ABSTRACT

Green mussel (Perna viridis) is one of the species in the Bivalve class which filter the food particles from their surroundings. As filter feeder, it captured anything including plankton, detritus, and even heavy metals. Heavy metals content in green mussels could become dangerous due to the bioaccumulation process. This research aimed to determine the levels of heavy metals Cadmium (Cd) and Chromium (Cr) not only in the tissues of green mussels but also in the sediments of Sawojajar waters, Brebes; current study also determined the feasibility of green mussels for consumption in the perspective of food safety. The method used in this research was a field survey method which conducted in June 2022. The analysis method used AAS refers to the American Public Health Association (APHA) with laboratory tests conducted at the Productivity and Aquatic Environment Laboratory of IPB. The results showed that the content of heavy metal Cd in green mussel samples was at 0.19 mg kg⁻¹ which was meet the threshold concentration. Whereas, the heavy metal Cr in green mussels’ tissue exceeded the threshold of chromium concentration at 0.21 mg kg⁻¹. In addition, the heavy metal content of Cd and Cr in sediment were still meet the threshold concentration at 3.2 mg kg⁻¹ and 0.13 mg kg⁻¹ respectively. In terms of food safety, the EDI (Estimate Daily Intake) value based on the results of heavy metals Cd and Cr concentrations was 0.11 and 0.13 µg kg⁻¹ day⁻¹ respectively. The result indicated that these heavy metals concentration had low risk in causing health issue. In the same way, the target hazard value (THQ) of Cd and Cr were not exceeded the RfD (Reference Dose) at 0.109 and 0.036 respectively. These value of THQ for both Cd and Cr indicated low risk in causing cancer and the green mussels are meet the food safety standard.

Keywords: Green mussels, Accumulation, Cadmium, Chromium, Sawojajar Waters

ABSTRAK

Kerang hijau (Perna viridis) merupakan salah satu spesies di kelas Bivalvia yang memiliki karakter menyaring partikel makanan dari lingkungannya. Sebagai filter feeder, kerang hijau menyaring apa pun termasuk plankton, detritus bahkan logam berat. Kandungan logam berat pada kerang hijau dapat menjadi berbahaya akibat proses bioakumulasi. Penelitian ini bertujuan untuk mengetahui kadar logam berat kadmium (Cd) dan kromium (Cr) tidak hanya pada kerang hijau tetapi juga pada sedimen di perairan Sawojajar, Brebes. Penelitian ini juga bertujuan untuk menentukan nilai kelayakan kerang hijau untuk dikonsumsi dari sudut pandang keamanan pangan. Metode yang digunakan dalam penelitian ini adalah metode survei yang dilakukan pada bulan Juni 2022. Metode analisis logam berat yang digunakan adalah AAS mengacu pada American Public Health Association (APHA) dengan uji laboratorium dilakukan di Laboratorium Produktivitas dan Lingkungan Perairan IPB. Hasil penelitian menunjukkan kandungan logam berat Cd pada sampel kerang hijau sebesar 0,19 mg kg⁻¹ nilai tersebut memenuhi ambang batas level Cd pada biota. Sedangkan kandungan logam berat Cr pada jaringan kerang hijau melebihi ambang batas konsentrasi krom sebesar 0,21 mg kg⁻¹. Selain itu, kandungan logam berat Cd dan Cr dalam sedimen masih memenuhi ambang batas konsentrasi, masing-masing sebesar 3,2 mg kg⁻¹ dan 0,13 mg kg⁻¹. Dari
INTRODUCTION

Sawojajar waters is located in Wanasari District, Brebes Regency and rich of marine products, one of which is green mussels (*Perna viridis*). This species of mussels have high economic value and also have good nutritional value, hence, they are favored by the community (Mahasri et al., 2019). The number of mussels in a body of water is often used as an eco-sentinel organism, i.e., as the marine environment monitoring (Yaqin et al., 2015). As this species are filter feeder which able to filter particles in their surrounding water. This ability may cause green mussels to accumulate heavy metal content from waters or sediments (Maharani et al., 2019). Heavy metals can be accumulated through the food chain, namely the higher the level of the food chain of trophic organism, the accumulation of heavy metals in its body also increases. Heavy metals have properties that easily bind to organic matter and the ability to settle to the bottom of the water. This causes heavy metals to fuse with sediments so that heavy metal levels in sediments are higher than in water (Rahmawati et al., 2015). Some of heavy metals which are abundant at the coastal area are cadmium (Cd) and chromium (Cr). Heavy metals pollution that occurs in a body of water will certainly be dangerous if consumed and accumulated by marine biota found in these waters. Food safety standards for food consumption containing heavy metals can be calculated based on EDI (*Estimated Daily Intake*) and THQ (*Target Hazard Quotient*). Therefore, it is necessary to conduct research which determine the levels of heavy metals Cd and Cr in sediments and the tissues of green mussels (*Perna viridis*) at Sawojajar waters, Brebes. This information may become a consideration in determining the feasibility of green mussels (*Perna viridis*) at Brebes for consumption (Kusuma et al., 2022). Hence, this study aimed to determine the levels of heavy metals Cd and Cr in the sediment and the tissues of green mussels (*Perna viridis*) at Sawojajar waters, Brebes and to provide information related to their food safety level.

MATERIAL AND METHODS

1. Green mussels and Sediment Collection

The green mussel (*Perna viridis*) sample were collected from Sawojajar Waters in June 2022. The green shell samples taken were weighed and their length were measured. The size of individual of the collected mussels were 5-6 grams with 4-7 cm of shell length which were then put into labeled plastic zip lock bag and stored in a cool box. The total sample of green mussels were 145 individuals.

Sediment samples were collected through 3 repetitions at the location of green mussels' cultivation area. The process used to collect sediments is by using Ekman grab, by means of the Ekman grab being tied with a rope then the grab was dropped from above the surface of the water until the grab fell and touched to the bottom of water, the Ekman grab was pulled up to the top, then the sediment was put in a Ziplock plastic bag. The sample required is around 50 grams in each repetition.

2. Heavy Metals Analysis in Green Mussels and Sediment

Analysis of Cd and Cr metals were carried out at the Productivity and Environment Laboratory, Bogor Agricultural University. Green mussels tissue samples were dissected and separated from the mussels, the tissue then dried in 60 °C oven for 24-48 h. The dried tissue of mussels was ground prior the heavy metals analysis.
Approximately 5 g of the dry green mussels tissue was weighed in a porcelain cup. The sample was added 1 mL of 65% HNO₃, then evaporated on a hot plate at 100°C until dry. After drying, it was put into the furnace and the temperature was raised gradually from 100°C every 30 minutes until it reached 450°C and maintained for 3 hours. The sample was cooled to room temperature then added 5 mL of 6 M HCl and then added 10 mL of 0.1 M HNO₃ and cooled at room temperature for 1 hour. The sample was transferred to a 50 ml polypropylene measuring flask and matrix modifier solution was added, adjusted to the mark with HNO₃0.1 M, then measured with AAS with a resonance wave of 228.72 nm (Noviansyah et al., 2021).

The heavy metals Cd and Cr content were measured by spectrophotometry using the APHA 3030 E, 3111B (2017) method. The sediment samples obtained were dried in an oven at temperature of 105°C, then the sediment was ground and sieved. Samples were weighed as much as 5 g then transferred to a 250 mL Erlenmeyer. Then the sample was added 5 mL of HNO₃ and 50 mL of distilled water. The sample was then destructed in a heat mantle to obtain a clear solution up to 10 mL in volume. Samples were filtered with Whatman No. filter paper. 41 and diluted with aquabides up to 50 mL. The levels of Cd and Cr metals in the samples were measured using an Atomic Absorption Spectrophotometer at a resonance wave of 228.72 nm (Noviansyah et al., 2021).

3. Estimation of EDI (Estimate Daily Intake) and THQ (Total Hazard Quotient)

**EDI (Estimate daily Intake)**

In green mussels where the concentrations of Cd and Cr are known, then the maximum limit values for food ingredients can be calculated. Accumulation values of heavy metals in mussel soft tissue were used to calculate estimated daily intake (EDI). The EDI value can be calculated according to the USEPA equation (2011).

\[
EDI = \frac{C \left( \frac{WAB}{7} \right) \times FIR (g)}{WAB (kg)}
\]

C = concentration of heavy metals; FIR = shellfish consumption rate assuming fish consumption (34.7 g/person/day (BPS 2018); WAB = average body weight for Indonesian adults 58 kg (Permenkes 2019).

**Target Hazard Value (THQ)**

The target hazard value (THQ) is the ratio of the potential exposure to a substance and the level, at which no adverse effects are expected. The THQ value is better known as a useful parameter for assessing human health risks for heavy metals contained in shellfish (Rayyan et al., 2019). If the calculated THQ value is less than 1.0, then there are no adverse health effects as a result of exposure to heavy metals. Conversely, if the THQ value is more than 1.0, then adverse health effects may occur. Based on (Ritonga et al., 2018) THQ values can be calculated using the equation according to (USEPA, 2011).

\[
THQ = \frac{EF \times Ed \times C \times FIR \times RfD \times WAB \times ATn}{RfD \times WAB \times ATn} \times 10^{-3}
\]

EF = frequency exposure to heavy metals (350 days/year) based on exposure to settlements (Ministry of Health 2012); Ed = duration of non-carcinogenic heavy metal exposure in Indonesia (30 years) (Ministry of Health 2012); RfD= is a reference dose for consumption of heavy metals per individual (1 μ.kg⁻¹.day⁻¹) for heavy metals Cd and Cr (USEPA 2011); ATn = average exposure time for non-carcinogenic (365 days.year⁻¹ x 30 years) (Ministry of Health, 2012).

4. Data analysis

The heavy metals concentration in the sediment and the tissue of green mussels were illustrated by using table. In the same way, the calculation of food safety characteristics (i.e., EDI and THQ) were illustrated by using table. All of the findings at present study were compared with quality standards and other references to draw the conclusions.
RESULTS AND DISCUSSION

1. Heavy Metals Concentration of Cadmium (Cd) and Chromium (Cr) in Green Mussels and Sediments

The results of the analysis of the heavy metal content of Cadmium (Cd) and Chromium (Cr) in green mussels (Perna viridis) and sediments in Sawojajar Waters, Brebes Regency are presented in Table 1 below.

The analysis of the heavy metal content in green mussels at Sawojajar Waters revealed that cadmium metal concentration was 0.19±0.02 mg.L⁻¹ and chromium metal concentration was 0.21±0.04 mg.L⁻¹ (Table 1). The results of the data showed that heavy metal Cd meets the predetermined quality standard of 1 mg.kg⁻¹ (SNI 7387, 2009) concerning the Maximum Limit of Heavy Metal Contamination in Food, whereas for heavy metal Cr exceeds the quality standard of 0.005 mg.kg⁻¹. This result indicated that the heavy metal Cd concentration in green mussels was still safe for consumption, while the heavy metal Cr was considered dangerous for consumption. However, the low levels of Cd concentration in the mussels were required to be monitored, remembering its impact on human health, if the seafood product is consumed (Arifin et al., 2021), because of the bioaccumulation effect.

The measurement of Cd concentration in sediments of Sawojajar waters was 3.20±0.04 mg.kg⁻¹ and Cr concentration was 0.13±0.05 mg.kg⁻¹. These results indicated that heavy metals Cd concentration in sediments of Sawojajar waters exceed the quality standard (ANZECC, 2000) which was 1.5 mg.L⁻¹. As for the results of heavy metal Cr concentrations in sediments still meet the quality standards (NOAAA, 1999) which was 52.3 mg. L⁻¹. Heavy metals that settle on the bottom of water column will form sedimentation. The source of heavy metals in sediments was usually from human activities or natural sources, hence, it will be distributed on sediment particles. Sedimentation which contained heavy metals could be due to the transfer of dissolved metals from water bodies into sediments. This may also be influenced by organic material on the sediment surface that will bind the heavy metals. Heavy metals in the body of water will accumulate more in sediments due to the deposition process (Maslukah, 1999). Green mussel filtered plankton (Sanjayasari, 2021) and other particles from the water, thus allowing the dissolved heavy metals to enter its body and may have the impact on the ecological imbalance of waters and the survival of biota that live in it (Liliandari & Aunurohim, 2013). On the other hand, the results of the analysis of heavy metal content of Chromium (Cr) in sediments of Sawojajar Waters was still below the maximum threshold < 52.3 mg.kg⁻¹ (NOAA, 1999). According to Palar (1994), heavy metal content which dissolved in water column at certain concentrations could potentially become source of poison and may be harmful for aquatic life. The heavy metals Cr have properties which easily bind to organic matter and tend to settle to the bottom of the waters and then merge with sediments, hence, if the waters have a high concentration of heavy metal chromium can also increase its accumulation in sediments (Wahyu et al., 2017). The concentration of heavy metal Cr in sediments of Sawojajar waters was still safe because it was below the threshold, but this requires to be monitored in order to maintain and minimize the heavy metal contamination.

Table 1. Mean concentrations of Cd and Cr Heavy Metals in Green Mussels and Sediments

<table>
<thead>
<tr>
<th>Type of metals</th>
<th>Green Mussels</th>
<th>Sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Values</td>
<td>Quality Standard</td>
</tr>
<tr>
<td>Cd</td>
<td>0.19±0.02</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Cr</td>
<td>0.21±0.04</td>
<td>0.005 (3)</td>
</tr>
</tbody>
</table>

(1) SNI 7387:2009 concerning Maximum Limits of Heavy Metal Contamination in Food
(2) ANZECC Year 2000
(3) PP RI No. 22 Year 2021
(4) NOAA (National Oceanic and Atmospheric Administration) 1999
The phenomena of the high concentration of heavy metals Cd in the sediment had the opposite value with the Cd concentration in mussels tissue and the high concentration of heavy metals Cr in the green mussels tissue, while the concentration in the sediment was still below the standard required an explanation. Some of possible reasons could be related to the mussels size which was used in this study and also the heavy metal characteristics. The size of green mussel which used in current study were above > 8 cm, at this range of size the mussels was considered in to adult or marketable size. The adult size of mussels is considered to have better physiological ability in rejecting toxicants such as heavy metals to the water during the filtration process compared to the smaller size of mussels (i.e., larvae or juvenile size) (Gosling, 2008). Whilst, for heavy metal Cr had higher concentration in the mussels tissue could be due to the characteristic of this metal was easily bind with other organic matters. The low value of the standard on heavy metals Cr (0.005 mg.kg\(^{-1}\)) could be caused by the toxic effect of the metals. However, since heavy metals Cr is widely distributed (i.e., soil, dust, gas, stone, animals and plant), therefore the standard level in the sediment is high (52.3 mg.kg\(^{-1}\)). Current finding was in line with previous study which showed the higher concentration of Cr was found in the sediment compared to mussels tissue (\textit{P. viridis}) in Mojosari waters. It explained that the distribution of the heavy metals was highly related to gravity wave action upwelling and other physical characteristics (Juharna et al., 2022).

2. EDI (Estimate Daily Intake) and THQ (Total Hazard Quotient) of Green Mussels

EDI (Estimated Daily Intake) is an estimate of the amount of heavy metals level which enter the human body through shellfish consumption per day. Whereas, the Target Hazard Quotient (THQ) value is used to determine the level of risk in humans that may be exposed by a hazard due to consuming food which contain heavy metals (Table 2).

The results of EDI calculation indicated that the heavy metals Cd and Cr obtained were 0.11 and 0.13 μg.kg\(^{-1}\).day\(^{-1}\) respectively (Table 2). The results in current study revealed that the calculation was below the RfD standard for heavy metals Cd which was 1 μg.kg\(^{-1}\) (USEPA, 2011). In the same way, the calculation of EDI for heavy metals Cr was also less than the RfD standard which was 3 μg.kg\(^{-1}\) (USEPA, 2011). If the ratio of heavy metal EDI to reference dose RfD is less than or equal to RfD, then the health risk will be minimum, if the ratio is > 1-5 RfD then the health risk is low, if the ratio is > 5-10 RfD, then the health risk will be moderate, but if ratio> 10 RfD, then the health risk will be high. Therefore, it could be seen that the calculation of EDI for both heavy metals Cd and Cr were < RfD, or could be categorized as minimum health risk. The results obtained are based on estimation, if people consume green mussel meat contaminated with heavy metals Cd and Cr in Sawojajar Waters, Brebes Regency based on the average amount of shellfish consumption in Indonesia with an average adult body weight, the health risk is low. However, if consumed in excessive amounts, these heavy metals may potentially affect human’s health.

Based on the results of the calculation of the target hazard value (THQ), the value of the Cd range is between 0.109 and the value of the Cr range is 0.036. This indicates that the THQ value for heavy metals in green mussels is less than 1, hence, based on the average consumption of mussel meat in Indonesia assuming consumption of green mussel meat exposed to heavy metals for 30 years. This shows that green mussels meat is still suitable for consumption and less effects on the health of human who consume it. This is in accordance with the statement of (Fathur et al., 2019), if the THQ value < 1 then the daily

<table>
<thead>
<tr>
<th>Green Mussels</th>
<th>Units</th>
<th>Cd</th>
<th>Cr</th>
<th>Quality Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDI</td>
<td>μg.kg(^{-1}).day(^{-1})</td>
<td>0.11±0.02</td>
<td>0.13±0.03</td>
<td>-</td>
</tr>
<tr>
<td>THQ</td>
<td>-</td>
<td>0.109±0.02</td>
<td>0.036±0.02</td>
<td>1</td>
</tr>
</tbody>
</table>
exposure value has not passed the RfD, thus, it indicated people who consume seafood contaminated with these two heavy metals presented in the study may be potentially to experience high non-carcinogenic health impacts. As non-carcinogenic heavy metal, cadmium may accumulate in the kidneys, liver, and some of it will be excreted through the digestive system. These heavy metals will affect smooth muscle either directly or indirectly through the kidneys. This will result in an increase in blood pressure in the body. Other impacts are damage to the brain, endocrine system, heart damage to cause death (Woei, 2016). Cadmium in humans will be excreted by the human body after 20-30 years. Apart from cadmium, the heavy metal chromium (Cr) also has a negative impact if consumed in excessive amount and may cause the damage in the kidneys and liver. One of these toxic effects of the chromium is being able to cause respiratory system disorders such as lungs function disorders in humans (BPOM No 14, 2021).

The calculation of EDI and THQ values are directly proportional to the heavy metal content. The higher the heavy metal content, the higher the EDI and THQ values. Previous studies showed that the more severe the marine pollution due to the accumulation of heavy metals, the more serious the risk to human health (Satriawan et al., 2021)

CONCLUSIONS

Based on the results obtained, it can be concluded that the heavy metal Cd in green mussels was still meets the established quality standards which was <1 mg.kg⁻¹, while the heavy metal content of Cr has exceeded the quality standards set. Meanwhile, the content of heavy metal Cd in the sediment of the waters exceeded the quality standards, while the heavy metal Cr it still met the quality standards that had been set. The standard of feasibility consumption for human’s based on the value of EDI and THQ on heavy metals Cd and Cr of green mussels in Sawojajar Waters is classified safe or low risk to human’s health.

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