter and saplings, and uses a destructive sampling method for undergrowth and litter (Hairiah *et al.,* 2011). Determining the location of the plot was carried out using the transect method using purposive sampling. Transects were made in 10 plots 100 m long with square plots measuring 10×10 m for trees, 5×5 m for saplings, and 2×2 m for undergrowth (Kusmana, C., 1997).

Tree biomass is measured by measuring each tree and sapling's DBH (Diameter at Breast Height) (+1.30 m above ground level). Primary data collected in the field, such as diameter at breast height and tree species names, will be used to determine biomass content. Next, samples of undergrowth were taken above the soil surface with plant scissors and weighed to determine the total wet weight, which was +300 grams. Litter samples were collected and weighed to yield a total wet weight of +300 grams (Hairiah *et al.,* 2011).

Tree sampling and sapling identification yielded species designations in Herbarium ANDA. Next, limestone rock samples were obtained using the chip sampling method, and %CaO was determined using the XRF method at the Chemistry Laboratory, UNP (Annisa, *et al.,* 2022). The Environmental Engineering Laboratory at UNAND conducted a %C analysis on soil. Environmental variables at the research location were measured at each research plot, including air temperature and humidity with a thermohygrometer, light intensity with a lux meter, and soil pH with a soil test.

Data Analysis of tree and sapling biomass measurement is calculated using the Allometric Coefficient Value Formula (a and b) (Kettrings *et al.,* 2001), for calculating upper biomass based on tree species using the calculation formula:

$$B = a\ \rho\ D^{b}$$

Description:
B = Tree and sapling biomass (kg)
D = Tree diameter and breast height (1.3 m)
ρ = density of wood each species (kg/m3)
a = constant with a value of 0.11
b = constant with a value of 2.62

To calculate the total tree biomass in one plot, use the formula:

Total biomass = B1 + B2 +….+Bn

Description:
Total biomass = total biomass of all species
B1 = biomass of spesies ke-1
B2 = biomass of species ke-2
Bn = biomass of species ke-n

Measurement of undergrowth and litter biomass is according to (Hairiah *et al.,* 2011), the formula for calculating undergrowth and litter biomass is:

$$\text{Total B (g)} = \frac{\text{BK subexample (g)}}{\text{BB subexample(g)}} \times \text{Total BB(g)}$$

Description:
Total B = total biomass (g)
Total BB = Total wet weight biomass (g)
BK subexample = Dry weight of the sample (g)
BB subexample = Wet weight of the sample (g)

Measurement of carbon stocks is calculation of carbon reserves uses the formula (Hairiah *et al.,* 2011):

$$C = 47\% \times B$$

Description:
C = Carbon content in biomass (ton/ha)
B = Biomass (kg)
47% = Carbon constant according to SNI 7724:2011

Measurement of Carbon Dioxide (CO_2) absorption calculations can be using the results of carbon stock (C-stock) calculations. The formula for calculating CO_2 absorption (IPCC, 2013) is as follows:

$$EC = 3.67 \times C$$

Description:
EC = CO_2 absorption (ton CO_2/ha),
3.67 = Relative molecular value (MR CO_2 to carbon: 44/12 (ton CO_2/ton C),
C = Stored carbon reserves (ton C/ha)

Measurement of inorganic carbon in limestone materials using the formula (Danardono *et al.,* 2019):

$$MC = \left\lfloor \frac{Ar\ C}{Mr\ CaCO3} \times \frac{Mr\ CaCO3}{Mr\ CaO} \times \%CaO \times BJ\ CaCO3 \times V\ CaCO3 \right\rfloor$$

Description:
MC = mass of carbon in limestone (kg)
Ar C = relative atomic mass of carbon (12 g/mol)
Mr $CaCO_3$ = relative atomic mass of $CaCO_3$ (100 g/mol)
Mr CaO = relative atomic mass of CaO (56 g/mol)
%CaO = percentage of CaO content in limestone resulting from laboratory analysis
BJ $CaCO_3$ = specific gravity of $CaCO_3$ (2.71 g/cm^3)
V $CaCO_3$ = volume of limestone

3. Results and Discussion

3.1. Results

Based on the research that has been carried out, a total of biomass, carbon reserves, and organic carbon absorption in organic plants. Data on the results of organic plants can be seen in Table 1.

Table 1. Biomass, Carbon Stocks, and Carbon Absorption in Organic Plants

Strata	Biomass (ton/ha)	Carbon Reserves (ton/ha)	Carbon Absorption (ton/ha)
Trees	303.963	142.863	524.306
Sapling	21.702	10.200	37.433
Undergrowth and Litter	1.083	0.509	1.868
Total	**326.748**	**153.571**	**563.607**

Table 2. Organic Carbon of Trees

No	Species name	Family	Biomass (ton/ha)	Carbon reserves (ton/ha)	Carbon absorption (ton/ha)
1.	*Ficus stricta* (Miq.) Miq.	Morac	220,471	103,622	380,291
2.	*Monocarpia euneura* Miq.	Annonac	17,919	8,422	30,909
3.	*Diospyros* sp.	Ebenac	15,857	7,453	27,351
4.	*Ficus variegata* Blume	Morac	10,051	4,724	17,337
5.	*Vitex pinnata* L.	Lamiac	8,360	3,929	14,420
6.	*Syzygium* sp.	Myrtac	5,198	2,443	8,966
7.	*Phoebe lucida* Blume	Laura	4,828	2,269	8,328
8.	*Pterospermum javanicum* Jungh.	Malva	4,443	2,088	7,663
9.	*Garcinia* L.	Clusia	3,756	1765	6,478
10.	*Croton argyratus* Blume	Euphorbi	3,258	1,531	5,619

No	Species name	Family	Biomass (ton/ha)	Carbon reserves (ton/ha)	Carbon absorption (ton/ha)
11.	*Paranephelium xestophyllum* Miq.	Sapind	2,692	1,265	4,643
12.	*Sterculia cordata* Blume	Malva	1,333	0,627	2,300
13.	*Alstonia* sp.	Apocyna	0,887	0,417	1,529
14.	*Eurycoma longifolia* Jack	Simarouba	0,789	0,371	1,361
15.	*Ficus sinuata* Tunb.	Morac	0,769	0,362	1,327
16.	*Artocarpus elasticus Reinw*. Ex Blume	Morac	0,695	0,326	1,198
17.	*Knema laurina (Blume)* Warb.	Myristi	0,600	0,282	1,035
18.	*Dialium* sp.	Faba	0,476	0,224	0,822
19.	*Nephelium lappaceum* L.	Sapind	0,474	0,223	0,818
20.	*Mallotus nudiflorus* (L.) Kulju & Welzen	Euphorbi	0,383	0,180	0,660
21.	*Aporosa benthamiana* Hook.f	Phyllantha	0,383	0,180	0,660
22.	*Ficus padana* Burm.f.	Morac	0,341	0,160	0,589
	Total (ton/ha)		**303,963**	**142,863**	**524,306**

Table 3. Organic Carbon of Sapling

No	Species name	Family	Biomass (ton/ha)	Carbon reserves (ton/ha)	Carbon absorption (ton/ha)
1.	*Diospyros* sp.	Ebena	2,317	1,089	3,996
2.	*Drypetes* sp.	Putranjiva	1,822	0,857	3,144
3.	*Syzygium* sp.	Myrta	1,397	0,657	2,410
4.	*Polyalthia* sp.	Annona	1,121	0,527	1,934
5.	*Pometia pinnata* J.R.Forst. & G.Forst.	Sapinda	0,960	0,451	1,656
6.	*Pterospermum javanicum* Jungh.	Malva	0,959	0,451	1,655
7.	*Artocarpus rigidus* Blume	Mora	0,840	0,395	1,449
8.	*Mallotus peltatus* (Geiseler) Muell. Arg.	Euphorbia	0,834	0,392	1,439
9.	*Croton argyratus* Blume	Euphorbia	0,792	0,372	1,366
10.	*Phoebe lucida* Blume	Laura	0,749	0,352	1,291
11.	*Dysoxylum alliaceum* Blume	Melia	0,731	0,344	1,261
12.	*Lithocarpus* sp.	Faga	0,709	0,333	1,222
13.	*Elaeocarpus angustifolius* Blume	Ealeocarpa	0,630	0,296	1,086
14.	*Macaranga aleuritoides* F.Muell	Euphorbia	0,626	0,294	1,080
15.	*Paranephelium xestophyllum* Miq.	Sapind	0,605	0,285	1,044
16.	*Monocarpia euneura* Miq.	Annona	0,585	0,275	1,010
17.	*Ficus stricta* Miq.	Mora	0,574	0,270	0,990
18.	*Ficus variegata* Blume	Mora	0,574	0,270	0,990
19.	*Vitex pinnata* L.	Lamia	0,529	0,249	0,913
20.	*Aglaia lawii* (Wight) C.J.Saldanha.	Melia	0,527	0,248	0,910
21.	*Annona* sp.	Annona	0,523	0,246	0,902

No	Species name	Family	Biomass (ton/ha)	Carbon reserves (ton/ha)	Carbon absorption (ton/ha)
22.	*Nephelium* sp.	Sapind	0,477	0224	0,823
23.	*Artocarpus elasticus* Reinw. Ex Blume	Mora	0,430	0,202	0,743
24.	*Sandoricum koetjape* Burm.f.Merr	Melia	0,375	0,176	0,646
25.	*Palaquium obovatum* (Griff.) Engl.	Sapota	0,339	0,159	0,585
26.	*Neonauclea calycina* (Bartl. Ex. DC.)	Rubia	0,269	0,127	0,465
27.	*Macaranga triloba* (BI.) Muell Arg.	Euphorbia	0,264	0,124	0,455
28.	*Dysoxylum* sp.	Melia	0,255	0,120	0,440
39.	*Polyalthia subcordata* Blume	Annona	0,214	0,101	0,370
30.	*Litsea* sp.	Laura	0,201	0,094	0,347
31.	*Alstonia scholaris* (L.) R. Br.	Apocyna	0,199	0,094	0,344
32.	*Sterculia rubiginosa* Vent.	Malva	0,174	0,082	0,299
33.	*Alstonia* sp.	Apocyna	0,099	0,047	0,171
	Total (ton/ha)		**21,702**	**10,200**	**37,433**

Table 4. Organic Carbon of Undergrowth and Litter

Plot	Biomass (ton/ha)	Carbon reserves (ton/ha)	Carbon absorption (ton CO_2/ha)
1	0,809	0,380	1,396
2	0,030	0,014	0,051
3	0,024	0,011	0,042
4	0,025	0,012	0,043
5	0,034	0,016	0,059
6	0,029	0,014	0,050
7	0,027	0,013	0,046
8	0,036	0,017	0,062
9	0,034	0,016	0,059
10	0,035	0,016	0,060
Total (ton/ha)	**1,083**	**0,509**	**1,868**

The relationship between biomass and diameter in the figure above 2 shows positive results.

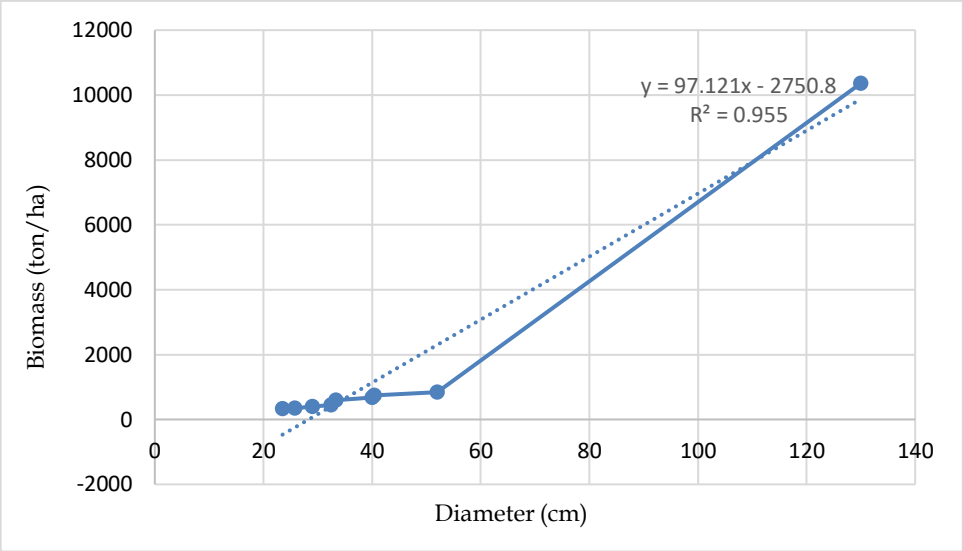


Figure 2. Relationship between biomass and diameter

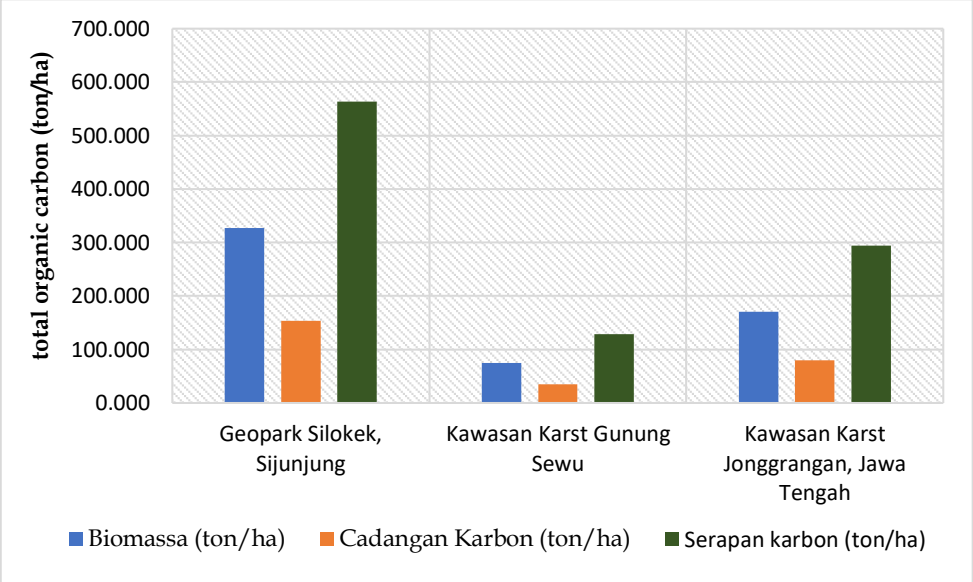


Figure 3. Comparison of biomass, carbon reserves, and carbon absorption from the three different locations

Based on the results of inorganic biomass in the karst *Geopark* Silokek area, that has been carried out the inorganic total carbon can be seen in Table 2.

Table 5. Inorganic Carbon Content

No	Sample	%CaO	Inorganic Carbon (ton/ha)
1.	Sample 1	90.087	1554.80
2.	Sample 2	96.879	1672.02
3.	Sample 3	97.414	1681.25
Total (ton/ha)			**4908.07**

Table 6. Environmental Factor Measurement

The research area	Environmental factor	Unit
Geopark Silokek Area, Sijunjung Regency	Temperature	27.9˚C
	Air humidity	75.9%
	Light intensity	8.86%
	pH	7

3.2. Discussion

Based on the research that has been carried out, a total of biomass, carbon reserves, and organic carbon absorption in organic plants. Data on the results of organic plants can be seen in Table 1. According to Table 1, the biomass of organic carbon in plants is 326,748 tons/ha, while in trees it is 303.963 tons/ha, saplings are 21.702 tons/ha, and undergrowth plants and litter are approximately 1.083 tons/ha. A tree biomass study was conducted because trees absorb more carbon than sapling strata, undergrowth, and litter, as evidenced by the fact that the older the tree, the bigger its diameter and height. Trees with diameters of more than 20-50cm contribute significantly to biomass analysis results, accounting for approximately 69% of total biomass.

The relationship between tree biomass (the total mass of organic material generated by a tree) can be understood as implying that the larger the tree's diameter, the greater the biomass produced. Apart from diameter, biomass correlates positively with specific gravity. This positive link was demonstrated in tree growth, with larger trees producing more biomass than smaller ones. This is because larger trees have more woody tissue, stems, and leaves, which all contribute to the tree's overall biomass. Trees take CO_2 from the atmosphere through photosynthesis and store it as biomass (Chave, *et al.,* 2014). A vegetation's stem diameter is connected to its biomass specific gravity is also related to biomass (Tuah, 2017).

The carbon reserves of organic carbon in plants are 153.571 tons/ha, while in trees it is 142.863 tons/ha, saplings are 10.200 tons/ha, and undergrowth plants and litter are approximately 0.509 tons/ha. Meanwhile, trees with a diameter of the research results show that the biomass and carbon reserves of trees and saplings are higher than those of undergrowth plants and litter because trees are photosynthetically active plants. The research area's average light intensity of 8.86% (table 6) allows trees to create new biomass throughout their lives. Based on research from Pan *et al.* (2011) trees with a strong structure and roots in the soil can grow larger and produce more biomass. In contrast to the undergrowth, the forest conditions at the research site are relatively dense, making it difficult for the undergrowth to absorb sunlight for photosynthesis, resulting in a modest biomass. Litter has a shorter life cycle compared to trees, because the process of decomposition and decomposition by microorganisms is relatively short.

Photosynthesis has an important role in determining the amount of carbon stores. Plants collect carbon from the air and convert it to organic compounds via photosynthesis (Syahrir *et al.,* 2019). Photosynthesis findings are utilized to promote vertical (height) and horizontal (diameter) growth in trees. The biomass contained in a forest area shows the

productivity strata in that forest, because the formation of plant body parts is related to biomass, where the longer the age of the plant, the greater the biomass produced. Biomass value is positively related to carbon reserves, where the higher the biomass value, the higher the carbon reserves.

According to table 1, the intake of organic carbon in plants is 563.607 tons/ha, while in trees it is 524.306 tons CO_2/ha, saplings are 37,433 tons CO_2/ha, and undergrowth plants and litter are approximately 1.868 tons CO_2/ha. This contrasts with research in the Gunung Sewu Karst area (Bantul, Gunung Kidul, Wonogiri, and Pacitan), which found a total biomass value of 74.780 tons/ha, carbon stocks of 35.147 tons/ha, and carbon intake of 128.988 tons/ha (Haryono *et al.,* 2016). Furthermore, studies were conducted in the Jonggrangan Karst area of Central Java, which had a total biomass value of 170.328 tons/ha, carbon reserves of 80.054 tons/ha, and carbon absorption of 293,799 tons/ha (Putro, 2010).

This research illustrates that trees can absorb more carbon than saplings, undergrowth, and litter. The value of carbon absorption in plants describes the plant's ability to absorb CO_2 from the surrounding air. The increase in diameter is driven by the storage of biomass produced by CO_2 conversion, which becomes greater as the tree absorbs more CO_2 (Manafe *et al.,* 2016). The largest part of a tree that can store more carbon is the trunk (Stephenson, *et al*., 2014).

The stem is a place to store food reserves from photosynthesis and is the woody part (Cahyaningrum *et al.,* 2014). Trees absorb carbon from the environment through photosynthesis. The carbon is absorbed by the leaves, which subsequently perform photosynthesis and spread to other parts of the tree. The number and density of trees, tree diameter, kind, and canopy, as well as climatic parameters including sunlight, water content, temperature, and soil fertility, all impact biomass values and CO_2 absorption in different research locations. The amount of biomass is determined by diameter, plant height, wood density, and soil fertility (Sedjarawan *et al.,* 2014).

Based on the analysis carried out by the REDD+ Task Force (2012), the classification of carbon stocks in forest ecosystems consists of low strata such as underground and litter (<35 tons/ha), medium strata such as sapling (35-100 tons/ha), and high strata such as trees (>100 tons/ha). The amount of carbon stocks is strongly influenced by biomass. Based on the results at the research location, it is known that the total carbon reserves are 153,571 tonnes/ha which is categorized as high. Biomass has a significant impact on carbon stock levels (Uthbah *et al.,* 2017).

Based on the comparison in the figure 3, it shows that the values of biomass, carbon reserves, and carbon absorption at the three research locations do not show significantly different total values. The difference in value depends on the volume and area of the area. The Silokek Geopark covers 1300 km^2 of karst terrain. This differs from the research by Haryono *et al.* (2016) in Mount Sewu Karst with karst volume area (29 km^2) and Jonggrangan Karst (1300 km^2). These differences in values indicate that although the values per unit area may be similar, the total biomass, carbon stock, and carbon

sequestration will differ significantly due to differences in area size. Therefore, it is important to consider factors such as area size, vegetation type, and environmental conditions in the analysis and comparison of biomass and carbon stocks in different karst areas.

Based on Table 5 it is known the differences in CaO content in the three rock samples in Table 2 above are due to differences in rock composition and influencing environmental factors. The carbon content value at the research location is approximately 4908.07 tons per hectare. Table 2 shows that the research location has an inorganic carbon content of 4908.07 tons/ha and a carbon absorption of 18012.60 tons/ha. This differs from other karst sites in Biduk-Biduk Karst, East Kalimantan, in that the inorganic carbon concentration is 9026.54 tons/ha and the carbon intake is 33127.40 tons/ha (Danardono *et al.,* 2019). The research area's inorganic carbon absorption values vary depending on volume and rock composition.

The results of the research show that the value of inorganic carbon content is higher than the value of organic carbon, that explains that the potential for carbon storage in rock formations is one of the highest carbon reserves on earth below ocean carbon reserves and organic carbon. Inorganic carbon rocks transform into a highly stable and durable form, playing a crucial role in long-term carbon storage. In the context of climate change, its influence on the carbon trapped in rocks can be significant. Due to its stability, carbon trapped in these rocks can persist on Earth for very long periods, functioning as a more durable carbon reserve compared to organic carbon, which is more easily decomposed (Schlesinger & Bernhardt, 2013). The higher value of inorganic carbon content is due to the influence of the volume and mass of this rock, which significantly surpasses the volume and mass of organic material on the soil's surface or in living species' biomass (a long-term geological process). In addition, the carbonate component in rocks is particularly chemically stable and is not easily degraded or changed by biological processes. In contrast, organic carbon is more susceptible to decomposition by microorganisms, oxidation, and other processes that convert it to carbon dioxide (CO_2) or methane (CH_4) (Schlesinger & Bernhardt, 2013).

Conclusion

Based on the results of research conducted regarding biomass analysis and organic and inorganic carbon reserves in the Silokek Geopark karst area, Sijunjung Regency, the following conclusions can be drawn: The total organic biomass in the Silokek Geopark karst area is 326,748 tons/ha. The total organic carbon reserves in the Silokek Geopark karst area are 153,571 tons/ha. The organic carbon absorption in the Silokek Geopark karst area is 563,607 tons/ha. The inorganic carbon content in the Silokek Geopark karst area is 4,908.07 tons/ha and the inorganic carbon absorption is 18,012.60 tons/ha. The organic and inorganic biomass in the Silokek Geopark area are categorized as high, thus contributing to reducing the amount of CO_2 in the atmosphere and mitigating climate change.

References

Annisa, A.A., Muh Said L., Ayusari W., & Rahmaniah. (2022). Analisis Kandungan Kalsium Karbonat ($CaCO_3$) Batu Gamping di Kelurahan Bontoa Kecamatan Minasate'ne Kabupaten Pangkajene dan Kepulauan. *JFT: Jurnal Fisika dan Terapannya,* Vol 9(2): 120-126. http://dx.doi.org/10.24252/jft.v%vi%i.23565.

Cahyaningrum S. T., Hartoko A., & Suryanti. (2014). Biomassa Karbon Mangrove pada Kawasan Mangrove Pulau Kemujan Taman Nasional Karimunjawa. *Jurnal Management Of Aquatic Resources,* 3(3): 34-42.

Cao J., Wu X., Huang F., Hu B., Groves C., Yang H., & Zhang C. (2018). Global significance of the carbon cycle in the karst dynamic system: Evidence from geological and ecological processes. *China Geology*, 1(1), 17–27. doi:10.31035/cg2018004.

Chave, J., et al. (2014). Improved allometric models to estimate the aboveground biomass of tropical trees. *Global Change Biology,* 20(10): 3177–3190. https://doi.org/10.1111/gcb.12629.

Danardono D., Haryono E., & Widyastuti M. (2018). The Nature Of Carbon Flux In Various Ecosystem Types In The Biduk-Biduk Karst Region, Berau District, East Kalimantan. *E3S Web Conf.,* 76, 1–7. doi:10.1051/e3sconf/20197604005.

Danardono D., Haryono E., & Widyastuti M. (2019). Potential of Carbon Stocks and Its Economic Values in Tropical Karst Landscape (Case Study in 41 Biduk-Biduk Karst, East Kalimantan, Indonesia). *Journal of Physics: Conference Series* 1373, 012030. doi:10.1088/1742-6596/1373/1/012030.

Danardono D., Haryono E., & Widyastuti M. (2022). Potensi Serapan Karbon Inorganik pada Kawasan Karst Tropis di Karst Biduk-Biduk, Kalimantan Timur. *Jurnal Wilayah dan Lingkungan*, Vol 10 Nomor 3. http://dx.doi.org/10.14710/jwl.10.3.257-281.

Hairiah K., Ekadinata A., Sari R.R., & Rahayu S. (2011). *Pengukuran Cadangan Karbon dari Strata Lahan ke Bentang Lahan*", Edisi Kedua. World Agroforestry Centre-ICRAF, SEA Regional Office, University of Brawijaya (UB), Malang, Indonesia XXp.

Hardiansyah & Gusti, et al. (2011). *REDD: Peluang HPH Menurunkan Emisi Global.* Pontianak: Untan Press.

Haryono, E., Danardono, D., Mulatsih, S., Putro, S. T., & Adji, T. N. (2016). The nature of carbon flux in Gunungsewu Karst, Java-Indonesia. *Acta Carsologica,* 45(2). doi:10.3986/ac.v45i2.4541.

IPCC. (2013). *Climate Change 2013: The Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press. Cambridge, United Kingdom and New York, NY, USA.

Karim M. A., Purwiyanto A. I. S., & Agustriani F. (2019). Analysis of carbon content (C) production rate of mangrove litter at Pulau Payung, Banyuasin District. *Marine Science Research Journal*, Vol 11(1): 1–8.

Kusmana, C., (1997). *Metode Survei Vegetasi.* Bogor: Institut Pertanian Bogor.

Keohane & Georgia Levenson. (2016). Climate Finance Fighting Deforestation and Climate Change: REDD Financing Lessons from Brazil and Indonesia (Excerpt). http://impactalpha.com.

Ketterings Q. M., Richard Coe, Meine van Noordwijk, Yakub Ambagau, & Cheryl A Plam, (2001). Reducing Uncertainty in The Use of Allometric Biomass Equations for

Predicting Above-ground Tree Biomass in Mixed Secondary Forest. *Forest Ecology and Managemet*, 146, 199-209.

Manafe G., Kaho M. R., & Risamasu F. (2016). Biomassa Permukaan dan Stok Karbon pada Tegakan pohon Avicennia marina dan Rhizophoa mucronate di Perairan Pesisir Oebelo Kabupaten Kupang. *Bumi Lestari Journal of Environment,* 16(2), 163. https://doi.org/10.24843/blje.2016.v16.i02.p09.

Pan, Y., et al. (2011). A large and persistent carbon sink in the world's forests. *Science*, 333(6045), 988–993. https://doi.org/10.1126/science.1201609.

Pertiwi V., & Chairul. (2024). Analisis Vegetasi Tumbuhan Strata Pohon di Kawasan Karst Geopark Silokek, Kabupaten Sijunjung. *Bioscientist: Jurnal Ilmiah Biologi*. 12(1), 367-379. https://doi.org/10.33394/bioscientist.v12i1.9231.

Putro S. T. (2010). Laju Pelarutan Batuan Karbonat di Karst Gunungsewu dan Karst Jonggrangan. *Skripsi*, Fakultas Geografi Universitas Gadjah Mada.

Rozak A. H., Mutaqien Z., & Destri. (2021). Biomass estimation of eaglewood (Aquilaria filaria (Oken) Merr.) in the karst ecosystem of West Papua. *Journal of Tropical Biodiversity and Biotechnology,* 6(1), 59221. https://doi.org/10.22146/jtbb.59221.

Satgas REDD+. (2012). *Strategi Nasional REDD+*.

Sedjarawan W., Akhbar, & Arianingsih I. (2014). Biomassa dan karbon pohon di atas permukaan tanah di tepi jalan Taman Nasional Lore Lindu. *Jurnal Warta Rimba*, 2(1), 105–111.

Schlesinger, W. H., & Bernhardt, E. S. (2013). B*iogeochemistry: An Analysis of Global Change.* Academic Press.

Stephenson, N. L., et al. (2014). Rate of tree carbon accumulation increases continuously with tree size. *Nature*, 507(7490), 90–93. https://doi.org/10.1038/nature12914.

Syachrir M., Subaedah St., & Parawansa A. K. (2019). Analisis Komposisi Jenis dan Potensi Simpanan Karbon pada Ekosistem Karst di Resort Tondong TallasaTaman Nasional Bantimurung Bulusaraung. *AGROTEK: Jurnal Ilmiah Ilmu Pertanian*. DOI:10.33096/agrotek.v2i2.61.

Tuah Nasib, Rudianda Sulaeman, & Defri Yoza, (2017). Perhitungan Biomassa dan Karbon di Atas Permukaan Tanah di Hutan Larangan Adat Rumbio Kab Kampar. *JOM Faperta UR,* Vol 4(1).

Uthbah Z., Sudiana E., & Yani E. (2017). Analisis biomassa dan cadangan karbon pada berbagai umur tegakan damar (Agathis dammara (Lamb.) Rich) di KPH Banyumas Timur. *Scripta Biologica*. 4: 119-124. https://doi.org/10.20884/1.sb.2017.4.2.404.

Van de Perre F., Willig M. R., Presley S. J., Andemwana F. B., Beeckman H., Boeckx P., Cooleman S., de Haan M., de Kesel A., Dessein S., Grootaert P., Huygens D., Janssens S. B., Kearsley E., Kabeya P. M., Leponce M., van den Broeck D., Verbeeck H., Würsten B., & Verheyen E. (2018). Reconciling biodiversity and carbon stock conservation in an Afrotropical forest landscape. *Science Advances* 4(3) 1-9. https://doi.org/10.1126/sciadv.aar6603.

Biota
Biologi dan Pendidikan Biologi
http://biota.ac.id/index.php/jb
DOI: http://dx.doi.org/10.20414/jb.v17i2.512

Research Article

Hatching Ratio and Larval Development of *Aedes aegypti* Eggs in Different Growth Media

M. Ali Azis Hasan Rizki[1*] Dadan Supardan[2]

[1]*Universitas Islam Al Azhar, Mataram, Nusa Tenggara Barat, Indonesia*
[2]*Institut Agama Islam Negeri Curup, Bengkulu, Indonesia*

**Corresponding author: hasanrizki@unizar.ac.id*

Abstract

Aedes aegypti is a mosquito species primarily known as the vector for dengue fever. The Hatching and development larval of *Aedes agypti* are crtical factors in mosquito control strategies and reducing dengue fever transmission. This study aims to investigate the effects of different water media on the hatching rate and development of *Aedes aegypti* from egg to adult stage. Three type of water from well water, treated tap water (PDAM) and staw soaked water were used in the experiment. The selection of water sources was based on their distinct physical and chemical properties, representing common environmental condition where *Aedes aegypti* may breed. Straw soak water was incorporated to simulate organic rich aquatic environments, often characterized by the presence of decomposing plant material. Such conditions are known provide a nutrient rich medium that facilitates mosquito egg hatching and larval development. Eggs were placed in each water type, and observations were carried out at hatching rate and larval development. The result revealed that the straw soaked water had the highest hatching rate 100% and the development time 6 days from egg to adult, well water show a hatching rate 67% with a longer development period of 7 days and no hatching and larval developmet in tap water (PDAM). This study shows that various types of water media affect the hatching success and developmental rates of *Aedes aegypti.* However, these effects are limited to the specific water types used in the experiment and should not be assumed to apply to all water quality conditions. Future research is recommended to explore other water quality parameters and their potential impact on the mosquito life cycle.

Keywords: *Aedes aegypti*, Hatching rate, larval development, Straw-soaked water, Water quality

1. Introduction

Vector-borne disease transmission dengue fever is a major concern for public health. Every year, 96 million cases of dengue fever occur worldwide causing approximately 9.110 deaths (Budiman et al. 2021). Indonesia has faced significant challenges with dengue fever, with case numbers fluctuating highly over recent years. In 2024, Indonesian ministry of health reported 88,593 cases and 621 deaths (WHO, 2024). Effective vector control strategies and a comprehensive understanding of the mosquito life cycle from egg to adult are essential to reduce dengue fever transmission. Knowledge of the mosquito life cycle is essential for developing effective prevention methods (Buhler et al. 2019).

The life cycle of *Aedes aegypti* includes several stages from egg , four larval instars, pupa and adult. Each stage is influenced by environmental factors such as water quality, temperature, humidity, survival, and population density (Arévalo-Cortés et al. 2022). The effective incubation of eggs and larval development is highly influenced by the type of water used for development, organic matter, and pH. Eggs of *Aedes aegypti* can remain

viable in dry conditions and hatch upon submersion in water, the quality of water significantly affects hatching success and development timing (Soares-Pinheiro et al. 2017). After hatching, the larvae pass through four stages to reach the instar before becoming to pupae. Larval ontogeny develops most rapidly and uniformly in media a with high organic matter (Tsegaye et al. 2023).

Recent study highlight the important role of water quality and type of water development *Aedes aegypti*. The type of water, such as rain water and treated tap water, which have different organic matter composition, has been shown to affect hatching success and larval survival (Thia Prameswarie et al. 2023). Furthermore, envronmental factors such as temperature and nutrient availability are crucial in determining how effectively mosquitoes develop at each stage(Souza et al. 2019).

This paper aims to explore how different types of water media affect the hatching rate and larval development of *Aedes aegypti*. This study investigates these dynamics to provide insights that can improve mosquito control strategies and reduce the risk of vector-borne disease transmission. The concept that various types of water affect larval development can lead to more effective control, ultimately reducing dengue incidence and enhancing public health outcomes.

2. Material and Method

Sample Collection

Aedes aegypti eggs were obtained and maintained in the Biology Education laboratory, UIN Mataram. The water types were kept under controlled conditions with a temperature of 29 ± 1°C and a Ph of ± 8.0.

Experimental Design

Three types of water were used in the development of *Aedes aegypti:* well water, tap water (PDAM) and Straw-soak water. Each type of water was tested in triplicate to ensure reproducibility. A total of 1 L experimental units was prepared, with each unit consisting of a container filled with 50mL of the respective water type.

Procedure

A standardized 10 number of eggs was placed into each container of water, the container were convered with mesh to prevent contamination and loss of eggs. The containers were observation, parameters recorded included the following. The number of eggs that hatched within a specified time frame. Then, the progression of larvae through the instars (1^{st} to 4^{th}) was observation. Measurement included the duration of each instar and survival rate. The transition from larva to pupa and to adult mosquito was recorded.

Statistical Analysis

The data collected from the experiments were analyzed using SPSS. Statistical tests, ANOVA and Tukey's HSD, were performed to compare hatching rates, development times,

and survival rates across different water media. A significance level of $p < 0.05$ was used for all statistical analyses

3. Results and Discussion

3.1. Results

Table 1. Hatching Ratio and Developmental stage *Aedes aegyptiI* (Analysis of Variance One Way)

Variable	Sum of Squares	df	Mean Square	F-Value	P-Value
Hatching Ratio	13333.34	2	6666.67	20.0	0.004
Duration Egg to Instar 1	6.802	2	3.401	5.3	0.05
Duration Instar 1 to Pupa	33.33	2	16.667	125	0
Duration Pupa to Adult	1.052	2	0.526	15.781	0.007

The ANOVA results reveal significant differences in several developmental variables of *Aedes aegypti* based on the type of water used. For the Hatching Ratio variable, an F Value of 20.0 with a p-value of 0.004 indicates that the type of water significantly affects the egg hatching ratio. This suggests that different water types impact the hatching rate of the eggs. For the Duration Egg to Instar 1 variable, the F Value of 5.3 and a p-value of 0.05 suggest marginal differences in the duration from egg to Instar 1 among the tested water types. Although this difference is close to the threshold of significance, further evaluation is warranted. The Duration Instar 1 to Pupa variable shows a very high F Value of 125.00 with a p-value of 0, indicating a highly significant difference in the duration of development from Instar 1 to pupa. This suggests that water type has a substantial impact on the rate of larval development during this phase. Finally, for the Duration Pupa to Adult variable, the F Value of 15.781 and p-value of 0.007 indicate significant differences in the duration from pupa to adult mosquito between water types. Overall, these results confirm that the type of water significantly influences both the hatching ratio and the duration of various developmental stages of *Aedes aegypti*.

Table 2. Hathing Ration and Developmental Stage (Posthoc Test Tukey-HSD)

Variable	**Comparison**	**Mean Difference**	**P-Value**
Hatching Ratio	Well Water - Staw Soak Water	33.33	0.69
	Well Water - PDAM Water	66.67	0.015
	Straw Soak Water - PDAM Water	100	0.06
Duration Egg to Instar 1	Well Water - Staw Soak Water	0.167	0.97
	Well Water - PDAM Water	1.84	0.08

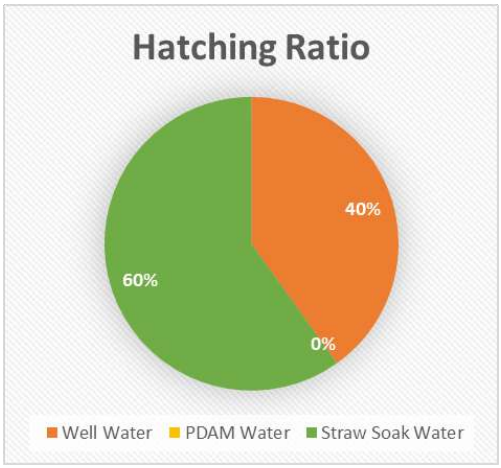
	Straw Soak Water - PDAM Water	2	0.08
Duration Instar 1 to Pupa	Well Water - Staw Soak Water	1.67	0.009
	Well Water - PDAM Water	4.67	0
	Straw Soak Water - PDAM Water	3	0.01
Duration Pupa to Adult	Well Water - Staw Soak Water	0.3	0.2
	Well Water - PDAM Water	0.8	0.006
	Straw Soak Water - PDAM Water	0.5	0.06

The post-hoc analysis for the hatching ratio reveals a significant difference between Well Water and PDAM Water, with a mean difference of 66.67 and a p-value of 0.015. This indicates that Well Water significantly supports a higher hatching ratio compared to PDAM Water. The comparison between Straw Soak Water and PDAM Water shows a mean difference of 100.00, though this difference is not statistically significant ($p = 0.06$). No significant difference was found between Well Water and Straw Soak Water (mean difference = 33.33, $p = 0.69$).

Regarding the duration from egg to Instar 1, no significant difference was observed between Well Water and Straw Soak Water (mean difference = 0.167, $p = 0.97$). However, there is a trend suggesting a longer duration in PDAM Water compared to Well Water (mean difference = 1.84, $p = 0.08$) and Straw Soak Water (mean difference = 2.00, $p = 0.08$), although these differences are not statistically significant. For the duration from Instar 1 to Pupa, significant differences are evident. Well Water shows a longer duration compared to Straw Soak Water, with a mean difference of 1.67 and a p-value of 0.009. Additionally, the duration in PDAM Water is significantly longer compared to both Well Water (mean difference = 4.67, $p < 0.001$) and Straw Soak Water (mean difference = 3.00, $p = 0.01$).

In terms of the duration from Pupa to Adult, Well Water exhibits a shorter duration compared to PDAM Water, with a mean difference of 0.8 and a p-value of 0.006, indicating a significant difference. The comparison between Straw Soak Water and PDAM Water approaches significance (mean difference = 0.5, $p = 0.06$), suggesting a trend towards a longer duration in PDAM Water. These results highlight significant differences in development rates and hatching ratios based on the type of water used, with Well Water generally supporting more favorable outcomes compared to PDAM Water.

Hatching Ratio



(**Figure 1.** Hatching Ratio)

The study reveals significant differences in the hatching ratios of *Aedes aegypti* eggs among various water media. In well water, the hatching ratio is 67%, indicating a relatively good rate of egg hatching and suggesting that well water supports egg hatching effectively. In contrast, no eggs hatched in PDAM water, resulting in a hatching ratio of 0%. This lack of hatching suggests that PDAM water does not support egg hatching, likely due to factors such as poor water quality or the presence of inhibiting substances. On the other hand, straw soak water demonstrated a hatching ratio of 100%, meaning all eggs placed in this medium successfully hatched. This indicates that straw soaking water is highly effective in supporting *Aedes aegypti* egg hatching, making it an exceptionally supportive medium for this purpose.

Larva Development Based on Medium Type

Table 3. Larvae Development Based on Medium Type

Medium Type	Stage	Average Duration (Day)
Well Water	Egg - Instar 1	2
	Instar 1- Instar 2	0.84
	Instar 2- Instar 3	0.84
	Instar 3 - Instar 4	1.34
	Instar 4 - Pupa	1.67
	Pupa - Adult	0.5
PDAM water	Egg - Instar 1	-
	Instar 1- Instar 2	-
	Instar 2- Instar 3	-
	Instar 3 - Instar 4	-
	Instar 4 - Pupa	-
	Pupa - Adult	-

Straw Soak Water	Egg - Instar 1	2
	Instar 1- Instar 2	1
	Instar 2- Instar 3	1
	Instar 3 - Instar 4	1
	Instar 4 - Pupa	0.5
	Pupa - Adult	0.5

In well water, the transition from egg to instar 1 takes 2 days, while the changes from instar 1 to instar 2 and from instar 2 to instar 3 each require 0.84 days. Moving from instar 3 to instar 4 takes slightly longer, approximately 1.34 days. The duration from instar 4 to the pupal stage is 1.67 days, and the pupal stage to adult mosquito is the shortest, at just 0.5 days. This indicates that the development of *Aedes aegypti* larvae in well water shows fairly consistent timing between instars, but requires a longer period for the transition from instar 4 to pupa compared to the transition from pupa to adult mosquito.

In contrast, PDAM water showed no data on larval development, suggesting that it is not suitable for *Aedes aegypti* larvae or contains factors that inhibit their growth. Concurrently, in straw soak water, the duration from egg to instar 1 is 2 days, consistent with well water. The transitions between instars (from instar 1 to instar 2, instar 2 to instar 3, and instar 3 to instar 4) all take 1 day each. The transition from instar 4 to pupa, as well as from pupa to adult mosquito, each takes 0.5 days. Thus, straw soak water provides more uniform development times between instars and quicker transitions from pupa to adult mosquito compared to well water. Overall, well water exhibits variable development durations with stable instar transitions but longer pupal periods, whereas straw soak water shows more consistent timing and faster transitions. PDAM water is unsuitable for larval development, suggesting it may hinder mosquito growth.

Comparasion of Development Time

Table 4. Comparasion of Development Time

Medium Type	Duration egg to Larva	Duration Larva to Pupa	Duration Pupa to Adult	Total Duration
Well Water	2	4.5	0.5	7
Straw Soak Water	2	3.5	0.5	6

The development of *Aedes aegypti* from egg to adult was evaluated in two types of water: well water and straw soak water. The duration from egg to larva was consistent across both media, with a time of 2 days. However, the duration from larva to pupa was significantly longer in well water, taking 4.5 days, compared to 3.5 days in straw soak water. This indicates that the transition from larva to pupa is slower in well water. Both media exhibited the same duration of 0.5 days for the transition from pupa to adult, showing no difference in this phase. Overall, the total development time from egg to adult

mosquito was shorter in straw soak water (6 days) compared to well water (7 days). This suggests that straw soak water supports faster development of *Aedes aegypti* compared to well water. Thus, while the initial and final stages of development are unaffected by the type of water, the intermediate stage from larva to pupa is more prolonged in well water, resulting in a longer overall development time.

3.2. Discussion

Study examines how different water media affect the development of *Aedes aegypti* from egg to adult. the results show of siginificant differences in hatching ratios and developmental times across various water types, which have implicaations for mosquito control and ecological research

Habitat Preferences for Hatching of *Aedes aegypti* Eggs

The results of this study indicate that *Aedes aegypti* eggs hatch effectively in well water, with a hatching ratio of 67%. This suggest that well water, although not fully sterile, possesses characteristics conducive to egg hatching. Suwartawan et al. (2022), in their study, it was established that *Aedes aegypti* generally prefers clean water for oviposition, and well water, which is relatively cleaner than domestic sewage, provides a more favorable environment for hatching. However, a hatching ratio that does not reach 100% may indicate the presence of inhibiting factors or less than optimal conditions in the well water that limit complete hatching.

The Inhibitory Effects in Treated Tap Water(PDAM)

The absence of hatching in tap water, hatching ratio: 0%, shows that the tap water does not support eggs hatching. Possibily, chemical substances such as chlorine act as inhibitors, preventing egg development even causing egg mortality. This experiment aligns with previous research, treated water containing inhibitory substance such as chlorine can significantly reduce hatching susccess (Fahri et al. 2019; Imam et al. 2014).

Organic-rich matter in straw soak water

With a hatching ratio of 100%, the characteristics of straw soak water demonstrate the highly supportive nature of this medium for eggs hatching. The presence of organic matter potentially creates an ideal environment for the development of *Aedes aegypti*. Nutriens released from straw supply additional resources that support egg development. The observation is consistent with research that suggests organic matter in water can enhance the attractiveness of such environments for oviposition and support higher hatching rates (Fahri et al. 2019; Pradani, Mutmainah, and Marlina 2023)

Effect of water characteristics on Hatching

The paper presents various significant findings in hatching ratios, depending on the characteristics of each water type. Organic-rich matter (Straw soak water) proved to be the most productive for egg hatching, while the tap water (PDAM) which contains

inhibitory substances, completely inhibited hatching. The result highlight effects of physical and chemical factors in water, such as oxygen, pH, organic matter, and inhibitory substances in determining *Aedes aegypti* egg hatching. (Imam et al. 2014; Suwartawan et al. 2022)

Larval development in well water

The results indicate that *Aedes aegypti* larvae in well water relatively stable development between instars, with a duration of 2 days from egg to instar 1. However, the development becomes variable in the next stage, especially during transition from instar 4 to pupae (1,67 days) and from pupa to adult, which only 0,5 days. The variation probably related to the availability of nutrients in well water (Araújo, Gil, and E-Silva 2012). The amount of available nutrients significantly influences the rate of larval development and survival. The nutrients in well water are probably sufficient to support growth but not as much as in straw soak water. The longer duration of the study from instar 4 to pupae suggest that larvae experience a nutrien deficit during the pupa stage. The temperature is an important factor influencing the rate of larval development. The optimal temperature for larvae is 32^0C. The consistency in developmental times between instars in well water suggests balanced environmental conditions, although slower pupal development probably related to reduced body size or adult survival due to suboptimal nutrition.

Larval Development in Straw Soak Water

In contrast, straw soak water accelerates development across all larval stages and the transision from pupa to adult involve 0,5 days. The development more accelerated psssibly caused by the high nutrient content from organic matter in straw soak water. Nutrien-rich water supply a more abundant food source, which is known to accelerated larval development (Fahri et al. 2019; Mackay et al. 2023). The studies have indicate that larvae with access to higher nutritional level exhibit higher developmental, reflected in a 100% hatching ratio and faster life progression in straw soak water.

Egg hatching and development in tap (PDAM) water: the absence of larval development data in tap water indicates that medium doesn't support the growth of *Aedes aegypti* larvae. The research consistent with the discussion that tap water containing chemicals such as chlorine inhibits larval development. The presence of inhibitory substances can disrupt the environmental conditions necessary for larval nutrient absorption with essential physiological processes for development (Couret, Dotson, and Benedict 2014; Mackay et al. 2023). Therefore, the tap water (PDAM) doesn't support laval growth and illustrates how external factors, such as water quality and chemical content inhibit life cycle.

Comparison of Development time: Table 4 presents a comparison of the development time of *Aedes aegypti* from egg to adult in two types of water: well water and straw soak water. The data indicate that the duration from egg to larva is consistent across both media, at 2 days. However, the transition from larva to pupa is significantly longer in well

water, 4.5 days, compared to 3.5 days in straw soak water. This indicates a slower larva to pupa transition in well water. Both media exhibit the same duration of 0.5 days for the transition from pupa to adult mosquito, with no difference observed in this final phase. Consequently, the total development time from egg to adult mosquito is shorter in straw soak water (6 days) compared to well water (7 days). This suggests that straw soak water facilitates faster development of *Aedes aegypti* relative to well water, particularly due to the differences observed during the larva-to-pupa transition.

Conclusion

This paper demonstrates that different different water have significant impact to development of *Aedes aegypti* from egg to adult. Straw soak water proved to be the most supportive medium, achieving a 100% hatching rate and faster development compared to well water, which only reached a 67% hatching rate. In contrast, PDAM-treated water did not support any hatching, likely due to the presence of chemicals such as chlorine that inhibit egg and larval development. These findings highlight the potential importance of water quality, particularly its organic content, in supporting the mosquito life cycle. However, Further research is needed to thoroughly investigate the effects of different water types, the interactions between environmental factors such as temperature and humidity and the development of natural substances that can inhibit mosquito development without harming the environment. These conclusions should be supported by more detailed results and discussions, accompanied by appropriate data and references.

References

Araújo, Maisa Da Silva, Luiz Herman S. Gil, and Alexandre De Almeida E-Silva. 2012. "Larval Food Quantity Affects Development Time, Survival and Adult Biological Traits That Influence the Vectorial Capacity of Anopheles Darlingi under Laboratory Conditions." *Malaria Journal* 11: 1–9.

Arévalo-Cortés, Andrea, Yurany Granada, David Torres, and Omar Triana-Chavez. 2022. "Differential Hatching, Development, Oviposition, and Longevity Patterns among Colombian *Aedes aegypti* Populations." *Insects* 13(6).

Budiman et al. 2021. "Essential Oil as a New Tool for Larvicidal *Aedes aegypti*: A Systematic Review." *Gaceta Sanitaria* 35: S459–62. https://doi.org/10.1016/j.gaceta.2021.10.074.

Buhler, Claudia et al. 2019. "Environmental Methods for Dengue Vector Control – A Systematic Review and Meta-Analysis." *PLoS Neglected Tropical Diseases* 13(7): 1–15.

Couret, Jannelle, Ellen Dotson, and Mark Q. Benedict. 2014. "Temperature, Larval Diet, and Density Effects on Development Rate and Survival of *Aedes aegypti* (Diptera: Culicidae)." *PLoS ONE* 9(2).

Fahri, Sukmal, Susy Ariyani, Gustomo Yamistada, and Eka S. Manulang. 2019. "Hatchability of the Eggs Aedesspp in Clean and Polluted Water." *KnE Life Sciences* 2019: 134–40.

Imam, Hashmat, Ghulamuddin Sofi, Zarnigar, and Seikh Aziz. 2014. "The Basic Rules and Methods of Mosquito Rearing (*Aedes aegypti*)." *Tropical Parasitology* 4(1): 53.

Mackay, Andrew J. et al. 2023. "Larval Diet and Temperature Alter Mosquito Immunity and Development: Using Body Size and Developmental Traits to Track Carry-over Effects on Longevity." *Parasites and Vectors* 16(1): 1–13. https://doi.org/10.1186/s13071-023-06037-z.

Pradani, Firda Yanuar, Rahayu Nirmatul Mutmainah, and Sela Marlina. 2023. "Description of the Behavior of *Aedes aegypti* Mosquito Laying Eggs in Water Contaminated with Animal Feces and Soil." *Mukhtabar : Journal of Medical Laboratory Technology* 1(1): 6–13.

Soares-Pinheiro, V. C., W. Dasso-Pinheiro, J. M. Trindade-Bezerra, and W. P. Tadei. 2017. "Viabilidade de Ovos de *Aedes aegypti* (Diptera, Culicidae) Em Diferentes Condições de Armazenamento Em Manaus, Amazonas, Brasil." *Brazilian Journal of Biology* 77(2): 396–401.

Souza, Raquel Santos et al. 2019. "Microorganism-Based Larval Diets Affect Mosquito Development, Size and Nutritional Reserves in the Yellow Fever Mosquito *Aedes aegypti* (Diptera: Culicidae)." *Frontiers in Physiology* 10(APR).

Suwartawan, I. Gede Dedy et al. 2022. "Life Cycle of *Aedes aegypti* Based on Acidity Variation of Domestic Sewage Water." *Southeast Asian Journal of Tropical Medicine and Public Health* 53(Supplement 2): 348–65.

Thia Prameswarie et al. 2023. "*Aedes aegypti* Hatchability and Larval Development Based on Three Different Types of Water." *Majalah Kesehatan Indonesia* 4(1): 27–32.

Tsegaye, Arega et al. 2023. "Anopheles Larval Habitats Seasonality and Environmental Factors Affecting Larval Abundance and Distribution in Arjo-Didessa Sugar Cane Plantation, Ethiopia." *Malaria Journal* 22(1): 1–12. https://doi.org/10.1186/s12936-023-04782-1.

World Health Organization. Dengue-Global Situation. 2024. https://www.who.int/emergencies/disease-outbreak-news/item/2024-DON51

Biota
Biologi dan Pendidikan Biologi
http://biota.ac.id/index.php/jb
DOI: http://dx.doi.org/10.20414/jb.v17i2.515

Research Article

Identification of the Heavy Metal Lead (Pb) in Red Macro Algae (Gracilia Sp) In the waters of Tanjung Luar, East Lombok

Almahera[1], Ahmad Aldi Kurniawan[1], B Firia Maharani[1], Yusfina Himayanti[2], Novia Dara Puspita[1], Baiq Repika Nurul Furqan[1*]

[1]*Departement of Pharmacy, Universitas Nahdlatul Ulama Nusa Tenggara Barat*
[2]*Departement of D-III Medical Records and Health Information, Universitas Nahdlatul Ulama Nusa Tenggara Barat*

**Corresponding author: repika.nf@gmail.com*

Abstract

Tanjung Luar Village is located in Keruak District, East Lombok Regency, West Nusa Tenggara, is a densely populated body of water human activity. In Tanjung Luar waters, household and ship waste dumped directly into the sea, polluting the local ecosystem. this condition important because people in the area consume a lot of produce marine catches, including red macroalgae (*Gracilaria Sp*). If *Gracilaria Sp.* is contaminated with levels of the heavy metal lead (Pb) that exceed threshold of 0.2 mg/kg, can be dangerous to human health impact on the nervous system, urinary system, endocrine system, problems gastrointestinal, and a very high risk of cancer. That thing based on BPOM Regulation No. 23 of 2017 (<0.2 mg/kg) for consumption materials. Therefore pollution and controlling the use of the heavy metal lead (Pb) important, especially in waters that play a supporting role life and food of local communities. The aim of this research is to determine the levels of the heavy metal lead (Pb) contained in the sample red macroalgae (*Gracilaria Sp.*) in Tanjung Luar Waters. Purposeful research to provide an overview and explanation of the issues discussed. This research uses the wet digestion method to identify heavy metal lead (Pb) of *Gracilaria sp* in waters Tanjung Luar, East Lombok. Next, levels of the heavy metal lead (Pb) were tested with ICP-OES spectrophotometry. With this method, researchers can identify the concentration of the heavy metal lead (Pb) in *Gracilaria Sp.* with a high level of accuracy. The results of the study showed that all sampling points of *Gracilaria sp.* did not exceed the threshold, which was less than 2 mg/kg. where at the sampling location point 1 had Pb concentrations of 0.072 ppm (location A), 0.063 ppm (location B), 0.057 ppm (location D), 0.051 ppm (location E) and 0.046 ppm (location F). for point 2 had Pb concentrations of 0.096 ppm (location A), 0.083 ppm (location B), 0.071 ppm (location C), 0.065 ppm (location E) and 0.059 ppm (location F). for point 3, the Pb concentrations were respectively 0.142 ppm (location A), 0.108 ppm (location C), 0.097 ppm (location D), 0.084 ppm (location E) and 0.070 ppm (location F).

Keywords: identification; heavy metals; red macroalgae; outer cape; east Lombok

1. Introduction

Metal pollution is a serious environmental concern in coastal areas. Metals, often referred to as heavy metals due to their density exceeding 5 g/cm^3, can accumulate in seawater from various sources. These metals may enter the ocean through human activities on land, such as industrial waste, agricultural runoff, and mining. They can also be deposited from the atmosphere or result from natural occurrences like volcanic eruptions (Hartati et al., 1993).

Once metals enter the water, they are persistent and difficult to break down, allowing them to accumulate in aquatic environments over time (Nontji, 1993). This accumulation

can harm marine life, both directly, through toxicity, and indirectly, by disrupting ecosystems. Some metals, including mercury, lead, and cadmium, can build up in the food chain, leading to higher concentrations in marine animals and, eventually, humans. This bioaccumulation poses significant risks to both ecological and human health.

The heavy metal lead (Pb) is a pure toxic substance that is commonly used in industry to stabilize certain compounds. In daily life, lead (Pb) is used in various fields such as mining, metal smelting and fuel oil. However, its use has the potential to become a toxic material when accumulates in living things. Lead (Pb) is usually used in various products, such as paint mixtures, pesticides, and as ingredients addition to vehicle fuel (Afandi, dkk 2017). Lead (Pb) is currently one of the primary pollutants in aquatic environments (Suhendrayatna, 2001). Lead is a neurotoxin that accumulates in organisms, causing harmful effects (Winarno, 1993). The main source of lead pollution comes from motorized vehicles, which release lead waste into the environment (Fardiaz, 1992). Lead enters water bodies through rainwater, following a crystallization process. Additionally, the corrosive action of waves and wind contributes to the presence of lead in marine environments (Palar, 2012).

West Nusa Tenggara, especially Lombok Island, is a producer of quite a lot of macroalgae (no. 5 in Indonesia), one of the locations where macroalgae are widely cultivated is the Tanjung Luar area, Keruak, East Lombok. The macroalgae that are widely cultivated are Kappaphycus alvarezii, Eucheuma sp. and Gracilaria sp. which are generally cultivated in the sea (Anggadiredja et al., 2008).

Gracilaria sp. is a type of red macroalgae that has a beta carotene content that is almost close to carrots (Phang et al, 2010). This makes it widely used as a thickener and stabilizer in the food, pharmaceutical, and cosmetic industries and is needed for its cell wall polysaccharides. In addition, it has been used in health drinks and anticancer nutraceuticals because of its antioxidant content and other nutritional compounds (Cornish and Garbary, 2010).

Based on data from the Ministry of Maritime Affairs and Fisheries' "REA CoFish Project 2022", a number of locations in the southern coastal area of East Lombok that are contaminated with heavy metal Lead (Pb) include Tanjung Luar (0.0163 ppm), Gili Maringkik (0.0367 ppm), Tanjung Sagui (0.0183ppm), Gili Linus (0.0343 ppm), Surga Beach (0.0193 ppm). This is based on the regulation in PP No. 22 of 2021 that the threshold for Pb content is <0.008 mg/L for sea water. The heavy metal that polluted the southern coastal area of East Lombok came from waste. This Environmental NGO figure suspects that PT. AMNT's tailings were dumped into the sea in Senunu Bay.

Therefore, it is necessary to identify the lead concentration in Glaciralia sp found on Tanjung Luar Beach, whether it is contaminated above the threshold or not, where based on BPOM Regulation No. 23 of 2017, the Pb threshold value is around <0.2 ppm for consumption materials

2. Material and Method

2.1 Materials

Red macroalgae, Hydrochloric acid (HCL), Nitric acid (HNO3), Aquabidest, ICP-OES, measuring cup, beaker, analytical balance

2.2 Methods

The method used in this study is the transect method with quadrant transects. This method is used to collect data on the distribution and presence of red macroalgae in the waters of Tanjung Luar, East Lombok. The sampling location is shown in Figure 1, which consists of 33 square points from three transects. Coordinates of the 33 square points. The squares used are rectangular with a size of 50 x 50 cm. The distance between squares on the same transect line is around 5 m, so there are 11 squares along the 50 m transect line. The distance between transects is around 25 m.

Location 0 indicates the shoreline (0 m), location A is indicated by 5 m which is the distance of the sampling location from the shoreline, location B is indicated by 10 m, location C for 15 m, location D for 20 m, location E for 25 m, location F is marked by 30 m and so on up to location J for 50 m. the same applies to sampling points 1, 2 and 3 (Figure 1).

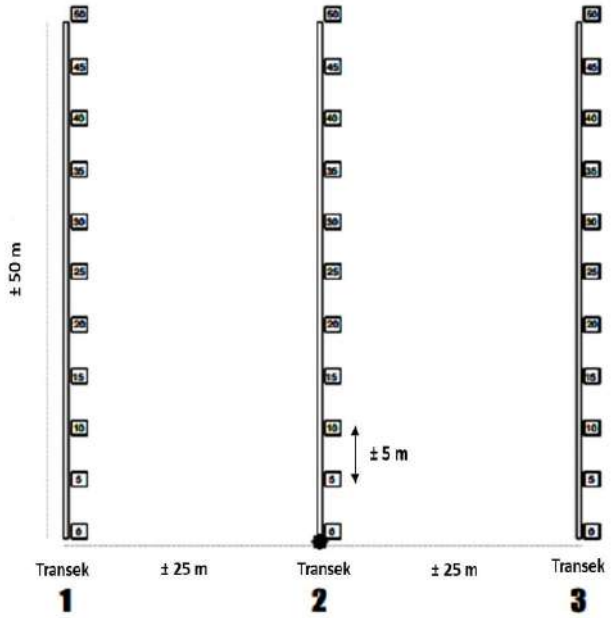


Figure 1. Sketch of the quadrant transect where samples of red macroalgae Gracilaria sp were taken.

Sample Determination

Morphological analysis of macroalgae samples was guided by an identification key (Jaasund, 1977; Koeman and Hoek, 1981; Atmadja et al., 1996; Ateweberhan et al., 2005; Lyer et al., 2013). Morphological analysis was carried out based on visual observation and documented using digital camera photos. Parameters observed included color, shape and

branching of the thallus. The morphology of macroalgae samples was evaluated and compared with the morphology of macroalgae found in the literature. Some of the literature used were Miller et al. (1998), Kogame et al. (2001), McKenzie et al. (2003), Uwai et al. (2005) and Guiry and Guiry (2017 and 2018).

Sample Preparation

The sample preparation process carried out on red macro algae is quite important to prepare samples before further analysis. Sampling: macro samples of red algae are taken in large quantities 500 grams. This sample is then dried to remove moisture and maintain stability during the analysis process. Sample cutting: after dried, the red algae macro samples were cut into small and thin pieces. The purpose of this cutting is to reduce the surface area of the sample, thus making the subsequent drying process easier. Sample grinding: The sample pieces were then crushed using mortar and Stamper until it becomes small and fine flakes. This erosion is purposeful to speed up the sample destruction process, namely decomposing the sample with strong acid to obtain the analyte in solution

Destruction

The sample destruction process was carried out at the University's Integrated Laboratory Nahdlatul Ulama West Nusa Tenggara is an important step in sample preparation before testing using ICP-OES. Sample preparation: 1 gram macro sample of red algae was put into a beaker measuring 100 ml. The purpose of this step is to prepare a sample will be destroyed. Addition of aqua regia: aqua regia, which is a mixture of nitric acid (HNO3) and concentrated hydrochloric acid (HCl), are added to the glass beaker with a ratio of 3:1 (HNO3:HCl). A total of 4 ml of aqua regia added to the sample. Aqua regia is a strong acid that can dissolve heavy metals in the sample, including the heavy metal lead (Pb). Warmup: the sample to which aqua regia has been added is heated using hot plate at 80°C for 30 minutes. This heating process aims to dissolve the sample and turn it into a solution so that the content the elements in it can be measured. Cooling: after heating process Once completed, the sample is taken from the hot plate and cooled. This is done for Cool the sample so it is ready for the next step. Addition aquabidest: after the sample has cooled, aquabidest (purified water) is added to it in the sample until the volume increases to 25 ml.

Addition

Aquabidest aims to dilute the sample solution so that it is ready for use tested using ICP-OES. iltration: samples that have been digested and form a solution and then filter it using filter paper. Filtration process This aims to separate the solution from debris or other particles may be present in the sample. Testing with ICP-OES: filtered filtrate and clear, ready to be tested for metal content using ICP-OES. ICP OES is a spectroscopic analysis method for measuring the concentration of elements in samples.

Testing for Heavy Metal Lead (Pb) Levels

After the digestion process is complete, the organic substances in the sample will be taken and tested with ICP-OES spectrophotometry. ICP-OES spectrophotometry can determine up to 70 elements simultaneously, in an inert environment and atomization temperature higher, and has a lower analyte detection limit than AAS instrument.

3. Results and Discussion

3.1. Results

Only 15 samples were obtained from 33 points in the quadrant transect, while at point 1 collection location 5 samples were obtained from 5 points, namely points A (5 m), B(10 m), D (20 m), E (25 m) and F (30 m), point 0 (shoreline), points C, G, H, I and point J did not find *Gracilaria sp* samples.

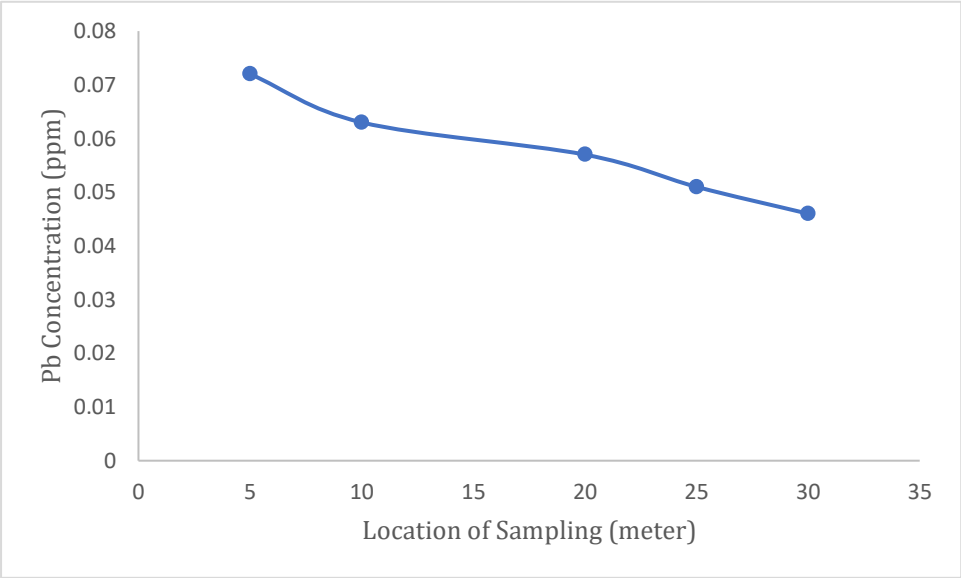


Figure 2. Graph of Pb concentration data in point 1 at each sampling location A (5 m), B (10 m), D (20 m), E (25 m) and F (30 m)

At Point 1, Gracilaria sp seaweed samples were taken from several distances starting from the shoreline. The following are the results of the concentration of heavy metal lead (Pb) at each sampling point, as seen in figure 2:

1. A distance of 5 meters (location A) obtained a concentration of heavy metal lead (Pb) of 0.072 ppm.
2. A distance of 10 meters (location B) obtained a concentration of heavy metal lead (Pb) of 0.063 ppm.
3. A distance of 20 meters (location D) obtained a concentration of heavy metal lead (Pb) of 0.057 ppm.
4. A distance of 25 meters (location E) obtained a concentration of heavy metal lead (Pb) of 0.051 ppm.
5. A distance of 30 meters (location F) obtained a concentration of heavy metal lead (Pb) of 0.046 ppm.

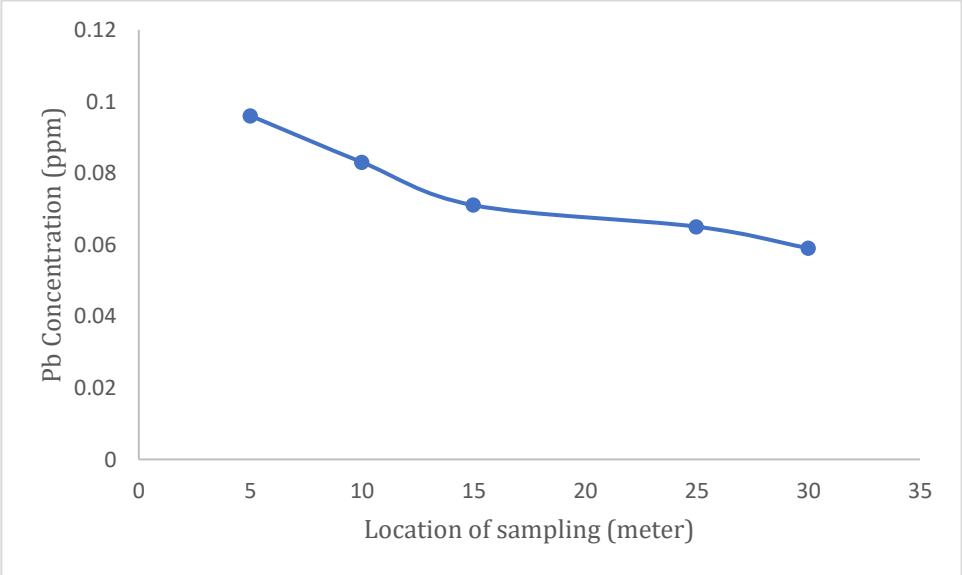


Figure 3. Graph of Pb concentration data in point 2 at each sampling location A (5 m), B (10 m), C (15 m), E (25 m) and F (30 m)

At Point 2, collection location 5 samples were obtained from 5 points, namely points A (5 m), B (10 m), C (15 m), E (25 m) and F (30 m), point 0 (shoreline), points D, G, H, I and point J did not find Gracilaria sp samples. The following are the results of the lead (Pb) heavy metal concentration at each sampling point, as seen in figure 3:

1. A distance of 5 meters (location A) obtained a lead (Pb) heavy metal concentration of 0.096 ppm.
2. A distance of 10 meters (location B) obtained a lead (Pb) heavy metal concentration of 0.083 ppm.
3. A distance of 15 meters (location C) obtained a lead (Pb) heavy metal concentration of 0.071 ppm.
4. A distance of 25 meters (location E) obtained a lead (Pb) heavy metal concentration of 0.065 ppm.
5. A distance of 30 meters (location F) obtained a lead (Pb) heavy metal concentration of 0.059 ppm.

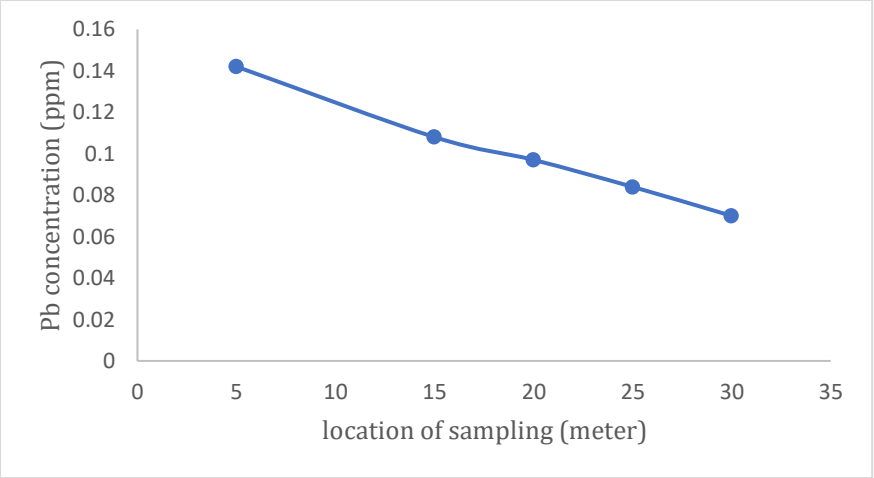


Figure 3. Graph of Pb concentration data in point 3 at each sampling location A (5 m), C (15 m), D (20 m), E (25 m) and F (30 m)

At Point 3, collection location 5 samples were obtained from 5 points, namely points A (5 m), C (15 m), D (20 m), E (25 m) and F (30 m), point 0 (shoreline), points B, G, H, I and point J did not find Gracilaria sp samples. The following are the results of the lead (Pb) heavy metal concentration at each sampling point, as seen in figure 4:

1. A distance of 5 meters (location A) obtained a lead (Pb) heavy metal concentration of 0.142 ppm.
2. A distance of 15 meters (location C) obtained a lead (Pb) heavy metal concentration of 0.108 ppm.
3. A distance of 20 meters (location D) obtained a lead (Pb) heavy metal concentration of 0.097 ppm.
4. A distance of 25 meters (location E) obtained a lead (Pb) heavy metal concentration of 0.084 ppm.
5. A distance of 30 meters (location F) obtained a lead (Pb) heavy metal concentration of 0.070 ppm.

3.2. Discussion

The heavy metal lead (Pb) in the waters of Tanjung Luar, East Lombok is suspected to come from PT. AMNT tailings waste which is dumped into the sea in Senunu Bay, based on data from the "REA CoFish Project 2022" of the Ministry of Maritime Affairs and Fisheries, their data states that a number of locations on the southern coast of East Lombok are contaminated with heavy metal Lead (Pb), one of which is located in Tanjung Luar with Pb contamination of 0.0163 ppm (where the threshold for Pb content in seawater is <0.008 ppm) (Hidayatullah, et al., 2023).

In addition, it is suspected that the lead (Pb) found in the waters of Tanjung Luar, East Lombok comes from natural sources, rainwater can crystallize lead (Pb) in the air, and the process of mineral rock corrosion is one way for lead (Pb) to enter the waters (Azizah et al., 2018). Neurotoxins are the properties of heavy metal lead (Pb). can enter and

accumulate in the human body, animals, and plants. Heavy metals can enter the bodies of aquatic organisms through the gills, body surface, digestive tract, muscles, and heart. The toxicity of the heavy metal lead (Pb) is more important as a therapy. The body absorbs lead (Pb) very slowly, which causes accumulation and triggers poisoning (Septriani et al., 2023).

The distribution graph of heavy metal lead (Pb) showing a decrease from each sampling point on red macroalgae shows variations in heavy metal concentrations in the waters. The decrease in lead (Pb) concentration along with the increasing distance from the coastline to the middle of the sea in the Tanjung Luar Waters of East Lombok can be interpreted that the distribution of tailings contamination that has polluted seawater affects the concentration of Pb in macroalgae Gracilaria sp. dimming each point. The closer the sampling location is to the coastline, the higher the concentration of heavy metal lead (Pb) in red macroalgae Gracilaria sp. Conversely, the further from the coastline, the concentration of heavy metal lead (Pb) in red macroalgae Gracilaria sp. is getting lower (Siaka, 2016).

This could be due to the lifespan of *Gracilaria sp*. At each point, where Gracilaria sp. is able to absorb heavy metals in high concentrations at the beginning of planting and is able to release them again before harvest, so that the older the age of Gracilaria sp. The lower the Pb content. This is in accordance with the results of a study that measured the Pb content in sampling Gracilaria sp. where 20 days in Pond 1 were 7.61 ppm and Pond 2 were 5.35 ppm. Analysis of Pb levels in the holdfast and talus of Gracilaria sp. obtained the highest Pb content at the age of 0 days before planting, which was 3.38 ppm and decreased until the post-harvest age of 40 days, which was 0.84 ppm (Tega et al, 2019). So it is still below the permitted threshold.

Factors that can affect the distribution of heavy metal lead (Pb) in waters include the amount and type of human activities that produce heavy metal waste, air discharge, wind direction, and physical and chemical conditions of the waters. Therefore, spatial monitoring of lead (Pb) heavy metal levels in waters is important to determine its distribution pattern and identify potential sources of pollution. Efforts to manage and overcome heavy metal pollution in waters need to be carried out to maintain environmental quality and human health (Siaka, 2016).

Conclusion

The results of the study showed that all sampling points of Gracilaria sp did not exceed the threshold, which was less than 2 mg/kg. where at the sampling location point 1 had Pb concentrations of 0.072 ppm (location A), 0.063 ppm (location B), 0.057 ppm (location D), 0.051 ppm (location E) and 0.046 ppm (location F). for point 2 had Pb concentrations of 0.096 ppm (location A), 0.083 ppm (location B), 0.071 ppm (location C), 0.065 ppm (location E) and 0.059 ppm (location F). for point 3, the Pb concentrations were respectively 0.142 ppm (location A), 0.108 ppm (location C), 0.097 ppm (location D), 0.084 ppm (location E) and 0.070 ppm (location F).

References

Affandi, A. S., Rahardja, B. S., & Suprapto, H. (2017). Pengaruh Kombinasi Biofilter Dan Zeolit Terhadap Logam Berat Timbal (Pb) Pada Media Air Laut , (2), 10-17.

Alhafizoh, F. (2021). Kandungan Logam Berat Pada Alga Hijau Di Perairan Way Ratai Kabupaten Pesawaran Provinsi Lampung.

Amanda, R., Nuraini, R. A. T., & Supriyantini, E. (2021). Korelasi Antara Daya Serap Terhadap Konsentrasi Logam Berat Cu di Media Pemeliharaan. Journal of Marine Research, 10(3), 363-368 .

Anggadiredja, J.T., A. Zatnika, H. Purwato, & S. Istini. 2008. Rumput laut, pembudidayaan, pengolahan dan pemasaran komoditas perikanan potensial. Penebar Swadaya: Jakarta.

Bila, V. S. (2022). Analisis Logam Berat Pb Dan Cd Pada Rumput Laut *(Eucheuma Cottonii),* Air Dan Sedimen Pada Daerah Budidaya Kabupaten Bantaeng Dengan Menggunakan Spektrofotometer Serapan Atom (SSA). (*Doctoral Dissertation*, Universitas Hasanuddin).

Cornish, M., D. Garbary. 2010. Antioxidants from macroalgae: potential applications in human health and nutrition. *Algae.* 25. 155171.

Guiry, M.D. dan Guiry, G.M. (2018): AlgaeBase, www.algaebase.org.

Harahap, M. R., Yulian, M., & Agusti, A. N. (2019). Analisis Logam Timbal Dan Tembaga Terhadap Daya Serap Rumput Laut *Gracilaria Sp*. Sebagai Biosorben. *Amina*, *1*(2), 45-58.

Hartati, R.I., Riyantini, & A. Djunaedi. 1993. Pemantauan Logam-Iogam Berat pada Kenangkerangan yang Dihasilkan dari Perairan Pantai Utara Gunung Muria, PPLH Undip, Semarang, 38 Hal.

Hidayatullah, N., Yasir, I., & Tahir, A. (2023). Heavy Metals Content of Copper (Cu) and Lead (Pb) in Codium Fragile Seaweed. *Jurnal Ilmu Kelautan SPERMONDE*, 27-32.

Iyer, R., De Clerck, O., Bolton, J. J., Coyne, V. E., & Sym, S. D. (2013): Morphological and taxonomic studies of Gracilaria and Gracilariopsis species (Gracilariales, Rhodophyta) from South Africa, *South African Journal of Botany*, **70**(4), 521–539.

Jaasund, E. (1976): *Seaweeds in Tanzania: A field guide*, University of Tromsø,Tromsø, 160.

Kementrian Kelautan dan Perikanan RI. REA Proyek Cofish. Kawasan Pesisir Selatan Lombok Timur Tercemar Logam Berat Timbal. November 2022. NTB Poss.

Kogame, K., Uwai, S., & Kawaguchi, S. (2001): First record of Asteronema rhodochortonoides (Phaeophyceae) for the Pacific Ocean. *Phycological Research*, **49**(4), 281–284.

Laut, A. (2016). Distribusi Logam Berat Pb Dan Cu Pada Air Laut, Sedimen, Dan Rumput Laut Di Perairan Pantai Pandawa.

Millar, A.(2011): Marcroalgae, New South Wales Department of Primary Industries, Sydney.

Nontji, A. 1993. Laut Nusantara: Djambatan, Jakarta.

Peraturan Badan Pengawas Obat Dan Makanan (BPOM) Nomor 23 Tahun 2017 Tentang Batas Maksimum Cemaran Logam Berat Dalam Pangan Olahan.

Peraturan Pemerintah Republik Indonesia Nomor 22 Tahun 2021 tentang Penyelenggaraan Perlindungan dan Pengelolaan Lingkungan Hidup.

Phang, S.M., H.Y. Yeong, P.E. Lim, A.R.M. Nor, K.T. Gan. 2010. Commercial varieties of *Kappaphycus* and *Eucheuma* in Malaysia.*Mal. J. Sci.* 29(3). 214-224.

Puspaningrum, D. A. D. (2021). Pengaruh waktu detensi tumbuhan eceng gondok (*Eichornia crassipes (Mart.) Solms*) terhadap penyerapan timbal (pb) sebagai sumber belajar biologi *(Doctoral dissertation*, Universitas Muhammadiyah Malang).

Saraswati, A. R., & Rachmadiarti, F. (2021). Analisis Kandungan Logam Berat Timbal (Pb) Pada Rumput Laut Di Pantai Sendang Biru Malang. Lenterabio: Berkala Ilmiah Biologi, 10(1), 67-76.

Tega, Y. R., Herawati, E. Y., & Kilawati, Y. (2019). Heavy Metal (Pb) and Its Bioaccumulation in Red Algae (Gracilaria sp.) At Kupang Village, Jabon Sub-District, Sidoarjo District. *The Journal of Experimental Life Science*, *9*(2), 139-146.

Biota

Biologi dan Pendidikan Biologi

Editorial Office:
Universitas Islam Negeri Mataram
Jalan Gajah Mada Nomor 100 Mataram, 83125
Website: https://biota.ac.id/index.php/jb
email: jurnalbiota@uinmataram.c.id
Telp: (0370) 621298