Bioconcentration of Chromium (Cr) on The Soft Tissue of Mussels (*Perna viridis, Linnaeus 1758*) in Tambak Lorok Waters, Semarang

Siti Rudiyanti*, Suryanti Suryanti, Churun Ain

Aquatic Resources Management Study Program, Faculty Fisheries and Marine Science, Universitas Diponegoro
Jl. Prof Jacub Rais, Tembalang, Semarang, Central Java 50275 Indonesia
Email: st_rudiyanti@yahoo.com

Abstract

Industrial development provides economic benefits, but also increases the concentration of waste pollution which affects the aquatic environment of Tambak Lorok on the North Coast of Central Java. The existence of this waste will affect the life of the biota in it, such as mussels (*Perna viridis*). The heavy metal Chromium is the focus of research, considering that its presence in large quantities which accumulates in the human body causes health problems. The aims of the research were to determine the content of heavy metal Cr in the waters, sediments, and soft tissue of mussels, to determine the bioconcentration of heavy metal Cr in mussels, and to determine the tolerable intake of weekly consumption of the mussels cultivated in Tambak Lorok waters. The content of Cr in water samples, sediment, and mussel soft tissue was measured the APHA 3111B-2012 method, US EPA SW-846/3050 B-1996 SM 3111B-2012 method, and SSA method. The calculation of bioconcentration mussels, using the bioconcentration factor and Maximum Tolerable Intake. The heavy metal content of Cr in the water column was 0.015 – 0.025 $mg.L^{-1}$ (upper the quality standard), in sediment was 25.12 – 28.72 $mg.kg^{-1}$(lower the quality standard), and in the soft tissue of mussels was 0.155 – 0.265 mg/kg (upper the quality standard). The bioconcentration factor of metal Cr in mussels is classified as low, which is around 10.35 – 14. The maximum tolerable intake for mussels was 6,078 kg/week, it is considered safe for consumption.

Keywords: Bioconcentration; heavy metal Chromium; *Perna viridis*; Tambak Lorok Waters, Maximum Tolerable Intake

INTRODUCTION

Industrial development results in two contradictory conditions. On the first side, it gives benefits economically, which was recorded by BPS in 2018 stated that in Semarang City there were 512 IBS (Large and Medium industries) which absorbed 123,888 workers. On the other side, it increases the concentration of waste pollution as recorded by the Semarang City Environment Service The number of water pollution locations that are classified as acute in 2022 is 27 and has increased compared to the previous year where only 3 locations were recorded. While in the light pollution category in 2022 there are 44 locations which have experienced a decrease from 2021 which was recorded as many as 55 locations. The need for good environmental management is because increase in the concentration of hazardous and toxic waste materials will decrease the environmental quality of Tambak Lorok on the North Coast of Central Java, due to several rapid industrial developments. One of them is the textile industry which produces of heavy metal Cr content waste from the dyeing process. The impact of wastes metallurgical industry potentially pollutes the surrounding water, soil and air (Izydorczyk *et al.,* 2021). The waste in the waters is from human activities, such as industrial management activity, domestic activity, and agricultural industry waste. However, not only organic material waste is produced from these activities, but also inorganic materials such as heavy metals are contained therein.

Mussels (*Perna viridis*, Linnaeus 1758) are native species from indo-pacific tropical waters in Asia (de Messano *et al.,* 2019). Mussels are included into dioecious species (males and females are differentiated) that reach their sexual maturity in 2 – 3 months and can live for 3 years. *P. viridis* has an annual reproductive cycle that is strongly interlinked and influenced by the seasonal variation of a set of environmental factors, feeding behaviors, and gonadal lipid and fatty acid contents (Asaduzzaman *et al.,* 2019). *Perna viridis* have special characteristics with speed growth and they

*) Corresponding author
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commonly have high tolerance toward environmental variables, such as temperature, salinity, and pollution (Ma *et al.,* 2020). They develop byssus thread that capable them to form a solid group to be able to adapt toward the ecosystem effectively and able to compete with other organisms. This species experiences bioaccumulation which occurs due to the tendency of heavy metals to form complex compounds with organic substances contained in the body of green mussels so that heavy metals are fixed and are not immediately excreted (Arifin *et al.,* 2021).

Mussels (*Perna viridis*) are invasive species that give negative impacts to native species in an environment. They grow rapidly and become competitors for native species for food, space, and ecosystem modification. They also give negative influences for fishery business (Dawson *et al.,* 2022). In the Southeast Asia, they can be harvested as food sources, but elsewhere, they are considered into pest as their rapid growth can cause biofouling on ships, industrial plants, and they can also cause a blockage of pipes and channels (Venugopalan, 2018). However, the consumption level has decreased because of the bioaccumulation of poisoneous substances that can cause health problems for humans (McGuire and Stevely, 2015).

Types of mussels, including mussels (*Perna viridis*) are the efficient bioindicators of pollution for heavy metal pollution, because they are filter feeder and they also have high tolerance toward high ecological pressure (Phaksopa *et al.,* 2023). Mussels (*Perna viridis*) are one of the mussels (mollusca, bivalve class) that are able to survive and breed in the condition with high ecological pressure. Their ability in accumulating heavy metal in mussels can be used to get a description of the heavy metal pollution level in an environment where the mussels live (Maznah *et al.,* 2018). According to Nacua *et al.* (2019), *Perna viridis* is a relatively inexpensive food source, but has high nutritional value such as amino acids, vitamins (A, B1, B2, B3, B6, B12, and C) and is rich in macro and micro mineral content.

Chromium is a type of heavy metals that are generally accumulated in mussels. Through the food chain, Chromium (Cr) can be deposited in living creatures' body parts and they can be toxic in certain amount. The Chromium that is accumulated inside the human body clearly cause health disturbance because Chromium has negative impacts as carcinogen (a cause of cancer), teratogenic (inhibits fetal growth) and mutagen (Sharma *et al.,* 2021). According to Sari and Soeprobowati (2021), the content of the heavy metal Cr in coastal waters is higher compared to the mangrove area which is the habitat of *Perna viridis*. Based on Donia and Yaakub (2022) research, it turns out that the most metals that accumulate in the dry season are Zn, Cu, Cr, Pb, and Ni, while in the rainy season they are Zn, Cr, Cu, Pb, and Ni. Through this research it turns out that the heavy metal Cr between 2 seasons is very influential so further research is needed.

Based on the observation results, there are many mussels (*Perna viridis*) from the Tambak Lorok waters. Many people living around the area consumes and sell them. In regard to those following conditions, there should be research that is aimed to determine the heavy metal content of Cr in waters, sediment, and soft tissue of mussels, determine how much of heavy metal bioconcentration of Cr in mussels, and determine the tolerable intake of weekly consumption of mussels that are cultivated in Tambak Lorok waters.

MATERIALS AND METHODS

This research was conducted with sampling an interval for once in 2 weeks. Samples of water, sediment, and soft tissue of mussels were collected from three research stations can be seen in Figure 1, which are areas around Tanjung Mas Port, ponds where many fishermen catch / cultivate mussels, and around settlement and creek that are assumed receiving industrial and domestic waste. The heavy metal content of Cr in the samples of water, sediment, and mussels were analyzed in Laboratorium Balai Besar Teknologi Pencegahan Pencemaran Industri (Laboratory of Center for Industrial Pollution Prevention Technology) Semarang. The content of Cr in the water sample was measured with APHA 3111B-2012 method, the sediment content was measured with the method of US EPA SW-846/3050 B-1996 SM 3111B-2012, and the soft tissue of mussels was measured with SSA method. The water quality was measured using DO-meter (DO, pH, and temperature), refractometer (salinity), current meter (stream speed), and Secchi disk (depth).

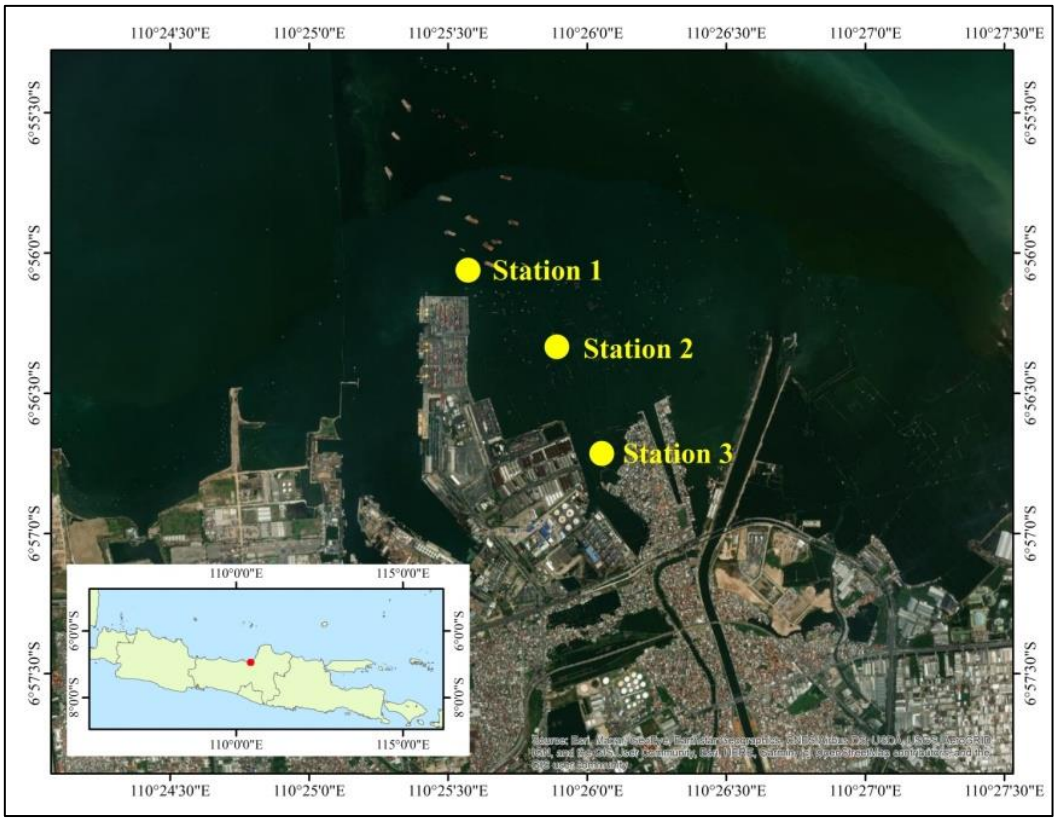

Figure 1. Research Location Map in Tambak Lorok Waters, Semarang

To determine the bioconcentration in mussel samples, the calculation of bioconcentration factors was conducted. Bioconcentration factors in animals tested were calculated with the formula (Van Esch, 1997 dalam Hidayah *et al.*, 2014) as follows :

BCF = C Biota / C Water.....(1)

Note: BCF = Bioconcentration Factor ; C Biota = Metal Concentration in Animals (mg/kg); C water = Metal Concentration in Water (mg/kg)

To determine the limited consumption of mussels that has been contaminated with heavy metal, can use the measurement of the Maximum Tolerable Intake. It is calculated based on the standards of the World Health International Food Institute Organization (WHO) and the Joint FAO/WHO Expert Committee on Food Additives (JEFCA), as follows:

MWI : BW x PTWI.....(2)

MTI : MWI / Ct.....(3)

Note: MWI = Maximum Weekly Intake (mg/week); BW = Weight (kg); PTWI = Provisional Tolerable Weekly Intake (mg/kg BW/week); MTI = Maximum Tolerable Intake (kg/week); Ct = Heavy Metal Concentration (mg/kg)

RESULT AND DISCUSSION

Based on the results of the research that was conducted in Tambak Lorok waters Semarang in three different station points, it is determined that the content of metal Cr in the water column shows results that are not varied, the average of 0.015 – 0.025 mg.L^{-1}. In the observation, the highest content

of Cr metal was in settlement and creek areas. They are assumed to receive industrial and domestic waste (Station 3) of 0.025 mg.L^{-1}. The lowest content of the Cr metal was in the Tanjung Mas Port area (Station 1), that was 0.015 mg.L^{-1}. The highest average of Cr metal content in sediment was in settlement and creek areas that are assumed to receive industrial and domestic waste (Station 3), that was 28.72 mg/kg. The lowest average was in the Tanjung Mas Port area (Station 1), that was 25.12 mg.kg^{-1}. In addition, based on research, the highest average of heavy metal Cr content in mussels (*Perna viridis*) was in Station 3 with 0.265 mg.kg^{-1}and the lowest one was in Station 1 with 0.155 mg.kg^{-1}(Table 1).

The content of heavy metal Cr in water column from three stations is still below the quality standard of sea water. It occurs because the heavy metal that enters the waters' area will experience dilution due to the influences of a tide, absorption, and absorption by aquatic organism (Bryan, 2011). If we pay close attention to those three stations, the creek area has a higher content of Cr that other locations. It is caused because the river streams brought the content of Cr that is from the creeks, such as waste from domestic waste containing Chromium, waste from industrial textil, and corrosion from water pipes containing Chromium metal. It indicates that the creeks have been more polluted by waste which contains Cr metal. Keshta *et al.* (2020), stated that industrial, domestic and agricultural activities around the waters will affect the amount of incoming pollutants which affect biodiversity of aquatic biota and plant in ecosystem.

Based on the observation, the condition of content value of heavy metal Cr in the sediment is much higher compared to those in the waters' column. It is presumed that there is a rapid process of sedimentation of heavy metal from anthropogenic factors industrial activities such as tanneries and textile that use Cr oxidant raw materials (Proshad *et al.,* 2019). Heavy metal in the water column will experience a compounding process with other substances; organic and inorganic materials. This will influence the process of sedimentation. It shows that sediment is a place for the heavy metal accumulation process around sea waters. It is in accordance with Jia *et al.* (2021) which stated that heavy metal concentration in sediment is much higher than those in the waters

Table 1. Heavy Metal Cr content in Water, Sediment, and Mussels (*Perna viridis*) Samples in Tambak Lorok Waters Semarang

Location	Heavy Metal Cr		Average	Quality Standard
	Repetition 1	Repetition II		
Station 1				
Water (mg.L^{-1})	< 0.01	0.02	0.015	0.005[a]
Sediment (mg.kg^{-1})	24.89	25.34	25.12	80[b]
Mussel (mg.kg^{-1})	0.16	0.15	0.155	0.005[c]
Station 2				
Water (mg.L^{-1})	0.02	0.02	0.02	0.005[a]
Sediment (mg.kg^{-1})	26.05	26.84	26.45	80[b]
Mussel (mg.kg^{-1})	0.15	0.41	0,28	0.005[c]
Station 3				
Water (mg.L^{-1})	0.02	0.03	0.025	0.005[a]
Sediment (mg.kg^{-1})	29.32	28.12	28.72	80[b]
Mussel (mg.kg^{-1})	0.18	0.35	0.265	0.005[c]

Note : a) PP No. 22 tahun 2021; b) ANZECC ISQG; c) a Decree of Minister of Environment Year 2004

The content of heavy metal Cr in mussels is higher if it is compared with the one in the waters. It is assumed as mussels (*Perna viridis*) is a filter feeder. They absorb their food by filtering the waters that enter inside their body so that heavy metal Cr compiled in the sediments will enter inside the mussels' body (*Perna viridis*) continuously and the heavy metal Cr will be accumulated inside their body. According to Siregar *et al.* (2020), metals inside biota's body influence by the concentration of metals in water. Darmono (2001) stated that the difference of mussels with other organisms is that mussels are able to accumulate metals than other aquatic animals. Mussels are settled so that they have slow influence towards pollution. In addition, they have high tolerance of heavy metal concentration. This fact is supported by Romero-Freire *et al*. (2020) that stated mussels cannot excrete metals well so they are accumulated continuously in the tissues by the increase of metals in the waters column. Mussels have slow mobility or immobilized and they cannot elicit metals. Based on research by Suprapto *et al.* (2021), the highest concentration of heavy metals in green mussel and in the water was found far away from industrial and human activities.

The content of Cr in the location of Tambak Lorok waters Semarang shows that the waters are contaminated by Cr metal since it is upper the quality standard applied by PP No. 22 tahun 2021. Furthermore, the content of Cr in sediment, that is collected from all points of research locations, does not exceed the quality standard that has been applied by ANZECC ISQG, where the quality standard for the content of Cr in sediment is 80 $mg.kg^{-1}$. the content of Cr in mussels from all points of research locations is between 0,155 – 0,265 $mg.kg^{-1}$. it has exceeded the quality standard applied by the Decree of the Minister of Environment Year 2004, where the quality standard of Cr in bivalve is 0,005 mg'kg.

The content of Cr in mussels between the three research stations showed different results, with the highest order from station 2 (0.28 $mg.kg^{-1}$), station 3 (0.265 $mg.kg^{-1}$), and station 1 (0.155 $mg.kg^{-1}$). Station 2 found a high content of heavy metal Cr in mussels due to high fishing activity and mussels cultivation waste at that location. then followed by station 3 around settlement and creek that are assumed to receive industrial and domestic waste. Industrial waste that contributes to the Cr content in mussels is the textile industry waste that is in the vicinity of the location. Station 1 (around Tanjung Mas Port) is farthest from the river which carries waste from human activities. The high content of Cr in mussels is greatly influenced by Cr in water, so it is necessary to carry out water management to reduce the impact on the aquatic ecosystem. Through good management, it is hoped that it can provide comparable benefits for human welfare. According to Sari and Wijaya (2019), water pollution control strategies can be carried out by reducing the pollution load, namely by supervising industrial waste management, managing domestic waste by involving the community, and the need for regular monitoring of river water quality and mapping potential pollutant sources at each location so that problems will be quickly resolved. Based on the measurement result of the bioconcentration factor on mussels, the average result is 10,35 – 14 that is in Table 2. It shows that the characteristic of pollutant in the research location is still considered low. This fact is supportednby Van Esch (1977) in Suyatno *et al.* (2021) that categorized the characteristics of pollutant into 3 levels, they are : high accumulative (BCF > 1000), medium accumulative (BCF 100 – 1000), and low accumulative (BCF < 100).

Table 2. The Measurement Result of Bioconcentration Factor

Location	BCF		Average
	Repetition 1	Repetition 2	
Station 1	16	7,5	11,75
Station 2	7,5	20,5	14
Station 3	9	11,7	10,35

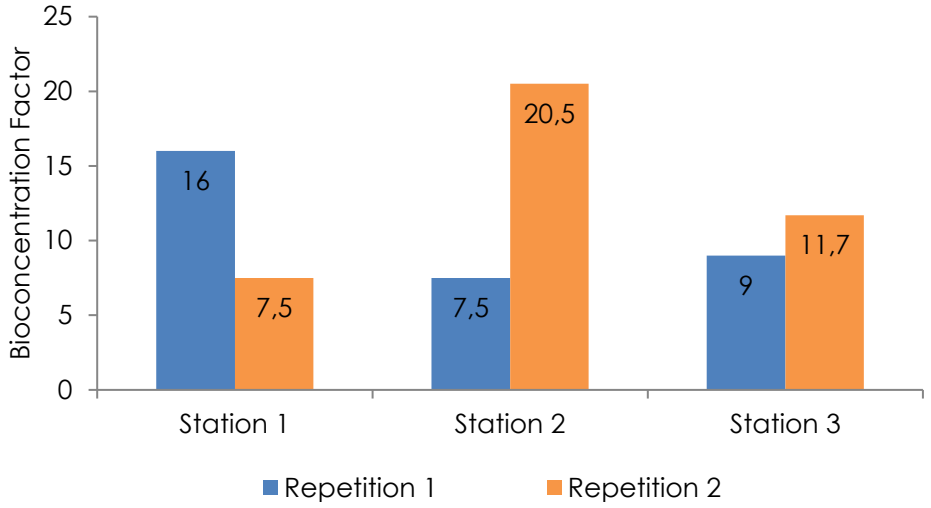

Figure 2. The Bioconcentration Factor of *P. viridis* from different station in Tambak Lorok Waters.

Based on the measurement result of maximum tolerable intake of mussels' consumption in the research location (Table 2), the result is 6,078 kg.week^{-1}.. Weight in calculating weekly intake, according to Barokah *et al.* (2019), it can be assumed that it is 60 kg and PTWI based on WHO is 0.0233 mg^{-1}.kg^{-1}.week.

Based on the criteria of WHO (1998), the content of Chromium in food that is permitted to consume is 0.5 ppm per week or 0.07 ppm per day. Thus, the content of Chromium in the blood of mussels (*Perna viridis*) exceeds the permitted threshold. If they are not consumed over 0.07 ppm perday, they will be harmless for consumers. When Chromium entering the human body exceeds the applied threshold, it will cause chronic toxicity. It is supported by Yin et *al.* (2021) who state that aquatic biota that is polluted by heavy metals when consumed by humans will cause health problems. The effect of heavy metal on human health depends on which part of the metal is attached to the body and how big the dosage of exposure. The research result of Palar (1994) shows that there was the accumulation of Chromium could cause damage to respiratory organ and also triggered the occurrence of cancer in humans. The experiment using marmot (*Cavia cobaya*) as a treatment with the dosage of 0,05 – 0,23 ppm for 45 days shows that Chromium is carcinogen. It can cause lung cancer. According to Sivaprakasam *et al.* (2022), the level of pollution in the waters is one of the causes of the number of heavy metals absorbed by biota. Overdose Effects from Consumption of biota containing Cr are toxic, ulceration, dermatitis, and allergic skin reactions.

Based on the result of Suseno's research (2007), the average can function as a bioindicator of Chromium pollution. It is based on its ability to accumulate by the value of concentration factor of 148.36 to 414.50, absorbing speed of 14.836 to 65.754 µg per day, releasing speed of Chromium of between 9.04 to 15.48% per day, and the remaining time in *Perna viridis*'s tissues to reaching half of the original concentration of 4.48 – 7.67 day. The results of the study by Mititelu *et al.* (2022), showed that bioconcentration factors varied based on the study area and the anatomical part of the shell. Based on the region, the content of heavy metals in shells was higher in samples collected from industrial areas (station 3) than from port (Station 1) and coastal areas (Station 2). Based on the measurement result of water quality parameter in the location, is presented in Table 3.

The heavy metal content of mussels is also influenced by the condition of the waters. Based on the measurement result of the quality parameter of Tambak Lorok waters, the temperature level in Station 3 are highest. The higher the temperature, the higher the level of heavy metal accumulation

Table 2. The Measurement Result of Maximum Tolerable Intake

Heavy Metal	BW (kg)	PTWI (mg.kg/ BW/week)	MWI (mg/week)	Ct (mg.kg^{-1})	MTI (kg/week)
Cr	60	0.0233	1.398	0.23	6.078

Note : The value of Ct is the average of heavy metal concentration in 3 stations

Table 3. The Parameter of Water Quality in Research Location

Parameter	Station 1			Station 2			Station 3		
	R 1	R 2	Average	R 1	R 2	Average	R 1	R 2	Average
DO (ppm)	5,2	4	4,6	3,8	4,3	4,05	4,7	3,6	4,15
pH	8	7,8	7,9	8	8	8	7,8	8	7,9
Temperature (°C)	28	30	29	28	30	29	30	31	30,5
Salinity (°/₀₀)	25	29	27	27	30	28,5	33	32	32,5
Stream Speed (m/s)	0,14	0,11	0,125	0,09	0,08	0,85	0,09	0,09	0,09
Depth (m)	6,7	6	6,35	5,2	4,7	4,95	4,8	4,5	4,65

temperature where tropical mussels can survive is 20 - 35°C, with the fluctuation that is nor more than 5°C. it shows that the average temperature in Tambak Lorok waters Semarang is still conducive for the growth of mussels (*Perna viridis*).

in sediment. According to Suprapti *et al.* (2016), the temperature's increase would result in the higher bioaccumulation level. That opinion was supported by Darmono (2001), stated that the higher the temperature in some waters, the higher the toxicity. This condition makes the content of Cr is more absorbable my mussels (*Perna viridis*) so that the content of Cr inside the mussels' body increases, and the lower the water temperature, the lower the toxicity. The increase in the water temperature will accelerate the reaction in forming heavy metal ions. According to Osei *et al.* (2021), the normal besides, the water temperature will also support the higher level of the Chromium solubility in the water. The measurement result in the field had noted that the temperature was around 29 – 30,5 °C. Dissolved oxygen level (DO) in the water are affected by temperature (Zhang *et al.,* 2019). The increase in the water temperature can decompose the mineral solubility level so that Cr solubility in the water is high (Ali *et al.,* 2022). Heavy metal solubility is also influences by the condition of DO in the waters.

Other influencing factors of mussels are pH and salinity. The fact was supported by Suprapti *et al.* (2016) who stated that the ability of *Perna viridis* to accumulate dissolved hexavalent Chromium in the waters depends on internal and external factors. In the internal factor, the accumulative capacity is the active site of the *Perna viridis* cell membrane which functions as a heavy metal bioligant bond, determined by the size of the animal. In the external factor, the condition of waters physical chemistry includes pH, salinity, particulate content, dissolved organic material content, and so on. This is confirmed by Pantea *et al.* (2020), that the concentration of heavy metals absorbed by mussels is influenced by the environmental conditions of the waters so mussels as a bioindicator of environmental pollution.

CONCLUSION

The content of heavy metal Cr in Tambak Lorok waters Semarang in the water column is between 0,015 – 0,025 mg.L^{-1} is indicated upper the quality standard. The content Cr in sediment it

is between 25,12 – 28,72 mg.kg^{-1} does not exceed the quality standard that has been applied by ANZECC ISQG. The content of Cr in mussels it is between 0,155 – 0,265 mg.kg^{-1} indicated upper the quality standard applied by the Decree of the Minister of Environment Year 2004. The bioconcentration factor of Cr in mussels in Tambak Lorok area Semarang is considered low, that is about 10,35 – 14. Based on the measurement of maximum tolerable intake of consuming mussels from research locations, it shows the result of 6,078 kg/week. It is considered safe to consume.

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