Research Article

Bioaccumulation and Health Risk Assessments of Heavy Metals in Mussels Collected from Madura Strait, Indonesia

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Abstract

Marine mussels are known as one of the biological indicators of heavy metals pollution in the aquatic environment and the sources of protein for human consumption. This study aimed to investigate the concentration of heavy metals (Cu, Zn, Pb, and Cd) in the seawater, sediment, and soft parts of the marine mussels (Perna viridis, Meretrix sp., and Anadara granosa), as well as to evaluate its health risk of consumption from Madura Strait, Indonesia. The seawater, sediment, and marine mussels were obtained from four sites and heavy metal concentrations were analyzed using Atomic Absorption Spectroscopy. In the seawater, heavy metals were in the order of Cu, Pb, Cd, and Zn from the highest to the lowest concentration respectively, while they went down in the order of Zn, Cu, Pb, and Cd in the sediment respectively. Excluding the Pb, higher Cu and Zn concentrations were observed in marine mussels than in seawater and sediments (BAF > 1). Furthermore, it was proven that Cu and Zn were considered as essential metals and required for metabolism, while Pb and Cd were non-essential metals which their biological functions were unknown. The Estimated Daily Intake (EDI) of the marine mussels was lower than the oral reference dose (ORD) and low values of target hazard quotient (THQ < 1) had shown that the consumption of mussels containing heavy metals would not cause significant health risks to humans. The health risk assessment indexes showed low values, which meant insignificant health hazard. However, long-term exposure and the impact of consuming mussels containing heavy metals may require additional investigation.

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1. Introduction

Heavy metals are defined as metallic elements with a density of over 5 g cm\(^{-3}\) which are associated with pollution and toxicity (Duffus, 2002; Tchounwou et al., 2012). It occurs naturally in low concentration. However, human activities have increased the concentrations significantly. Heavy metals enter aquatic environment from domestic, industrial, and fisheries activities (Keshta et al., 2020; Nowrouzi and Pourkhabbaz, 2014; Truchet et al., 2021; Yona et al., 2018). Due to its persistence and hardly degraded characteristics, an accumulation of heavy metals have been observed in the waters (Hoang et al., 2020; Karaouzas et al., 2021; Yona et al., 2020c), sediments (Sari et al., 2018; Wang et al., 2020; Yona et al., 2018), and living organisms (Krupnova et al., 2018; Mangalagiri et al., 2020; Yona et al., 2020a, 2020b) in the aquatic environments. The accumulation of heavy metals in these environments might reach toxic concentration levels and pose a hazard not only to the organisms but also to human health.

Mussels are benthic organisms that are often used as bioindicators for heavy metal pollution in coastal areas (Loder et al., 2016; Pinto et al., 2015; Sudsandee et al., 2017; Yona et al., 2016, 2020b). They are at high risk of accumulating contaminants since they are filter feeder and sessile. The accumulation of metals in its tissues depend on many factors, such as body size, body weight, age, and food acquisition capability (Honig et al., 2020; Mubiana et al., 2006; Yap et al., 2009). The heavy metal accumulation in the mussels can be assessed either from their hard shells or soft tissues (Dar et al., 2018; Proum et al., 2016; Yap et al. 2008). Heavy metals have been observed to accumulate in the gills, digestive gland, liver, and muscle of the mussels (Kouali et al., 2020; Ruiz et al., 2018; Vasanthi et al., 2017).

Mussels are also one of the most famous food sources that contain omega-3 rich fatty acids and proteins to improve human health (Carboni et al., 2019). Therefore, consuming mussels that contain heavy metals can pose a risk to human health. The health risk assessment of heavy metals consumption in marine mussels have been measured using several indexes by many studies. Yap et al. (2016) used Estimated Daily Index (EDI), Target Hazard Quotient (THQ), and Hazard Index (HI) to evaluate the consumption of wild and farmed Asian green mussels containing heavy metals from the Strait of Malacca and the Strait of Johore. Another study conducted by Sudsandee et al. (2017) in the upper Gulf of Thailand used Hazard Quotient (HQ) to calculate the non-carcinogenic risk of heavy metals consumption in blood cockles. Those indexes are determined using the concentration of each metal in the soft tissue of the mussels combined with various other parameters, such as body weight, exposure time, intake rate, and also oral reference dose.

Green mussel (Perna viridis), blood cockle (Anadara granosa), and Asiatic hard clam (Meretrix sp.) are highly consumed by local people in the village of Sukolilo, Bangkalan, Madura Island, Indonesia. The mussels are harvested from the wild in the Madura Strait by the fishermen and sold in the market. Madura Strait separates Java and Madura Island and it is highly influenced by the industrial and domestic activities from both islands. Furthermore, Putri et al. (2016) and Ahyar et al. (2018) reported high concentrations of heavy metals in this area. Therefore, this study was conducted to investigate the concentrations of Cu, Zn, Pb, and Cd in the seawater, sediments, and three species of mussels obtained from the Madura Waters as well as to evaluate the health risk exposure of heavy metals in the mussels in association with human consumption.

2. Materials and Methods

2.1 Study Area and Sample Collection

The sampling was performed in August, 2020 in one of the estuary areas of Madura Strait, near the end of Pande River, Sukolilo Village, Bangkalan, Madura, Indonesia. The village is a home to many small-scale fishing activities, such as catching fish around Madura Strait, producing salted and smoked fish, and also collecting wild marine mussels. A total of four sampling sites were used to obtain seawater, sediment, and mussel samples for the heavy metal analysis (Figure 1).

At each site, 500 ml of seawater samples were collected into polyethylene water bottles and acidified with 5 drops of HNO\(_3\). The sediments samples were obtained using hand shovel from surface to 5 cm depth. In order to avoid contamination from the shovel, only the inner part of the sediments was kept in a plastic bag. About 10 individuals with approximately 5 cm long mussels from every species were collected from each sampling site. The mussels were rinsed with distilled water to remove the dirt. On site, the soft tissues of the mussels were separated from the hard shells and placed in zipper bag. All samples were taken to the laboratory for heavy metal analysis. Cu, Zn, Pb, and Cd were analyzed in the seawater, sediments, and P. viridis; only Zn and Cd were found in the A. granosa; and Cu and Pb were found in the Meretrix sp. (Table 1).

2.2 Laboratory Measurements

The analysis of heavy metal in the seawater and sediment samples (n=4) were performed according to the instruction of Ali et al. (2016). Seawater samples
were filtered using 0.45 μm Whatman filter paper and 50 ml of sample were placed in 100 ml size of beaker glass. The acid digestions were carried out by adding 5 ml HNO₃ to the water sample and evaporating it to an almost dry state on a hot plate. Digestion processes were continued until the light-colored residue remained and filtered through Whatman filter paper.

The sediment samples were dried overnight using oven at 70° C and crushed as fine as possible using pestle and mortar. The acid digestions were then performed by adding 10 ml HNO₃ and continued until the solution became clear. Furthermore, the solution was filtered through Whatman filter paper and made to 100 ml volume with deionized water.

Heavy metal analysis for mussel samples was performed according to Yap et al. (2016). Soft tissue of mussels was dried overnight at 105° C using oven and about 0.5 g was digested using 10 ml HNO₃. The digested mussel samples were filtered through Whatman filter paper and diluted with 40 ml deionized water. The concentration of Cu, Zn, Pb, and Cd were determined using Atomic Absorption Spectrophotometer (Shimadzu type AA. 6200) using standards of each metal. In order to monitor the performance of the instrument, blank and standard solution were used to obtain data quality by developing calibration curves.

2.3 Data Analysis

The health risk assessment indexes were calculated using two levels of consumption rates, namely the average level mussel (ALM) and the high-level mussel (HLM). The values of ALM and HLM are 17.86 g/day and 35.71 g/day, respectively (Yap et al., 2016). The Estimated Daily Intake (EDI) was measured to determine daily limit of the consumption of mussels using the following equation:

$$ EDI = (C_{metal} \times CR)/BW $$

The EDI was presented in μg/kg/day; CR is the consumption rates (ALM and HLM) in g/day and BW is adult body weight (60 kg). The Target Hazard Quotient (THQ) was used to understand the non-carcinogenic effect of heavy metals in the mussel’s soft tissues. It was calculated using the following equation:

$$ THQ = \frac{(EF \times ED \times CR \times C_{metal})}{(ORD \times ABW \times AET)} \times 10^{-3} $$

EF is the frequency exposure (365 days/year); ED is the exposure duration (equivalent to 70 years); CR is the consumption rate (ALM and HLM); C_{metal} is the concentration of metal in the mussel (mg/kg); ORD is Oral Reference Dose (400 and 300 μg/kg/day for Cu and Zn, respectively; and 1.5 mg/kg/day for Pb); ABW is the average body weight (60 kg), AET is the average exposure time for non-carcinogens (365 days/year × ED); and 10⁻³ is unit conversion factor.

Figure 1. Study area and sampling location in the Madura Strait to collect seawater, sediments and mussels’ samples
3. Results and Discussion

3.1 Heavy metal concentrations in the seawater, sediments, and mussels

Heavy metal in the seawater, sediments, and different types of mussels were discovered in different concentrations (Figure 2). In the seawater, heavy metals concentrations were in the order of Cu, Pb, Cd, and Zn from the highest to the lowest; while in the sediments were Zn, Cu, Pb, and Cd respectively. Zn was not detected in the seawater, while Cd was not detected in the sediment (detection limit of Zn was 0.031 ppm and Cd was 0.0028 ppm). In natural seawater, the concentration of dissolved Zn is very small and anthropogenic input increases its concentration significantly (Thuróczy et al., 2010). In addition, Zn is a type of metal needed for the growth of organisms, therefore, the concentration on the water surface may be depleted. In contrast, although with very low values, Cd concentrations were observed in the seawater (0.01 ± 0.004 ppm) and it was not detected in the sediment samples. A low anthropogenic input that can easily dissolves in the water, resulted in low concentrations of Cd in the surface water and very little would be accumulated in the sediments (Gerringa et al., 2001). The results were in agreement with some other studies. The sediments accumulated more heavy metals than seawater (Abdel Gawad, 2018; Baltas et al., 2017; Hoang et al., 2020) because metals have the ability to settle, precipitate, accumulate, and bind to the sediment (Hoang et al., 2020; Schertzinger et al., 2018). Heavy metals in the coastal environment are highly influenced by the anthropogenic activities. Sukolilo village is a small fishing village with lots of fishing activities. The accumulation of Cu, Zn, and Pb in this study area might be the result of the use of antifouling paints on boats, river discharge, gas, and oil discharge from the fisheries ship, the same reasons for many other studies (Pinto et al., 2015; Vasanthi et al., 2012; Youssef et al., 2017).

It was reported that heavy metals accumulate more in the mussels than in the seawater and sediments. An accumulation over a longer period of time can lead to higher concentration of heavy metals in the body of organisms than in the seawater or sediments (Ibrahim and El-regal, 2014). Accumulation of heavy metals in the soft tissue of the mussels were higher than in the seawater and sediments except for Pb. Furthermore, Cu concentrations was discovered to be the highest in P. viridis (11.08 ± 4.43 ppm), followed by Meretrix sp. (9.46 ± 7.90 ppm), sediments (4.35 ± 2.33 ppm), and seawater (1.11 ± 1.59 ppm). The highest concentration of Zn was also observed in P. viridis (18.43 ± 2.17 ppm), followed by A. granosa (13.50 ± 5.04 ppm) and sediments (8.17 ± 4.47 ppm). Meanwhile, the highest concentration of Cd was in A. granosa (0.37 ppm), followed by P. viridis (0.08 ± 0.057 ppm) and seawater (0.007 ± 0.004 ppm). Sediment contained the highest concentration of Pb (2.01 ± 0.95 ppm), followed by P. viridis (0.89 ± 1.02 ppm), Meretrix sp. (0.45 ± 0.49 ppm), and seawater (0.02 ± 0.005 ppm). The heavy metal concentrations in this study are comparable with other studies from other localities worldwide (Table 2).

The accumulation of Cu and Pb was higher in the P. viridis than in the Meretrix sp. and the accumulation of Zn was higher in P. viridis compared to that in A. granosa. The results showed that P. viridis accumulated more heavy metals than the other two mussels. Conversely, the accumulation of Cd was higher in A. granosa than in P. viridis. In addition, the Cd concentrations was low in the seawater and sediment, but it was accumulated quite high in A. granosa from one study site and below the detection limit for the other study sites. More investigation in term of sampling sites and periods are needed to understand the distribution and accumulation of Cd in this study area. Despite that, bioaccumulation of Cd has been observed to be very low in many studies (Rashida et al., 2015; Vasanthi et al., 2012). Moreover, Yap et al., (2008) reported higher concentration of Cd in the hard shells of the mussels rather than in the soft tissues.

3.2 Bioaccumulation of heavy metals in the mussels

The bioaccumulation factor (BAF) was calculated to determine the accumulation of heavy metal from the environment in the body of the organisms. Furthermore, BAF in P. viridis was calculated for Cu, Zn, and Pb while in Meretrix sp., it was calculated for Cu and Pb. The BAF of Cd could not be determined in the P. viridis and A. granosa because the concentrations of Cd in the sediments were below the detection limit.

Table 1. Type of metals analyzed in the samples

| Sample   | Cu | Zn | Pb | Cd |
|----------|----|----|----|----|
| Seawater | √  | √  | √  | √  |
| Sediment | √  | √  | √  | √  |
| P. viridis | √  | √  | √  | √  |
| A. granosa | –  | √  | –  | √  |
| Meretrix sp. | √  | –  | √  | –  |
The BAF values of heavy metals varied between the mussels (Table 3). *P. viridis* was considered to be a macro-concentrator of Cu and Zn, as well as micro-concentrator of Pb. In contrast, *Meretrix* sp. was observed as macro-concentrator of Cu and deconcentrator of Pb. Micro-concentrator type was

**Table 2.** Comparison the results of this study and other studies

| Location                      | Sample   | Heavy metal concentrations | References                      |
|-------------------------------|----------|-----------------------------|---------------------------------|
|                               |          | Cu  | Zn  | Pb   | Cd   |
| Gulf of Suez, Egypt           | Seawater | 0.26±0.62 µg/g   | 0.2±20.25 µg/g   | 0.08±1.80 µg/g   | 0.013±0.13 µg/g   | (Nour & El-Sawy, 2022) |
|                               | Sediment | 0.81±13.2 µg/g  | 4.33±59.4 µg/g  | 5.3±28.3 µg/g  | 0.12±1.23 µg/g | |
|                               | Seashells| 3.33±7.62 µg/g  | 13.4±32.4 µg/g  | 3.23±7.51 µg/g  | 0.82±1.90 µg/g  | |
| Coastal area of Eastern Black Sea, Turkey | Seawater | 9.17±0.73 µg/g  | 4.33±0.43 µg/g  | 6.20±0.61 µg/g  | –               | (Baltac’s et al. 2017) |
|                               | Sediment | 357.02±9.02 µg/g | 357.02±9.02 µg/g | 97.33±3.20 µg/g | –               | |
|                               | Mussel   | 276.53±7.97 µg/g | 1555.24±41.01 µg/g | 76.46±2.61 µg/g | –               | |
| Eastern coastal waters of Guangdong Province, South China | Seawater | 2.39±0.40 µg/l | 9.10±3.92 µg/l | 1.52±0.92 µg/l | 0.03±0.11 µg/l | (Zhang et al. 2016) |
|                               | Sediment | 3.12±0.99 µg/kg | 49.13±19.99 µg/kg | 41.56±16.74 mg/kg | –               | |
|                               | Mussel   | 41.30±16.79 mg/kg dry weight | 38.20±16.38 mg/kg dry weight | 3.02 mg/kg dry weight | 1.15±1.04 mg/kg dry weight | |
| Madura Waters, Indonesia      | Seawater | 1.11±1.59 ppm | Not detected | 0.02±0.05 ppm | 0.01±0.004 ppm | This study |
|                               | Sediment | 4.35±2.33 ppm | 4.17±4.47 ppm | 2.01±0.95 ppm | Not detected | |
|                               | Mussel   | 10.27±5.99 ppm | 15.96±4.46 ppm | 0.67±0.78 ppm | 0.18±0.17 ppm | |

**Table 3.** Bioaccumulation factor (BAF) of heavy metals from the surface sediments in the mussels

| Species   | Cu       | Zn       | Pb       |
|-----------|----------|----------|----------|
| *P. viridis* | 4.24±4.49 | 2.66±1.00 | 1.11±1.89 |
| *Meretrix* sp. | 3.14±2.89 | *        | 0.55±0.93 |
| *A. granosa* | *        | 1.95±1.10 | *        |

*No data available for heavy metals in the soft tissues of the mussels

**Table 4.** Estimated Daily Intake (EDI) of heavy metals for average (ALM) and high level (HLM) consumption rate of the mussels

| Species   | Average Estimated Daily Intake (µg/kg/day) | Cu | Zn | Pb |
|-----------|------------------------------------------|----|----|----|
|           | ALM               | HLM             | ALM  | HLM |
| *P. viridis* | 3.30±1.32   | 6.59±2.64   | 5.48±0.65 | 10.96±1.29 | 0.27±0.30 | 0.53±0.61 |
| *Meretrix* sp. | 2.82±2.35   | 5.63±4.70   | –   | –   | 0.13±0.14 | 0.27±0.29 |
| *A. granosa* | –           | –             | 4.02±1.50 | 8.03±3.00 | –     | –           |

**Table 5.** Target Hazard Quotient (THQ) of heavy metals for average (ALM) and high level (HLM) consumption rate of the mussels

| Species   | Cu       | Zn       | Pb         | HLM |
|-----------|----------|----------|------------|-----|
|           | ALM   | HLM | ALM  | HLM    |
| *P. viridis* | 0.08 | 0.16 | 0.02 | 0.04 |
| *Meretrix* sp. | 0.07 | 0.14 | – | – |
| *A. granosa* | – | – | 0.01 | 0.03 |
more essential metals in their soft tissue from the sediments. In contrast, BAF < 1 for Pb meant the mussels restricted the accumulation of Pb, since it was considered as non-essential metal. Pinto et al. (2015) reported high accumulation of Cu in P. viridis related to the reproduction process and metabolic regulation. Zahir et al. (2011) in their study of accumulation of Zn, Cu, and Pb in A. granosa from Langkawi Island, Malaysia discovered the limited ability of the mussels in accumulating the metals (< 1) except for Zn and the accumulation was related to the concentration of metals in the environment.

3.3 Health risk assessment of heavy metals in the mussels

The Estimated Daily Intake (EDI) value varied between the metals and the mussels (Table 4). The daily consumption rates of P. viridis containing Cu and Pb were higher than the consumption rates for Meretrix sp. containing the same metals for both ALM and HLM. The daily consumption rates of P. viridis containing Zn were also higher compared to the consumption rates of A. granosa with the same metals for both ALM and HLM. The lowest daily intake of Pb was observed in Meretrix sp. for the average level consumption of 0.13±0.14 μg/kg/day and the highest was observed in P. viridis for high level consumption rate of 0.53±0.61 μg/kg/day. The HLM of Pb for Meretrix sp. (0.27±0.29 μg/kg/day) was in the same number with the ALM for P. viridis (0.27±0.30 μg/kg/day). The EDI of heavy metals for ALM and HLM consumers in the P. viridis for Cu were 3.30±1.32 μg/kg/day and 6.59±2.64 μg/kg/day, respectively, and for Zn were 5.48±0.65 μg/kg/day and 10.96±1.29 μg/kg/day, respectively. The daily intake of A. granosa containing Zn and the average level consumption was 4.02±1.50 μg/kg/day while for the high level was 8.03±3.00 μg/kg/day. Meretrix sp. containing Cu could be consumed daily for the average level of 2.82±2.35 μg/kg/day and high level of 5.63±4.70 μg/kg/day. The values of EDI were compared to ORD for each metal in the mussels, and the results showed that all the ALM and HLM values were lower than the ORD guidelines. It was indicated that the daily consumption of the mussels containing Cu, Zn, and Pb in the study area would not cause significant health risks to human.

The potential non-carcinogenic health risk from consuming mussels containing heavy metals was calculated using the Target Hazard Quotient (THQ). Furthermore, the THQ for all metals in the three mussels were less than 1, which meant that there was no potential carcinogenic health risk in consuming the mussels from

Figure 2. Concentration of Cu (a), Zn (b), Pb (c) and Cd (d) in the seawater, sediment and soft tissue of mussels. Zn concentration in the seawater and Cd concentration in the sediment were below detection limits.

observed in A. granosa for metal Zn, while macro and micro-concentrator types occurred in the mussels with higher concentration of heavy metals in its soft tissues compared to the concentration of heavy metals in the sediment. The bioaccumulation is related to the ability of the organisms to absorb metals from the medium they live (Szefer et al., 1999; Pinto et al., 2015). The BAF > 1 for Cu and Zn proved that the mussels accumulated
the study area (Table 5). A THQ less than 1 has been observed in mussels of Bulgarian Black Sea (Peycheva et al., 2019), marine mussels from the Strait of Malacca and the Strait of Johore (Yap et al., 2016), and mussels from Boka Kotor'ska Bay Montenegro (Perosevic et al., 2018). The health risk assessment indexes showed low values, which meant insignificant health hazard, but long-term exposure and consuming mussels containing heavy metals might require additional studies.

4. Conclusion

This study reported the variabilities of heavy metal concentrations (Cu, Zn, Pb, and Cd) in seawater, sediments, and mussels in Madura water. Cu, Zn, and Cd accumulated more in the soft tissues of the mussels, while it was observed that the Pb concentrations were higher in the sediments. The result of bioaccumulation factor (BAF > 1) showed that mussels accumulated more Cu and Zn in its body and restricted the accumulation of Pb from the sediments. The Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ) showed that the consumptions of mussels containing heavy metals in this study area were within the safe limit and have not yet caused potential health hazard. However, long-term studies are needed to monitor the concentrations and other elements in mussels and their environment, as well as the potential effect on human health.

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Authors' Contribution

Defri Yona; designed the sampling process and drafted the manuscript. Aida Sartimbul, Muhammad Arif Rahman and Syarifah; Priyanka Mondal; proofread the manuscript. Abdullah Hamid and Tsania Humairoh; designed the sampling process and collected the data. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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