

Assessment of Copper and Zinc Concentrations in *Anadara granosa*

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Abstract. Due to economic growth and increased human activities, large amounts of metal pollutants have been discharged into the aquatic environment. *Anadara granosa*, which is a filter feeder cockle species, can act as an indicator to evaluate the presence of metal elements. It is a prominent cockle species and an important protein source in Southeast Asia. Thus, this study aims to evaluate the distributions of metal elements in commercialised *Anadara granosa* sampled from Jeli wet market, and to assess the safety of consumption using Target Hazard Quotients (THQ). The concentrations of Cu, and Zn in those samples were determined using the acid digestion method, which has been verified with standard reference material. Based on the findings, metal concentrations in *Anadara granosa* followed the order of; Zn > Cu. The differences in metal accumulation observed in *Anadara granosa* were probably due to the differences in their environmental conditions. Results from preliminary risk assessments suggest that the risks posed by metals via consumption of *Anadara granosa* for Cu and Zn were within the tolerable region. The Hazard Quotient (HQ) of, Cu and Zn was < 1, indicating no potential human health risk. However, it is advisable to limit the intake of *Anadara granosa* to reduce the risk of potential health effects.

1 Introduction

Metals in the environment can come from a variety of sources, including both natural geologic sources and human-made sources. As a result of economic growth and human activities, large amounts of metals have been discharged into the aquatic environment [1]. Metals are among the pollutants that must be addressed due to their long-term persistence potential. Following their introduction into the aquatic environment, metals may be bioaccumulated by aquatic species and biomagnified along the food chain, culminating in elevated levels in predators and the human body. Essential metals, such as Cu and Zn, required in deficient amounts, can have adverse biological consequences at higher

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concentrations Others, such as Cd, are toxic and harmful even at low concentrations, which can cause toxicity effects [2].

Metals in surface water, suspended waste, and sediment, and interstitial water, for example, are constantly in contact with cockles [3]. The cockles, *A. granosa* is recognised as a low-priced source of protein in tropical areas, particularly in the Indo-Pacific region and economic importance in Malaysia. In Malaysian fishing villages, gathering *A. granosa* from beach beds is a popular activity due to edible species. Since they are sessile, filter or deposit feeders with a high filtering rate, restricted in their capacity to biotransform toxins, and have a high tolerance to several forms of pollution [4], *A. granosa* were often utilised in previous study as biomonitoring organism [5, 6].

Therefore, it is of great concern to know the possibility of these contaminants to accumulating in the *A. granosa* to ensure the intake stays within acceptable limits. Because of their tendency to deposit metals inside their tissues, filter feeder species such as *A. granosa* have long been used as biomonitoring organisms to detect metal pollution [7]. Thus, a study on *A. granosa* was conducted in order to assess risk to human consumption.

2 Materials and Methods

The *A. granosa* (n=30) was purchased in July to September 2022 from Jeli wet market. The samples were packed in low-density polyethylene sampling bags, refrigerated in an ice box, and transported to the laboratory the same day. Before dissection, the specimens were placed in a 20 °C freezer and their length and weight were measured. Tissue of *A. granosa* that had been removed from their shells were cleaned several times with deionized water before dried in the oven at 110 °C to achieve constant weight. Each dried tissue was pulverised with a pestle and mortar to improve sample homogeneity, and then kept in a desiccator.

2.1 Laboratory procedure

All chemicals used were of analytical quality, where concentrated nitric acid (HNO₃, 65%, (w/w)) solution were of Suprapur[®] quality. Certified Reference Materials ERM[®]-BB422 (trace elements in fish muscle) from Institute for Reference Materials and Measurements (IRMM, Belgium) was used to assess the accuracy of measurement methods for metals in *A. granosa*. Approximately, 1.0 g of dried samples were directly weighed into a 50 ml beaker and digested for the first hour on a hot plate at 40 °C with 10 ml of concentrated HNO₃. The temperature was then increased to 140 °C and kept there for another 3 hours. The digestion process was completed when all tissue samples were completely dissolved in acid. Then, allow the sample to cool to room temperature before being added to a 250 ml volumetric flask of double-distilled water. The clear sample was filtered via a 0.45 m PTFE membrane. Diluted sample solutions were then kept in centrifuge tubes at temperatures below 8°C, and concentrations were determined using an atomic absorption spectrophotometer.

2.2 Calculation and analyses

All statistical calculations were carried out using the software packages, Microsoft[®] Excel 2020 and SAS[®]JMP[®] version 9. An analysis of variance (ANOVA) was employed to analyse the significant differences between the studied elements [8]. In this study, the methodology for estimating target hazards provides an indication of human health risks related to pollutant exposure. As a result, the equation used to estimate the hazard quotient is as follows [9] :

$$THQ = \frac{EDI}{RfD} \quad (1)$$

The target hazard quotient (THQ) is a complicated variable that is frequently used to evaluate the possibility of non-carcinogenic risks related to long-term exposure to heavy metals from dietary sources such as water and aquatic life. Furthermore, THQ merely displays a risk level associated with pollutant exposure but does not assess the dangers. If the ratio is less than 1, the exposed population is not at danger and it is safe. However, there is a chance that the investigated metal will have negative health impacts when the ratio is larger than 1 [9]. The hazard analysis was utilised as the initial stage in a procedure to determine the risk associated with *A. granosa* ingestion.

3 Results and Discussion

To ensure the accuracy of the acid digestion process, certified reference material (ERM®-BB422) was used. Table 1 shows that the elemental concentrations determined in the certified reference materials were in a good agreement.

Table 1. Analysis of certified reference material.

	Certified / mg kg ⁻¹	Found / mg kg ⁻¹	Recovery / %
Cu	1.67	1.23	74
Zn	16.0	18.0	112.5

(*n* = 5)

In order to address the potential variability associated with size variation in the present study, an effort was made to sample *A. granosa* of comparable size, and their morphometric data are shown in Table 2. This is also meant to minimize the possible effect of size and age variation on metal accumulation. However, a previous study by Riani [10] stated that small shells have a higher tendency to accumulate heavy metals. The buildup of heavy metals will continue as the age of cockles increases. Thus, the large size of *A. granosa* tends to accumulate higher concentrations of metals [10].

The mean concentrations of heavy metals in the studied tissue of *A. granosa* are summarised in Table 2, expressed in mg/kg on a dry weight basis. Generally, the results demonstrated that the studied *A. granosa* contained higher concentrations of Zn. Essential metals such as Zn are needed in marine organisms' metabolism. As an important component of numerous enzymes, zinc is kept at a reasonably high level in the bodies of many species, including cockles. Due to different patterns of storage, feeding habits, internal regulation, and environmental factors, *A. granosa* shows significant differences ($p < 0.005$) in metal accumulation rates [6].

Table 2. Biometric parameters and metal concentrations of *Anadara granosa*

Biometric parameters		
Length (cm)	2.69 ± 0.14	
Weight (g)	1.72 ± 0.28	
Metal concentration (mg/kg)		Permissible limit by FAO (1983) (mg/kg)
Cu	6.08 ± 1.0	30
Zn	94.83 ± 0.5	30

(n=30)

However, concentrations of Zn exceeded the maximum permissible limit set by FAO (1983). This could be an alarming finding, and it is advisable to limit consumption of *A. granosa* in order to lessen the likelihood of adverse health effects. Generally, filter-feeding bivalve such as *A. granosa* are shown to accumulate the highest levels of Zn from aquatic environments. The results found in this study are compatible with the findings reported at Juru and Jerawi Rivers, Penang, due to industrial effluent [11].

As shown in Table 3, the THQ values are much lower than one, showing that the metals studied in *A. granosa* soft tissue pose no health risks to consumers. However, it is observed that THQ of Cd and Zn are near to one. Based on the THQ values, it indicates that consuming *A. granosa* throughout a lifetime is likely to have an adverse effect on Malaysians.

Table 3. The target hazard quotient and estimated daily intake for metal ingestion.

	THQ (mg/kg)	EDI (mg/kg)	PTWI mg/kg [12]
Cu	0.38	1.7×10^{-2}	245
Zn	0.80	2.2×10^{-1}	490

The Daily metal intake (EDI) was calculated based on the average metal concentrations in *A. granosa* soft tissues and the daily consumption rates. Current metal intakes were compared with the respective recommended daily dietary allowance recommended by JECFA as shown in Table 3. The findings indicated that *A. granosa* intake is less susceptible to environmental contamination in general, as the EDI were found to be lower than the PTWI reference values. Thus, the results revealed that the studied species consumption might not have any adverse effect on human health. Both the amount of metal present in food and how much of it is eaten each day affect the daily intake of metals. Additionally, a person's body weight might affect how well they tolerate pollution. [12]. The current study's EDI calculation solely considers *A. granosa*, which contributes just a small portion of contamination intake through daily food consumption. As a result, metal concentrations in other aspects of the aquatic environment, such as water or sediment samples, must be considered. However, based on the risk assessment for *A. granosa*, daily consumption may not generate any adverse health effects.

4 Conclusion

A study on the Cu and Zn concentrations in *A. granosa* has been undertaken. Based on THQ values, the findings indicate that metals assessed on the edible parts of *A. granosa* pose no health risks. However, because the THQ values of Zn in *A. granosa* are approaching one, extra consideration should be given to its consumption. Thus, to avoid the possibility of adverse health consequences, it is advisable to limit intake of *A. granosa*.

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