Heavy metals accumulation and risk assessment of *Anadara granosa*
from eastern water of Java Sea, Indonesia

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Abstract

Blood cockle (*Anadara granosa*) has been known as one of the high economic value clams and an important marine product in many different countries including Indonesia. Its filter feeder life cycle resulted in the vulnerability of this organism in accumulating metal pollutants from the sediment. Consumption of blood cockles contaminated with heavy metals might cause potential health risk to human. This study was conducted to assess heavy metals Fe and Zn accumulation in sediments and blood cockle tissues from eastern water of Java Sea and to investigate its health risk potential. Eastern water of Java Sea is polluted with heavy metals from high industrial and domestic activities. The results showed that Fe concentrations were found higher than the concentrations of Zn in both sediments and blood cockle tissues. Higher concentrations of Fe were observed in the sediments, while the concentrations of Zn were found higher in the cockle tissues. These distribution patterns resulted in higher bio-concentration factors (BCF > 1) of Zn compared to Fe (BCF < 1) indicating blood cockle accumulated more of Zn from the environment. The estimated daily intake (EDI) of blood cockles was found to be lower than oral reference doses (ORDs) for both
metals, thus showing no potential human health risk in consuming the cockles. However, attention on the rate of consumption is still needed due to the ability of blood cockles to accumulate heavy metals from the contaminated environment that will bring hazard to human.

**Keywords**: Blood cockle (*Anadara granosa*), bio-concentration factor (BCF), estimated daily intake (EDI), heavy metal, Java Sea.

1. **Introduction**

   Blood cockle or in Indonesian known as “kerang darah” is one of the important marine molluscs as food sources in Indonesia. It can be used as an alternative protein source because it contains 9.64 % protein and some minerals such as zinc, iron, copper and calcium (Solang, 2017). In Indonesia, blood cockle can be harvested either from the natural sources or from aquaculture.

   The habitat of blood cockle is soft muddy bottom sediment of shallow waters (Mzighani, 2007) and this habitat is subjected to pollution such as heavy metals. Mollusc are well known to accumulate metals in its soft tissues (Alina et al., 2012; Hossen et al., 2014; Yap et al., 2016; Yona et al., 2016). As one of the food sources, this metal accumulation in the mollusc might bring harm to human (Tchounwou et al., 2012). The study of heavy metals accumulations in the blood cockle have been conducted in several countries such as Indonesia (Amriarni et al., 2012; Selpiani et al., 2015), Malaysia (Hossen et al., 2014; Yap et al., 2008; Zahir et al., 2011) and Thailand (Sudsandee et al., 2017). Health risk assessments of mollusc have also been studied by Yap et al. (2016) and Suddandee et al. (2017) and they found that heavy metals accumulated in the mollusc were not likely to cause harm to human.
Eastern water of Java Sea is known with the mollusc aquacultures. However, the water is also contaminated with pollutant from the heavy industrial activities from the surrounding cities. Thus, this study was conducted to assess heavy metals Fe and Zn accumulations in sediments and blood cockle tissues from eastern water of Java Sea and to investigate its health risk potential.

2. Material and Methods

Study sites and sample collection

The study sites were located in the eastern water of Java Sea in the Gresik City. Gresik has been known as one of the center of industrial activities, home to many factories, in Indonesia. It is also famous with the blood cockle aquacultures. Sampling was conducted in February 2018. There were six sampling stations, two stations were near the river mouth and four stations were located about 100 m seaward (Figure 1). About 500 g of surface sediment samples were collected using core sampler and placed in zip lock plastic bag. Blood cockle samples were collected from the water with the help of fishermen using their traditional device. In each sampling sites, 30-50 individual blood cockles with shell lengths of 2-3 cm were roughly rinsed with seawater to remove attached particles and mud and stored in plastic bags. Both sediment and blood cockle samples were placed in an ice box and transferred to the laboratory.

Heavy metal analysis
Figure 1 Sampling location of blood cockle *Anadara granosa* from the eastern water of Java Sea.

In the laboratory, sediment samples were dried in an oven at 60 °C for 48 h and then crushed to the finest possible fraction using pestle and mortar. About 2 g of the samples were digested with 10 ml of 70 % reagent grade nitric acid overnight. Blood cockles were rinsed with distilled water before dissected and the soft tissues were oven dried at 60 °C for 48 h. The digestion of blood cockles were conducted by placing about 0.5 g dried and grinded mussel tissues in a beaker glass filled with 10 ml HNO₃ overnight. Iron and zinc concentrations from both digested sediment and blood cockle samples were measured using a Shimadzu Flame Atomic Absorption Spectrophotometer Model AA-6800. The performances of the instrument were monitored using blank and standard solutions to obtain data quality by developing calibration curves. The heavy metal analysis was conducted according to method from Yap et al. (2016).
Data analysis

Bioconcentration factors (BCF) were calculated by dividing the concentration of the metal in the tissue blood cockle to that in the sediment. BCF > 1 indicate the mussels accumulated more metals in its tissues than the accumulation in the sediment. On the other hand, BCF < 1 shows more metals in the sediments than in the blood cockle tissues (DeForest et al., 2007).

Estimated daily intake (EDI) was used to assess the safety of the consumption rate of the blood cockle using the following equation:

$$\text{EDI} = \frac{(\text{Mc} \times \text{consumption rate})}{\text{body weight}}$$

Mc is the metal concentration in the blood cockle tissues; consumption rate is 17.86 g/day for ALM (average blood cockle) and 35.7 g/day for HLM (high level blood cockle); and body weight is 60 kg. The results of EDI is then confirmed with the oral reference dose (ORD in µg/kg/day) to evaluate the safety level in consuming the blood cockle.

3. Result and Discussion

The distribution patterns of Fe and Zn in the study areas varied widely both in the sediments and blood cockle tissues (Figure 2). The concentrations of Fe were observed higher in the sediments compared to the concentration in the blood cockles (Figure 2A). On the other hand, Zn concentrations were found higher in the blood cockle tissues than the one in the sediments (Figure 2B).

Fe concentrations in the sediments were in the range of 74.82–91.66 mg/kg, while Zn concentrations were very much lower in the range of 1.73–2.94 mg/kg. The concentration values were observed in the similar patterns among sampling stations for both metals. Most studies have found that the concentrations of Fe were higher than the
concentrations of Zn (EL-Bady, 2014; Nethaji et al., 2017; Nour and El-Sorogy, 2017; Saher and Siddiqui, 2016). Both metals are released to the environment from natural and anthropogenic sources. However, the input of Zn has been well known from the human activities, while the input of iron is from the lithogenic origin (Serrano et al., 2011; Winton et al., 2016). Moreover, higher concentrations of Fe might also be the result of broad use of this metal in daily life.

**Figure 2** Heavy metal concentrations of Fe (A) and Zn (B) in the sediment and blood cockle in the study area.

The concentrations of Fe in the blood cockle were in the range of 3.39–8.95 mg/kg and the concentrations of Zn were 1.54–5.48 mg/kg. Despite the similar values of metals concentration presented in the blood cockle, compared to the metal concentrations in the sediments revealed that zinc was absorbed more by the organisms and resulted in the difference of bio–concentration factors (Table 1). BCFs of Fe were very small (< 1) for most sampling stations, while BCFs of Zn were observed more than 1 except at Station 5. According to Dallinger (1993), the accumulation of Zn in the blood cockle tissues is classified as macroconcentrator (BCF > 2) and the accumulation of Fe is considered deconcentrator (BCF < 1). Although, iron and zinc have been known as
essential metals that are required by the organisms for various biochemical and physiological functions (Tchounwou et al., 2012), but the metabolism processes of both metals inside the organisms might be different. Zinc can be accumulated and stored over long periods of time, while iron might be absorbed and readily discharge by excretion (Dallinger, 1993). Higher accumulation of Zn compared to the accumulation of Fe has also been observed in the study of Yap and Cheng (2013) in the tissues of mangrove snail *Nerita lineata*. Moreover, the accumulation processes of heavy metal in marine organisms are influenced by several factors such as water temperature, salinity, organic matter, heavy metal bioavailability and also biological parameters of the organisms (Belabed et al., 2013).

Estimated daily intakes (EDI) show both metals were in the lower values than oral reference dose (ORD) guidelines (Table 1). The EDI for Fe for ALM and HLM were in the range of 1.01–2.66 and 2.02–5.33 µg/kg/day, respectively. The EDI for Zn for ALM were in the range of 0.56–1.63 µg/kg/day and 0.92–3.26 µg/kg/day for HLM. The results show that the intake of both metal to human consumptions are still in the permissible tolerance for human health. Similar results were also obtained in the study of Yap et al. (2016) and Jovic and Stankovic (2014).
Table 1 Bioconcentration factor (BCF) and estimated daily index (EDI) for Fe and Zn

| Sampling station | Bioconcentration Factor (BCF) | Estimated Daily Index (EDI) |
|------------------|-------------------------------|-----------------------------|
|                  | Fe ALM | Zn ALM | Fe HLM | Zn HLM | Fe ALM | Zn ALM | Fe HLM | Zn HLM |
| 1                | 0.07   | 2.53   | 1.55   | 3.10   | 1.43   | 2.86   |
| 2                | 0.10   | 2.81   | 2.32   | 4.64   | 1.44   | 2.89   |
| 3                | 0.05   | 3.00   | 1.06   | 2.12   | 1.63   | 3.26   |
| 4                | 0.09   | 2.63   | 2.29   | 4.57   | 1.37   | 2.74   |
| 5                | 0.04   | 0.86   | 1.01   | 2.02   | 0.56   | 0.92   |
| 6                | 0.10   | 2.05   | 2.66   | 5.33   | 1.25   | 2.49   |

Oral Reference Dose (µg/kg/day) 700 300

ALM (Average Level Mussel); HLM (High Level Mussel)

4. Conclusion

The distributions of Fe and Zn in the sediments and in the blood cockle tissues showed the opposite patterns in which Fe was found higher in the sediment, while Zn was found higher in the blood cockle tissues. This pattern resulted in the higher BCF values of Zn compared to the BCF of Fe. Both metals are essential, however Zn might be accumulated in longer period of time than Fe that is readily be used and excreted by the organisms. Estimated Daily Intake (EDI) showed both metals present in the blood cockles are still in the permissible limit to be consumed by human.

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