# Bioconcentration of lead (Pb) and cadmium (Cd) in green-lipped mussels (*Perna viridis*) in the coastal waters of Semarang Bay, Indonesia

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**Abstract**. The utilization of the coastal areas around Semarang Bay is very intensive, having the potential to cause pollution to coastal waters. Pb and Cd are 2 types of heavy metals that cause pollution in the waters of Semarang Bay. Both types of metals are found in various types of aquatic biota in the Semarang Bay, such as plankton, fish and green shellfish. This research was conducted to determine the concentration, bioconcentration (BCF), maximum acceptable concentration (MTI) of Pb and Cd in the waters and soft tissue of green mussels from the Semarang Bay. Green shells were collected from 2 locations in Semarang Bay, namely the waters around Tambak Mulyo and Bedono. The concentrations of Pb and Cd in water and soft tissue were analyzed by Atomic Absorption Spectrophotometry (AAS). Furthermore, the BCF and MTI values were determined. The results showed that the concentrations of Pb and Cd metals in seawater around the Mulyo Pond had exceeded the seawater quality standards for marine biota. The concentration of Pb in seawater around Tambak Mulyo was higher than in Bedono. The concentration of Cd around Tambak Mulyo was lower than in Bedono. Pb concentration in the soft tissue of green mussels has exceeded the limit of Pb concentration in fish meat and mollusks, according to the Indonesian National Standard. The bioconcentration factor of Cd in green mussels was higher than that of Pb.
**Key Words**: BCF, green clams, heavy metals, MTI, Semarang Bay.

**Introduction**. Semarang Bay stretches along the coastal waters of Kendal Regency to Demak Regency, Central Java Province. It is bordered by Tanjung Korowelang, Kendal Regency to Morodemak waters, Demak Regency, with a length of approximately 23 miles (Suhariyono 2003). The three districts and cities on the edge of Semarang Bay have a variety of activities that are quite intensive, including housing, industries, seaports, steam power plants, coastal tourism and fish/shrimp ponds (Haeruddin 2006; Haeruddin et al 2019a,b). The intensive utilization of coastal areas has the potential to cause pollution in coastal waters. Various studies have shown that the coastal waters of Semarang Bay have been polluted. Lead (Pb) and cadmium (Cd), which are 2 types of heavy metals, pollute the waters of Semarang Bay (Arifin 2001; Haeruddin 2006; Adinugroho et al 2015; MMFA 2015). Both types of metals are often found in the waters (Stankovic & Jovic 2012) and have a negative impact on aquatic biota (Haeruddin 2006; Adinugroho et al 2014).

Metals found in animals or humans can come from the consumption of shellfish. Mollusks like shellfish can accumulate heavy metals in their bodies and can move to other living things through the food chain (Azhar et al 2012; Chalghmi et al 2016; Khusnia et al 2019). Several studies have found that heavy metal concentrations are quite high in the soft tissue of shellfish (Cevik et al 2008; Karadede-Akin & Unlu 2007; Bartolome et al 2010; Azhar et al 2012; Chalghmi et al 2016).

The coastal communities around Semarang Bay have long cultivated green mussels, which are sold in local markets in the city of Semarang and its surroundings. Cultivated green shells can reproduce and grow into consumption-size shells (Chou & Lee 1997). Pb and Cd were found in plankton, fish and green shellfish in Semarang Bay (Adinugroho et al 2014; Khusnia et al 2019).

Heavy metals found in waters can accumulate quickly in the shells because they can pass through the soft tissue easily (Baldwin & Marshall 1999). Metal accumulation is directly related to bioavailability, which is related to the life cycle of shellfish, especially age and maturity (Cossa 1989). Besides, it is influenced by several other factors: body size (Riget et al 1996), internal cavity volume (Mubiana et al 2006), and physiological conditions (Soto et al 2000). Another factor that needs to be considered is the fat content of shellfish. The results of the research by Khusnia et al (2019) show that the Pb concentration in the soft tissue of green mussels from the coastal waters of Semarang City ranges from 0.29 to 0.55 mg $kg^{-1}$. Metal pollutants need attention because they can pollute the aquatic environment and cause public health problems (Stancovic et al 2012). Metal concentrations in the human body can have various effects on the human body (Stankovic et al 2012; Stankovic & Jovic 2012).

The main challenge for ecotoxicology is providing an approach to forecasting hazards and evaluating the risks facing an ecosystem (Bacci 1993). The residual levels of various toxic substances in various species that are consumed can be used as a tool to determine the potential risks received by consumers (Liu et al 2010). The chances of having an adverse effect as a result of human consumption of seafood can be calculated by using the Acceptable Daily Intake (ADI) or Maximum Weekly Intake (MWI). This research was conducted to analyze the concentrations of Pb and Cd in seawater and soft tissue of green mussels, distribution, bioconcentration and risk analysis for humans.

**Material and Method**. Water and green mussel samples were collected from two investigated green mussel cultivation locations, namely: the waters around Tanjung Mas and Tambak Mulyo (TM), Semarang City and Bedono Village, Sayung District, Demak Regency (Figure 1).

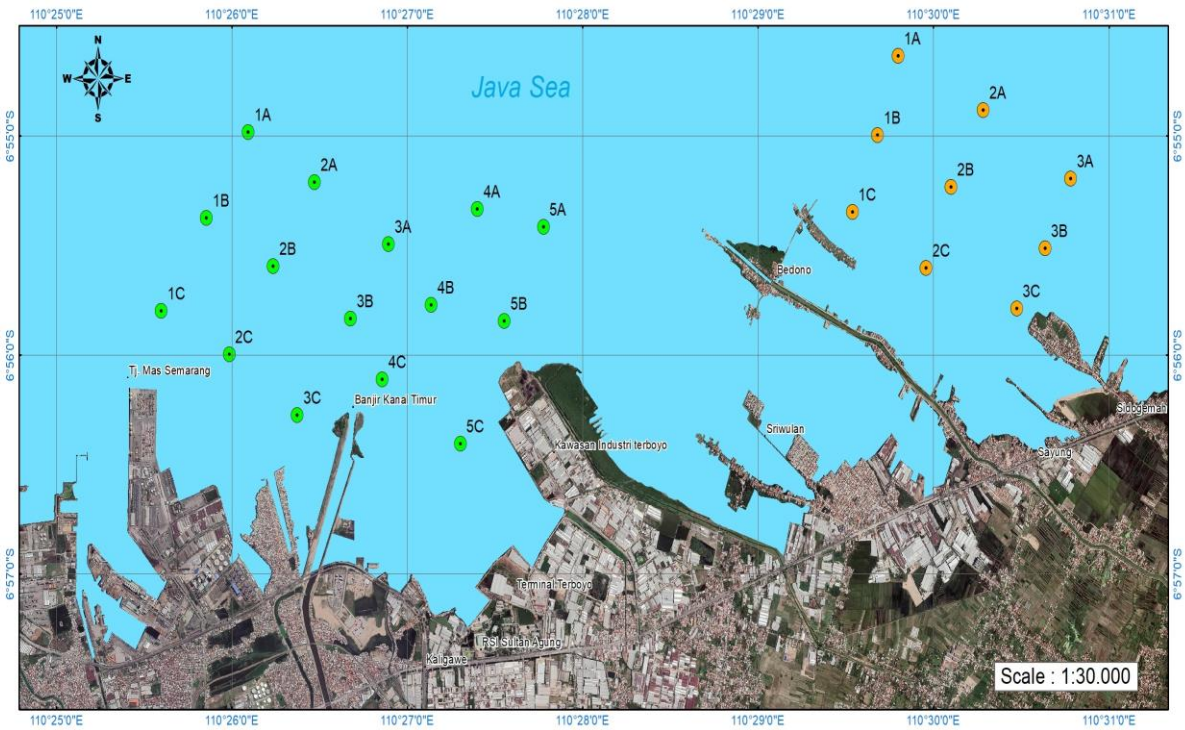

Figure 1. The sampling location in Tambak Mulyo and Bedono waters.

Sampling was carried out at 5 stations in the waters around Tanjung Mas and Tambak Mulyo, while in Bedono it was carried out only at 3 stations. There are more stations at

Tambak Mulyo since there are more people who cultivate green shellfish and the area of their cultivation business is wider. Sampling was carried out at each station with 3 replications. Water samples were obtained by collecting seawater, using a water sampler, then stored in labeled PE bottles. The sample water in the bottle is given 15 drops of 60% nitric acid ($HNO_3$) solution to keep the metal fixed or can dissolve into the sample water, due to the acidic pH (Hutagalung 1997). Green-lipped mussels are collected by harvesting directly from the bamboo where the shells are attached, then storing them in labeled plastic bags. Then the water samples and green shells are put into a cold box and cooled with ice cubes or blue ice or a mixture of both. The concentrations of Pb and Cd in sample water and soft tissue of green shells were analyzed using Shimadzu Inductively coupled plasma (ICP-MS) spectrometry. Preparation and analysis of metal concentrations in seawater and soft tissues of green-lipped mussells were carried out according to APHA (1989). Before the soft tissue of the green mussels is mashed, the weight of the shells is carried out (the weight of the soft tissue of the mussels and shells), the soft tissue weight of the mussels, and the shell weights. The bioconcentration factor of metals in the soft tissues of green mussels is calculated using modifications of Potipat et al (2015) formula as follows:

$$BCF = \frac{CMst}{CMw}$$

Where:
BCF – bioconcentration factors;
CMst - the metal concentration in soft tissues (mg $kg^{-1}$);
CMw - the metal concentration in the water media of mussels's culture (mg $L^{-1}$).

The analysis of the risk of health problems as a result of consuming green shellfish containing Pb and Cd originating from the study location was carried out using the Maximum Tolerable Intake (MTI). According to Hidayah et al (2014), the formula is as follows:

$$MTI = \frac{MWI}{Ct}$$

Where:
MWI - maximum weekly intake (g);
Ct - heavy metal content in the soft tissues of green mussels (mg $kg^{-1}$).

The MWI value is determined according to the following formula (Cahyani et al 2016):

$$MWI\ (g) = Body\ weight \times PTWI$$

Where:
Bodyweight – 50 kg for adult Indonesian humans and 15 kg for children;
PTWI – provisional tolerable weekly intake for metal Cd and Pb of 25 μg $kg^{-1}$ body weight $week^{-1}$ (INS 2009; EFSA 2011).

The examination of differences in metal concentrations in seawater and the soft tissue of green-lipped mussels between the sampling locations was carried out using the Kruskal-Wallis test and the Median Mood test. The pattern and closeness of the relationship between the metal concentration in seawater and the metal concentration in the soft tissue of green mussel were analyzed by regression-correlation analysis, and then presented in a curve form.

**Results**. The concentration of Pb in seawater is higher in the marine waters around Tambak Mulyo than in the waters around Bedono. The highest Pb concentration was observed at the TM-5 station, and the lowest was at Bedono-2, as shown in Figure 2.

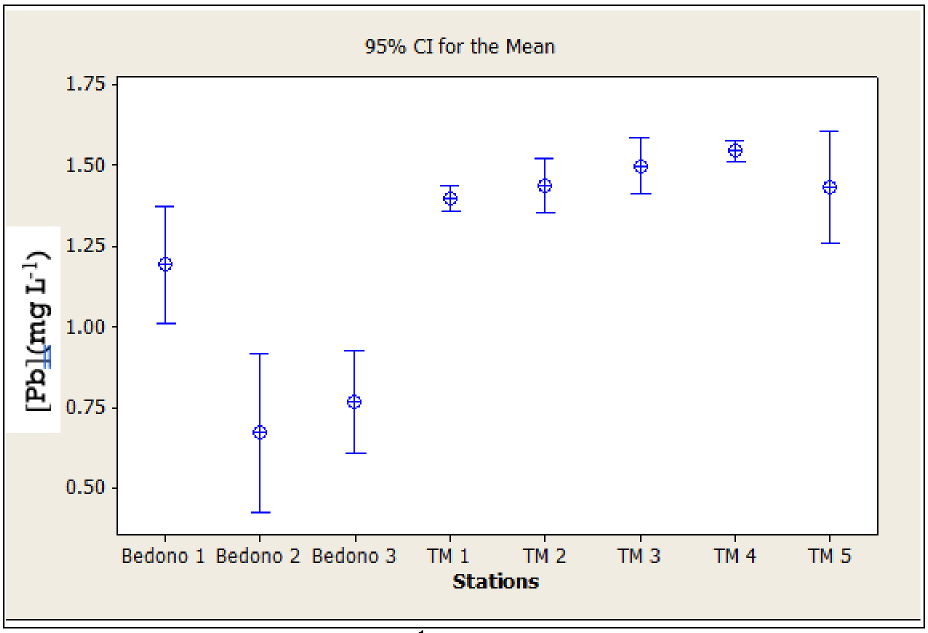


Figure 2. Concentrations of Pb (mg $L^{-1}$) in seawater at various sampling locations.

The concentration of Cd in seawater is higher around Bedono waters than in the waters around Tambak Mulyo. The highest concentration of Cd was at the Bedono-2 station and the lowest was at the TM-2 station (Figure 3).

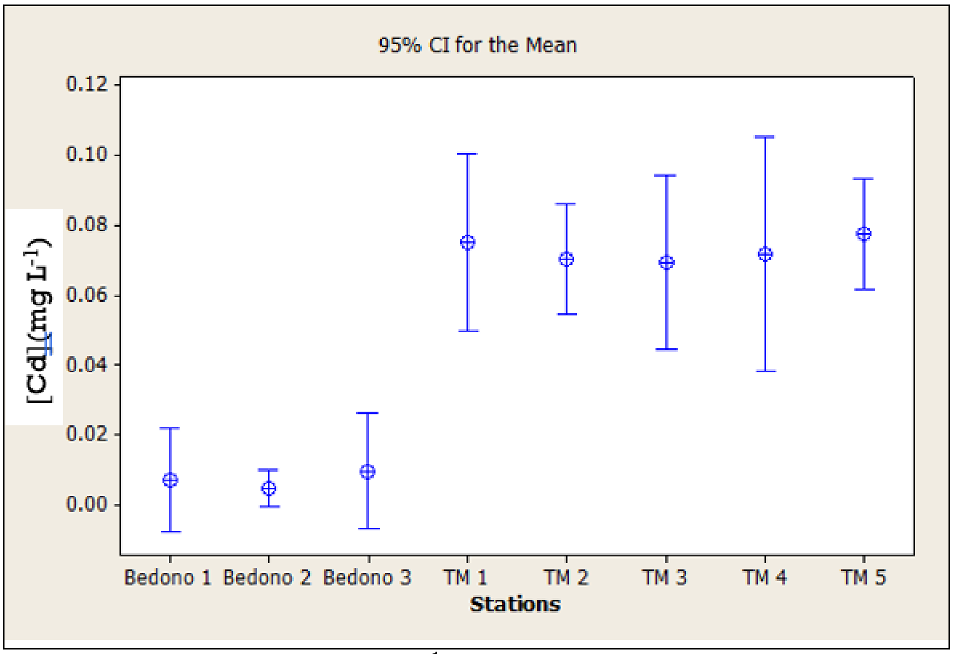


Figure 3. Concentrations of Cd (mg $L^{-1}$) in seawater at various sampling locations.

The concentration of Pb in the soft tissue of green mussels was higher in the specimens collected from marine waters around Tambak Mulyo than in Bedono (Figure 4). In contrast, the concentration of Cd in the soft tissue of green mussels was lower in shells collected from marine waters around Tambak Mulyo than in Bedono (Figures 5). The Kruskal-Wallis test results showed a difference between the Pb and Cd concentrations in the soft tissue of the green mussels between observation stations (p=0.016).

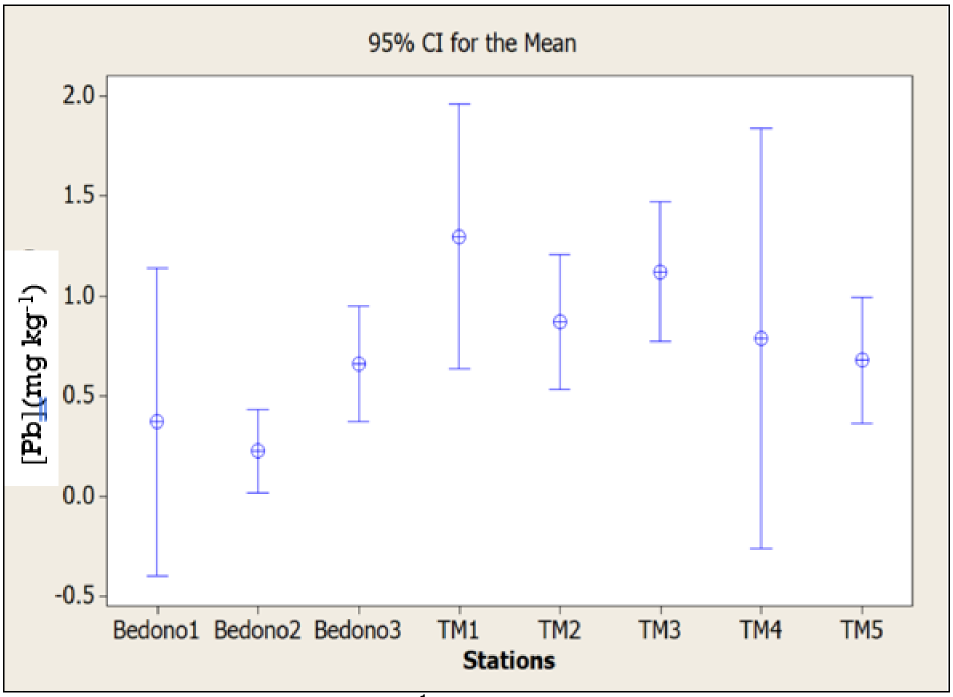


Figure 4. Concentrations of Pb (mg kg$^{-1}$) in the soft tissue of green mussels at various sampling stations.

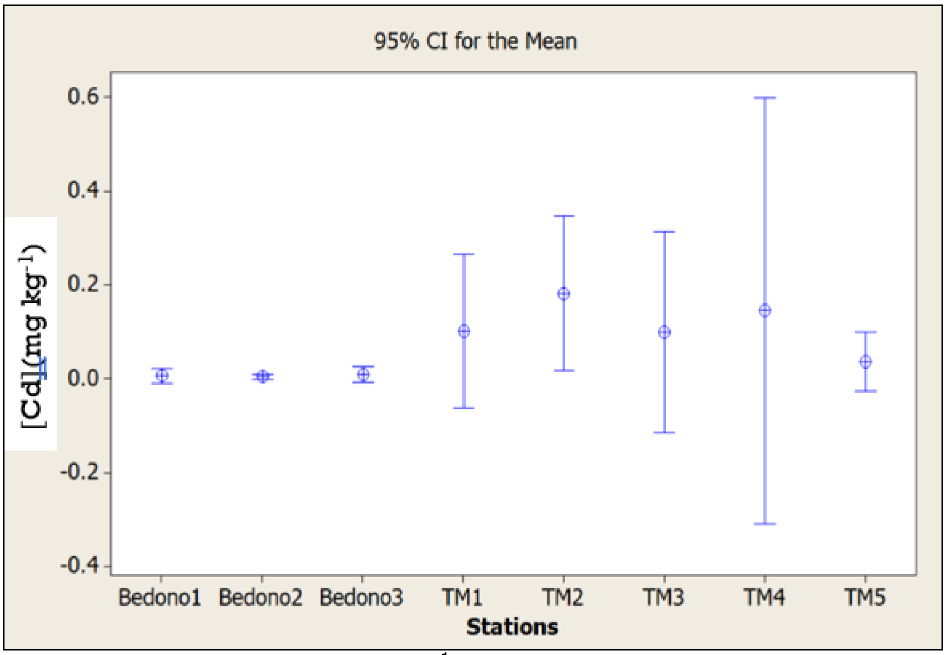


Figure 5. The concentration of Cd (mg kg$^{-1}$) in the soft tissue of green mussels at various sampling stations.

The spatial distribution of Pb and Cd concentrations in water and green mussel soft tissue at the research location is presented in Figure 6.

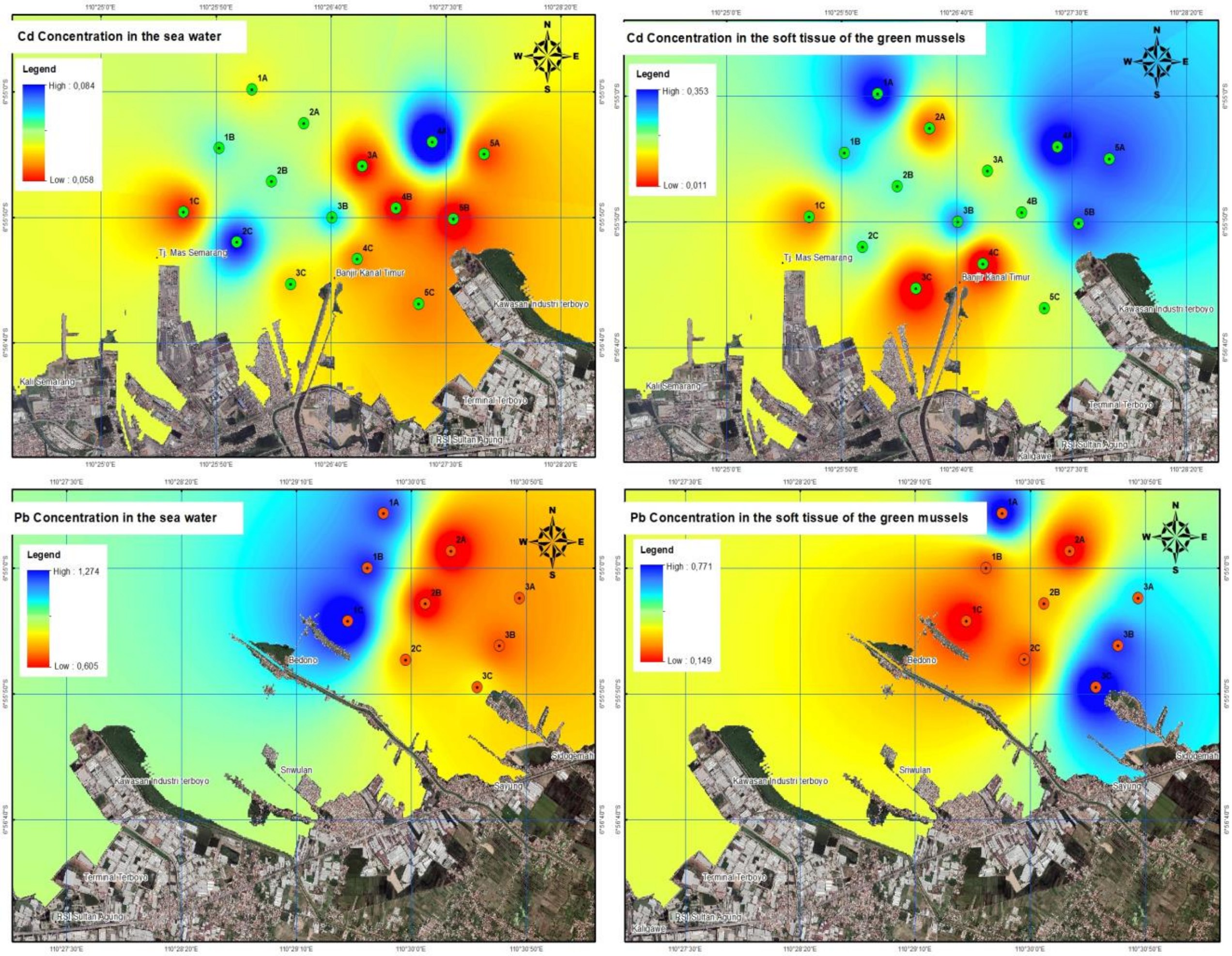

Figure 6. The spatial distribution of Pb and Cd concentrations in water and green mussel soft tissue.

BCF Pb was highest at TM-1 station, and lowest at Bedono-1 station (Figure 7).

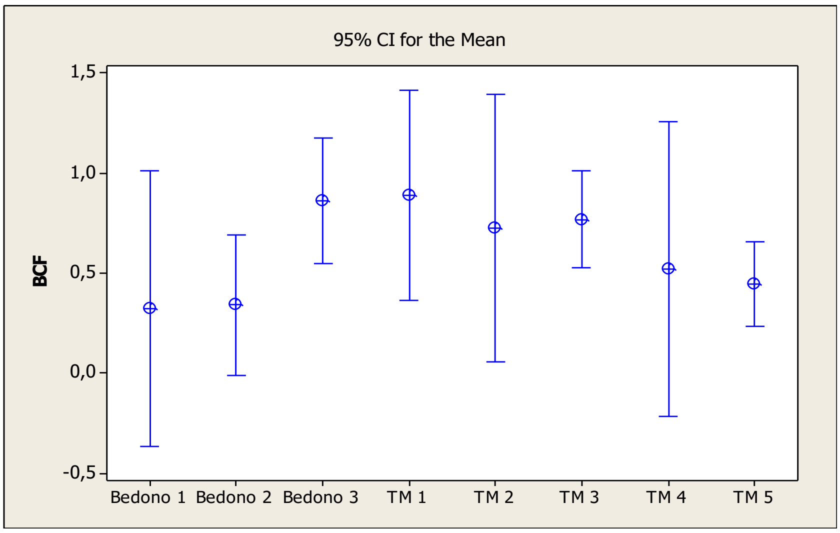

Figure 7. Bioconcentration factors of Pb at various sampling stations.

The value of BCF Cd is higher than the value of BCF Pb. The highest BCF Cd is at the Bedono-2 station, and the lowest is at the TM-5 station (Figure 8).

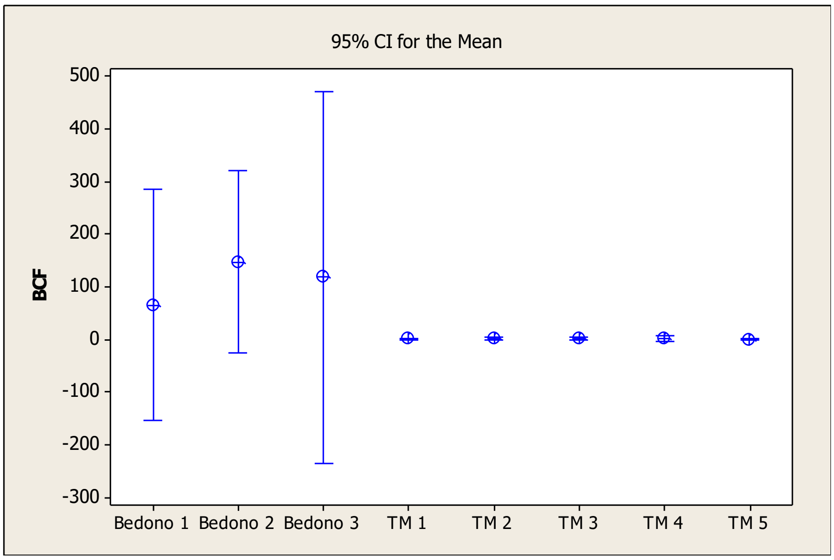


Figure 8. Bioconcentration factors of Cd at various sampling stations.

The relationship between the bioconcentration factor (BCF) of Pb with the concentration of Pb in water ($X_1$) and the soft tissue of blood clams ($X_2$) at the sampling location in Tambak Mulyo is expressed by the equation: $Y=1.93-1.25\ X_1+0.608\ X_2$ ($R^2=0.921$). Pearson correlation coefficient and p-value are shown in Table 1.

Table 1

Correlation coefficient and p-value between Pb concentration in water ($Pb_w$), the soft tissue of green-lipped mussels ($Pb_{st}$), and bioconcentration factor ($BCF_{Pb}$) in Tambak Mulyo

| *Parameter* | $Pb_w$ | $BCF_{Pb}$ |
|---|---|---|
| $BCF_{Pb}$ | -0.690<br>0.004 | |
| $Pb_{st}$ | -0,513<br>0.050 | 0.927<br>0.000 |

The data above shows that the Pb concentration in the shell soft tissue ($X_2$) is more closely correlated with the Pb bioconcentration factor. Compared to the concentration of metals in water, the relationship between the concentration of Cd metal in water ($X_1$) and in the soft tissue of blood clams ($X_2$) and the BCF metal Cd in Tambak Mulyo is expressed by the equation: $Y=1.40-18.2\ X_1+12.7\ X_2$ ($R^2=0.993$). Pearson correlation coefficient and p-value are shown in Table 2.

Table 2

Correlation coefficient and p-value between Cd concentration in water ($Cd_w$), the soft tissue of green-lipped mussels ($Cd_{st}$), and bioconcentration factor ($BCF_{Cd}$) in Tambak Mulyo

| *Parameter* | $Cd_w$ | $Cd_{st}$ |
|---|---|---|
| $Cd_{st}$ | 0.368<br>0.177 | |
| $BCF_{Cd}$ | 0.250<br>0.369 | 0.989<br>0.000 |

The correlation coefficient data above shows the problem of regression collinearity on the relationship between the concentration of Cd metal in water ($X_1$) and the soft tissue of blood clams ($X_2$), namely a different sign between the regression coefficient and the correlation coefficient on the variable concentration of Cd metal in water p value>5%, so the effect of this variable can be ignored. Thus, the only relationship that occurs is between the concentration of Cd in soft tissue and BCF in the form of the equation $Y=0.147+12.1\ X_2$ ($R^2=0.978$). The relationship between the concentration of Pb in water ($X_1$) and in the soft tissue of blood clams ($X_2$) at the sampling location in Bedono is expressed by the equation: $Y=0.383-0.393\ X_1+1.12\ X_2$ ($R^2=0.96$). Pearson correlation coefficient and p-value are shown in Table 3.

Table 3

Correlation coefficients and p values between Pb concentration in water ($Pb_w$), green-lipped mussels soft tissue ($Pb_{st}$), and bioconcentration factor ($BCF_{Pb}$) in Bedono

| *Parameter* | *$Pb_w$* | *$BCF_{Pb}$* |
|---|---|---|
| $BCF_{Pb}$ | -0.055<br>0.889 | |
| $Pb_{st}$ | -0.362<br>0.338 | 0.929<br>0.000 |

The results of the above analysis indicate that there is no contradictory correlation-regression coefficient, but p-value >0.05 on the correlation of Pb concentration in water to BCF Pb, so the factor considered after the regression-correlation analysis was only the Pb concentration factor in the soft tissue of green mussels, with the regression equation $Y=0.0303+1.14X$($R^2=0.86$), where X=the Pb concentration in the soft tissue of green mussels.

Meanwhile, the concentration of Cd correlation with BCF is expressed by the regression equation $Y=161-15836\ X_1+153\ X_2$ ($R^2=0.86$), where $X_1$=the concentration of Cd in seawater and $X_2$=the concentration of Cd in the soft tissue of green-lipped mussels. Pearson correlation coefficient and p-value are shown in Table 4.

Table 4

Correlation coefficients and p values between the concentration of Cd in water ($Cd_w$), the soft tissue of green-lipped mussels ($Cd_{st}$) and the bioconcentration factor ($BCF_{Cd}$) in Bedono

| *Parameter* | *$Cd_w$* | *$Cd_{st}$* |
|---|---|---|
| $Cd_{st}$ | -0.109<br>0.780 | |
| $BCF_{Cd}$ | -0.861<br>0.003 | 0.433<br>0.244 |

Although the regression and correlation coefficients were not in opposite directions, the correlation coefficient of the metal concentration in shellfish tissue with the BCF has a p-value >0.05, so the effect was negligible. Thus, the regression equation becomes $Y=229-16556\ X$ ($R^2=0.74$), where X=Cd concentration in seawater.

The regression and correlation analyses conducted show that BCF is directly proportional to the metal concentration in the soft tissue of green mussels and inversely proportional to the metal concentration in seawater. The exception is for Cd in the Tambak Mulyo (TM) sampling location, where the concentration of Cd in water is also directly proportional to BCF, but it can be ignored due to the collinearity problem in the regression equation. The various Pearson correlation coefficients above indicate that metal concentrations in the soft tissue of green mussels have a stronger relationship with the BCF than the metal concentrations in water. The relationship between the soft tissue weight of green mussels and the concentration of Pb in the soft tissue of green mussels at the Tambak Mulyo sampling location is presented in Figure 9.

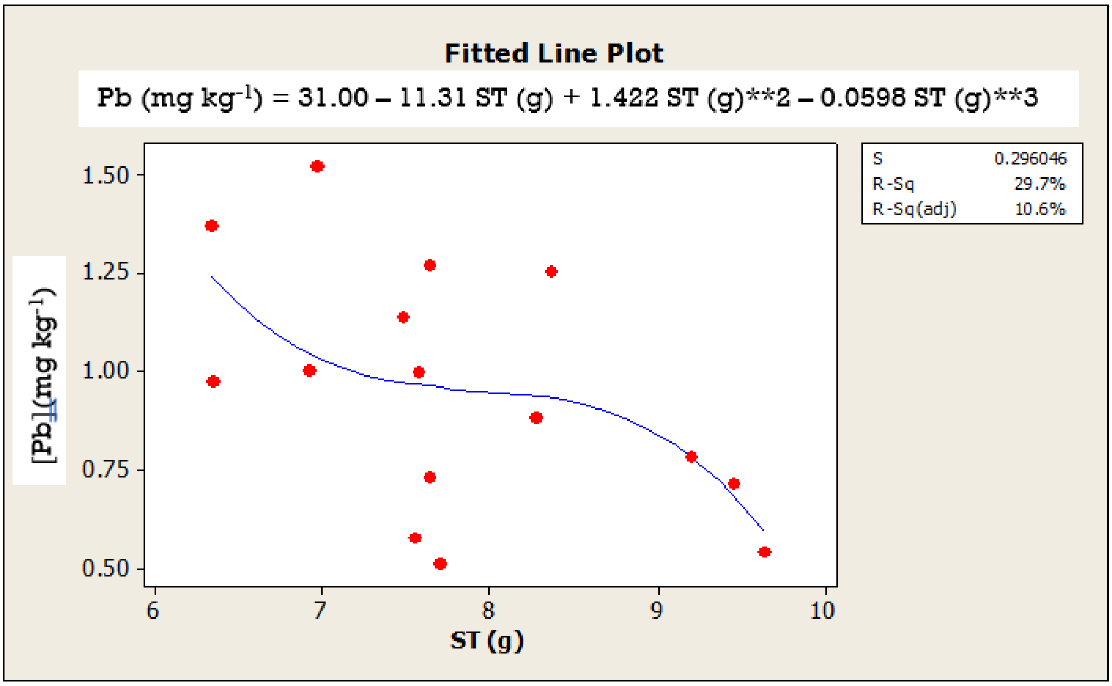


Figure 9. The relationship between weight (ST in g) and Pb metal concentration (mg $kg^{-1}$) in the soft tissue of green mussels from Tambak Mulyo waters.

Figure 10 presents the relationship between the soft tissue weight of green mussels (g) and the concentration of Cd in soft tissue (mg $kg^{-1}$). The relationship pattern found was similar to the pattern of the relationship between the soft tissue weight of green mussels and the Pb concentration in soft tissue green mussels. The metal concentration was higher in shellfish with a lighter soft tissue weight than in those with a heavier soft tissue weight.

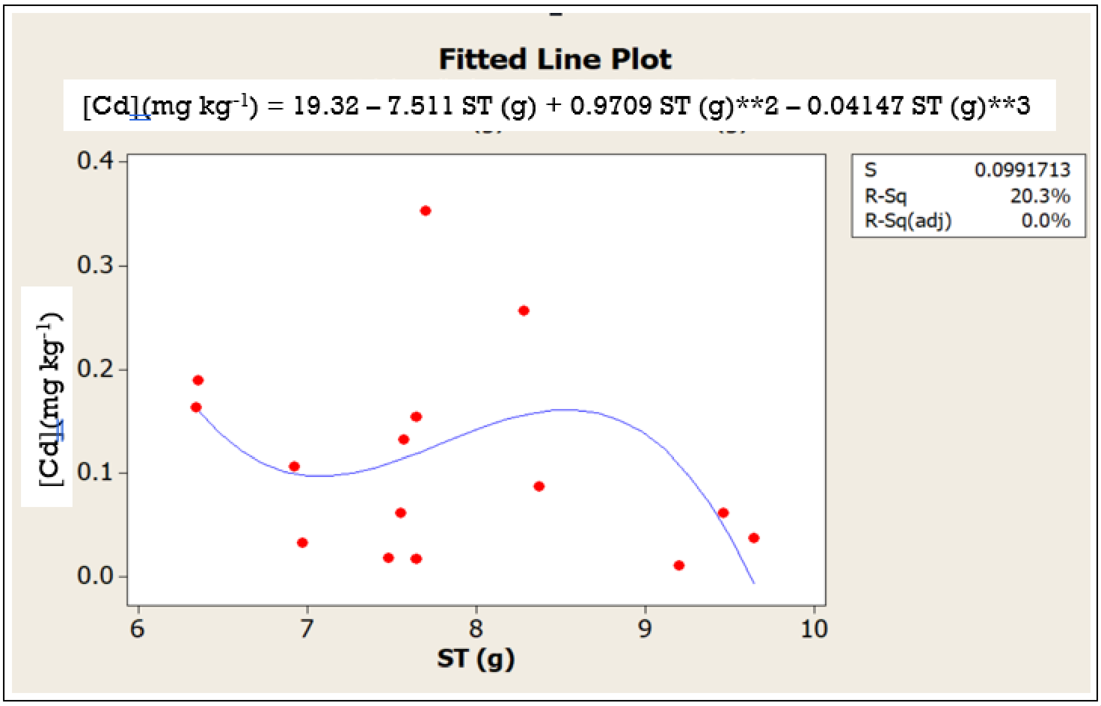


Figure 10. Relationship between weight (ST in g) and Cd metal concentration (mg $kg^{-1}$) in the soft tissue of green mussels from Tambak Mulyo waters.

The relationship between the soft tissue weight of green mussels (g) collected from Bedono and the Pb metal concentrations is presented in Figure 11. The Pb concentration in the soft tissue tends to be higher in small than in medium-sized shells and it also tends to be higher in large green mussels than in any other size.

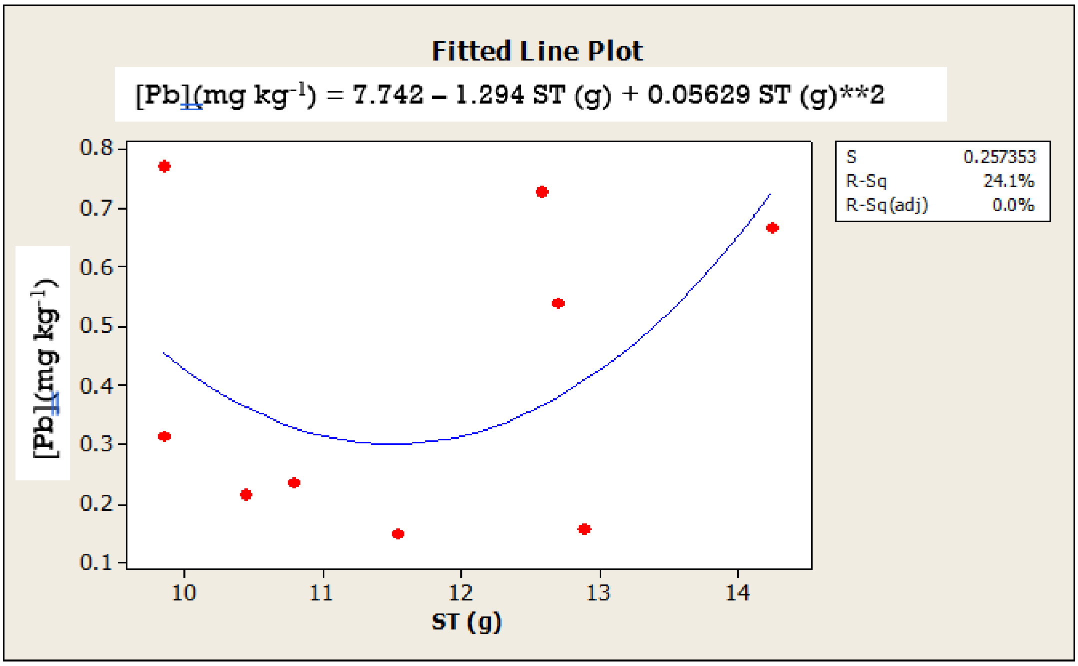


Figure 11. Relationship between weight (ST in g) and Pb metal concentration (mg $kg^{-1}$) in the soft tissue of green mussels from Bedono waters.

The mussels collected from Bedono tended to have a higher concentration of Cd in the soft tissue of the heavier green clams (Figure 12).

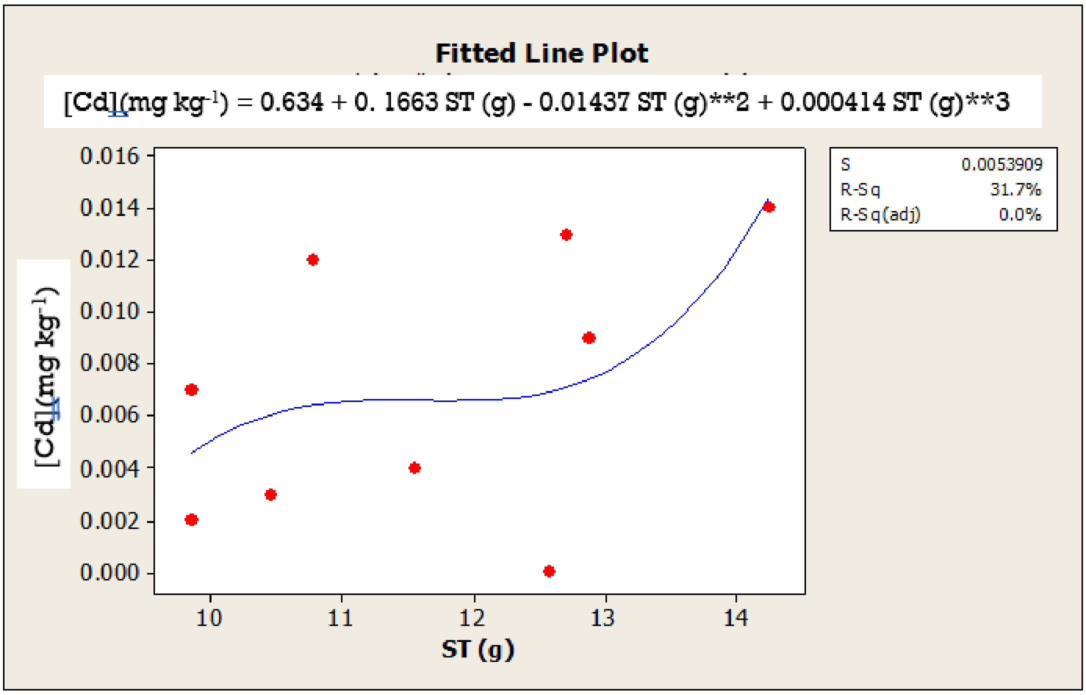


Figure 12. Relationship between weight (ST in g) and concentration of metal Cd (mg $kg^{-1}$) in the soft tissue of green mussels from Bedono waters.

The MWI value for Cd and Pb is 1,250 μg $week^{-1}$ (=50 kg bodyweight x 25 μg $kg^{-1}$ $week^{-1}$) for adult humans and 375 μg $week^{-1}$ (=15 kg bodyweight x 25 μg $kg^{-1}$ $week^{-1}$) for children. The MTI values for Cd and Pb of green clams from Bedono are presented in Table 5.

Table 5
The MWI and MTI values of green shellfish from Bedono for adults and children

| *Parameter* | *MWI (μg week$^{-1}$)* | *Ct (mg kg$^{-1}$)* | *MTI (g week$^{-1}$)* |
|---|---|---|---|
| Cd | 1,250 | 0.064 | 0.0195 |
| Pb | 1,250 | 0.420 | 0.0030 |
| Cd | 375 | 0.064 | 0.0059 |
| Pb | 375 | 0.420 | 0.0009 |

The MTI values for Cd and Pb of green mussels originating from the Tambak Mulyo are presented in Table 6.

Table 6
The MWI and MTI values of green mussels from Tambak Mulyo for adults and children

| *Parameter* | *MWI (μg week$^{-1}$)* | *Ct (mg kg$^{-1}$)* | *MTI (g week$^{-1}$)* |
|---|---|---|---|
| Cd | 1,250 | 0.113 | 0.0111 |
| Pb | 1,250 | 0.98 | 0.0013 |
| Cd | 375 | 0.113 | 0.0033 |
| Pb | 375 | 0.98 | 0.0004 |

**Discussion**. The concentrations of Pb and Cd measured in the studied coastal waters of Semarang Bay varied between concentrations which did not exceeded the seawater quality standard for marine biota, as recommended in the Regulation of the State Minister for the Environment No. 51 of 2003, Annex 3. The maximum concentration of Cd in seawater is 0.001 mg $L^{-1}$ and 0.008 mg $L^{-1}$ for Pb. The same values were found for the Pb and Cd concentrations measured in the soft tissue of green mussels. The concentration of Pb in the soft tissue of green mussels was measured at concentrations below to above (Figure 4) Indonesian National Standard 7387 (2009), with the maximum metal concentration allowed in foodstuff category number 9 (fish and fishery products, including mollusks, crustaceans, echinoderms, amphibians, and reptiles) of 1.0 mg $kg^{-1}$. The concentration of Cd was also below the maximum allowable limit (1.0 mg $kg^{-1}$).

Figures 6 and 7 depict the values of BCF Pb and Cd, showing that the two metals, which are found in the waters of Semarang Bay, can accumulate in the soft tissue of green mussels cultivated in these waters. Bioconcentration of Cd was higher than bioconcentration of Pb, although Pb concentration in marine waters was higher than Cd concentration. The bioconcentration of pollutants in animal or plant soft tissues is determined by the ability of pollutants to penetrate the soft tissues of animals or plants and accumulate in these tissues, and also by the concentration of pollutants in the water. Pollutants with higher concentrations in water or soil will flow into plant or animal tissues, due to differences in concentration gradients, until they become saturated.

The toxicity of Pb to aquatic animals was higher than the toxicity of Cd. Although Cd is toxic to most microorganisms, Cd in the form of salts and organic Cd are easily soluble in water, so they can reduce toxicity. On the other hand, lead salts are difficult to dissolve in water, so they are more toxic in living things. Therefore, Pb metal is used as an exterminator of sea snails (*Cerithidea alata*, *Cerithidea diariensis*, *and Cerithidea autodorata*) in fish and shrimp ponds in Indonesia under the brand name Brestan, at a dose of 0.5-2.5 mg $L^{-1}$. Brestan pesticide 60 WP (wettable powder) is an organotin pesticide. Organotin pesticides in the aquatic environment hydrolyzed become fentin hydroxide, which is very effective in killing mollusks, tridents, and snails. Because Pb is more toxic, it can only accumulate in low concentrations. At high concentrations it causes death in mollusks. The Pb concentration in the soft tissue of the green mussels studied tends to be higher in small green mussels from the waters around Tambak Mulyo and in green mussels from the waters around Bedono.

The opposite situation occurs in Cd metal. Green mussels were able to accumulate Cd in a higher concentration than Pb, without experiencing Cd poisoning, so that the value of the Cd BCF was higher than the Pb BCF in green mussels. The concentration of Cd in the soft tissue of the mussels was proportional to the weight of the studied green mussels (Figure 10).

Analysis of the more dominant factors influencing metal bioconcentration in the network showed variations between sampling locations. At the Tambak Mulyo sampling location, the BCF was determined more by the concentration of Pb in the soft tissue of green shells than by the concentration of Pb in seawater. Meanwhile, for the Cd, the BCF was only determined by the concentration of Cd in the soft tissue of green shells. At the Bedono sampling location, the BCF of the Pb was only determined by the concentration of Pb metal in the soft tissue of green shells, and the BCF of the Cd was only determined by the concentration of Cd metal in seawater.

Metal accumulation in shellfish is determined by several factors, among other things, metal diffusion rate into body tissues, metal bioavailability, age, body size, internal cavity volume and the physiological condition of the shellfish. The results of the analysis of the dominant factors affecting the BCF showed that the Pb bioconcentration in green clams was mostly determined by internal factors, namely: the process of metal accumulation in soft tissue, the Pb metabolism and its excretion by green clams. Pb metal tends to be more concentrated in smaller shells than in shells of other sizes, at the 2 sampling locations. Besides, being caused by the Pb toxicity factor, this is likely to be related to the diffusion rate in shellfish at small sizes, which is greater than their rate of excretion. The metal that will be excreted out of the body must first be metabolized by shellfish by involving detoxification enzymes in the body so that it becomes more polar and can dissolve into body fluids, eventually being excreted with urine. More enzymes are available of in larger shells than in smaller shells.

The Cd bioconcentration tends to vary due to internal factors in the green mussels from the Mulyo pond and to external factors, namely the concentration of Cd which tends to be higher in the sea waters around Bedono than in the Mulyo pond, resulting in a stronger absorption process into the network, through the diffusion process. The process is continuous, so that the large green shells tend to accumulate more Cd than the small ones (Figure 11).

The studied green mussels met the harvest size and were fit for consumption. According to the WWF-Indonesia Fisheries Team and Silfester Basi Dhoe (2015), the size for consumption is similar to a shell length of 6-8 cm. The length of the green clam shells from Tambak Mulyo was 7.37±0.76 cm, while in those from Bedono it reached 8.33±0.79 cm. Tables 1 and 2 show the limit of a safe consumption for human health for blood clams from Mulyo and Bedono ponds. For shellfish from Tambak Mulyo containing Cd, the safe consumption limit is 0.0011 g week$^{-1}$ for adults, and 0.0033 g week$^{-1}$ for children, while from Bedono it is 0.0195 g week$^{-1}$ for adults and 0.059 g week$^{-1}$ for children. For shellfish containing Pb, the maximum safe consumption from the Tambak Mulyo is 0.0013 g week$^{-1}$ for adults and 0.0004 g week$^{-1}$ for children. Meanwhile, for the shellfish from Bedono, the limits were 0.0030 g week$^{-1}$ for adults and 0.0009 g week$^{-1}$ for children. Consumption of shellfish which accumulate excess Pb, exceeding the safe consumption limit, can cause a decrease in the number of red blood cells, shortening the life of blood cells and increasing the content of Fe and protoproteins in the blood. Meanwhile, the consumption of shellfish that accumulates Cd excessively and exceeds the safe consumption limit can cause health problems, including disorders of the nervous system, reproductive system, kidney damage and the risk of cancer.

**Conclusions**. The concentration of Pb and Cd in the waters of Semarang Bay and in the soft tissue of the green mussels under study ranged from below to above the applicable quality standard in Indonesia. Both types of metals can accumulate in the soft tissue of green mussels, as shown by the value of the bioconcentration factor. The bioconcentration factor of Cd was greater than Pb. Although the green mussels studied and cultivated in the waters of Semarang Bay have accumulated Pb and Cd metals in their soft tissues, the shellfish is still safe for consumption as long as it does not exceed the MTI value. The recommended MTI values for adults are ≤0.0013 g $week^{-1}$ for Pb and ≤0.0011 for Cd, respectively. Meanwhile, for children the recommended MTI values are ≤0.0004 g $week^{-1}$ for Pb and ≤0.0033 g $week^{-1}$ for Cd.

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**Conflict of interest**. The authors declare no conflict of interest.

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