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Characterization of Mineral Absorption in Freshwater Mussel Gills (*Pilsbryoconcha exilis* Lea 1883) Using Scanning Electron Microscopy (SEM): Implications for Environmental Remediation

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Abstract

Background: Freshwater mussels (*Pilsbryoconcha exilis*) are known as filter feeders with high survival resilience and can be utilized to address water pollution caused by various pollutants, including heavy metals.
Aims: This study aims to identify and characterize the minerals absorbed in the gills of freshwater mussels using Scanning Electron Microscopy (SEM) techniques.
Result: The research results show that, on average, SEM analysis of the freshwater mussel gills identified five metal elements: Carbon (C), Oxygen (O), Phosphorus (P), Sulfur (S), and Calcium (Ca). Quantitative analysis revealed the following concentrations of these elements: C: 52.70±0.12; O: 31.78±0.20; P: 5.18±0.06; S: 0.65±0.02; Ca: 9.70±0.10.
Conclusion: Based on quantitative analysis, these elements are present in varying proportions, with Carbon being the dominant element. This finding suggests that these metal elements, classified as micronutrients, are necessary for freshwater mussels (kijing), although they can be potentially harmful at certain concentrations. Further research on the potential use of freshwater mussels in environmental remediation is needed, considering their ability to absorb heavy metals. This could be part of a strategy for managing pollution in freshwater systems.

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1. Introduction

Water is one of the essential components for humans and other living beings as a source of life. One source of water can come from surface water, which can be defined as water found on the ground's surface, either stagnant or flowing, such as rivers (Bigas *et al.*, 2001; Hamidah & Anggereini, 2021). The availability of surface water in the form of rivers in Indonesia has a significant volume. Rivers are often utilized as a source of drinking water, for rice irrigation needs, aquaculture, tourism, and transportation. The quality of river water in Indonesia is still categorized as insufficient. In 2019, Indonesia had 98 rivers, with pollution distribution showing 54 rivers with light pollution, 6 rivers with light to moderate pollution, and 38 rivers experiencing heavy pollution (Firmansyah *et al.*, 2021). This has serious impacts on the surrounding environment, especially the ecosystems within them (Adam *et al.*, 2018).

In addition to the decline in aquatic biota populations due to pollution, this also affects the nutrient content of aquatic organisms that are continuously consumed by humans (Gassim, 2023). This issue can be addressed by improving water quality through the use of filters, whether mechanical, chemical, or biological (Nataraj *et al.,* 2020; Parsons, 2022). Biological filters are natural filters that utilize aquatic animals, one of which is clams/mussels as filter feeders, combined with a recirculation system for efficient water use (Komarawidjaja, 2006). The local freshwater mussels (*Pilsbryoconcha exilis*) or kijing in Indonesian language is known as a filter feeder with high survival resilience, and its abundance can be utilized to mitigate water pollution caused by pollutants, including heavy metals (Muliari *et al.,* 2018; Odey *et al.,* 2020). Thus, this organism can assist in water purification efforts; freshwater clams can utilize leftover food that fish do not consume and can serve as biofilters (Utami *et al.,* 2018).

Pilsbryoconcha exilis is a freshwater clam belonging to the family Unionidae and the genus Pilsbryoconcha. This clam has filtration capabilities (Wedsuwan *et al.,* 2016), making it a common agent for biofiltration in contaminated aquatic areas, and it has the potential to accumulate hazardous minerals such as heavy metals (Sativa *et al.,* 2017). This species is often harvested by local communities for daily food due to its taste and the nutritional content of the clam meat, which is essential for human health. Identification of the contents in clam meat reveals proteins and amino acids that support body maintenance and growth, including eicosatetraenoic acid (EPA) and docosahexaenoic acid (DHA), which can enhance brain function (Komarawidjaja, 2006). Consuming clams from polluted water can have adverse health effects. Further research on the potential use of freshwater clams in environmental remediation is needed, considering their ability to absorb heavy metals. This could be part of a strategy for managing pollution in freshwater systems. Analyzing the mineral content in clams is crucial to minimizing unwanted negative impacts. Identifying the absorption by clams is necessary, as many studies have discussed this organism, but studies on the absorption of micro and macro minerals in clam meat have not yet been identified. One technique for analyzing minerals in clam meat is Scanning Electron Microscopy (SEM). This study aims to identify and characterize the minerals absorbed in the gills of freshwater clams using the Scanning Electron Microscopy (SEM) technique. Through mineral analysis, this research seeks to identify and characterize the minerals in this species. The data obtained is expected to provide new insights into the nutritional aspects of the clam *Pilsbryoconcha exilis*.

2. Methods

The research materials used were freshwater clams *Pilsbryoconcha exilis*, obtained from a cultivation pond at the Fish Cultivation Center (BBI), Fisheries Department, East Lombok Regency, West Nusa Tenggara.

Sample Preparation

The samples of *Pilsbryoconcha exilis* are sliced thinly and then dried under direct sunlight for 3 days to reduce moisture content and to avoid structural damage due to improper drying. The clams were cut into smaller pieces using a microtome as a cutting tool, and the samples are mounted on SEM stubs using conductive tape. Ensure that the samples are securely and stably attached.

Inspection and Calibration

Turn on the SEM and allow the vacuum chamber to stabilize. Place the stub with the mounted sample inside the SEM working chamber. Conduct an initial inspection using low observation mode to locate interesting areas on the sample. Adjust the SEM parameters, such as acceleration voltage to 15.0 kV, electron beam current, and lens aperture. Once the settings are complete, take images at various magnifications. Pay attention to the contrast and resolution of the resulting images.

Data Analysis

Analyze the SEM data and images obtained using image analysis software to examine the morphology and structure of the sample.

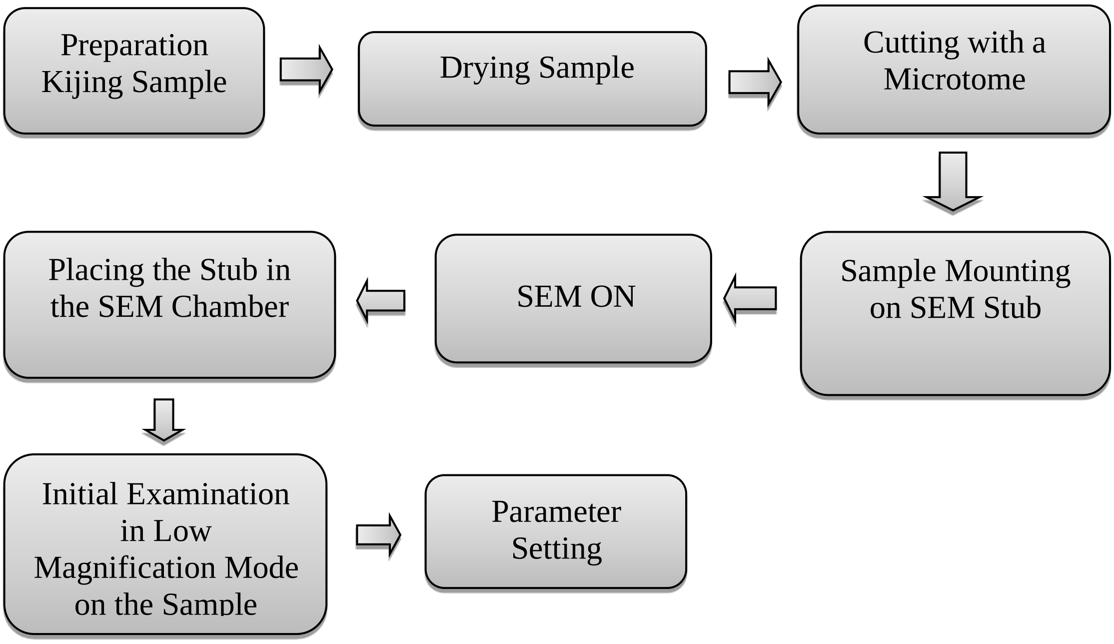

Figure 1. Procedure for Identification and Characterization of Minerals in Kijing Samples.

3. Results and Discussion

Results of Scanning Electron Microscopy (SEM) Analysis on the Histological Images of *Pilsbryoconcha exilis* Gills (Figure 2)

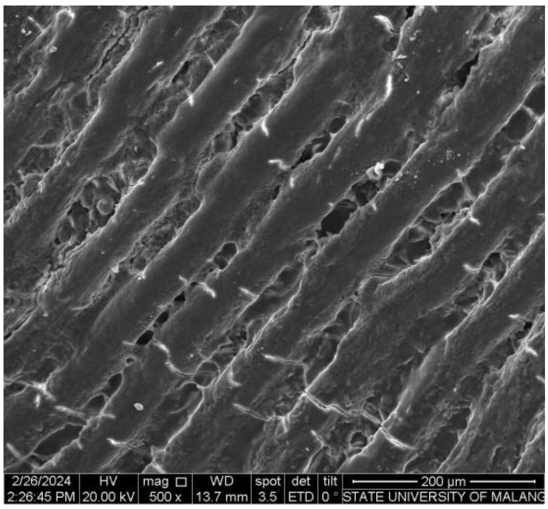

Figure 2. Description of Gill Structures of *Pilsbryoconcha exilis* as Observed in 500x SEM Magnification

Histological Analysis of *Pilsbryoconcha exilis* Gills characterized by Scanning Electron Microscopy (SEM). The histological examination of the gills of *Pilsbryoconcha exilis* was conducted using Scanning Electron Microscopy (SEM) at a magnification of 100x, as shown in Figure 2. The SEM analysis aimed to determine the pore size present in the adsorbent and to observe the morphological structure of the particle surface. The results are presented in the form of images that illustrate the physical processes involved in the interaction between atoms and materials with electrons (Sujatno *et al.*, 2015). Based on Figure 2, the SEM analysis reveals filamentous structures of the gills, which are essential for respiration and oxygen uptake. The images also show the presence of debris or particles adhering to the gill surface, providing insights into the environmental conditions where the clam resides. Previous research by (Sujatno *et al.*, 2015) indicated that the local clams exhibit a needle-like structure that is more organized, characteristic of aragonite. According to (Saksono *et al.*, 2010) calcite crystals are strongly adhered to surfaces (most stable), aragonite tends to detach easily from walls, while vaterite is unstable and can transform into calcite through solvent mediation.

Table 1. Percentage of Mineral Absorption in the Gills of *Pilsbryoconcha exilis*

No	Chemical Coumpounds	Formula	Value
1	Carbon	C	**52.70**±0.12
2	Oksigen	O	**31.78**±0.20
3	Fosfor	P	**5.18**±0.06
4	Sulfur	S	**0.65**±0.02
5	Calsium	Ca	**9.70**±0.10

The quantitative analysis results from Scanning Electron Microscopy (SEM) of the gills of *Pilsbryoconcha exilis* identified the presence of five different minerals with varying percentages. Based on the data obtained, the five identified minerals include Carbon, Oxygen, Phosphorus, Sulfur, and Calcium. The quantitative analysis revealed the following metal element values: C: 52.70 ± 0.12; O: 31.78 ± 0.20; P: 5.18 ± 0.06; S: 0.65 ± 0.02; Ca: 9.70 ± 0.10. According to the quantitative analysis, these elements are present in different proportions, with Carbon being the dominant element, which falls under the category of heavy metals (Firmansyah *et al.,* 2021). These heavy metal types are classified as micronutrients required by the kijing, but at certain levels can be harmful (Utami *et al.,* 2018). At the molecular level, the mineral composition in kijing can be influenced by several factors, including their living environment, diet, and internal biological processes. Calcium is the element with the highest percentage value. This is due to the numerous important roles that calcium plays in kijing. In addition to shell formation, calcium also plays a role in muscle contraction (Szent-Györgyi, 1975). The muscles of the shell, driven by calcium, regulate functional activities, body metabolism, and the movement activities of the kijing. Adequate availability of calcium will accelerate the growth and development of the shellfish (Shita & Sulistiyani, 2010).

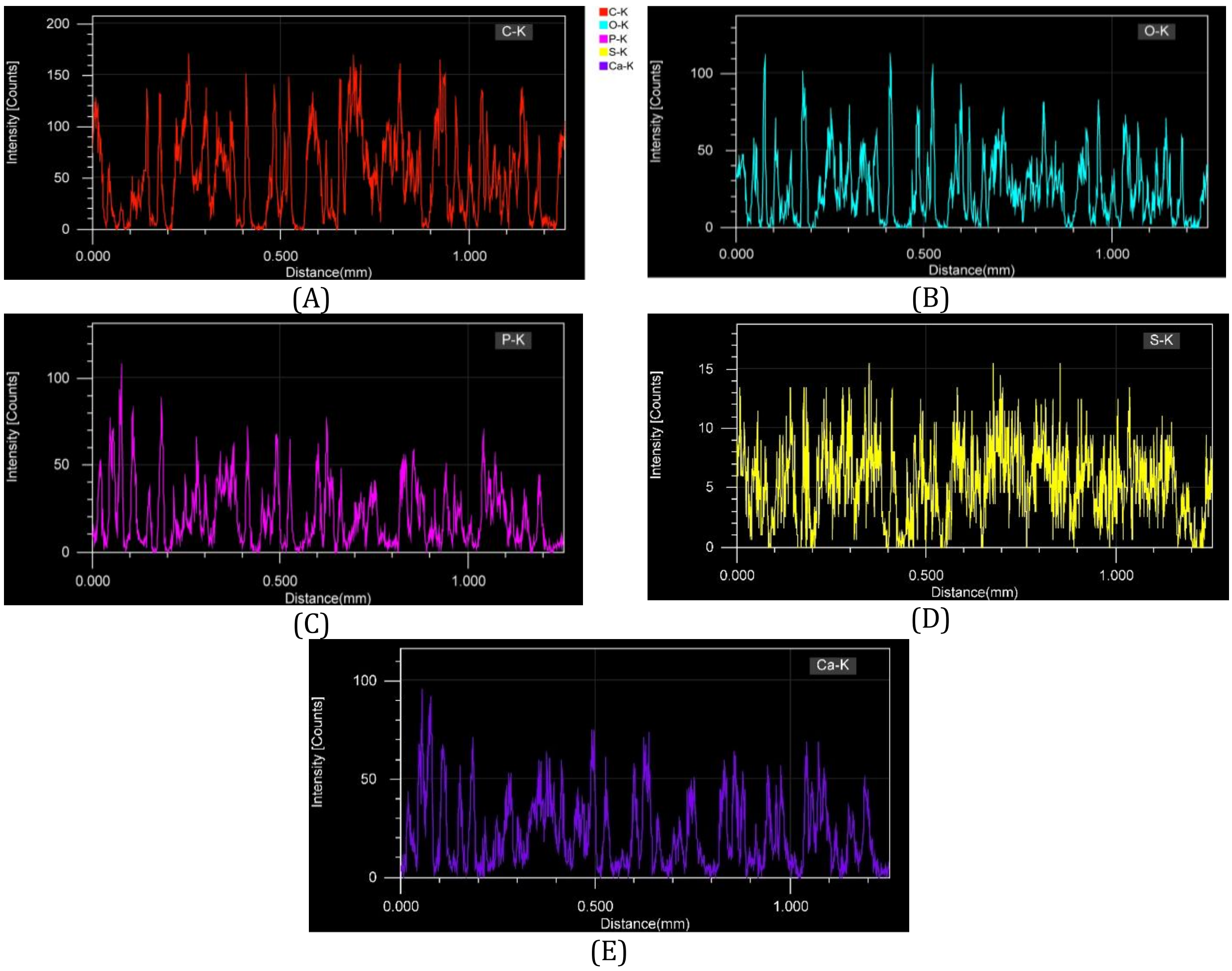

Figure 3. Graph of Mineral Absorption Intensity; (A) Carbon; (B) Oxygen; (C) Phosphorus; (D) Sulfur; (E) Calcium

In Figure 3, the mineral absorption intensity in the kijing shellfish is shown, where five types of minerals—Oxygen, Phosphorus, Sulfur, and Calcium—exhibit different absorption percentage values. Among these, the highest mineral absorption is observed for Sulfur (S), as indicated by the graph showing that the absorption intensity of Sulfur (S) is denser compared to the other minerals. According to (Hutagalung & Syamsu, 1987), shellfish have the ability to accumulate heavy metals in their bodies, meaning that the concentration of heavy metals in kijing will continue to increase as the shellfish remain in water contaminated with heavy metals.

Research conducted by (Aliza *et al.,* 2021; Bigas *et al.,* 2001) states that heavy metals can accumulate in shellfish from water, sediments, and phytoplankton. Therefore, one alternative to maintain the sustainability of water resources is through environmental bioremediation, where freshwater kijing can filter particles between 0.1-50.0 μm from bodies of water, and for particle sizes > 4.0 μm, filtration efficiency can reach up to 100% (George R C *et al.,* 1998; Wisha *et al.,* 2019). Based on this filter feeder characteristic, (Ningrum et al., 2024; Sartika et al., 2024) suggests that kijing shellfish can be used as a water purifier. Freshwater kijing tend to grow faster in stagnant water compared to flowing water (Shita & Sulistiyani, 2010).

4. Conclusions

The histological images of the kijing gills reveal the filamentous structure responsible for respiration and oxygen uptake. Additionally, debris or particles observed on the gill surfaces can provide insights into the environmental conditions in which the shellfish live. Because of that As decomposers, freshwater mussels play an important role in environmental restoration by helping to improve air quality. The presence of freshwater mussels in an ecosystem can help restore contaminated areas through natural bioremediation methods.

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