Study of bioaccumulation and heavy metal depuration of cadmium in green mussel (Perna viridis)

Budiawan¹, R Bakri¹, S A Aziz¹ and H Suseno²

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia
²Center for Technology Radiation Safety and Metrology, National Nuclear Energy Agency (BATAN), Jakarta 12710, Indonesia

Corresponding author’s email: dr.budiawan@gmail.com

Abstract. Green mussel (Perna viridis) is a favorite seafood, which has good protein content and economical price. The shellfish habitat is in a polluted area in the Jakarta Bay caused by industrial waste. Presently, the consumption of green mussels is very problematic because the habitat of green mussels has experienced pollution. Bioaccumulation of heavy metal study was conducted to determine the process of heavy metal accumulation in a biota by using a certain dosage. The heavy metal cadmium was used to be exposed to a biota as much as half LC₅₀ is 0.1 ppm. The depuration process was performed to reduce the levels of cadmium metal in green mussel. A seven-day drainage depuration method was used by immersing acetic acid and citric acid with variations of concentration 0.75 %, 1.5 %, and 2.25 % in 24, 48, 72, 96, 120 min variations. Levels of cadmium in green mussel were measured using atomic absorption spectrophotometry. Concentration of cadmium decreased to 3.05 mg.Kg⁻¹, 1.7 mg.Kg⁻¹ and 0.65 mg.Kg⁻¹ after depuration process that used immersing by water, acetic acid and citric acid, respectively.

Keywords: Green mussel, Perna viridis, heavy metal accumulation, cadmium

1. Introduction
The estuarial community of Kamal has been working on the fisheries sector since the 1970's. Presently, less than 40 % of fishermen conduct marine cultivation including fish, shrimp, and mussel. The Jabotabek surrounding area that have 20.3 million of inhabitants cause the pollution in Jakarta bay [1]. In 2003, the Regional Environmental Management Board of the Province of Jakarta Capital Special Region mentioned the industrial waste that generates 2050 B₃. This B₃ waste is a major source of pollution in waters in Jakarta. The increase in the concentration of pollutants, both organic or inorganic, resulting in the occurrence of eutrophication and a decrease in the number of fauna as well as a decline in water quality and sediment on the waters [2]. The main polluters in a waste waters source have hazardous substances and components that are toxic, such as heavy metals. Heavy metals are classified based on the nature of the toxicity and the source of its existence which is divided into the low, medium and high categories. One example of a heavy metal which belongs to a high toxicity i.e. metal cadmium [1]. The Ministry of Environment and Forestry describes the value of a threshold based on Raw Water Quality Metal Cadmium in waters at 0.001 ppm. The metal content of the dissolved cadmium in Kamal estuary is as much as 0.02 ppm. In 2001, an advanced research was done to measure the content of the
metal cadmium which was increased to 0.046 ppm [3]. In 2002, the research on back metal dissolved cadmium reached 0.785 ppm. Heavy metals contamination in waters can be accumulated in marine biota. Green mussel (Perna viridis) is a rich biota in the waters of the estuary of Kamal. Mussels is a favorite seafood commodity having a good protein content and economical price. Two hundred grams of mussels contained 21.9 %, protein 14.5 %, fat 4.3 %, carbohydrate 18.5 % and the remaining water 40.80 % [4]. Mussels have a filter feeder, capable of eating anything that exists in the environment and stuck in the body. Consuming shellfish became a public concern because it has been contaminated with heavy metal and gave an effect to the human metabolism.

Some bioaccumulation studies have conducted on tilapia fish [5], milk fish [6, 7] and shrimp [8]. In this study, Cd bioaccumulation and depuration by green mussel were conducted.

2. Method

2.1. Preparation of aquarium and sea water
The aquarium of 300 L was used, filled with 250 L of clean sea water. The salinity of sea water was determined by refractometer and a temperature of 25 °C was tailored in the laboratory using a thermometer.

2.2. Retrieval of biota
Green mussels (Perna viridis) as bio indicator were used and taken from the waters of the Jakarta bay, Muara Kamal. Biota samples obtained were cleaned from dirt. Furthermore, the biota was collected in a plastic sack and ice gel was added to maintain the freshness while headed to BATAN PTKMR aquatic laboratory for acclimatization. Mussels used were approximately 1–3 of the month, with a length of about 3 cm.

2.3. Acclimatization biota
The Biota samples taken from Kamal were put into a clean sea water as much as 2–3 times. After that, the biota were bound with a rope and hung with a raffia string in the aquarium for acclimatization. The aquarium were aerated in dark and light continued to 12:12 h. During the acclimatization, biota was fed with Artemia.

2.4. Bioaccumulation of metal cadmium
The aquarium with 50 L capacity was filled by approximately 40 L sea water. The aquarium was equipped with filtration, aeration and skimmer systems. Thirty of shells Perna viridis were immersed in this aquarium system.

2.5. Depuration
Depuration were conducted in 2 types i.e., drainage and acid immersion. Depuration drainage was carried out using the aquarium contained 40 L sea water. Aquaria systems equipped with modeling the flow of water will be drained and filtrated. Sea water was replaced every day. Fourteen biotas were used in this experiment. Depuration acid soaking was conducted on citric and acetic acid. Variation of the acid concentrations were 0.75 %, 1.5 % and 2.25 %. The time variation were 48 h, 72 h, 96 min, and 120 min. This experiment was conducted in three different containers based on concentration. Each container contains 10 shells, which will be taking as much as two in every variation of the time.

2.6. The determination of metals content
Mussel dissection was conducted to separate parts of the mussel. Each part was destructed with USGS Test Method B-9001-95. Sulfuric and nitric acid were used for destruction process. The composition of the acids was 1:5. The cadmium content in each part was determined by Atomic absorption Spectrophotometer.
3. Results and discussion

3.1. Description of location
Industrial areas around the estuary are galvanizer, metal casting, garment and Pharmaceuticals [1]. Density of industry around Muara Kamal leads to potentially disposal of waste containing heavy metals into the residential areas. The wastes were disposed directly without being processed in advance. It can interfere with the quality of the water and the ecosystem also reside in these waters. A lot of industrial waste containing heavy metals can have an impact on living things. The impact can be felt through the human by consuming the seafood that has been polluted and also the water. As the supporting data, the recording conditions (table 1) at the mouth of Kamal are presented.

3.2. Retrieval of biota
The sampling conducted in the estuary of Kamal, was taking place in the middle of the ocean for cultivation of biota. Biota is the mussels. Green shells used in this research were 1–3 months, with long range of 3 cm, 2 cm width, 1.5 cm thickness.

The chosen green mussels still have a substrate. The living mussels have the characteristics of shells, and are green to brown color. When in the water, living mussels will sink. Generally, living mussels are attached to a substrate using a byssus thread. Byssus is present on the lower leg foot clams adapted to attach to the substrate.

3.3. Acclimatization of biota
Acclimatization is the adjusting process of individuals to the changes in environmental conditions. Acclimatization aims to relieve stress in the animal experiments in conditions of the aquarium so that it can be used in the experiment of bioaccumulation as well as to reduce the risk of death in biota. Acclimatization in the form of media is used in aquariums. Preparation of the aquarium is done a day earlier, with aeration and filtration system already ensuring a well mounted. Filtration components, namely cotton filter, rocks are to filter the impurities bioball insoluble. Impurities insoluble fibers such as fibers that attached to the body of the shellfish. Also, a skimmer is used to filter the impurities dissolved in the aquarium. Soluble impurities resulting from the process of excretion of the mussels. The working principle of the skimmer is to create air bubbles that make contact with colloids and particles–solid particles. This tool is used to create such conditions in the sea produces froth–effervescence.

The use of the mussels still affixed to the substrate is to approach the green mussel habitat to its original condition. Previously, the mussels were cleaned by dipping the clams in a clean sea water, so that the mud or dirt does not contain in the aquarium. Clean mussels are suspended inside the aquarium. The process of acclimatization is done for seven days without giving the contaminants. During the first seven days, the nutrients around it will be absorbed. Green mussel byssus will use to move and some stick to the walls of the aquarium.

3.4. Bioaccumulation of cadmium metal
Measurement of content of the metal cadmium is performed using atomic absorption spectroscopy. By calculating the radio wave electromagnetic absorbance by the free atoms an element. Contaminant uptake observations was done by analyzing the content of the metal cadmium (table 2) in a certain period. The higher levels were obtained on the seventh day. The process of bioaccumulation was reached viewing the cadmium levels that tend to reach a steady state.

Taken control mussels originating from aquarium acclimatization. Cadmium levels were measured using atomic absorption spectroscopy of 0.99 mg/Kg cadmium levels may be seen. On the seventh day (table 3) of 7.21 mg/Kg, experienced a rise of 86.27 %. The condition tends to reach a steady state starting on the sixth day and the seventh day, at cadmium level during bioaccumulation. Cadmium levels in biota of the conversion can be done on the parameters of the biokinetic CF (Concentration Factor).
Table 1. Muara Kamal data condition

| Parameter      | Value |
|----------------|-------|
| Temperature (°C) | 32    |
| Salinity (‰)    | 33    |
| pH              | 6-7   |

Table 2. The content of cadmium metal in the green mussels.

| Time (Day) | The content of cadmium metal in The Green Mussels (mg/Kg) |
|------------|----------------------------------------------------------|
| 1          | 3.15                                                     |
| 2          | 3.98                                                     |
| 3          | 4.78                                                     |
| 4          | 6.03                                                     |
| 5          | 6.66                                                     |
| 6          | 6.91                                                     |
| 7          | 7.21                                                     |

Table 3. CF’s value

| Time (Day) | CF (mL.g⁻¹) |
|------------|-------------|
| 1          | 31.5        |
| 2          | 39.77       |
| 3          | 47.78       |
| 4          | 60.4        |
| 5          | 66.63       |
| 6          | 69.1        |
| 7          | 72.1        |

Figure 1. Bioaccumulation rate

It can be seen also from the process of concentration rate of bioaccumulation. The rate of this concentration can use a principle of first order. Depicted in a graph (figure 1) where the x is the length of exposure to contaminants and the y axis is the ln (concentration). It can be seen the influence of rate
depends on the time of exposure to the contaminants. The value of the rate will be even greater at the longer time.

The ability of bioaccumulation in biota is also represented by the rate of uptake of contaminants (k_u). In a single compartment, the K_u value is assumed as the mechanism of contaminant uptake by biota throughout the body. However, the speed of its distribution into the various types of organs is ignored. The K_u values (mL g^{-1} day^{-1}) is a constant rate of uptake are calculated based on the slope of the curve C_Ft against t (from t = 0 to t at steady state conditions).

3.5. Bioaccumulation model
The equations in this model refers to a single compartment, bioaccumulation approach to the value that is used, to the C_Fss and derived from the experimental results. Form change to steady state was reached on day seven, and shown with the value of the C_Fss. Physiological factors which include metabolic processes contaminants in the body is assumed to be constant [9]. Retrieval rate constants determined by changing the equation becomes a linear equation so that equation is obtained as follows:

\[
\ln (C_{Fs} - C_{Ft}) = K_u \cdot t
\]

From equations, it is plotted into a graph of today vs. \( \ln (C_{Fs} - C_{Ft}) \) where t is the x axis (day) while the y-axis is \( \ln (C_{Fs} - C_{Ft}) \). The result of the equation will produce an equation of a line and the slope of the equation of a line is the value from the process of contaminant uptake of green mussels. Bioaccumulation modeling is shown in figure 2.

3.6. Depuration
These methods use the system of flow circulating. The depuration process was conducted for seven days, and replaced with clean water every day. During the depuration process, Cd were determined using atomic absorption spectroscopy. Cadmium levels during the process of depuration has decreased. Decrease in low cadmium metal is obtained on the seventh day (table 4), by the amount of 3.058 mg/Kg cadmium Levels. Declining the most at 57.59 % compared with levels of cadmium at present day bioaccumulation.

Retrieval process (uptake) and redemption (depuration) contaminants can be done in a single compartment (figure 3). Bioaccumulation process, when the levels of cadmium is increasing and likely to reach steady state. The release of cadmium levels indicated at any given time. Rate of depuration in this study was conducted in the order of one (figure 4). Refers to the rate of bioaccumulation, the rate of water loss depuration was obtained. The decline in the rate based on the concentration of cadmium at any time that is increasingly low. From the rate of depuration, biological half-life can be known that illustrates the ability of mussels to eliminate Cd^{2+} contaminants accumulated in its body of 3.55.

Figure 2. Bioaccumulation model
The measurement levels of cadmium on depuration was performed by acid immersion. The levels of cadmium that is stuck in the body of biota after a depuration was measured. Cadmium levels decline at any concentration. Cadmium levels decrease occurred in the body of the green shells, along with the increasing time of immersion in acetic acid solvent. The decrease levels of cadmium can be seen, too, in the retention values (table 5 and 6).

Depuration rate in acetic acid (figure 5) and citric acid (figure 6) were declined. The kinetic of reaction rate of depuration followed the first order one. The depuration reached the steady state after 120 min.

3.7. Determination of BCF value

BCF or Bioconcentration Factor is the ratio between the concentration in the organism and in the water during a state of stable equilibrium [10]. The BCF is determined by comparing the values of

| Table 4. Content and retention of cadmium metal. |
|------------------------------------------------|
| Time (Day) | Content of metal cadmium in Mussle (mg/Kg) | Retention (%) |
| 1          | 6.031                                      | 0.97          |
| 2          | 5.315                                      | 0.87          |
| 3          | 5.07                                       | 0.84          |
| 4          | 4.647                                      | 0.78          |
| 5          | 4.42                                       | 0.75          |
| 6          | 3.35                                       | 0.6           |
| 7          | 3.058                                      | 0.56          |

![Figure 3. Uptake and release cadmium metal](image)

![Figure 4. Water depuration rate](image)

| Table 5. Content and retention of cadmium by green mussel with acetic acid depuration. |
|-------------------------------------------------------------------------------------|
| Time (minute) | Acetic Acid 0.75 % Cd’s content in mussle (mg/Kg) | Retention (%) | Acetic Acid 1.5 % Cd’s content in mussle (mg/Kg) | Retention (%) | Acetic Acid 2.25 % Cd’s content in mussle (mg/Kg) | Retention (%) |
|---------------|---------------------------------------------------|---------------|-----------------------------------------------|---------------|-----------------------------------------------|---------------|
| 24            | 6.17                                              | 0.99          | 5.98                                          | 0.97          | 6.15                                          | 0.99          |
| 48            | 3.16                                              | 0.58          | 5.73                                          | 0.93          | 5.59                                          | 0.91          |
| 72            | 2.56                                              | 0.49          | 5.46                                          | 0.9           | 5.26                                          | 0.87          |
| 96            | 2.18                                              | 0.44          | 4.77                                          | 0.8           | 3.8                                           | 0.66          |
| 120           | 2.15                                              | 0.43          | 1.98                                          | 0.4           | 1.7                                           | 0.37          |
the constants of uptake (ku) with constant release (to). The constant taking of value is obtained from the slope equation of a line on the rate of uptake on the process of bioaccumulation of 0.5782. The slope value of the line equation in the chart represents the constant value, the rate of contaminants (ku) by the mussels.

To analyze the concentrations of chemical substances contained in certain ecosystems/waters used a value of BCF in table 7. Can be determined with equation, i.e. $BCF = \frac{K_u}{K_e}$.

### Table 6. Content and retention of cadmium in green mussels with citric acid depuration

| Time (minute) | Citric Acid 0.75 % | Citric Acid 1.5 % | Citric Acid 2.25 % |
|---------------|--------------------|--------------------|--------------------|
|               | Cd’s content in mussle (mg/Kg) | Retention (%) | Cd’s content in mussle (mg/Kg) | Retention (%) | Cd’s content in mussle (mg/Kg) | Retention (%) |
| 24            | 6.15               | 1.11               | 6.48               | 2.6           | 7.27               | 1.04           |
| 48            | 5.28               | 0.87               | 4.64               | 3.57          | 5.42               | 0.78           |
| 72            | 4.78               | 0.8                | 4.18               | 3.72          | 3.22               | 0.72           |
| 96            | 3.08               | 0.56               | 2.27               | 4.16          | 0.85               | 0.45           |
| 120           | 2.25               | 0.45               | 1.75               | 4.25          | 0.65               | 0.38           |

### Figure 5. Acetic acid depuration rate

### Figure 6. Citric acid depuration rate

### Table 7. BCF value

|                      | Ku    | Ke     | BCF   |
|----------------------|-------|--------|-------|
| Water Depuration     | 0.5872| 0.1954 | 3.01  |
| Acetic Acid Depuration 0.75 % | 0.5872| 0.0141 | 41.64 |
| Acetic Acid Depuration 1.5 % | 0.5872| 0.0141 | 41.64 |
| Acetic Acid Depuration 2.25 % | 0.5872| 0.0169 | 34.75 |
| Citric Acid Depuration 0.75 % | 0.5872| 0.3861 | 1.52  |
| Citric Acid Depuration 1.5 % | 0.5872| 0.3097 | 1.896 |
| Citric Acid Depuration 2.25 % | 0.5872| 0.5028 | 1.17  |
Table 8. Content of protein in green mussle

| Sample                          | % Protein |
|---------------------------------|-----------|
| Control                         | 16.56     |
| After Water Depuration          | 9.995     |
| After Acetic Acid Depuration 0.75 % | 15.169   |
| After Acetic Acid Depuration 1.5 % | 14.963   |
| After Acetic Acid Depuration 2.25 % | 12.304   |
| After Citric Acid Depuration 0.75 % | 15.818   |
| After Citric Acid Depuration 1.5 % | 12.364   |
| After Citric Acid Depuration 2.25 % | 10.516   |

3.8. Determination of protein with kjehdahl method
Kjehdahl method based on the calculation of the total nitrogen contained in the shellfish meat then the value converted to protein levels is obtained. This protein test is done on the shellfish control, bioaccumulation and depuration. Protein levels can be seen in table 8.

4. Conclusion
Mussels are able to accumulate cadmium metal with a value of CF to 72.09 mL g\(^{-1}\). The ability of the cadmium bioaccumulation was 7.21 mg Kg\(^{-1}\). The lowest concentration of cadmium metal is obtained on the mussels after the depuration on drainage method of 3.058 mg Kg\(^{-1}\), 2.25 % acetic acid immersion during 120 min of 1.7 mg Kg\(^{-1}\), and 2.25 % citric acid immersion during 120 min of 0.65 mg Kg\(^{-1}\).

Acknowledgments
This research were sponsored by Dikti grant Project and performance in Aquatic Laboratory Center Technology for Radiation Safety, National Nuclear Energy Agency (BATAN).

References
[1] Wendling C C et al. 2013 Mar. Pollut. Bull. 71 222-9
[2] Koropitan A F and Ikeda M 2016 Procedia Environ. Sci. 33 532-52
[3] Riani E, Cordova M R and Arifin Z 2018 Mar. Pollut. Bull. 133 664-70
[4] Al-Usmani S M P, Jagtap T G and Patil D N 2015 Mar. Pollut. Bull. 99 328-31
[5] Suseno H, Hudiyono S, Budiawan B and Wisnubroto D S 2010 Aust. J. Basic Appl. Sci. 4 792-9
[6] Prihatiningsih W R, Suseno H, Zamani N P and Soedharma D 2016 Mar. Pollut. Bull. 110 647-53
[7] Prihatiningsih W R, Suseno H, Zamani N P and Soedharma D 2016 Atom Indo. 42 129-35
[8] Suseno H, Hudiyono S and Muslim M 2016 HAYATI J. Biosci. 23 117-20
[9] Landrum P F and Fisher S W 1998 Influence of Lipids on the Bioaccumulation and Trophic Transfer of Organic Contaminants in Freshwater Ecosystems (New York: Springer) pp. 203-34
[10] Opperhuizen A and Schrap S M 1987 Environ. Toxicol. Chem. 6 335-42