https://doi.org/10.1051/bioconf/202414701025

Quality and Safety of Green Mussel (*Perna viridis*) at Ketapang, Tangerang Regency, Indonesia

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Abstract. This study aimed to determine the chemical and microbiological qualities of raw green mussels of different sizes in Ketapang Village, Tangerang Regency, Indonesia. The research method used was a single-factor treatment design of green mussel size with small (51-60 mm), medium (61-70 mm), and large (71–80 mm) sizes with environmental design and Complete Random Design. The parameters used were the content of heavy metals Pb, proximate, and microbiology. ICP-MS was used for heavy metal testing. The method of analyzing the proximate content and microbiology refers to the Indonesian National Standard (SNI). The results of the study showed that green mussels with shell sizes of 51-60 mm and 71-80 mm significantly affected the lead (Pb) content. The different sizes of green mussel shells significantly affected the protein and fat content. As for the carbohydrate content, a significant difference was only shown by green mussels with shell sizes 51-60 mm and 71-80 mm. The results of microbiological tests showed that the Total Plate Count in green mussels did not exceed the SNI, the *Escherichia coli* content exceeded the SNI limit, and there were green mussel samples that were positive for *Salmonella* and *Vibrio parahaemolyticus*.

1 Introduction

Ketapang is a village located in Mauk District, Tangerang Regency, Banten Province. The Tangerang Regency Government has established a mangrove education center within the mangrove conservation area of Ketapang Village. The development of fish cultivation and processing in this area aims to support the growth of the “Eduwisata” or educational tourism zone. This requires enhancing the quality and safety of fishery products to ensure that they remain safe and high-quality for the local community and visitors to the Eduwisata area. One

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of the key cultivation activities practiced by the people of Ketapang Village is the farming of green mussels (*Perna viridis*).

The production of green mussels (*Perna viridis*) is processed and marketed both locally and outside the Tangerang Regency area. According to research by Raryanti [1], the waters of Ketapang Village are suitable for supporting the life of green mussels (*Perna viridis*) and can serve as a green mussel cultivation area. However, studies on the risk assessment of green mussels (*Perna viridis*) in various locations along the north coast of Java, conducted by [2-12], indicate that green mussels (*Perna viridis*) contain heavy metals and may pose health risks.

Heavy metals are among the dangerous substances that are difficult to degrade, causing aquatic organisms to easily absorb these metals from polluted waters, which then accumulate in their bodies—green mussels [4] being one example. Falah [2] stated that green mussels (*Perna viridis*), as filter feeders and sessile organisms, absorb heavy metals from the water column and sediment through the feeding process. Green mussels (*Perna viridis*) can survive in polluted waters, including environments with heavy metal concentrations exceeding specified thresholds [13]. Heavy metals are toxic, with the most biotoxic metals being Hg, Pb, Cd, and Cr [14]. Mercury (Hg) poisoning leads to neurotoxic diseases, while lead (Pb) poisoning can affect almost all human organ systems. Cadmium (Cd) poisoning causes damage to the lungs, kidneys, liver, and bone. According to Andayani [11], most green mussels harvested from the northern coast of Java are contaminated with heavy metals and are unsafe for consumption.

Industrial waste, domestic waste, and garbage entering the waters where green mussels (*Perna viridis*) are cultivated not only contribute to heavy metal contamination but also lead to bacterial contamination of mussel meat. This study aimed to identify the chemical and microbiological quality of raw green mussels of various sizes in Ketapang Village, Tangerang Regency, Indonesia.

2 Materials and Methods

2.1 Samples

This research began by collecting live, freshly harvested green mussels (*Perna viridis*) from the aquaculture area in Ketapang Village, Tangerang Regency, Indonesia. The samples were sorted by size and cleaned of any impurities.

2.2 Heavy Metal Testing

The heavy metal content of Pb, Hg, and Sn was determined in both raw and boiled green mussel meat using ICP-MS (Inductively Coupled Plasma Mass Spectrometry). The heavy metal testing procedures followed the methods outlined in previous studies [15, 16].

2.3 Proximate composition

The proximate content of raw and boiled green mussels was tested, including water, protein, fat, and ash content, following the Indonesian National Standard (SNI). Testing was conducted in triplicate at the laboratory of the Jakarta Technical University of Fisheries. The water content of raw green mussels was tested according to SNI 2354.2:2015 [17] and the protein content was tested according to SNI 01-2354.4:2006 [18]. Additionally, the fat content of raw green mussels was determined by referring to SNI 2354-3:2017 [19], while the ash content was tested in accordance with SNI 2354.1:2010 [20]. The carbohydrate content was determined by difference, ensuring that the calculated value represented the overall proximate composition. The formula for calculating carbohydrate content is presented below:

$$\text{Carbohydrate content} = 100\% - (\%\ \text{protein content} + \%\ \text{fat content} + \%\ \text{ash content} + \%\ \text{water content}) \quad (1)$$

2.4 Data Analysis

The research method employed a single-factor treatment design based on green mussel size: small (51-60 mm), medium (61-70 mm), and large (71-80 mm), within a Completely Randomized Design (CRD) environmental framework. The measured parameters included the heavy metal (Pb) content, proximate composition, and microbiological quality. Data analysis was conducted using Analysis of Variance (ANOVA), followed by Duncan's multiple range test for further comparison.

3 Results and Discussions

3.1 Lead (Pb) Content

The subject of this heavy metal testing was raw green mussels (*Perna viridis*) collected from the aquaculture area in Ketapang Village. The lead (Pb) content in these raw green mussels was tested to assess their safety for consumption. The average results of lead (Pb) content testing in raw green mussels are presented in Table 1.

Table 1. Lead (Pb) content of green mussels' meat (*Perna viridis*) with different shell sizes

Green Mussel Shell Sizes (mm)	Lead (Pb) (mg/kg)
51–60	0.17 ± 0.01^{b}
61–70	0.17 ± 0.05^{b}
71–80	0.25 ± 0.02^{a}

Note : The difference in letters in superscript indicates a significant difference

Lead (Pb) heavy metal testing was conducted on three different shell sizes. Table 1 shows that the lead (Pb) content in raw green mussels of varying shell sizes from the aquaculture area in Ketapang Village remains below the threshold specified in SNI 3450.1:2009 [21]. Therefore, green mussels from this aquaculture area are considered safe and fit for consumption. However, consumers should still observe the maximum consumption limits for green mussels, as presented in the safe consumption guidelines [3].

Based on the ANOVA results, the p-value for lead (Pb) content was 0.018 (< 0.05), leading to the rejection of H0. In other words, the three shell size groups had significantly different effects on lead (Pb) content. Due to these significant differences among the sizes, Duncan's post hoc test was conducted to determine the effects of each shell size group. The results indicated that green mussels with shell sizes of 51-60 mm and 61-70 mm did not have a significantly different effect on lead (Pb) content, whereas green mussels with shell sizes of 71-80 mm showed a significantly different effect, exhibiting the highest lead (Pb) content among the groups.

According to Andayani [11], the accumulation of heavy metal concentrations in mussels depends on factors such as size, sex, food habitat, reproductive condition, season, and pollution levels. Salman [22] also notes that heavy metal accumulation in aquatic organisms is influenced by age, body weight, species, and the concentration of heavy metals in water and sediment. Green mussels, being sessile marine organisms, experience higher heavy metal concentrations in their bodies the longer they live in waters with high levels of heavy metals. This extended lifespan often results in larger green mussels, with size closely linked to the level of accumulated heavy metals in their bodies. Sulistiyaningsih [23] stated that green mussel growth can be observed by increasing shell size, with shell length generally serving as a key parameter for assessing their growth.

Falah [2] reported that lead pollution primarily originates from motor vehicle exhaust and industrial waste. Lead (Pb) is commonly used as a gasoline additive, with an estimated 65% of motor vehicle emissions contributing to overall lead pollution. This air pollution eventually accumulates in the aquaculture area of Ketapang Village. Similarly, Zhang [24] suggested that lead (Pb) pollution in the air originates from emissions due to transportation, industrial activities, battery manufacturing, and coal combustion.

Green mussels can serve as bioindicators of heavy metal pollution in aquatic environments. They live on the seabed and obtain food through filter feeding, which involves filtering both sediment and seawater [6, 25, 26]. This feeding behavior enables heavy metals to readily enter and accumulate in the bodies of green mussels over time [10].

Lead (Pb) can be absorbed by filter-feeding species in aquatic environments or through their food sources, such as phytoplankton, zooplankton, and microscopic plants, which also contain accumulated lead and eventually bind it to their body tissues [9]. Additionally, according to Barokah [10], lead (Pb) can accumulate in waters due to various sources, including community activities, the disposal of used batteries in water bodies, paint peeling from pipes, and residue from the combustion of fuel in boat engines used by fishermen for transportation.

Eshmat [9] stated that heavy metal concentration is influenced by the chemical and physical factors of water. The quality of water and sediment in the habitat of live green mussels directly affects the heavy metal content within them. This means that if the concentration of heavy metals is high in the water, the concentration in sediments will also be high. High concentrations of heavy metals in sediments can lead to increased accumulation within marine organisms, particularly sessile species [22]. Additionally, water currents play a role in dispersing heavy metals from the surface to deeper areas. Current patterns influence the distribution of heavy metals in water, eventually leading to their entry into green mussel bodies. If water current velocity is too high, green mussels struggle to filter their food, whereas slow currents can impede growth and lead to harmful substance deposits in the water [1].

Heavy metals are among the hazardous materials that can gradually accumulate in green mussels and pose risks to those who consume them. The waters of Ketapang Village are near the river mouth, where pollutants may be carried downstream at specific current velocities. Raryanti [1] explains that, based on measurements, the water current velocity in Ketapang Village ranges from 1 m/s to 3.5 m/s (considered medium), which supports green mussel cultivation. This aligns with testing results indicating that lead (Pb) content in green mussels remains below the SNI safety threshold.

Green mussels can reduce heavy metal levels in their bodies through excretion in feces and urine. While this reduction is limited, it helps to minimize the heavy metal content in green mussels [27]. Additionally, lead (Pb) levels in green mussels can be further reduced through immersion treatments at specific concentrations and durations. For example, Mahardika [28] found that lead (Pb) content decreased significantly by 59.79%, from 0.97 ppm to 0.39 ppm, after a 90-minute immersion treatment with tomatoes (*Lycopersicum esculentum*).

3.2 Proximate Content

The proximate content, or chemical composition, of foodstuffs reflects the quantity and quality of nutrients they provide, contributing to human nutritional intake [29]. Proximate content testing aims to determine the nutritional composition of green mussels (*Perna viridis*) sourced from the aquaculture area in Ketapang Village, Mauk, Tangerang, Banten. The nutritional information obtained from proximate testing can serve as a reference for the community in handling and further processing [30]. The average results of proximate content testing on green mussels are presented in Table 2.

Table 2. Proximate content of green mussels meat (*Perna viridis*) with different shell sizes

Sizes (mm)	Water (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
51–60	81.36±0.05^a	10.27±0.12^a	2.11±0.06^a	2.47±0.09^a	3.78±0.11^a
61–70	82.19±0.48^a	9.53±0.05^b	1.37±0.03^b	2.55±0.06^a	4.56±0.25^a
71–80	83.53±0.11^a	9.19±0.11^c	0.23±0.02^c	2.57±0.04^a	4.47±0.24^a

Note : The difference in letters in superscript indicates a significant difference

3.2.1 Water Content

Water or moisture content represents the amount of water in a material necessary for digestion and metabolism [31]. Water content analysis in raw green mussels was conducted based on different shell sizes. The average results indicated that green mussels with shell sizes of 51-60 mm had the lowest water content, while those with shell sizes of 71-80 mm had the highest. According to the ANOVA results, the p-value for the water content in raw green mussels was 0.383 (> 0.05); therefore, H0 was accepted. This indicates that shell size does not significantly affect water content in green mussels.

The findings of the water content analysis align with previous research. For instance, Purwaningsih [29] reported a water content of 82.25% in green mussels, which was higher than the results obtained by Mahardika [28] and Pebrian [32], who reported values of 80.75% and 78.86%, respectively. This variation in water content across studies is likely due to differences in water content among fish species, among individuals within a species, and even among different body parts within a single individual [33].

3.2.2 Protein Content

The protein content analysis showed that green mussels with shell sizes of 71-80 mm had the lowest protein content, while those with shell sizes of 51-60 mm had the highest. According to the ANOVA results, the p-value for protein content in raw green mussels was 0.000 (< 0.05), leading to the rejection of H0. This indicates that shell size significantly affects protein content in green mussels. Due to these significant differences, Duncan’s post hoc test was conducted to assess the effects among each shell size group. The results revealed that green mussels with shell sizes of 51-60 mm, 61-70 mm, and 71-80 mm all showed significant differences in protein content. Specifically, green mussels with shell sizes of 51-60 mm had the highest protein content.

These findings are consistent with previous research by Mahardika [28], Purwaningsih [29], and Pebrian [32], who reported protein contents of 9.17%, 9.98%, and 11.84%, respectively, for green mussels with shell sizes of 71-80 mm. Additionally, the protein content found in this study falls within the ranges reported by Murdinah [34] (7.06–16.78%) and by other researchers [35] (7.14–13.1%).

According to Akbar [36], the crude protein content in mussels ranges from 9% to 13%. Suwandi [37] reported that factors such as food type, habitat, and food availability influence protein levels in fish. Variations in protein content among green mussels likely result from differences in the availability of microalgae, their primary food source, as filter feeders (*Bivalvia Mollusca* and *Perna viridis*) rely on microalgae [35]. Microalgae are autotrophic organisms that convert carbon dioxide into biofuels, food, and bioactive compounds using sunlight. They contain proteins, fats, unsaturated fatty acids, pigments, and vitamins [38].

The protein content in green mussels depends on the availability of food in their habitat, as they feed by filtering particles from the water using their gills. However, their filtration ability may be impaired if their gills become covered in mucus. Green mussels secrete mucus in response to the accumulation of lead (Pb) in their gill tissues. As a sessile species, green mussels remain in lead-contaminated waters for extended periods, leading to increased lead concentrations in their gills. This build-up stimulates mucus production, which further covers the gill surface. As a result, the filtration and respiration abilities of green mussels decline, reducing their intake of food and oxygen [39].

The research findings indicate that as green mussels grow larger, their protein content decreases. Thus, smaller green mussels are more efficient at obtaining food. Furthermore, lead (Pb) content testing shows that larger mussels accumulate more heavy metals. This increased heavy metal content disrupts the filtration process, which in turn limits the intake of nutrient-rich food sources, such as protein and fat, as evidenced by the test results [39].

3.2.3 Fat Content

Excess energy stored by animals is in the form of fat, and an animal's energy balance determines the amount of fat in its body available as food [31]. Belitz [40] noted that fat is valuable as a source of essential fatty acids and vitamins (A, D, E, and K).

The fat content analysis results indicated that green mussels with shell sizes of 71-80 mm had the lowest fat content, while those with shell sizes of 51-60 mm had the highest. According to the ANOVA results, the p-value for fat content in raw green mussels was 0.000 (< 0.05), leading to the rejection of H0. This means that shell size has a significant effect on the fat content in green mussels. Due to these significant differences, Duncan's post hoc test was conducted to analyze the effects among each shell size group. The results showed that shell sizes of 51-60 mm, 61-70 mm, and 71-80 mm all had significantly different effects on fat content, with green mussels of 51-60 mm having the highest fat content.

The fat content observed in this study falls within the range reported by Murdinah [34], where the fat content in fresh green mussels was 0.40–2.47%. However, Suwandi [37] suggested that differences in fat content values can result from various factors, including habitat, sex, and diet.

Green mussels obtain nutrients from their aquatic habitat primarily through microalgae and plankton. *Tetraselmis chuii* and *Skeletonema costatum* are examples of natural food sources with high nutritional value, providing protein, fat, and essential HUFA (High Unsaturated Fatty Acid) content, which supports growth [41,42]. HUFA omega-3 fatty acids, such as C20:5n-3 eicosapentaenoic acid (EPA) and C22:6n-3 docosahexaenoic acid (DHA), are crucial for most marine organisms, playing an important role in their survival and normal growth. A deficiency in HUFA omega-3 can lead to high mortality rates and slow growth [43].

High levels of heavy metals in green mussels disrupt their filtration process. Research findings indicate that larger green mussels contain the highest heavy metal levels. This suggests that the nutritional needs of larger green mussels are not fully met, as shown by the inverse relationship between mussel size and protein content in this study.

This aligns with the research findings of Widasari [43], which indicate that extended culture time or a longer lifespan in mussels leads to decreased levels of essential EPA and DHA fatty acids. The decline in EPA content may be due to its utilization in early stages for normal organ development, while the reduction in DHA is likely because mussels use most of the DHA obtained from natural food sources to meet daily needs, such as growth, membrane formation, osmoregulation, prostaglandin synthesis, and supporting immune function.

Water temperature and salinity variations are key environmental factors that influence the growth and development of marine bivalve gonads, contributing to differences in fat content [44]. Slight differences in fat content in *Perna viridis* tissues may be due to variations in food availability, temperature, and microclimatic conditions [45].

3.2.4 Ash Level

The ashing process is conducted to determine the mineral content in a material [52]. Analysis of the ash content in raw green mussels of different shell sizes yielded an average result of 2.47%–2.57%. Based on the ANOVA results, the p-value for ash content in raw green mussels was 0.304 (> 0.05), indicating that H0 was accepted. This means that shell size does not significantly affect the ash content in green mussels.

Pebrian [32] reported that ash content in green mussels can reach 3.6%. The relatively high ash content is likely due to the abundant minerals in green mussels. These minerals may be beneficial or harmful, depending on the mussels' habitat. Clean habitats are more likely to provide beneficial mineral content, whereas polluted aquatic environments may lead to the accumulation of harmful minerals [44].

Similarly, Winarno [46] found that variations in ash content can be influenced by environmental factors and different organisms. Each aquatic environment provides unique mineral intake opportunities for aquatic organisms, and each organism varies in its ability to regulate and absorb minerals or metals, which impacts the ash content in their bodies [47]. According to Setiawan [48], the types of food and mineral content in the habitat of fish also affect the ash content in their bodies.

3.2.5 Carbohydrate Content

Okzus [49] found that carbohydrates in fishery products are primarily in the form of glycogen and do not contain fiber. Glycogen content in fishery products is approximately 1% in fish, 1% in crustaceans, and 1-8% in mussels.

The average carbohydrate content (calculated by difference) in green mussels based on shell size in this study was within the range reported by Murdinah [34], which was 2.36–4.95%. According to the ANOVA results, the p-value for carbohydrate content in raw green mussels was 0.078 (> 0.05), indicating that H0 was accepted. This means that shell size does not have a significant effect on the carbohydrate content in green mussels.

Murdinah [34] found that carbohydrate content can increase when the levels of protein, fat, and ash decrease in fish. The average carbohydrate content in green mussels in this study was higher than that reported by Poedjiadi [50] (3.6%) but lower than those found by Purwaningsih [29] and Pebrian [32], which were 4.70% and 4.99%, respectively.

According to Laxmilatha [51], an examination of the proximate composition of the surf clam *Mactra violacea* revealed that the average water content in females (80.6%) was higher than in males (79.7%). The average fat content in females (3.5%) was also higher than in males (3.1%).

The results of proximate content testing in this study were within the range reported by Sulistiyaningsih [23], where mussels had a protein content of 7.062%, a fat content between 0.40 – 2.47%, and a carbohydrate content of 2.36 – 4.95%. However, the proximate content values found in this study differ from those reported in other studies, despite focusing on the same species. These differences likely arise due to various internal and external factors that affect nutritional content, including age, species, sex, diet, season, gonad maturity, and water quality in the habitat [34,51,53]. This aligns with Fallah [53], who stated that fish habitat influences the chemical composition of fish, including proximate content, amino acids, and fatty acids.

3.3 Microbiological content of green mussels (*Perna viridis*)

Microbiological testing was conducted to identify the microorganisms present in raw green mussels (*Perna viridis*), including the total bacterial count and specific types of harmful bacteria. The microbiological tests performed included Total Plate Count (TPC), *E. coli, Salmonella* sp., and *Vibrio parahaemolyticus*. The test results are presented in Table 3.

Table 3. Microbiological content of green mussel meat (*Perna viridis*) with different shell sizes

Sizes (mm)	TPC (col/g)	*Escherichia coli* (MPN/g)	*Salmonella*	*Vibrio parahaemolyticus* (MPN/g)
51 – 60	4.5×10^4	13.0 ± 2.83	Positive	5.10 ± 2.97
61 – 70	6.0×10^3	11.6 ± 3.39	Positive	4.50 ± 2.19
71 – 80	2.6×10^3	13.5 ± 3.54	Positive	5.20 ± 3.11

3.3.1 Total Plate Count (TPC)

Based on Table 3, the highest Total Plate Count (TPC) was found in green mussel meat with the smallest shell size. However, the TPC results for green mussels still meet the quality requirements for frozen clams, which is a maximum of 5.0 x 10^5 colonies/g according to SNI 3460:2009 [21]. Microbiological contamination in green mussels can occur during capture, handling, and processing [54]. One method for maintaining the quality of fishery products is the application of a cold chain. Proper handling involves implementing a cold chain system, which keeps the clams at low temperatures to preserve their freshness.

3.3.2 Escherichia coli

The number of *Escherichia coli* bacteria in green mussels exceeds the SNI 3460:2009 standard, which sets a maximum value of <3 MPN/g. This indicates that *E. coli* levels in green mussels are high; therefore, if they are to be consumed, they must first undergo a processing stage. *E. coli* is typically eliminated through cooking at high temperatures (>100°C), which destroys proteins in the bacterial cells, rendering them inactive, as refrigeration or freezing is insufficient to kill these bacteria [55]. The presence of *E. coli* in green mussels is likely due to contamination during fishing and handling, as well as from domestic waste directly dumped into the ocean.

Waste pollution arises from residents living close to the shore who dispose of urine, feces, or food waste directly into the waters, leading to coliform bacterial contamination of fishery products [56]. Green mussels, being filter feeders, are particularly susceptible to ingesting, absorbing, and accumulating pollutants, including heavy metals and microbes. According to Sutiknowati [55], *E. coli* originates from animal or human feces. Additionally, high *E. coli* levels in the water can result from changes in the physicochemical properties of the environment [54]. For example, *E. coli* levels can increase after rainfall owing to higher concentrations of organic matter (N and P) as well as fluctuations in temperature, salinity, and light intensity [55].

3.3.3 Salmonella sp.

Based on Table 3, it is evident that green mussel meat across all shell sizes contains *Salmonella* sp. The presence of *Salmonella* bacteria in raw green mussels is likely due to waste pollution, as residents living close to the beach often discard urine, feces, or food waste directly into the waters, leading to bacterial contamination of live fishery products [56]. Additionally, waste pollution occurs in polluted river areas in Ketapang Village, where household waste from nearby residential areas flows into the waters. Contamination by *Salmonella* can be exacerbated by the availability of nutrients and environmental conditions that support bacterial growth [57]. To mitigate *Salmonella* contamination in green mussels, it is essential to maintain proper sanitation and hygiene around cultivation sites and ensure cleanliness of equipment used during harvesting [58].

3.3.4 Vibrio parahaemolyticus

The levels of *Vibrio parahaemolyticus* bacteria found in green mussels of various shell sizes exceed the Indonesian National Standard (SNI) 2332.5:2006, which sets a maximum value of <3 MPN/g. *Vibrio parahaemolyticus* is a bacterium commonly found in brackish and coastal waters, making it a natural organism in shellfish, including green mussels. It belongs to the *Vibrio* genus, which includes species pathogenic to both green mussels and humans, contributing to its high prevalence and spread in the environment [59]. The high sea temperatures (25-35°C) in Indonesia contribute to the consistent presence of *Vibrio parahaemolyticus* throughout the year [59]. Contamination in green mussels by *Vibrio parahaemolyticus* can occur due to cross-contamination from mussel feces that are subsequently reabsorbed by the mussels [60]. Indonesia's tropical climate, with sea temperatures often reaching 25-35°C, creates favorable conditions for the survival and proliferation of *Vibrio parahaemolyticus* bacteria in coastal waters [59].

4 Conclusions

Based on the results of this research, it can be concluded that the lead (Pb) content in green mussels is still below the threshold of SNI 3460.1:2009, so it is safe and fit for consumption. The different sizes of green mussel shells (51 – 60, 61 – 70, and 71 – 80 mm) significantly affected the protein and fat content. As for the carbohydrate content, a significant difference was only shown by green mussels with shell sizes 51-60 mm and 71-80 mm. The results of microbiological tests showed that the Total Plate Count (TPC) in green mussels did not exceed the National Standard Indonesia (SNI), the *E. coli* content exceeded the SNI limit and there were green mussel samples that were positive for *Salmonella* and *Vibrio parahaemolyticus*.

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