Depuration Study of Heavy Metal Lead (Pb) and Copper (Cu) in Green Mussels *Perna viridis* through Continues-discontinues and Acid Extraction Methods

**Budiawan, Ridla Bakri, Intan Cahaya Dani, Sri Handayani, Rizki Ade Kurnia Putri, Riska Tamala**

Chemistry Department, Faculty Mathematics and Sciences, Universitas Indonesia 16424
E-mail: dr.budiawan@gmail.com

**Abstract.** Green mussel or *Perna viridis* is filter feeder, which is very susceptible to heavy metals. It takes an effort to release heavy metal contents on the green shell, one of method that can be used to release heavy metal from green shell is depuration process. In this research, the depuration process was conducted by continues method of depuration, discontinues method by using various kind of water and acid extraction. The optimum time of continues depuration method is 1.5 hours, with circulation speed 250 L/h and result of Pb metal content decreased is equal to 30.048% and 29.748% for Cu. In the discontinues method, the optimum result was reached at 100°C by using PAM water as the media at 3 h immersion period with decrease of Pb metal content 35.001% and Cu metal content 39.015%. In the acid extraction method, the optimum condition was achieved by 11% acetic acid solvent with decreasing of Pb and Cu levels are 88.224% and 76.298%. For the determination of protein content, the decrease of protein content obtained by treatment with 11% acetic acid extract showed decrease of protein content 36.656% with Kjeldahl method.

1. **Introduction**

Based on the Decree of the Minister of Environment in 2004, the value of copper metal standard in seawater is 0.008 mg/L and if excess will be toxic and may cause disturbance to living organisms [1]. The content of Cu metal in green shells from the Jakarta bay is 0.0619-2.9065 ppm [2]. This value has not exceeded the maximum level of Ditjen POM No.03725/B/SK /1982 which is 20 ppm for copper [3]. The development of industrial area around the Jakarta bay, and the indication of metal pollution to the aquatic, it is necessary to monitor the accumulation of copper metal in the body of the shell. The prevention and efforts to reduce the level of heavy metal pollution in sea food products need to be conducted, one of the efforts is through the process of depuration. In this research the depuration process was conducted for lead (Pb) and copper (Cu). The depuration process is carried out by water flow recirculating method, discontinues water method and extraction (acetic acid, and citric acid). The water flow recirculating used sea water and PAM water as media depuration by variations of water speed and duration of flow. It was conducted by variations type of water, immersion time and variations of temperature. The extraction method was carried out using acetic acid solvent (8,9,10 and 11% ), and citric acid (0.8, 1, 1.5 and 2%) at 30°C with stirring 120 rpm for 4 hours. In addition, the determination of protein content contained in green shells by Kjeldahl method to determine the protein content in the green shell before and after depuration due to depuration process using acid can cause
the decreased of protein levels due to interaction between protein and metal disruption by the addition of other substances.

2. Experimental Method

The sample was obtained from Muara Kamal, North Jakarta. The green mussel samples high have taken based on 3 parameters which are age, body length, and sampling location (only at one location). After the shellfish samples were taken, the samples were storage in the cooler box with temperature ± 4°C [4]. This research was conducted by several stages, namely determination of Pb and Cu levels of samples without treatment, depuration process, determination of metal content after depuration, and determination of protein content.

2.1 Acclimatization

The acclimatization process was conducted by immersing the green shellfish in sea water which has been filtered with filter paper and change every 12 hours to maintain green shell life.

2.2 Depuration with Continuous Streaming Method (Water flow recirculating)

In this continues water method, a 35 x 30 x 30 cm aquarium was equipped with pumps and filters. The design of the aquariums have pump capacity 250 L/h and 500 L/hr. The variation of flow time were 6, 12, 18, and 24 hours, in which time the test sample were taken and measured the heavy metal content on the mussel meat using SSA instrumentation.

2.3 Depuration with Discontinues Method

This method was conducted by two types of water that are sea water and water PAM. Variation of immersion time were 1.5 h, 2 h and 3 h. After obtaining optimum result for variation of immersion time, the temperature variation was conducted in 80°C, 90°C, 100°C. In order to optimize the depuration process, it is stirred by 120 rpm.

2.4 Depuration with Acetic Acid Extraction

The extraction with acetic acid solvent was conducted by variation of concentration 8; 9; 10; 11% (v / v). The process of depuration was conducted by immersing the green shell in acetic acid solution. Further stirring 120 rpm at a temperature of 30°C for 4 hours. After the depuration process is complete, the green shells are rinsed and the metal content was measured with Atomic Absorption Spectrophotometer (SSA).

2.5 Depuration with Citrate Acid Extraction

The variation concentration of citric acid solution used was 0.8; 1; 1.5; 2% (w / v). The process of depuration is also done by immersing the mussels using citric acid solution with stirring 120 rpm at 30°C for 4 hours.

2.6 Determination of Protein content

Determination of protein content by Kjeldahl method was conducted by subtilize the dried sample meat. Furthermore, about 1 gram sample was put into a Kjeldahl flask and Selenium mixture was added to the flask. The concentrated H₂SO₄ solution was added and the mixture was heated until the solution was clear with the flask in a tilted state and then closed by short funnel. After the sample solution was cooled, the mixture was diluted in a 25 mL water. About 10 mL of sample solution was put from the solution. PP indicator and 30% NaOH were added to the solution until the solution was alkaline. After that, the solution was moved to the distillation apparatus. Then 10 mL of 5% H₃BO₃ solution and BCG-MM indicator on 10 mL erlenmeyer were prepared to accommodate the distillate. The distillation process ends when the distillate shaft changes color from red to green. The resulting destillates were then titrated with a standard 0.1 N HCl solution and recorded the required volume of HCl for the calculation of protein content, based on the formula:

\[
\text{Protein Content} = \frac{(\text{ml titration} \times \text{HCl}) \times 14 \times 6.25}{1000 \times \text{sample mass}} \times 100\%
\]
3. Results and Discussion

3.1 Determination of metal in seawater samples
Measurement of metal content is carried out on seawater samples. It was obtained the results of Pb metal content in seawater samples before filtration was 0.109 mg/L while Cu content was 0.089 mg/L. From the above results it is known that the metal content of Pb and Cu has exceeded the standard quality determined by the Decree of the Minister of Environment in 2004 regarding the quality of seawater for marine biota, which is 0.008 mg/L for metal Pb and Cu. Cu metal is more stable to form complexes in sediments, based on Moore and Ramamoorthy (1984), the Cu complex bonds that occur in marine sediments are the most stable complexes, while the most complex in seawater, the lowest stability [5].

3.2 Depuration with Continues Method (Water Flow Recirculating)
In continues method, there are some variations of circulation velocity with two pumps in capacity 250 L/h and 500 L/h. The purpose of this variation of circulation velocity is to know the effect of time contact in depuration media on the process of releasing heavy metals in the body of green shells. Here are the results of the measurement of the initial metal content of Pb and Cu:

![Figure 1 Pb and Cu content in green shells](image1.png)

![Figure 2 Percentage of Pb and Cu content decreases (continues method)](image2.png)

Based on the picture above (Figure 4.1), the initial lead metal content is in the range of 8.931-14,147 g/kg, with an average of 11.443 mg/kg. From this result, the accumulation of Pb in the body of the green shell has been categorized as dangerous. Meanwhile for Cu metal is in the range of 3,541-8,826 mg/kg, with a mean of 5.233 mg/kg. In this study, the depuration process was carried out within 24 hours in which the variations of water flow duration were 6, 12, 18 and 24 hours and variation of water circulation speed were 500 L/h and 250 L/hr. Here is the result of decreasing levels of Pb and Cu metal after depuration.

From the data above, by using the variation of circulation speed 500 L/h, the longer the drainage time, the higher percentage of lead and copper metal decrease. This is due to the water drainage assisting heavy metal excretion on the body of the green shell. The result of excretion in the form of
faeces also contains metal, can be drawn with pump which has a stronger suction power and enter the filtration system. Optimal depuration are obtained at 24 hours with lead decrease of 23.971% and copper of 25.28%. This result is less efficient because it takes a long time to detoxify the metal.

At a circulation of 250 L/h, the highest percentage decrease in metal content at 12 hours with 30.047% decreases for lead metal and 29.748% decreases for copper metal. However, after 12 hours, the metal content has increased again, this may be due to the metal that has been excreted back into the shell body. From the graph above, when compared to the decreasing of heavy metal content at 500 L/hr and 250 L/h at 6-18 h, it was found that, both of metal decreasing at circulation of 500 L/h is smaller than circulation of 250 L/h, this is due to the existence of self-defence mechanisms by the green shell while in an environment that is less conducive to shell life such as heavy currents and extreme water conditions. One way to defend oneself is to close the shell and produce mucus [6]. This condition can be observed when the process of depuration is done with a faster circulation, shells are not fully open as in shells with a circulation of 250 L/hr. When the shell is not fully open, the heavy metal present in the mucus found in cleft of organ of the shell, is not eliminated properly.

3.3 Depuration of Discontinues Method

In this study, discontinues method was done on temperature variations of 80, 90 and 100°C and immersion time of 1.5 hours, 2 hours and 3 hours. Media used for immersion is PAM water and seawater that has been treated first. The average result of lead metal measurement is 11.0528 mg/kg and 4.9515 mg/kg for copper metal. The yield of heavy metals in the sample of clam meat after the depuration process using discontinues method with PAM water medium, for each of heavy metals, showed the optimum results at temperature 100°C with the immersion time was 3 hours. The decreases of protein content of Pb was 35.008% and copper was 39.016%.

From the results of sea water immersion, it was found that the highest decrease of heavy metal and copper was affected by treatment condition of temperature 100°C and immersion for 3 hours with 19.15% lead metal decay and 30.64% for copper metal. While the longer of the immersion time, obtained the decreases of heavy metals. Other metallic substances in seawater are likely to affect the process of releasing heavy metals in the body of the shell [7].

3.4 Depuration of Acid Extraction

The average of Pb metal content in initial shellfish was 9.684 mg/kg and for Cu metal 6.4141 mg/kg. In this study acetic acid concentration used for depuration is 8.9, 10 and 11% (v/v). Based on the data, optimal results were obtained when using acetic acid 11% with decrease of Cu metal equal to 76.29% and decrease of metal Pb equal to 88.24% (Figure 4.4). Acetic acid can interfere with the interaction between metal and cysteine because acetic acid has the ability to protonate metal that binds to amino acids such as cysteine by donating H+ ions and this results in bonds between amino acids and metals being able to break. According to Sari and Keman (2005), the bond between acetic acid and metals makes the metal lose its ionic properties, resulting in less toxicity [8].

The result of decrease of heavy metal content after depuration process with citric acid as in Figure 3. The percentage of the decrease in lead and copper metal content is equal to the increased concentration of citric acid. From the data above (Figure 4.3), the largest percentage of the decrease in both of metal content is on 2% citric acid with yield of 35.009% for Cu metal and 42.927% for lead metal. This is because citric acid is a polydentate ligand. In ligands that have two or more donor atoms, the donor atoms may bind to the same central atom forming a chelate complex [9]. The thing that makes citric acid can be metal because citric acid is able to donate 2 free electron pairs to the metal acting as Lewis acid.
3.5 Determination of Protein content

In this study, the determination of protein content is only done on the depuration process which gives the percentage of the most optimal metal content decreases. Here are the results of protein content in green mussel samples:

Figure 3 Percentage of Pb and Cu metal content decreases by Acetic Acid and Citric Acid Extraction

Figure 4 Percentage of Protein Content

From the data above (Figure 4), showed that the protein content of green shell after depuration with acetic acid 11% was 63.34%. The results of this Kjedahl method showed that there was a decrease of nitrogen content from clam meat samples after depuration treatment. The loss of these nitrogen levels can be affected by several factors. The first factor is stirring, according to Wu and Ling, mechanical treatment such as stirring can cause the isoelectric point to reach faster. Generally, denaturation speed is slower than stirring. The interaction between depuration media such as water and acid, while the depuration process takes place, also causes loss of nitrogen content in the sample of clam meat. The depuration process using acetic acid, causes the decreasing of metal content. Meanwhile, according to the protein content measurement, the increasing of acid can also alter the conformation of protein structure and protein damage [10].
4. Conclusion
From the depuration process of lead and copper as heavy metal in green shell (Perna viridis), it was obtained that optimum time of depuration by continues method is 1.5 h, with circulation velocity 250 L/h and obtained the decrease of Pb metal content as much as 30.048% and 29.748% for Cu. In discontinu method, optimum result was achieved at temperature 100°C and using PAM water as the media for 3 hours immersion period with decreasing Pb metal content of 35.001% and Cu metal content 39.015%. In the extraction method, the optimum condition was achieved in the extraction using 11% acetic acid with the decrease of Pb and Cu concentration of 88.243% and 76.298%. The depuration process by using water flow recirculating has a decrease percentage of the protein content as much as 13.267% by Kjedahl method. The immersion treatment which was accompanied by heating on the discontinues method, has a percentage of the decrease in protein content as much as 21.491% by Kjedahl method. Acetic acid extraction showed a decrease in protein content for about 36.655% based on the Kjeldahl method.

References
[1] KLH. 2004 Keputusan Menteri Negara Kependudukan dan Lingkungan Hidup No Kep-02/MENKLIH/1998 Tentang Pedoman Penetapan Baku Mutu Air Laut. Jakarta
[2] Inswiasri 1995 Kadar Logam Cu, Pb, Cd Dan Cr Dalam Ikan Segar dan Kerang. Jakarta: Badan Penelitian dan Pengembangan Kesehatan
[3] BPOM 2013 Peraturan Kepala Badan Pengawas Obat dan Makanan Republik Indonesia Nomor 8. Jakarta.
[4] Budiawan and Neera 2012 Biomonitoring Logam Berat In Diktat Biomonitoring (p. 18) Jakarta
[5] J.W. Moore S. Ramamoorthy 1984 Organic Chemicals in Natural Waters- Applied Monitoring and Impact Assessment.New York-Berlin-Heidelberg-Tokyo. Springer Verlag. 289 S., 81 Abb, DM 129
[6] Hughes, R. 1986 A functional biology of marine gastropods. London: Croom Helm.
[7] Hutagalung, H. P. 1991 Pencemaran Laut oleh Logam Berat dalam Status Pencemaran Laut di Indonesia dan Teknik Pemantauannya. Jakarta: LIPI.
[8] Sari, F. I., Keman, & Soedjadi. 2005 Efektifitas Larutan Asam Cuka untuk Menurunkan Kandungan Logam Berat Cadmium dalam Daging Kerang.
[9] Effendy 2007 Perspektif Baru Kimia Koordinasi Ilid 1. Malang: Bayumedia Publishing.
[10] Purnomo T, M. 2007 Analisis kandungan timbal (Pb) pada ikan bandeng (Chanoschanos Forsk.) di Tambak Kecamatan Gresik. Surabaya: Universitas Negeri Surabaya
[11] Power, A. R. 2004 First Occurrence of The Nonindigenous Green Mussel, Perna viridis in Coastal Georgia. 2004: United States: Journel of Shellfish Research 23
[12] Setyono, D. E. 2006 KARAKTERISTIK BIOLOGI DAN PRODUK Kekerangan Laut. Osca, Volume XXXI, Nomor 1, 1-7
[13] Skoog, D., Holler, F. J., & Crouch, S. R. 2007 Principles of Instrumental Analysis. USA: Thomson Brooks/Cole
[14] Soedarmadjadi, Haryono, & Suhardi. 1997 Prosedur Analisa Bahan Makanan dan Pertanian. Yogyakarta: Liberty
[15] TAN, W. (1975). Eggs and larva development in the green mussels, Mytilus viridis Linnaeus. The Velinger, 151-155
[16] WHO 1977 Environmental Health Criteria 2 : Copper. World Health Organization
[17] WHO 1977 Environmental Health Criteria 3: Lead. World Health Organization
[18] WHO 1977 International Programme on Chemical Safety Food Additives Series No. 12. Inchem
[19] Wibisono, M. S. 2005 Pengantar Ilmu Kelautan. Jakarta: PT. Grasindo
[20] Widowati, W. S. 2008 Efek Toksik Logam. Yogyakarta