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Research Article

Analysis of Heavy Metals in Shellfish in Tanjung Balai Waters

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Abstract: Shellfish is a source of toxic heavy metals and the maximum levels of heavy metals allowed in shellfish by BPOM RI are 1.5 mg/kg for lead (Pb), 1 mg/kg for cadmium (Cd) and 20 mg/kg for copper (Cu). Heavy metal content in shellfish is influenced by environmental pollution in which the shellfish live. The purpose of this study was to determine the levels of heavy metals in three types of shellfish in Tanjung Balai Asahan waters. Shellfish samples were taken from 3 different locations in Tanjung Balai in July 2021. Determination of the levels of heavy metals, Pb, Cd and Cu, in green mussels, mussels and blood clams was carried out using an atomic absorption spectrophotometer (AAS). The results showed the levels of Pb, Cd and Cu in green mussels in Tanjung Balai, as follows: 0.273 mg/kg – 0.406 mg/kg (Pb), 0.089 mg/kg – 0.106 mg/kg mg /kg (Cd), 1,864 mg/kg – 2,011 mg/kg (Cu). In mussels Pb was 0.051 – 0.095 mg/kg, Cd was 0.077 – 0.091 mg/kg, and Cu was 1.024 mg/kg – 1.064 mg/kg. In blood clamps : 0,184-0,317 mg/kg (Pb) , 0,081 – 0,127 mg/kg (Cd) , 1,564 – 1,744 mg/kg. It was concluded that Cu in green mussels had the highest heavy metal content. The levels of heavy metals in the analyzed shellfish samples were still below the maximum levels permitted by BPOM RI.

Keywords: *Lead, Cadmium, Copper,Atomic Absorption Spectrophotometry, Shellsfish*

1. INTRODUCTION

Heavy metal is one of the toxic pollutants that can cause death (lethal), and non-death (sublethal) such as impaired growth, behavior and morphological characteristics of various aquatic organisms [1]. If marine biota has been contaminated with these heavy metals consumed within a certain period of time, the heavy metals can become toxic materials that will poison the bodies of living things [2]. Heavy metals such as; Cadmium (Cd), Plumbum (Pb), Copper (Cu) are very dangerous if the dissolved levels in the human body are high enough or exceed the permissible threshold. The main cause of heavy metals being hazardous pollutants because heavy metals cannot be destroyed (non-degradable) by living organisms in the environment and accumulate in the environment, especially settling on the bottom of the water to form complex compounds with organic and inorganic materials by absorption and combination [3]. These heavy metals are very toxic which can enter the human body through several ways, namely food such as fish, shellfish, processed foods, drinking water and can interfere with breathing and skin penetration [4]. One type of marine life is clams. Shellfish is one type of shellfish that has the potential and high economic value to be developed as a source of protein and minerals to meet the food needs of the Indonesian people.

Shellfish are one of the most efficient marine animals to accumulate heavy metals. This is because mussels live in the sediment layer of the bottom of the water, moving very slowly and their food is detritus at the bottom of the water, so the chance for heavy metals to enter the body is very large. These biota are sessile (settled) biota and filter feeders, which take their food by filtering water.This way of eating makes various material components enter the shell's body [5]. Heavy metal pollution is one of the problems that often occurs in coastal waters. Andayani et al (2020) stated that green mussels (*Perna viridis L.*) from Jakarta Bay were not suitable for consumption due to heavy metal pollution (Pb, Cd) [6]. The results showed the metal content of Pb and Cd in green mussels in four adjacent locations, namely Jakarta Bay, Brebes and Cirebon, and Panimbang. The results of the analysis from Jakarta Bay were 0.42mg/kg – 29.4 mg/kg, Brebes were 0.01 mg/kg – 3.52 mg/kg and Cirebon were 0.01 mg/kg – 2.66 mg/kg which had passed the maximum permissible threshold according to SNI (2009).

The waters of Tanjung Balai Asahan, currently developing with rapid development around the mouth of the Asahan River, have built residential areas for residents and companies/factories such as mining, timber industry, palm oil mills and water transportation [7]. The wastes from these activities flow to the mouth of the Asahan River, so that they can disrupt the development of ecosystems and organisms around the waters. To prevent the occurrence of bioaccumulation of heavy metals in the human body, a study is needed to analyze the heavy metal content. This study aims to analyze the content of heavy metals Pb, Cd and Cu in the types of shellfish that are often consumed in Tanjung Balai waters namely in green mussels *(mytilus viridis)*, mussels (*meretrix meretrix*) and blood clams (*anadara granosa*) in Tanjung Balai rivers. Determenition of heavy metal content in shellfish was carried out using the AAS (Atomic absorption spechtrophometer) method.

2. MATERIALS AND METHODS

2.1. Materials

The materials used to carry out heavy metal testing were mussel meat, standard solution of Pb, Cd, Cu (Merck), deionized water, HNO3 (Merck), 1.5% HNO3 (15 ml concentrated HNO3 dissolved in 1 L deionized water) , HClO4 (Merck), NaBH4 3% (3 grams of sodium tetra borate dissolved in 100 ml of 1% NaOH), 1% NaOH (0.4 grams of NaOH dissolved in a 1 L volumetric flask with deionized water, Shellfish from Tanjung Balai rivers, AAS (PerkinElmer PinAAcle 900T and VARIAN) in LAKESDA of Sumatra Utara.

2.2. Analysis of Heavy Metal Content

The heavy metal analysis procedure was carried out in 3 stages. The first stage procedure is the dry ashing process. The second stage procedure is the process of making a standard solution of heavy metals. The third step is the process of making a heavy metal calibration curve and reading the concentration of heavy metal samples of mussel meat on an atomic absorption spectrophotometer (AAS) [8].

2.2.1. Dry Destruction

Destruction is carried out like the method of Riani et al., (2017) with modifications. The mashed sample was carefully weighed as much as 5 g in a porcelain crucible and then repeated 6 times. The porcelain exchange containing the sample was heated on a hot plate with a temperature of 100°C, then ashed in a kiln with an initial temperature of 100°C and slowly the temperature was raised to a temperature of 500°C with 25°C intervals every 5 minutes. Ashing is carried out for 72 hours (calculated when the temperature reaches 500°), then after the furnace temperature is ±27°C, the porcelain crucible is removed and left to cool in a desiccator

2.2.2. Producing a sample solution

The cold digested sample was dissolved with 5 ml of HNO3 (1:1) then transferred to a 50 ml volumetric flask and the porcelain crucible was rinsed 3 times with demineralized water. The results of the rinsing were put into a volumetric flask, then the solution was made up to volume with demineralized water to the marking line and filtered using Whatman filter paper No. 42, the first filtrate was discarded as much as 5 ml to saturate the filter paper and then the filtrate was then collected in a bottle. This filtrate is used as a sample solution for heavy metal analysis [8].

2.2.3. Determination of Metal Rates on Samples

Determination of metal content by making a calibration curve, then the absorbance is measured from various concentrations of standard solutions of Pb, Cd and Cu with the wavelength of each metal and then obtained the concentration. The procedure carried out in accordance with SNI 2011 was taken ± 50 ml of the sample solution from the destruction and put into the sample bottle and placed into the sample holder on the atomic absorption spectrophotometer. The absorbance of the sample solution was measured using an atomic absorption spectrophotometer at a wavelength of 217.0 nm, Cadmium 228.8 nm and Copper 324.8 nm. The absorbance and concentration obtained are plotted into a calibration curve.

According to Rohma et al., (2007), the determination of the levels in the sample can be calculated from the regression equation which is then used to calculate the levels in the sample.

$$Y=aX+b$$

The metal content in the sample can be calculated in the following way:

$$\text{Metal Content } (\mu g/g) = \frac{\text{concentration } (\mu g/ml) \text{ Volume } (ml)}{Weight\ of\ sample\ (g)}$$

2.3. Analytical Method Validation

Validation of the analytical method is an action to determine the accuracy (validity) of the analytical method applied, to prove that the results obtained are in accordance with the analyte content in the sample. In this method, the metal content in the material is determined first, then the metal content in the material is determined after the addition of a standard solution with a certain concentration. The scallops that had been mashed were carefully weighed as much as ± 5 grams in a porcelain crucible, then 0.1 ml of standard solution of Pb and Cd 10 g/ml were added, while for Cu each 0.5 ml of standard solution of Cu 10 g/ml was then continued. with the destruction procedure as previously done. According to Harmita, (2004) Percent recovery can be calculated by the following formula:

$$\text{Recovery } (\%) = \frac{(c)sample+spike-(c)sample}{(c)spike} \text{ x } 100\% \text{ [10]}$$

2.4. Determination of the Maximum Limit for Heavy Metal Consumption

Shellfish exposed to heavy metals will be dangerous if consumed by the community continuously because it will accumulate in the body and have an impact that endangers human health.To reduce this impact, it is necessary to calculate the maximum allowable metal consumption using the following formula Cahyani, (2016):

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

Description:

MWI : Maximum Weekly Intake is the maximum limit of heavy metal content of foodstuffs which may be consumed per week (mg / week)

Weight : Weight average adult mm 50 kg [11]

PTWI : Provisional Tolerable Weekly Intake or numbers Maximum tolerance limit per week issued by WHO (2011) in (mg/kg body weight/week).

3. RESULTS AND DISCUSSION

3.1. Calibration Curves Pb, Cd and Cu

Calibration curves were obtained by measuring the absorbance of Pb, Cd and Cu standard solutions at their respective wavelengths.

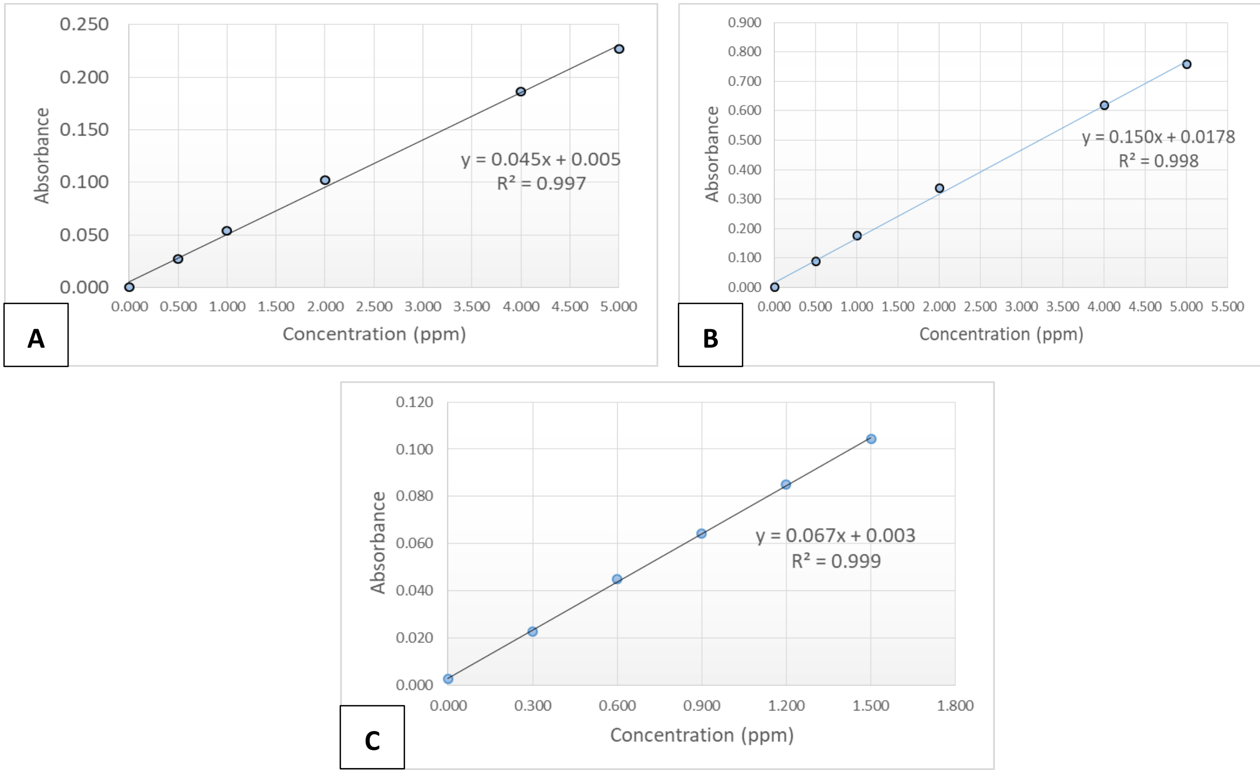

Figure 1. A. Calibration Curve Pb; B. Calibration Curve Cd; C. Calibration Curves Cu

This curve shows a positive correlation between concentration (X) and absorbance (Y), The value of $r \geq 0.97$ indicates a linear correlation which states that there is a relationship between X (concentration) and Y (absorbance) which means that the increase in concentration is proportional to the increase in absorbance [12] .

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

3.2. Concentration of Pb, Cd and Cu in Shellfish

Determination of levels of Pb, Cd and Cu in shellfish by atomic absorption spectrophotometry. The results of heavy metals Pb, Cd, and Cu can be seen in Figures 2,3 and 4. The results of testing the value of the highest heavy metal content is found in green shells with Cu content of 2, 011 mg // kg and the lowest value of Cd is found in mussels that is 0.027 mg/kg

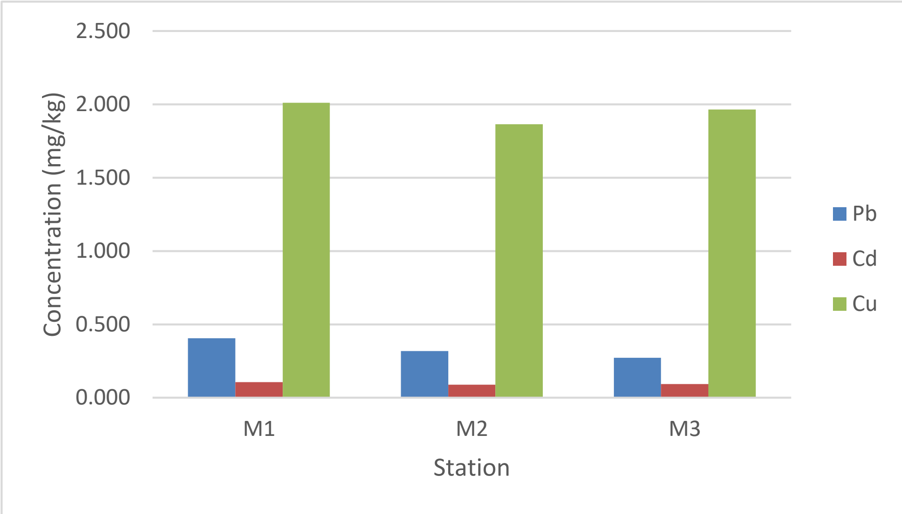

Figure 2. Pb, Cd and Cu content of green mussel (*perna viridis*) (M1: Station 1, M2 : Station 2, M3 : Station 3)

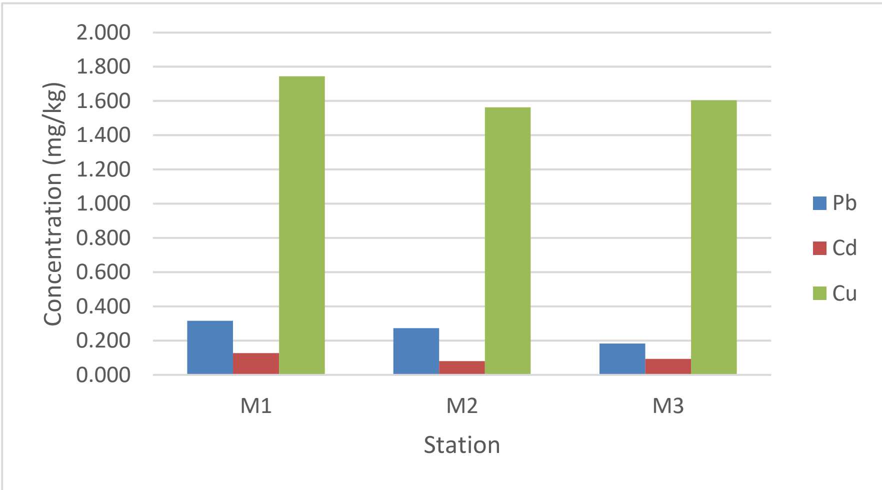

Figure 3. Pb, Cd and Cu content of blood clams (*anadara granosa*) (M1 : Station 1, M2 : Station 2, M3 : Station 3)

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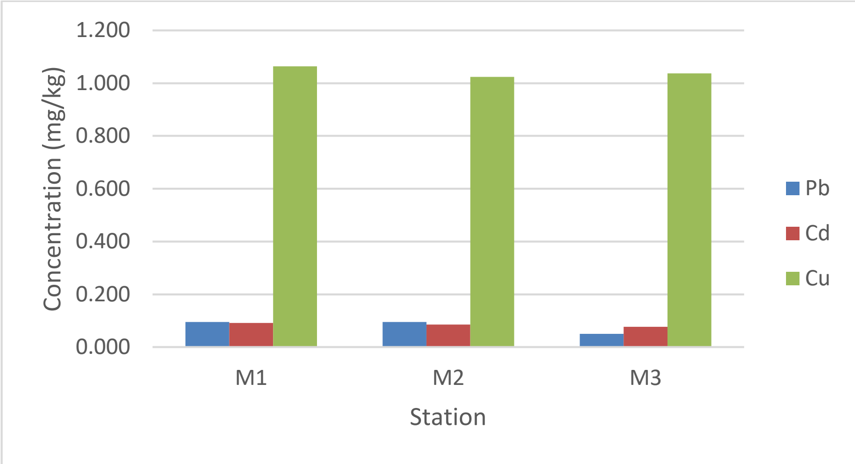

Figure 4. Pb, Cd and Cu conten of mussels (*Meretrix meretrix*) (M1 : Station 1, M2 : Station 2, M3 : Station 3)

The figure above shows that all samples at three stations in Tanjung Balai waters gave positive results for Pb,Cd, and Cu metals with different levels. absorb these metals into the body and can be due to differences in metal levels contained in the place of origin of the sample which can be affected by the presence of pollution of the surrounding environment [4].

Each organ of the shellfish body has a different role either in its metabolic function or physiological function. This affects the distribution of metals in different tissues of shellfish, as a result the metal detoxification process in the body of shellfish can also be different [13]. Differences in metal levels in shellfish at different stations indicate differences in the amount of harmful metals found in the aquatic environment where the shellfish habitat is located. The coastal waters of Belawan and Tanjung Balai have a relatively high degree of similarity in the accumulation of Pb heavy metal in shellfish bodies and other variables measured [14]. The results showed the levels of Pb, Cd and Cu in green mussels in Tanjung Balai, as follows: 0.273 mg/kg – 0.406 mg/kg (Pb), 0.089 mg/kg – 0.106 mg/kg mg /kg (Cd), 1,864 mg/kg – 2,011 mg/kg (Cu). In mussels Pb was 0.051 – 0.095 mg/kg, Cd was 0.077 – 0.091 mg/kg, and Cu was 1.024 mg/kg – 1.064 mg/kg. In blood clamps : 0,184-0,317 mg/kg (Pb) , 0,081 – 0,127 mg/kg (Cd) , 1,564 – 1,744 mg/kg. It was concluded that Cu in green mussels had the highest heavy metal content.

Based on the figure above, green mussels, blood clams and mussels still meet the standard threshold set by BPOM RI. The maximum limit of Pb contamination is 1.5 mg/kg and for Cd is 1 mg/kg. Based on the Decree of the Director General of POM No. 03275/B/SK/VII/89 concerning the maximum limit of metal contamination in shellfish, the maximum limit set for Cu is 20 mg/kg.The figure above shows that all samples at three stations in Tanjung Balai waters gave positive results for Pb, Cd and Cu metals with different levels shellfish to absorb the metal into its body. For bivalves it appears that the pattern of metal accumulation tends to depend on the metabolism and ability of the shellfish to excrete or store these metals [13].

Shellfish have a feeding habit by preying on particles in the form of microorganisms or the remains of organic matter (detritus). This is because shellfish have a diet that is a filter feeder , namely filtering all types of food around it so that it can accumulate microorganisms (including bacteria and viruses) and other foreign materials including heavy metals that are absorbed and stored in their digestion [14]. In addition, the nature of this shellfish is more sessile and not migratory. This makes it easy for heavy metals to concentrate and accumulate in the shellfish[14]. The entry of contaminants into the body of these biota can be through water and feed routes, allowing these contaminants to accumulate and undergo biomagnification in each food chain. The pattern of accumulation of heavy metals in the body of marine organisms is not only influenced by body size, external factors such as food choices and internal factors such as metabolic rate also affect the accumulation of heavy metals. Dietary factors in shellfish greatly determine the accumulation of heavy metals in meat, therefore the ability of shellfish to accumulate heavy metals differs according to species ([4].

3.3. Validation of Analysis Methods

Table 1. Validation methods of Pb

Sample	concentration (mg/kg)	Spike concentration (mg/kg)	Spike + sample concentration (mg/kg)	Percent Recovery(%)
1	0.410	0.200	0.610	101
2	0.430	0.180	0.620	106
3	0.430	0.180	0.630	110
4	0.410	0.200	0.610	104
5	0.440	0.170	0.620	106
6	0.410	0.200	0.610	103
Σ				630
$\bar{X}$				105
SD				3.063
RSD				2.918 %
LOD				0.003 mg/L
LOQ				0.01 mg/L

Table 2. Validation methods of Cd

Sample	concentration (mg/kg)	Spike concentration (mg/kg)	Spike + sample concentration (mg/kg)	Percent Recovery(%)
1	0.100	1.000	1.100	100
2	0.110	0.960	1.070	97
3	0.100	0.960	1.060	96
4	0.100	0.990	1.090	99
5	0.100	0.940	1.040	94
6	0.110	0.980	1.090	99
Σ				586
$\bar{X}$				98
SD				2.064
RSD				2.115 %
LOD				0.001 mg/L
LOQ				0.004 mg/L

Table 3. Validation methods of Cu

Sample	concentration (mg/kg)	Spike concentration (mg/kg)	Spike + sample concentration (mg/kg)	Percent Recovery(%)
1	1.060	0.200	1.270	101
2	1.070	0.190	1.270	103
3	1.060	0.200	1.280	105
4	1.060	0.200	1.270	103
5	1.040	0.220	1.280	108
6	1.050	0.210	1.270	101
Σ				622
$\bar{X}$				104
SD				2.570
RSD				2.481 %
LOD				0.003 mg/L
LOQ				0.01 mg/L

According to ICH (1994) for the determination of the recovery, the spiking technique was used, i.e the known concentration of Pb, Cd, and Cu solution were added to shellfish, and the resulting spiked samples were measured, calculated, and compared to the known value of Pb, Cd and Cu. The indicators for detection limit are absolute LOD (instrument LOD) and relative LOD (method LOD)[15]. Absolute LOD represents the smallest concentration of analyte that can still be detected by the instrument, while relative LOD is the smallest concentration of an analyte in the sample that can still be detected. Limit detection testing was carried out by using a calibration curve with the formula 3[syx/b]. LOQ value constitutes the lowest limit of the calibration curve. LOD values obtained were 0,003 mg/l (Pb), 0,001 mg/l (Cd), and 0,003 mg/l (Cu). LOQ values obtained were 0.01 mg/L for Pb, 0.004 mg/L for Cd, and 0.01 mg/L for Cu. The results of heavy metal (Pb, Cd, and Cu) concentrations from green mussels , blood clams and mussels were still below the LOD and LOQ. It means the methods have good sensitivity. The RSD value obtained were 2,918 %(Pb), 2,115% (Cd), and 2,48 % (Cu). The maximum RSD values acceptable for the analyte of 1 ppm is 16 %. Therefore, it can be stated that the developed method exhibited a good precision [16].

The test results of recovery meets the accuracy requirements that have been established, if the average results of recoveryis in the range of 85-115% [11]. Based on the table, it can be seen that the average recovery test results from shellfish for Pb, Cd, and Cu were 105 %, 98%, and 108%. Percent recovery shows satisfactory work accuracy at the time of inspection of the sample. This indicates that the result of the method is acceptable for Pb, Cd, and Cu .

Table 4. Maximum Tolerable Intake

Types of Shellfish	Station	Heavy Metal	Concentration (mg/kg)	PTWI (µg/kgBB/ week)	MWI (mg/week)	MTI (kg/week)
Green mussels	1	Pb	0,406	25	1,25	3,08
	2		0,317	25	1,25	3,94
	3		0,273	25	1,25	4,58
	1	cu	2,011	3500	175,00	87,02
	2		1,864	3500	175,00	93,87
	3		1,964	3500	175,00	89,09
	1	cd	0,106	7	0,35	3,29
	2		0,089	7	0,35	3,95
	3		0,094	7	0,35	3,74
Mussels	1	Pb	0,095	25	1,25	13,15
	2		0,095	25	1,25	13,15
	3		0,051	25	1,25	24,71
	1	cu	1,064	3500	175,00	164,44
	2		1,024	3500	175,00	170,85
	3		1,038	3500	175,00	168,66
	1	cd	0,091	7	0,35	3,85
	2		0,085	7	0,35	4,11
	3		0,077	7	0,35	4,57
Blood clamps	1	Pb	0,317	25	1,25	3,94
	2		0,273	25	1,25	4,59
	3		0,184	25	1,25	6,80
	1	cu	1,744	3500	175,00	100,32
	2		1,564	3500	175,00	111,88
	3		1,604	3500	175,00	109,09
	1	cd	0,127	7	0,35	2,75
	2		0,081	7	0,35	4,33
	3		0,094	7	0,35	3,74

Notes : PTWI = Provisional Tolerable Weekly Intake, MWI= Maximum Weekly Intake

The MTI of heavy metals in Tanjung Balai waters is 2.75 kg/week because according to Cahyani (2016) the maximum consumption limit is determined by choosing the smallest value. This is because food ingredients that contain heavy metals even if they contain a small amount if consumed continuously will accumulate in the human body and tend to be toxic.

4. CONCLUSION

The test results showed that the highest value of heavy metal content was found in green mussels with concentration of Cu was 2.011 mg//kg and the lowest value of cadmium was found in mussel shellfish, which was 0.027 mg/kg. The metal content of Pb, Cd and Cu found in mussels showed different levels for each metal where the Cu content of green mussels was higher than the levels of other metals. This level is still safe because had not exceeded the limit set by BPOM RI.

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