Study of Bioaccumulation and Depuration of Pb Metal Ions in Green Mussels (*Perna viridis*)

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Abstract: In this study, bioaccumulation and depuration studies of Pb in green mussels were performed. The bioaccumulation process was carried out using flowing water method for 7 days. The Pb ion concentration used was 1.225 ppm. During bioaccumulation, Pb contained in mussels was determined every 24 hours. Two depuration methods were applied in this study, flowing clean water method for 7 days and immersing in acid solution for 2 hours. Two variations of acid solutions used were acetic acid and citric acid with variations concentration of 0.75%; 1.5%; and 2.25%. Pb contained in mussels was analyzed using AAS. The research showed that the highest value of Pb contained was reach after 7 days exposure with concentration of 41.92 mg/kg and concentration factor (CF) value of 32.15 L/kg. The lowest content of Pb was reached after depuration by immersing in 2.25% citric acid for 2 hours. Pb content after depuration was 16.96 mg/kg with the decrease of Pb by 59.5%. Bioaccumulation ability was expressed by Concentration Factor (CF). Based on this experiment, green mussel can be classified as low category bioindicator biota for Pb accumulation.

1 INTRODUCTION

The development of industrial sector around Jakarta impact on pollution in the region. This is due to the Jakarta Bay area that accommodate the waste generated by 13 rivers that bring millions of waste every year to the sea. So level of polution from year to year increase. (Hutagalung, 1991). Water pollution in this area causes a decrease in water quality, so the water can no longer be used as intended. Liquid waste is entering the Jakarta Bay often carries hazardous pollutants, such as heavy metal. The impact of heavy metal pollution due to its nature that cannot decompose and easily absorbed by marine organisms, so it can accumulate in the body. Heavy metals can also indirectly damage fisheries and human health (Supriharyono, 2000).

One method that can be used to monitor pollution in aquatic environments is by using marine organisms called bioindicators. In choosing the bioindicator should be based on laboratory research to obtain a mechanism of pollutant or biokinetic behaviour. The data acquisition can be used as a reference for data interpretation in the real aquatic environment (Suseno, 2006). The phenomenon may indicate the potential of aspecies as bioindicator in detecting heavy metals pollution (Hamed, 2006). One of marine organism that can be used as a bio-indicator is a green mussel. Green mussels (*Perna viridis*) belong to the Bivalvia class that is widely consumed by humans, because it is rich in protein. (Ismail, 2006). The edible portion of the shell is all parts of the body, including the digestive tract. This aquatic biota is highly susceptible to heavy metal contamination due to its filter feeder intake and relatively immobile (sessile) (Gosling, 2004). This makes it easy for heavy metals to accumulate in mussels because heavy metals are readily bonded to particles in the water and difficult to dissolve, thus settling on the bottom of the waters or feeding phytoplankton and marine organisms (Siddall, 1980). Aquatic plants and soft animals such as shells, snails, etc., which are immobile or slow in mobility, cannot regulate metals like other aquatic animals (Darmono, 1995). Therefore, it is important to know how much metal content in an organism before it is consumed by humans. Prevention or effort to reduce the level of metal pollution needs to be done, among others by depuration method.

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In this research, bioaccumulation and depuration study of lead (Pb) was conducted using green mussels as bioindicator. Two depuration methods were applied, i.e by continuous flowing clean water method and immersing in acid solution. Pb contained in mussels were then analysed by using Atomic Absorption Spectroscopy.

2 MATERIAL AND METHODS

2.1 Material

Materials used in this reasearch were Green Mussel, Aquabidest, Boiling Stones, *Arthemia* sp., Sea Water, Aquadest, HNO_3 65%, H_2SO_4 97%, Lead Solution 1000 µg/mL, $CH_3COOH_{(l)}$ 25%, Citric Acid, Selenium, $NaOH_{(l)}$ 30%, H_3BO_3 5%, Phenolpthalein, BCG-MR Indicator, MR Indicator, Hydrocholric Acid 1,0 N, $Na_2B_4O_7.10H_2O$.

2.2 Methods

2.2.1 Acclimatization

The acclimatization was performed for seven days in an aquarium of clean seawater. Green mussels were fed with Artemia sp. every day. During feeding, the filtration system was switched off for 1 hour. Research can be further conducted if the number of dead green mussels were less than 20%.

2.2.2 Process of Bioaccumulation

Lead (Pb) exposure was carried out in an aquarium with a capacity of 80 L. The concentration of Pb ions presented is 1.225 ppm, which is half of the LC_{50} value (Dobson, 1991). Bioaccumulation was conducted for 7 days.

2.2.3 Process of Depuration

Two variation depuration methods were performed. The first method was the recycling of water using aquarium filled with seawater and equipped with fitration and aeration system. Previously also carried out measurements of temperature, pH, salinity, and Pb^{2+} concentration in seawater were measured. Depuration was performed for 7 days.. Samples of green mussels were taken daily to determine the level of Pb. The second method was immersion in acid solution. Two variations of acid solution used were acetic acid and citric acid. The variations of concentration of the two acid solutions used were 0.75%, 1.5%, and 2.25%, with variations of sampling time 24, 48, 72, 96, and 120 minutes.

2.2.4 Pb Content Analysis

The determination of Pb content, the green mussels meat were destructed using 5 mL of HNO_3 and 1 mL of H_2SO_4. Pb levels were then analyzed using Atomic Absorption Spectrometer (AAS).

3 RESULT AND DISCUSSION

3.1 Process of Bioaccumulation

The process of accumulation on green mussels body can occur because heavy metal ion entering into the body of the green mussel form a complex with the cell follows several steps, including metal diffusion from solution to the biological surface, metal adsorption/complexation on the passive side of the bond in a protective layer or spesific binding side of the outer surface of the plasma membrane and internal metal picking transported along the plasma membrane. One of the passive diffusion processes experienced by metals passes through epithelial tissue located in the green mussel's tissue, especially in the gills that are the most significant limbs associated with the outflow of substances derived from the aquatic environment. Heavy metals entering the gills will tend to form complexes with proteins in glycoprotein constituents of gill mucus (Palar, 1994). In mussels, heavy metal like Pb can replace the essential metal and induce changes in protein conformation that caused protein denaturation. Heavy metals may bind to sulfhydryl (-SH), carboxyl (-COOH), hydroxyl (-OH), or amino groups of proteins. One of the ligands present in the green mussels body is the sulfihydryl (-SH) group of cysteine (Grant, 2008).

The formation of metal-protein complexes can be attributed to hard of acid base (HSAB) concept which describes the tendency of hard or soft an acids and base. The metal which is the Lewis acid will act as an electron acceptor and a protein that is Lewis base will act as electron donor. The -SH group of cysteine belongs to the soft-base group. Therefore heavy metals have a tendency to form complexes with soft base groups as well. The complex forming reaction is presented in Figure 1.

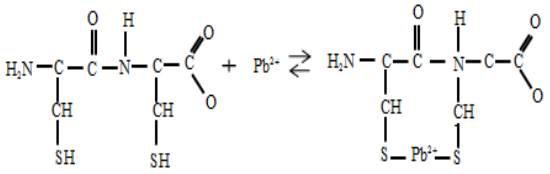


Figure 1. Reaction of Complex Formation

The form of equilibrium equation is a bond with heavy metal deprecating the bonding amide of a nitrogen atom of a peptide. The metal is electrophile with d orbitals which having number of unfilled electrons, while the thiol (sulfhydryl) group in the amino acid cysteine is nucleophile, so that between heavy metals and thiols have a tendency to form bonds. In the process of exposure is used metal in nitrate salts, it allows nitrate ions in the water to form nitric acid compounds that can reduce the pH of the solution, there by triggering in increased toxicity of the green mussel, so the toxicity of heavy metals increasingly great (Hutagalung, 1990). In bioaccumulation process will get the value of CF (Concentration Factor). This value proves the ability of accumulation of green mussel with contaminants. To obtain the value of CF by comparing the metal ion content in green mussel with the metal ion content in water using equation 1.

$$\text{Concentration Factor (CF)} = \frac{\text{concentration of contaminants in organism}}{\text{Concentration of pollutant in water}} \quad (1)$$

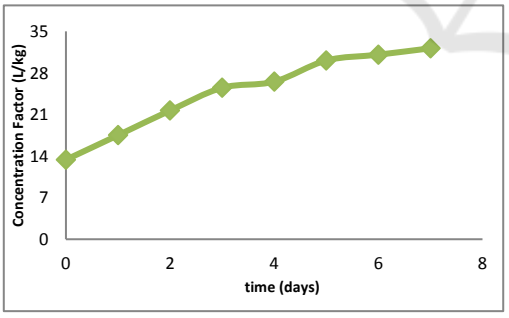


Figure 2. Pb Content in Green Mussels During Bioaccumulation

The value of CF continues to increase and tend to reach the state of steady state was started from the 7 days was 32.15 L/kg. Condition based on the data, indicating that green mussel can accumulate as much as 32 times the concentration of lead ion in the sea water. The implementation of the experimental resuts, if there is lead ion pollution in Jakarta bay then after 1 day the concentration of lead reaches 13 times compared with concentration in sea water. If the pollution is still going on, then in 7 days the concentration wil increase to 32 times compared with concentration in sea water. Then a 30-days was modeled in Figure 3.

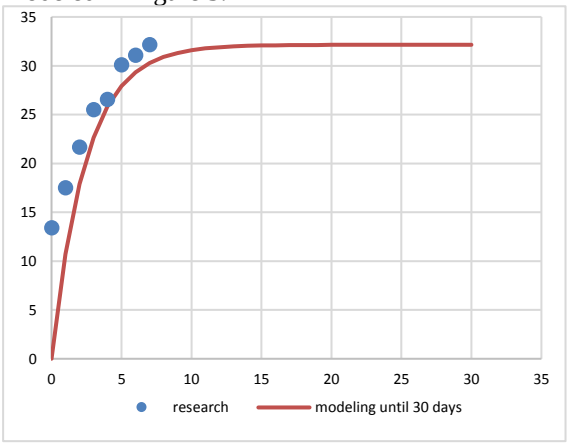


Figure 3. Modeling of CF values if observed until 30 days

When the bioaccumulation is done for 5 days, there is steady state and it can use to get CF_{ss} value. It is a maximum capability of green mussel when accumulate lead ion. It is 32.15 mL/g. The bioaccumulation capasity of the green mussel was also represented by the rate of taking the contaminant (k_u). In a single compartement, value of k_u is assumed as a contaminant uptake mechanism by the entire body of the green mussel. However, the speed of distribution into various type of organs was neglected. Value of k_u (mL/g.day) was an uptake rate calculated based on the slope of the CFt curve to t (from t = 0 to t in steady state (Umbara, 2007). The value of k_u was the influence of physiological factors of green mussel, chemical species of contaminants and the interaction between physiological factors and chemical species. The rate constant is determined by converting equation (2) to a liniear equuation so that (3) is obtained:

$$CF_t = CF_{ss} (1-e^{-ku.t}) \quad (2)$$

$$\ln(CF_{ss}-CF_t) = -k_u.t \quad (3)$$

Then pass the equation (3) in the graph where the x axis is the duration of exposur to the contaminant (day) and the y axis is ln(CFss-CFt). The slope value obtained from the line equation in the graph represents the constant value of the uptake constanta (k_u) by the green mussel. Based on the experiment, the uptake constanta (k_u) was 0.403 mL/g.day.

3.1 Process of Depuration

In this method is done by transferring samples derived from the bioaccumulation aquarium to a contaminant free aquarium to allow the green mussel to continue the filter feeding process. Therefore, it's important to keep the green mussel to stay alive. The process is carried out for 7 days and for 7 days also done replacement of seawater. Factors that can weaken the complex bonds between metals and protein is in the presence of other ligands that can form more stable complexes with metals, such as H_2O. Therefore, a water solvent is used as a provider of H_2O ligands in the depuration process which will disupt the stability of the metal complex with –SH group in the amino acid cysteine protein constituent. In this water-treatment is done with the replacement of seawater media everyday with the speed of flow from water made constant.

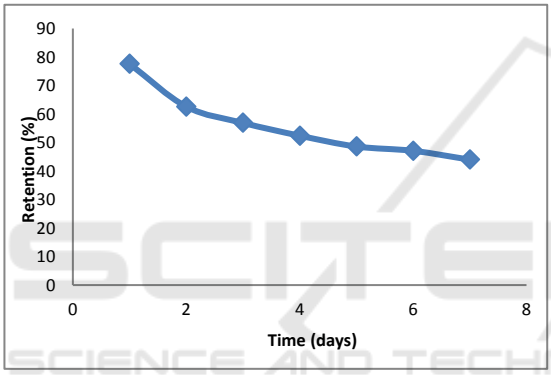


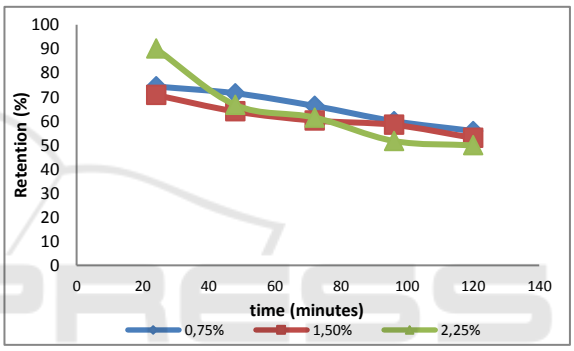
Figure 4. % Retention Value on Depuration Using Continuous Flowing Water

From figure 4, it can be seen that the percentage (%) retention of metal ion of lead retained on the green mussel body decreases during depuration time as the replacement of water is repeated. Retention rate is percentage in the body of the green mussel against the predicted time (Sari, 2005). The decrease in metal ion of lead content may be due to the H_2O ligand which will form a complex with metal ion of lead to $[Pb(H_2O)_6]^{2+}$ and it will cause the bond between the –SH group in sistein and it cause the metal bond to be disturbed. The H_2O ligand is a group of monodentic ligands which can donate one pair of free electrons to fill the empty d orbitals from metal ion of lead. The process of release or excretion from metal ion of lead is one of the processes to maintain electrolyte balance in the body of green mussel. The ability to release contaminants by the green mussel bodies is represented by the value of the realease constant (t_0). The value to be obtained by changing the equation of model depuration (eq. 4) into linear equation (eq. 5).

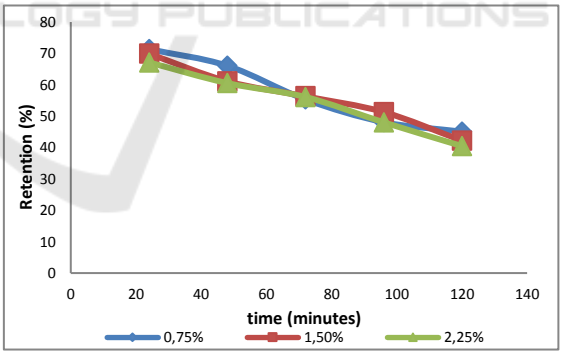
$$C_t = A_0.e - k_e.t \quad (4)$$

$$\ln (A_0-A_t) = k_e.t \quad (5)$$

Equation 5 is plotted into graph and a line equation of the graph is determined. The slope of the line equation represent the value to the green mussel. The value obtained is 0.129 day^{-1}. This may indicate a decrease in the ability of the green mussel to eliminate contaminants from the body. In the other depuration method by using acid solution, that is asectic acid and citric acid. The use of immersion media with that acid is expected to interfere with the bond between metal and protein. The observed results are presented in Figure 5-a and Figure 5-b.



(a)



(b)

Figure 5. % Retention on Depuration Using (a) Acetic Acid, (b) Using Citric Acid

From figure 5 is seen that retention (%) of Pb metal ions retained on the green mussel body decrease during depuration time along with the addition of cencentration to the acid. The determination of heavy metals in green mussel can be caused of insoluble acetat salts from the metals (Suprapti, 2016). The ability to realease contaminants by the biota bodies is represented by

the value of the realease constant (k_e). The gain of the value to be derieved from equation 5 is plotted into a graph and the line equation of the graph is determined. The slope of the line equation represents the k_e value of the green mussel. This may indicate a decrease in the ability of the green mussel to eliminate contaminants from the body. The values to those obtained are presented in Tabel 1.

Table 1. Biokinetic of Lead In Green Mussel

	CF_{ss}	k_u (day^{-1})	k_e (day^{-1})	BCF	$T_{1/2(b)}$ (day)
Depuration Using Water Drainage	32.15	0.41	0.13	3.12	5.37
Depuration Using Acetic Acid 0,75%	32.15	0.41	0.06	6.88	11.75
Depuration Using Acetic Acid 1,5%	32.15	0.41	0.05	8.83	15.07
Depuration Using Acetic Acid 2,25%	32.15	0.41	0.02	27.07	46.20
Depuration Using Citric Acid 0,75%	32.15	0.41	0.07	5.64	9.63
Depuration Using Citric Acid 1,5%	32.15	0.41	0.06	6.34	10.83
Depuration Using Citric Acid 2,25%	32.15	0.41	0.06	6.66	11.36

Biokinetic data is a mode that can be implemented in sea water conditions. The rate of taking lead by green mussel is 0.41 times per-day from the concentration in seawater.release rate of 0.02-0.13 perday from the animal cody with several different realease methods. Biological residence time so that the concentration become half time ($T^{1/2}$). The lead ion in the body of green mussel is 5.37 to 46.20 days. The bioaconcentration factor (BCF) lead ion the animal is 3.12 to 27.07 times compared to the concentration in seawater with several different dischange methods.

4 CONCLUSIONS

Heavy metal ion of Lead can accumulate in green mussels seen from CF (Concentration Factor) value obtained that is equal to 32.15 L/kg.day, the value of k_u (Uptake Constanta) obtained from treatment of exposure of heavy metal ion of Lead foor 7 days equal to 0.403 L/Kg.day, the value of k_e (Elimination Constanta) for depuration treatment with a recurrent water-draining method equal to 0.129 day^{-1}, the smallest value of depuration with immersion of acetic acid is 0.0015 day^{-1} when the variation of concentration in 2.25%, and the smallest value of depuration with immersion of citric acid is 0.0061 day^{-1} when variation of concentration in 2.25%, the value of %retention obtained by 44% in reccurent water drainage, 49.87% when immersion of acetic acid with variation of concentration in 2.25% and 40.46% when immersion of acetic acid with variation of concentration in 2.25%.

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