

Original Research

# Evaluation of Heavy Metal Contamination and Nutritional Value of Mollusks in Ex-Tin Mining Areas on Bangka Island, Indonesia

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## Abstract

Heavy metal contamination in coastal environments affects the nutritional quality of marine organisms. Bangka Island, a major tin-producing region in Indonesia, has ex-tin mining areas where post-mining activities contribute to heavy metal runoff. This study aims to determine levels of Cadmium (Cd) and Copper (Cu), amino acid profiles, and proximate composition of *Laevistrombus canarium*, *Anadara granosa*, and *Geloina erosa* collected from three former tin mining sites (Anak Air Island, Batu Belubang, and Kurau). Sediment from Anak Air Island was dominated by silt (46.45%), while Batu Belubang and Kurau were sandy (94.61% and 94.03%). Batu Belubang had the highest TOM (17.94%) and heavy metal concentration (Cu:5.70; Cd:0.03 mg/kg). *A. granosa* had the highest Cd (0.21 mg/kg), and *L. canarium* was dominated by Cu (5.70 mg/kg); all levels remained below safety thresholds. Nutritional analysis showed *L. canarium* had the highest protein content (16.29%) and carbohydrate content (5.27%). Lysine (5.25%), phenylalanine (6.85%), and leucine (4.07%) were dominant essential amino acids (EAA) in *L. canarium*, *A. granosa*, and *G. erosa*, respectively. Non-essential amino acids (NEAA) were dominated by glutamate (8.97-10.79%) in all species. Prevalence of metal-binding amino acids (e.g., glutamate, lysine) may reflect a biochemical adaptation

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to contaminated environments. These findings suggest that environmental factors influence both the accumulation of heavy metals and the nutritional profiles of mollusks.

**Keywords:** amino acid, ex-mining, heavy metal, mollusks, proximate

## Introduction

Indonesia, as one of the world's leading tin producers, has extensive areas of ex-mining sites, particularly on Bangka Island [1]. However, illegal mining activities, both on land and at sea, have caused significant environmental impacts, such as sediment contamination by heavy metals. Affected areas include Batu Belubang, Anak Air Island, and Kurau [2-4]. These regions are rich in benthic biodiversity and serve as habitats for economically valuable species such as *L. canarium*, *A. granosa*, and *G. erosa*. Heavy metal contamination raises concerns about the safety and quality of these organisms as food sources.

In recent years, global concern has increased regarding ecological impacts and public health risks associated with environmental contamination by heavy metals. In this study, Cd and Cu were selected to represent non-essential and essential heavy metals. Cd is a non-essential metal that poses toxicological risks to aquatic organisms even at low concentrations and tends to bioaccumulate in tissues [5]. Although Cu is an essential metal, excessive levels can be toxic, causing oxidative stress, growth impairment, and tissue damage [6]. Moreover, tin mining activities in Bangka Island are known to contribute to the release of metals into aquatic ecosystems [7, 8]. According to Ahmad [7], although most metals were within safe limits, Cd and Cu showed localized pollution based on the geoaccumulation index in several coastal areas of Bangka Island, making the monitoring of Cd and Cu particularly relevant in this region.

Sediment plays a crucial role as a primary medium for heavy metal accumulation in aquatic environments due to its ability to bind various pollutants, including heavy metals such as Cd and Cu [9]. Heavy metal concentrations in sediments can increase due to anthropogenic activities and sediment resuspension caused by water currents [10]. Additionally, TOM influences the distribution and availability of heavy metals in sediments, playing a crucial role in their absorption and binding [11].

Previous studies in Bangka have shown heavy metal accumulations, including Lead (Pb), Zinc (Zn), and Tin (Sn), at moderate to high concentrations in coastal sediments, primarily due to tin mining activities and other anthropogenic sources [2]. A chronosequence study of former tin mining ponds also revealed increasing concentrations of heavy metals such as Pb, Cu, Zn, Arsenic (As), and Manganese (Mn) in older ponds, indicating long-term accumulation and elevated ecological risks [12]. Excessive accumulations of heavy metals can negatively impact the physiological and

morphological conditions of organisms, altering their nutritional value, including amino acid profiles and proximate composition [13].

Furthermore, heavy metals can interact with nutrients in aquatic organisms, potentially altering their physiological and biochemical functions. For instance, Cd can disrupt the absorption and metabolism of essential minerals such as Ca, Zn, and Fe, and may bind to amino acids or proteins, thereby causing dysfunction in biological systems [14]. Similarly, elevated levels of Cu may induce oxidative stress, leading to lipid peroxidation and decreased protein stability, particularly under conditions of environmental stress [15].

Many studies have focused on measuring heavy metal concentrations in marine organisms. However, studies investigating the impact of heavy metal contamination on the nutritional quality of seafood remain limited, particularly in Indonesia. This aspect is critical for evaluating the safety and nutritional value of mollusks intended for human consumption. This study aims to determine levels of heavy metals (Cd and Cu), amino acid profiles, and proximate composition in tissues of *L. canarium*, *A. granosa*, and *G. erosa* collected from former tin mining sites on Bangka Island, Indonesia. It provides baseline data for understanding the nutritional quality and potential metal exposure risks in benthic mollusks from areas influenced by tin mining activities.

## Material and Methods

### Sediment and Mollusks Sampling

Sediment samples were collected using an Eckman grab, while mollusks were manually gathered by walking in shallow waters or diving (snorkeling). Sediment and mollusk samples were immediately placed in Ziplock bags and stored on ice until taken to a laboratory for analysis. Three mollusk species were collected from different locations on Bangka Island, Indonesia. *L. canarium* was obtained from Anak Air Island, *A. granosa* was collected from Batu Belubang, and *G. erosa* was taken from Kurau. These locations represent diverse environmental conditions, with sampling coordinates as follows: Batu Belubang (106°12'55.978"E, 2°10'24.041"S), Kurau (106°15'48.33"E, 2°20'12.951"S), and Anak Air Island (106°40'10.261"E, 2°56'40.466"S).

Batu Belubang is a coastal area close to artisanal tin mining sites, located approximately 2-3 km from residential areas and fishing docks. Kurau is near mangrove ecosystems and a river mouth,

with high exposure to domestic wastewater inputs due to its proximity (~20 m) to a fishing settlement and boat landing site. In contrast, Anak Air Island is a remote and uninhabited islet located in open waters, far from the mainland. It has sandy-silty substrates, seagrass beds, and no mining activity. The area is used for seasonal mollusk harvesting by local fishermen but otherwise has minimal anthropogenic disturbance. Sampling stations are illustrated in the following image (Fig. 1).

#### Heavy Metals and TOM Analysis, Sediment Characterization

Cu and Cd concentrations were analyzed at the Regional Health Laboratory of DKI Jakarta using an inductively coupled plasma optical emission spectrophotometer (ICP-OES) Thermo Scientific iCAP 7000 in accordance with laboratory procedures. Sediment samples (0.5 g) were placed into digestion tubes and treated with 5 mL of concentrated nitric acid (HNO<sub>3</sub>) and 1 mL of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The tubes were then allowed to stand for 20-30 minutes. The mixtures were subsequently digested in a microwave system at 190 °C for 1 hour. After cooling, each digested sample was transferred into a 50 mL volumetric flask and diluted to volume with deionized water. If any precipitate remained, the solution was filtered through a 42 µm filter paper prior to analysis. Tissue samples (approximately 1 g of homogenized dry mollusk tissue)

were digested following the same procedure using concentrated HNO<sub>3</sub> and 1 mL of H<sub>2</sub>O<sub>2</sub>, with microwave-assisted digestion applied to ensure complete breakdown of organic matter and to minimize matrix interferences from salinity and organic residues. Method blanks (reagent-only samples) were included with all test samples to identify any potential contamination during preparation and to ensure analytical accuracy. Method detection limits (MDLs) were 0.015 mg/kg for Cd and 0.02 mg/kg for Cu.

Sediment organic matter analysis was conducted using the loss-on-ignition (LOI) method [16]. Samples were dried in an oven at 105°C for 12-24 hours and subsequently combusted in a muffle furnace at 550°C for 2 hours. Organic matter content was calculated using the formula:

$$\text{LOI}_{550} = \left( \frac{DW_{150} - DW_{550}}{DW_{150}} \right) \times 100$$

LOI<sub>550</sub> represents the weight lost at 550°C, DW<sub>105</sub> is the sample weight at 105°C, and DW<sub>550</sub> is the sample weight at 550°C.

Granulometry analysis was conducted to determine the size and type of sediment. Sand samples were analyzed using the dry sieving method for particles >0.0625 mm, while mud samples were examined using the pipetting method with a hydrometer [17]. A total of 50 g of the sample was dried in an oven at 100°C before

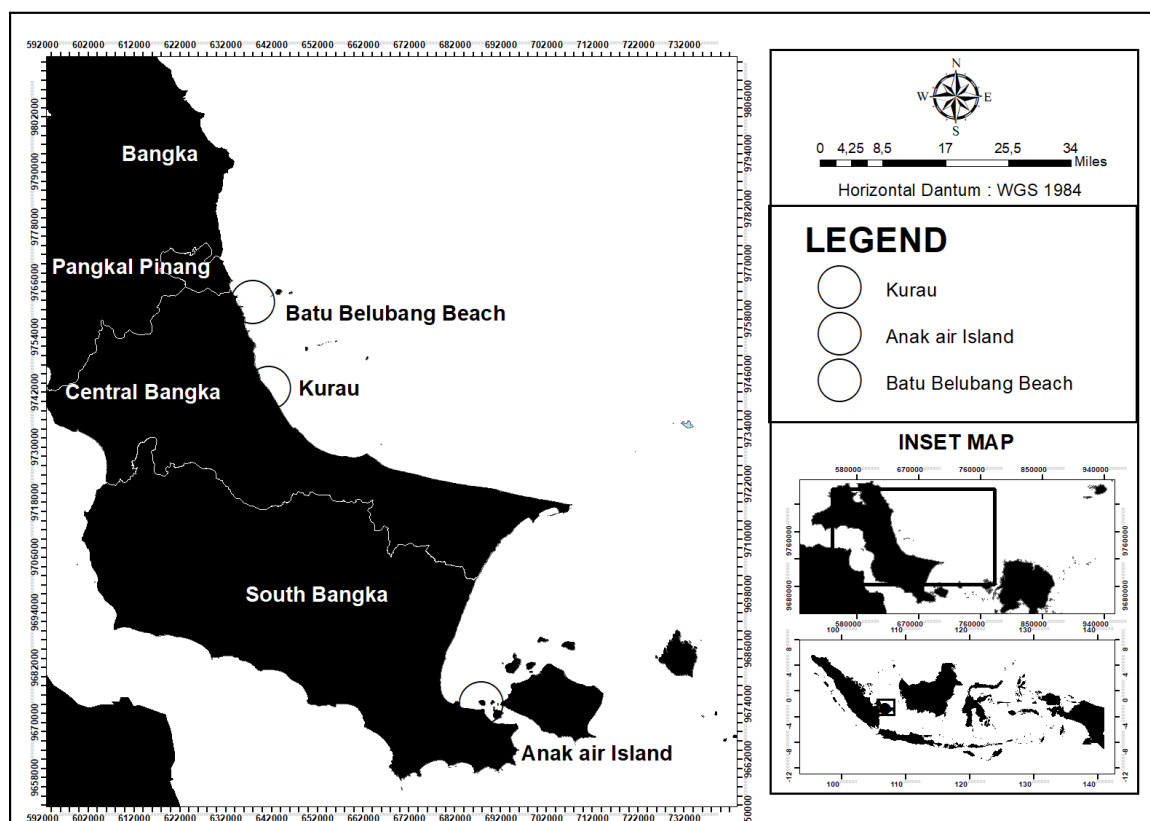


Fig. 1. Sampling areas on Bangka Island.

dry sieving with a sieve shaker to determine the coarse fraction. Wet sieving with a hydrometer was used for mud and silt fractions. Sediment classification followed Wentworth's method (1922) [18].

### Proximate Composition and Amino Acid Profile Analysis

Proximate analysis was performed following the AOAC 2015 method and conducted at the Biotechnology Laboratory of the National Research and Innovation Agency [19]. Moisture and ash content were determined gravimetrically, protein by the Kjeldahl method, fat through Soxhlet extraction, and carbohydrates using the by-difference method.

Amino acid analysis was conducted at the Integrated Laboratory, IPB University, using High-Performance Liquid Chromatography (HPLC) RF20A (Shimadzu, Japan) with a C18 ODS column. A 3 mg sample was hydrolyzed with 1 ml of 6 N HCl at 110°C for 24 hours. After hydrolysis, the sample was dried using a rotary evaporator, filtered through a 0.45 µm Whatman filter, and mixed with a derivatization buffer containing potassium borate and ortho-phthalaldehyde (OPA). The sample mixture was incubated at 55°C for 10 minutes before HPLC analysis. Amino acid content was determined by comparing chromatogram peak areas with  $\alpha$ -aminobutyric acid (AABA) standards.

### Data Analysis

Data analysis was conducted using Analysis of Variance (ANOVA), followed by the Least Significant Difference (LSD) test to determine significant differences among groups based on habitat characteristics. Descriptive statistics were used to summarize heavy metal content, amino acid profiles, and nutritive values of mollusk species. Additionally, Pearson correlation analysis was used to examine the relationships between environmental parameters (e.g., TOM, sediment texture, and heavy metal concentrations) and the nutritional composition and amino acid profiles in mollusk tissues.

### Results and Discussion

Environmental characteristics based on Fig. 2. Sand and mud content on Anak Air Island differed significantly ( $p < 0.05$ ) from other locations. The sand content was the lowest (42.25%), while the mud content was the highest (46.45%). TOM content in Anak Air Island was 10.72%, showing a significant difference ( $p < 0.05$ ) compared to Batu Belubang. Muddy sediments effectively trap organic matter due to their high capacity for binding essential nutrient ions (nitrogen, phosphorus, and potassium), which support organism growth. Additionally, the negative charge on mud particles attracts and retains positively charged substances,

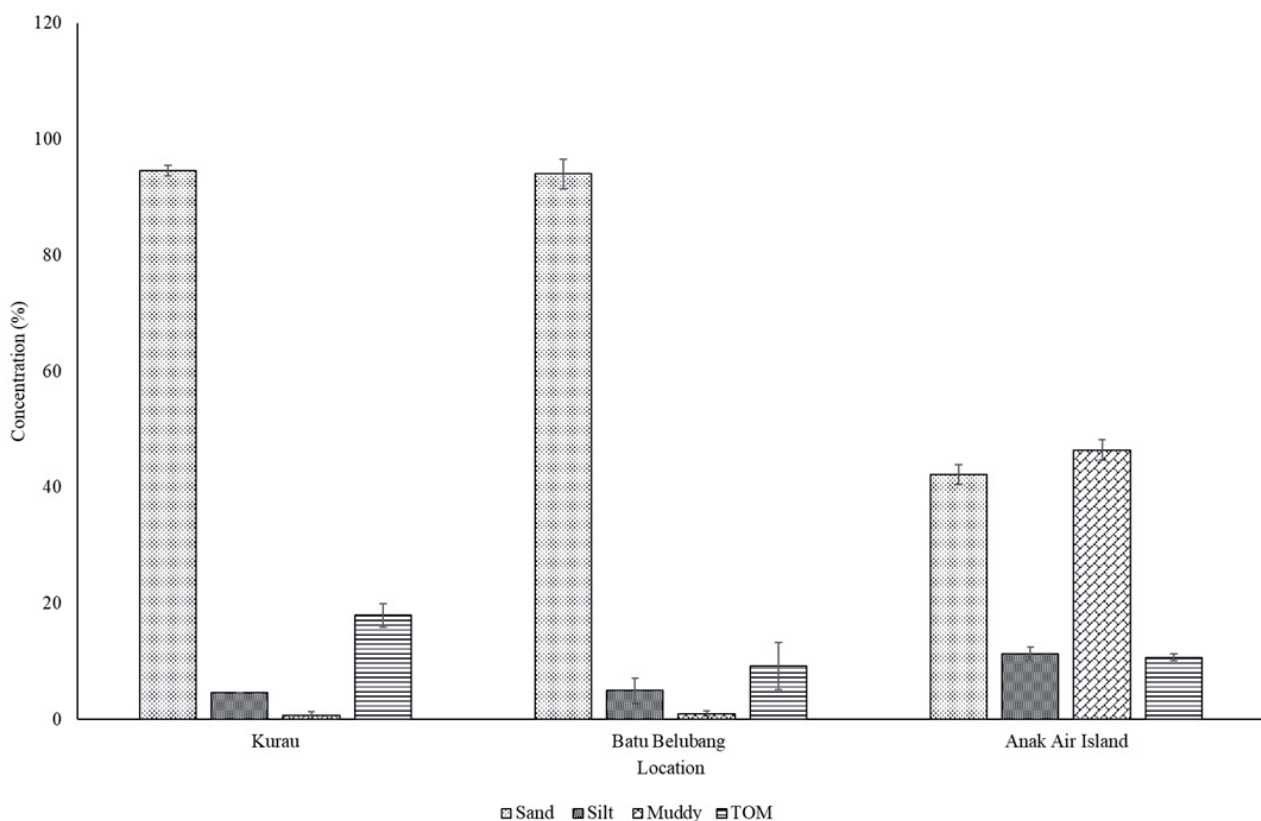


Fig. 2. Sediment composition and total organic matter (TOM) of different stations.

such as metal ions (cations) [20]. Dense and cohesive properties of mud enhance organic matter retention, prevent nutrient leaching, and improve pH and cation exchange capacity [21]. A study in intertidal waters of Teluk Penyau, Cilacap, reported that TOM content in muddy sediments ranged from 3.72-5.86% [22]. A previous study in the Normandy estuary, France, found that the mud content increased from 50% to over 60% between September and April, resulting from hydrodynamic stability, which allowed more fine particles to settle in the sediments, along with a high remineralization rate of organic matter within the muddy sediments [23].

Batu Belubang was predominantly composed of sand (>94%) but had the highest TOM content (17.94%) and the highest concentrations of heavy metals Cu (5.30 mg/kg) and Cd (0.03 mg/kg) compared to other sites compared to other sites (Fig. 3). Statistical analysis revealed a positive correlation between TOM and Cu ( $r = 0.998$ ). Meanwhile, the correlation between TOM and Cd could not be further analyzed due to the limited number of data points. Organic matter can bind with metal ions to form stable organo-metallic complexes, thereby increasing their persistence in the sediment matrix and reducing their mobility [24].

Batu Belubang is dominated by sandy sediment and is located near artisanal tin mining areas, residential neighborhoods, and fishing docks. These anthropogenic sources, along with local hydrodynamic conditions, contribute to the accumulation of organic matter and increased levels of Cu and Cd in the sediment. The proximity of the site to ongoing artisanal tin mining activities has been linked to the release of heavy metals, such as Cu, Cd, Zn, Pb, and Hg, into the aquatic environment [25]. Similar results were found in Betahwalang waters, Central Java, Indonesia, where a location dominated by sandy sediment (91.4%) had the highest TOM (66.99%-74.87%); this was attributed to

rainfall and abundance of shell fragments on the surface of sandy sediment [26]. Estuaries, as active transition zones, play a role in biogeochemical cycles where organic matter is processed before entering marine ecosystems. A study in southeastern China showed an interaction between terrestrial and marine sources; sediment texture affects organic matter retention and heavy metal accumulation, as both muddy and sandy sediments may store high TOM based on local conditions [27].

In contrast, Anak Air Island showed higher mud content (46.45%) and lower Cu (1.37 mg/kg) and Cd (0.01 mg/kg) levels, which may reflect limited exposure to anthropogenic sources. Meanwhile, Kurau had the highest sand content (94.03%) but showed no detectable levels of Cu. This suggests that sandy substrates may have a reduced capacity to bind metal ions compared to fine-grained sediments, and hydrodynamic factors may also influence sediment deposition and metal accumulation [28, 24].

In a similar study, research on the Coromandel Coast of India showed a significant correlation between mud content and higher concentrations of heavy metals (Cr, Cu, Ni, Pb, and Zn) found in areas with elevated levels of sediment organic matter [29]. A similar result was observed in a study on Ranokomea Coast, Southeast Sulawesi, Indonesia, which showed a strong correlation ( $R = 0.99$ ) between high TOM content and increased concentrations of Cu and Zn in sediments [30]. These findings were similar to results from Batu Belubang, which showed the important role of sediment texture and organic matter content in influencing the accumulation of trace metals in coastal environments.

Previous studies reported that Cd concentrations in Batu Belubang were at undetectable levels (below 0.001 mg/kg) [31]. However, the results of this study indicated that Cd concentrations (0.03 mg/kg) in the area had increased. Increasing Cd levels in Batu

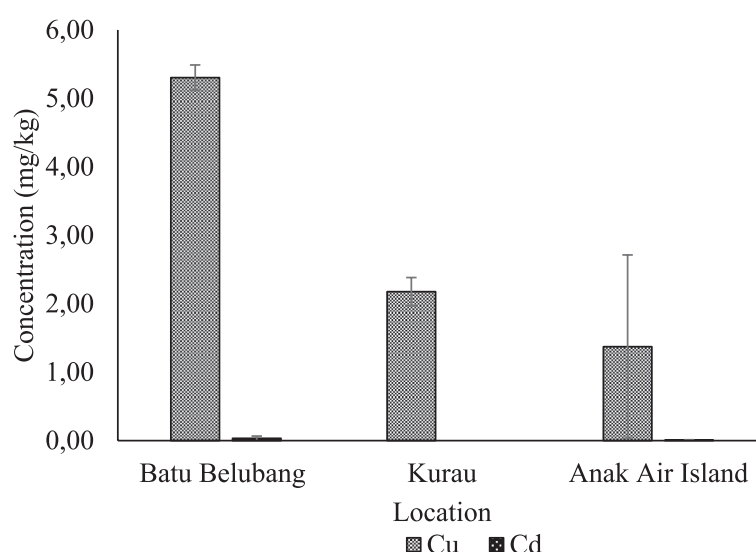


Fig. 3. The concentration of heavy metals at three locations.



Belubang were attributed to anthropogenic activities, particularly small-scale tin mining. Meanwhile, another study reported that some former tin mining areas in Bangka Island had Cu concentrations ranging from 3.08-8.94 mg/kg [12]. Nevertheless, Cu and Cd concentrations in all three locations remained below environmental quality standards set by the Australian and New Zealand Environment and Conservation Council, which were 65 mg/kg (Cu) and 1.5 mg/kg (Cd) [32]. Comparison with previous studies indicates that artisanal mining activities are a long-term source of heavy metal contamination. Although detected concentrations remained below the quality standard threshold, the measurable presence of Cd suggests an increasing accumulation, highlighting the need for continued monitoring in the future.

#### Heavy Metal Content in Mollusks

Heavy metal concentrations in mollusk tissues showed significant variation among species ( $p < 0.05$ ). *L. canarium* had the highest Cu content (5.70 mg/kg) among species. This species was collected from Anak Air Island, a remote island located in open waters far from the mainland, which is predominantly composed of muddy-sandy sediment. Cu levels in this location were lower than those in other locations; the presence of mud plays a key role in the retention of heavy metals in sediment. Cu concentrations in *L. canarium* may be

associated with differences in feeding strategies between gastropods and bivalves. *L. canarium*, a gastropod that actively searches for food in substrate, tends to absorb Cu directly from sediment, whereas a bivalve such as *G. erosa*, as a filter feeder, absorbs food from water through its gills. This difference leads to variations in the accumulation of heavy metals among species. Han et al. [33] found that sediments contain various adsorptive components, including organic matter, oxides, sulfides, carbonates, and aluminosilicate minerals, which can bind heavy metals through physicochemical processes such as precipitation, dissolution, adsorption, desorption, oxidation, and reduction. Additionally, Cavalletti et al. [34] note that the body actively regulates copper levels as an essential micronutrient to prevent oxidative stress and, along with Zinc (Zn) and Iron (Fe), tends to accumulate more easily in tissues than other heavy metals.

Higher Cd levels in the muscle of *A. granosa* from Batu Belubang (0.21 mg/kg) were linked to the higher Cd concentration in sediment (0.03 mg/kg) at that site. Batu Belubang is located near various human activities, making it more susceptible to anthropogenic pollution, including heavy metal runoff. Cd accumulation in Batu Belubang plays a significant role in bioavailability and subsequent transfer to aquatic organisms through trophic pathways. Cd has no biological function and becomes toxic when accumulated in the body [35]. A study in Zhelin Bay (South China) found that while most metals

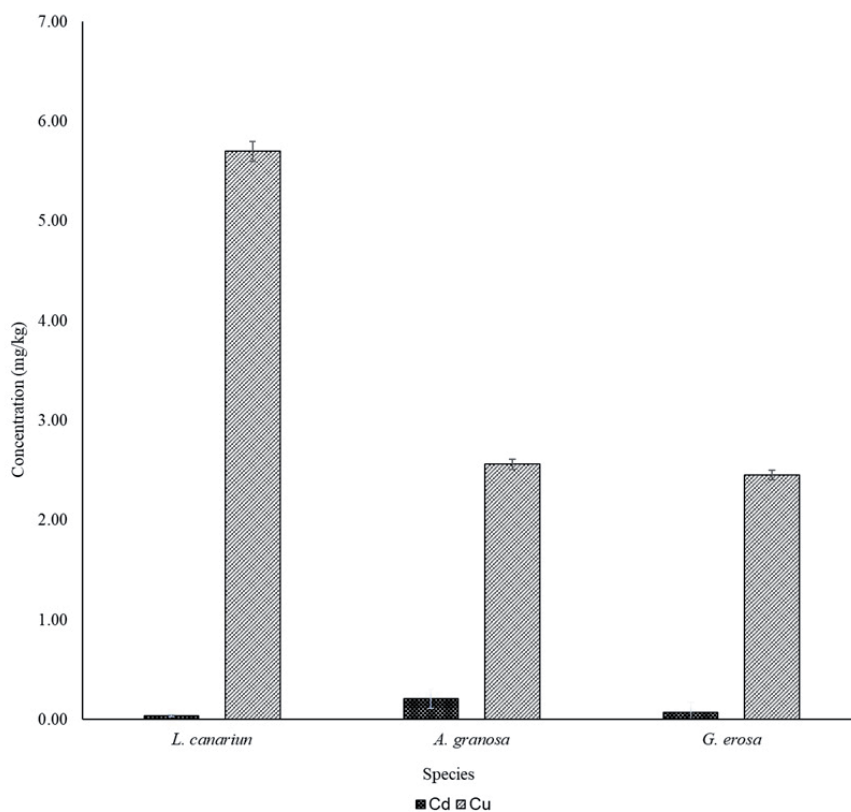


Fig. 4. Heavy metal content (Cd and Cu) in mollusk species.

in intertidal surface sediments were present in residual fractions, Cd and Mn had the highest bioaccessibility [11]. Furthermore, research has shown that mollusk muscle tissue continues to absorb Cd during low-tide exposure, as Cd gradually transfers from the mantle to the muscle, allowing for continuous uptake even in the absence of water [36].

A study by Lisna et al. [37] found that Cd levels in *A. granosa* from Jambi, Indonesia, ranged from 0.16-0.25 mg/kg. Another mollusk species from the Chinese Bohai Sea, *Chlamys farreri*, contained Cd (0.36-112.06 mg/kg), while *Mya arenaria* had Cu (15.25-1891.34 mg/kg) [38]. Heavy metal bioaccumulation depends on species type, invasion pathways, tissue metabolic profiles, and environmental conditions [39]. While Cu is not considered acutely toxic or carcinogenic, long-term exposure can still be harmful. It can disrupt enzymatic systems, trigger oxidative stress, and cause nutrient imbalances, with long-term ingestion linked to DNA damage and liver cirrhosis [40, 41].

The Indonesian government has established threshold limits for the content of Cu and Cd in edible shellfish. In this study, Cu levels ranged from 2.45-5.70 mg/kg, while Cd levels ranged from 0.04-0.21 mg/kg (Fig. 4). According to the Indonesian National Standard (SNI), the maximum allowable limit for Cd is 1.0 mg/kg [42], while the Indonesian Food and Drug Authority (BPOM) Regulation determines the Cu limit at 20 mg/kg [43]. Based on these limits, Cu and Cd levels measured in this study were within the acceptable range according to Indonesian food safety standards.

Moreover, the presence of heavy metals may not only pose toxicological risks but also impact the nutritional quality of mollusks. Heavy metal accumulation has been linked to oxidative stress in molluscan tissues, resulting in protein denaturation, lipid peroxidation, and disruption of essential metabolic pathways [44-46]. High Cu concentrations can damage muscle proteins through oxidative stress, which reduces protein digestibility and bioavailability for human consumers [47]. Similarly, Cd exposure can disrupt amino acid profiles and enzymatic activities, potentially altering their nutritional value, due to increased protein catabolism and reduced amino acid availability [6, 48]. A study by Yu et al. [49] reported that in *Clarias gariepinus*, exposure to Cd stress increases plasma glucose levels by converting amino acids and glycerol into glucose through chronic stress-induced gluconeogenesis. This indicates that environmental contamination by heavy metals can reduce both the safety and nutritional value of seafood.

### Nutritional Value of Mollusks

Proximate analysis of the three shellfish species showed variations in their nutritional composition (Fig. 5). Pearson correlation analysis revealed a significant relationship between crude protein and carbohydrate content ( $p < 0.05$ ), indicating that species

with higher protein levels also tend to have higher carbohydrate content. Although correlations between moisture and crude protein, as well as crude fiber and carbohydrate, were not statistically significant ( $p > 0.05$ ), the results suggest a potential relationship between lower moisture content and higher nutritional value. A previous study found that *Cochlea japonica* showed the highest moisture level (89.0%) and the lowest protein content (38.1%) among the species, indicating an inverse relationship between moisture content and nutritional density in mollusk tissues [50]. The highest moisture content was observed in *A. granosa* (85.20%). This is consistent with the general characteristic of bivalve soft tissues, which typically have high water content [51]. Water transport plays a vital role in maintaining cellular functions. In certain cell types, this process is regulated by specialized proteins known as aquaporins, also referred to as water channels [52].

The highest protein content was observed in *L. canarium*, at 16.29% (dry weight base). Protein is a key component that influences the nutritional value of food, with its biological value and quality depending on the amino acid composition, digestibility, absorption, and utilization by the body. Various studies have shown that protein concentrates and isolates are often used in the production of functional and nutritious foods [53]. Inthe et al. [54] reported that *A. anadara* from Pinrang waters, Indonesia, contained 12.51% protein (dry weight). Moniruzzaman et al. [55] documented variations in protein composition (wet weight base) among four species from Bangladeshi waters: *A. granosa* (75.4%), *Meretrix meretrix* (59.3%), *Crassostrea virginica* (67.2%), and *Saccostrea cucullata* (65.3%).

Lipids play a crucial role in biological functions, including energy storage, cell membrane structure, and signaling processes [56]. Generally, mollusks contain high-quality lipids that are beneficial to human health, although these vary depending on the species and habitat [57]. In this study, the highest crude fat content was found in *G. erosa* (0.85%). Erniati et al. [58] found total fat content in shellfish species *Crassostrea* sp. (10.83%) and *A. granosa* (3.04%) from North Aceh waters. Crude carbohydrate content was highest in *L. canarium* (5.27%). In mollusks, glycogen is the primary source of carbohydrates, providing energy and supporting gamete formation, as well as helping to maintain their condition during times of nutritional deficiency [59]. A different study reported that carbohydrate content in snow clam meat from Muara Angke, Jakarta, was 33.55% [58].

The highest total ash content was found in *L. canarium* (2.75%). Ash content in marine organisms generally correlates positively with heavy metal content, because metals like Cd and Pb tend to associate with minerals such as Ca and P [60]. Ash content reflects inorganic materials that support biological functions, such as bone formation, metabolic regulation, and enzymatic activities [61]. A previous study on *A. anatina*, which was exposed to various levels of Pb, Cu, and Cr in water, also showed a significant increase in ash

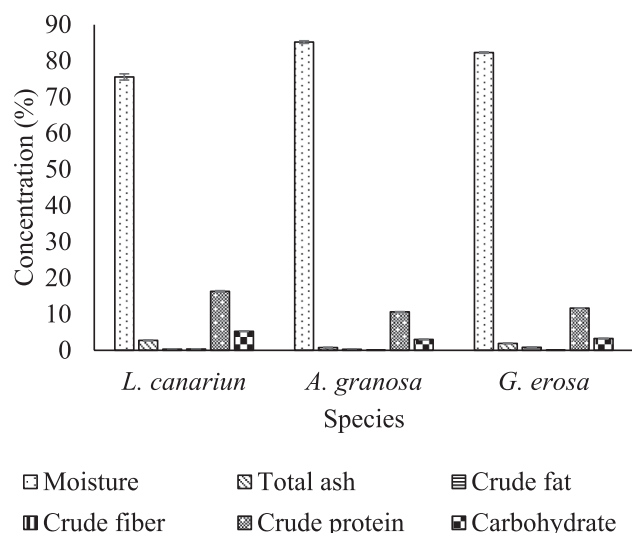


Fig. 5. Proximate composition of mollusk species.

content [62]. The highest crude fiber content was found in *L. canarium* (0.37%). In general, marine organisms have lower fiber content due to their composition, which is primarily dominated by protein and minerals. Previous studies have shown that mollusks, particularly bivalves and gastropods, tend to have low fiber levels. The species *Teredo navalis* from Halmahera waters, Indonesia, contained 0.056% crude fiber [63].

Proximate composition parameters are primarily determined by intrinsic biological characteristics and environmental conditions [64]. Additionally, the ash fraction typically comprises alkali and alkaline earth metals (AAEMs), heavy metals, non-metallic elements, and other inorganic compounds [60]. Furthermore, protein content and amino acid composition may not be quantitatively altered; they can be associated with physiological responses such as metallothionein synthesis, which plays a role in metal detoxification [28, 65]. Therefore, nutritional profiles may offer indirect insights into an organism's adaptive response to contaminated environments.

Statistical analysis revealed significant differences ( $p < 0.05$ ) in both essential (EAA) and non-essential (NEAA) amino acid contents among the three mollusk species. *L. canarium* exhibited high levels of glutamic acid and lysine, while *A. granosa* had the highest concentration of phenylalanine. Elevated levels of glutamic acid and glycine in mollusks may reflect species-specific biochemical adaptations to environmental stress and also contribute to the umami flavor profile.

Based on the amino acid profile (Table 1), it showed that EAA in *L. canarium* was dominated by lysine (5.25%), while *A. granosa* had the highest phenylalanine content (6.85%), and *G. erosa* had the highest leucine content (4.07%). Branched-chain amino acids (BCAAs) such as leucine, isoleucine, and valine were crucial for

protein synthesis, energy production, and cell signaling through the mechanistic target of rapamycin (mTOR) pathway, which regulated cell growth and provided nitrogen for the synthesis of NEAA that supported body metabolism [66]. Amin et al. [67] reported that *L. lambis* from the Red Sea Coast contained approximately 3.05% valine, 4.27% lysine, and 4.26% leucine. In contrast, Nguyen et al. [68] reported that BCAA composition in *Tegillarca granosa* from South Korean waters ranged from 0.13-0.17% (valine), 0.32-0.48% (leucine), and 0.12-0.15% (isoleucine).

Phenylalanine, a precursor to tyrosine, plays a significant role in cognitive function and dopamine production. Other studies reported phenylalanine content in various mollusks, including *Paphia malabarica* (28.20 mg/100 g), *Villorita cyprinoides* (33.11 mg/100 g), and *Crassostrea bilineata* (110.00 mg/100 g) [69]. The nutritional content of shellfish varies depending on several factors, including species type, individual condition, body part analyzed (e.g., tissue, shell, or meat), sex differences, season, and species morphological characteristics, as well as the ecological and geographical structure of their ecosystem [70, 65]. In this study, *L. canarium* showed the highest essential amino acid (EAA) content at 28.19%, which is higher than that of other species. Total essential amino acid content in *L. canarium* was above the WHO's recommended daily intake of 152.46 mg/kg body weight. [71, 66].

NEAA in *L. canarium* and *G. erosa* were dominated by glutamic acid, with contents of 10.79%, 8.97%, and 9.12%, respectively. Glutamic acid was recognized as a neurotransmitter essential for the nervous system and played a significant role in protein breakdown [72]. The higher glutamic acid content in these mollusks indicated its vital role in energy production and maintaining the body's acid-base balance [73]. Additionally, glutamic acid contributes to the umami, or savory taste, of



food. According to Ma et al. [74], L-glutamate and 5' ribonucleotides resulted in an increased intensity of umami taste, a characteristic feature of savory flavors. Dominance of glutamic acid was also observed in abalone (*Haliotis discus hannai* Ino) from Nanri Island, China, with a content of approximately 17.02% (dry weight) [75].

Additionally, the interaction between amino acids and heavy metals in mollusks is influenced by the ability of amino acids or proteins to bind these metals. This interaction is often associated with stress-response proteins, such as metallothionein, which are rich in specific amino acids (e.g., cysteine) with thiol (-SH) groups capable of binding heavy metals, including Cd, Cu, or Zn [76]. *L. canarium* showed the highest essential amino acid content and also accumulated elevated concentrations of Cd and Pb, suggesting that it may activate protein- or peptide-based detoxification mechanisms, such as expression of metallothionein. Free amino acids (FAAs), metallothionein (MTs), and chemical forms of heavy metals are closely associated with the mechanisms of metal accumulation and detoxification in mollusks [77]. Although cysteine, a key amino acid involved in MT synthesis, was not directly quantified in this study, the presence of other

amino acids, such as lysine and glutamic acid, may support protein biosynthesis under environmental stress. These amino acids could indirectly contribute to the detoxification response through their role in maintaining redox balance and cellular metabolism [78].

A positive correlation was observed between TOM and both carbohydrate content ( $r = 0.98$ ) and protein content ( $r = 0.95$ ), indicating that high levels of organic matter in the environment may enhance the availability of nutrients for mollusks. Additionally, Cd concentrations in sediment showed a positive correlation with carbohydrate ( $r = 0.99$ ), protein ( $r = 0.96$ ), and Cu levels in mollusk tissues ( $r = 0.96$ ). When mollusks are exposed to heavy metals, they may respond by increasing nutrient production and creating special compounds that help protect their cells [79]. The habitat of mollusks plays a crucial role in the bioaccumulation process, as elevated heavy metal concentrations are often detected in deeper waters or fine-grained substrates, which are commonly inhabited by a variety of mollusk and gastropod species. Protein compounds, including free amino acids, possess the capacity to bind heavy metals due to chemical interactions between metals and functional groups of proteins or amino acids. Biochemical parameters such as metallothioneins,

Table 1. Amino acid profiles of muscle mollusk species.

Amino acid profile (% w/w)	<i>L. canarium</i>	<i>A. granosa</i>	<i>G. erosa</i>
Essential amino acids (EAAs)			
Arginine (Arg)	4.32±0.03	2.95±0.05	2.81±0.03
Histidine (His)	1.79±0.02	0.94±0.02	1.46±0.03
Tryptophan (Try)	3.20±0.05	2.27±0.03	2.53±0.03
Isoleucine (Ile)	5.24±0.05	3.77±0.03	4.07±0.03
Leucine (Leu)	5.25±0.05	2.74±0.02	2.57±0.03
Lysine (Lys)	1.77±0.03	1.09±0.02	1.37±0.03
Methionine (Met)	3.57±0.03	6.85±0.05	3.37±0.03
Phenylalanine (Phe), Tyrosine (Tyr)	3.04±0.05	1.85±0.03	1.79±0.03
Total	28.19±0.29	22.46±0.23	19.97±0.23
Non-essential amino acids (NEAAs)			
Alanine (Ala)	4.55±0.05	3.68±0.03	4.99±0.03
Aspartic acid (Asp)	8.68±0.13	5.78±0.03	5.74±0.04
Glutamic acid (Glu)	10.79±0.10	8.97±0.03	9.12±0.03
Glycine (Gly)	5.70±0.05	3.12±0.03	3.91±0.04
Serine (Ser)	3.35±0.05	2.33±0.03	2.46±0.03
Threonine (Thr)	3.25±0.05	2.52±0.03	2.69±0.03
Valine (Val)	3.75±0.05	2.42±0.03	2.97±0.03
Total	40.07±0.48	28.82±0.18	31.89±0.22

Values are means (±SD) of three replicates.

catalase, phosphatases, and lipid peroxidation in *Perna canaliculus* have been used as biomarkers to detect exposure to heavy metals (As, Cd, Cu, Pb, Ni, and Zn) [80]. These parameters reflect biological responses to oxidative stress and the detoxification ability resulting from metal accumulation in shellfish tissues. Hertika et al. [65] reported a significant correlation between heavy metal concentrations (Pb, Cd, and Hg) and metallothionein levels in gill and stomach tissues of *Crassostrea* species, indicating that metallothionein expression functions as a biomarker for heavy metal exposure in mollusks. Similarly, Zhang et al. [28] demonstrated that exposure to Cd and Zn induces metallothionein expression in freshwater mollusks, highlighting the role of metallothionein in metal detoxification processes. These findings suggest that the amino acid profile may be linked to metal detoxification processes in mollusks exposed to contaminated environments.

Furthermore, amino acid composition also reflected environmental influences. Glutamate and lysine, two amino acids known for their metal-binding capabilities [81], were found to be dominant in Batu Belubang. Existence of biochemical adaptation mechanisms in mollusks in response to environmental stress caused by heavy metal exposure. Amino acids such as glutamate, lysine, and histidine play roles in metal binding and detoxification [82-83]. Moreover, TOM and fine sediment fractions (e.g., silt or mud) have been shown to increase metal bioavailability, consequently affecting bioaccumulation in benthic organisms [28]. Results showed that environmental factors affected both the amount of heavy metals in mollusks and their nutritional quality, which are important for seafood safety and the health of coastal ecosystems.

## Conclusion

In conclusion, three mollusk species (*L. canarium*, *A. granosa*, and *G. erosa*) demonstrated significant differences in their nutritional content and heavy metal content. Despite varying habitat characteristics, including sediment composition and organic matter content, the species showed generally safe levels of heavy metals, remaining within established consumption limits. High-quality lipids, proteins, and amino acids found in these mollusks indicate their potential as valuable sources of nutrition, particularly for human health. Moreover, the presence of EAA and NEAA, which are potentially involved in metal-binding processes, such as glutamic acid and lysine, suggests a biochemical response mechanism in mollusks exposed to metal contamination. This suggests that nutritional content, particularly the amino acid profile, may be indirectly related to mollusk detoxification of metals, such as through the production of metallothionein. These findings highlight the importance of understanding the nutritional

composition and safety of mollusks, especially in regions affected by heavy metal contamination, such as Bangka Island.

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## Conflict of Interest

The authors declare no conflict of interest.

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