Available online: journal.ipb.ac.id/index.php/jphpi http://dx.doi.org/10.17844/jphpi.v25i3.39813

MAXIMUM TOLERABLE INTAKE OF MANGROVE OYSTER (*Crassostrea rhizophorae*) FROM THE TAPAK RIVER, SEMARANG CITY, INDONESIA, WHICH CONTAINS Cd AND Pb METALS

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Diterima: 4 Februari 2022/Disetujui: 11 November 2022
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Cara sitasi (APA Style 7th): Haeruddin, Ayuningrum, D., & Oktaviani, J. (2022). Maximum tolerable intake of mangrove oyster (*Crassostrea rhizophorae*) from the tapak river, Semarang city, Indonesia, which contains Cd and Pb metals. *Jurnal Pengolahan Hasil Perikanan Indonesia,* 25(3), 418-427. http://dx.doi.org/10.17844/jphpi.v25i3.39813

Abstrak

Mangrove oysters are a type of oyster typical for human consumption in Semarang City. The Tapak River in Semarang is a popular fishing ground for mangrove oysters, but it is also contaminated with cadmium (Cd) and lead (Pb) metals. This research was determined to analyze the concentration of Cd and Pb metals in mangrove oyster meat from the Tapak River and determine the maximum tolerable intake for humans. Oysters were collected from 3 stations with 3 replicates per station. Metal concentrations in mangrove oyster meat were analyzed with a wet digestion method followed by atomic absorption spectrophotometry. Most of the meat samples contained Cd exceeding standard concentrations for shellfish and all meat samples contained Pb exceeding the standard concentrations for shellfish. The maximum tolerable intake of Cd and Pb for human adults is 0.954±0.431 mg week^{-1} and 0.164±0.014 mg week^{-1} respectively.

Keyword: cadmium, lead, mangrove oyster, MTI, tapak river

Asupan Maksimum Tiram Bakau (*Crassostrea rhizophorae*) yang Dapat Ditoleransi dari Sungai Tapak, Kota Semarang, Indonesia, yang Mengandung Logam Cd dan Pb

Abstract

Tiram bakau merupakan salah satu jenis tiram yang dikonsumsi masyarakat Kota Semarang. Salah satu lokasi penangkapan adalah Kali Tapak Semarang, yang telah tercemar dengan logam Cd dan Pb. Penelitian ini dilakukan untuk menganalisis konsentrasi logam Cd dan Pb dalam daging tiram bakau dan menetapkan MTI. Tiram dikumpulkan dari tiga stasiun dengan tiga kali ulangan setiap stasiun. Konsentrasi logam dalam daging tiram bakau dianalisis dengan ASS, setelah didestruksi basah. Hasil penelitian menunjukkan bahwa sebagian besar sampel daging yang dikumpulkan mengandung logam Cd yang melampaui standar kandungan Cd dalam kekerangan. Semua contoh daging yang dikoleksi mengandung logam Pb yang melampaui standar kandungan Pb dalam kekerangan. MTI Cd dan Pb utuk dikonsumsi oang dewasa berkisar 0,954±0,431 mg minggu^{-1} untuk Cd dan 0,164±0,014 mg minggu^{-1} untuk Pb.

Kata kunci: kadmium, MTI, timbel, tiram bakau, sungai tapak

INTRODUCTION

Oysters are bivalves discovered in the 14th century. Mangrove oysters belong to the Ostreidae family, the true oysters. This family of oysters is edible and mainly belongs to the genera Ostrea, Crassostrea, Ostreola, Magallana, and Saccostrea (Hautmann *et al*., 2017). The origin of the true oyster is not known (Márquez-Aliaga, 2005). Oysters from the genus *Crassostrea* are particularly popular for consumption by the world community (Duncan 2003; Pedrouso-José, 2020), including Indonesians. Although cultivation is possible (Duncan, 2003; Pedrouso-José, 2020), in Indonesia, oysters are mostly obtained from the wild (Food and Agriculture Organization [FAO], 1996) and especially from rocks, roots, or mangrove trunks to which they attach themselves. *Crassostrea rhizophorae* is a species of bivalves belonging to the Ostreidae family often found living attached to the roots and trunks of mangroves (iNaturalist, 2022).

In 2017, the world oyster production was approximately 5.9 million tons with harvest production being 97.5 % of the total production. Oysters are considered the fourth most important seafood group globally, by volume of production, after cyprinid fish, seaweed, and clams (FAO, 2020). According to data from Indonesia Oysters Market Insights published by Selinawamucii (2022), in 2019 Indonesia shipped 135 tonnes of oysters, a significant decrease from 2017, which reached 1500 tons.

Oyster meat contains essential nutrients such as protein, vitamins, minerals, and omega-3 fatty acids. Oyster meat contains a lot of vitamins B12, zinc, and copper (Kubala, 2022). Each 100-gram oyster meat dish contains nutrients (Food Data Central United States Department of Agriculture [FDC-USDA] 2019; Kubala, 2022): 8.87 g proteins, 12.9 μg Vitamin B 12 (538% of the Daily Value (DV), 61 mg Zn (555% of the DV), and 4.44 mg Cu (493% of the DV).

Protein accounts for about 8 % of shellfish body weight (Muchtadi, 2008; Abdullah, 2017). Oyster meat is a source of omega-3 fatty acids, which play an important role in the body to overcome inflammation, support liver, and brain health, and prevent 2 types of diabetes (Krupa *et al*., 2022; Shahidi, 2018). Each 100 g oyster meat dish contains 0.82 g of total PUFA (FDC-USDA, 2019). Vitamin B12 is necessary for the maintenance of the nervous system, metabolism, and the formation of blood cells. Elderly humans usually experience vitamin B12 deficiency (Wong, 2015). Zinc plays an important role in maintaining immunity (Fukada, 2015; Skrajnowska & Bobrowska-Korczak, 2019) and cell growth, especially in cellular metabolism. Zn is needed in supporting the catalytic activity of approximately 100 types of enzymes (Trumbo *et al.,* 2001; Zastrow *et al.* 2014). Taurine is also quite abundant in shellfish (Sokolowski *et al*., 2003). Taurine is an important functional nutrient for the human body and plays a role in several physiological processes, namely central nervous neuromodulation, energy production, protection against antioxidants, and immunomodulation in nerve cells (Oktawia *et al*., 2010). The iron contained in oyster meat is needed by the body for the formation of hemoglobin and myoglobin, the transport of oxygen, and the production of cell energy (Moustarah & Mohiuddin, 2021). Selenium is necessary for maintaining thyroid function and metabolism (Nessel & Gupta, 2021). The iron and selenium content of 100 g of cooked oyster meat (FDC-USDA, 2019; Kubala, 2022) is 7.16 mg (40% of the DV) and 30.7 micrograms (56% of the DV) respectively.

However, oysters are sometimes harvested from aquatic environments polluted by waste such as pesticides and heavy metals (Budiastuti *et al*., 2016). If consumed in large enough amounts, this can cause serious health problems such as cancer (American Public Health Association [APHA], 1992). Therefore, this research analyzed the cadmium (Cd) and lead (Pb) concentrations in oyster meat harvested from the Tapak River, Tugu District, Semarang City. The Tapak River found in Semarang City is surrounded by industries resulting in high levels of water pollution by the river's liquid waste (Marjanto, 2005; Br. Sinurat, 2021). This study was conducted to determine the concentration of Cd and Pb metals in mangrove oyster meat and set limits on the safe consumption of oyster meat, which contains Cd and Pb metals, for health using the Maximum Tolerable Intake (MTI) approach.

MATERIALS AND METHODS

Material and Tools

The materials used in the study were meat/soft tissue of mangrove oysters, machetes for prying oysters from mangrove roots or trunks, clean water and aquadest for cleaning and rinsing oyster meat, nitric acid (HNO_3) 65%, hydrogen peroxide (H_2O_2), Beaker Glass 500 mL, electric heater, magnetic stirrer, 50 mL measuring flask, 10 mL pipettes, sample bottles for oyster meat containers, Whatman filter paper size 180 µm, cuvettes and Atomic Absorption Spectrophotometer (AAS) Shimadzu AA-6200 for analysis of Cd and Pb content in oyster meat.

Methods

Mangrove oysters were collected from mangrove roots or trunks from three different locations representing the upstream, middle, and estuaries of the Tapak River (Figure 1), by gouging using machetes. At each sampling location collected three samples of meat were from different mangrove trees.

Meat that had been separated from the shell was cleaned using clean water and rinsed with aquadest (Figure 2). After that, the meat was included in an example bottle to analyze the Cd and Pb content in the meat using AAS Shimadzu AA-6200. Preparation of oyster meat samples to be analyzed with AAS was carried out with wet destruction, using a mixture of 65% HNO_3 and 30% H_2O_2 (Wulandari & Sukesi, 2013). 5 g of meat sample was put into a 100 mL Beaker Glass, then

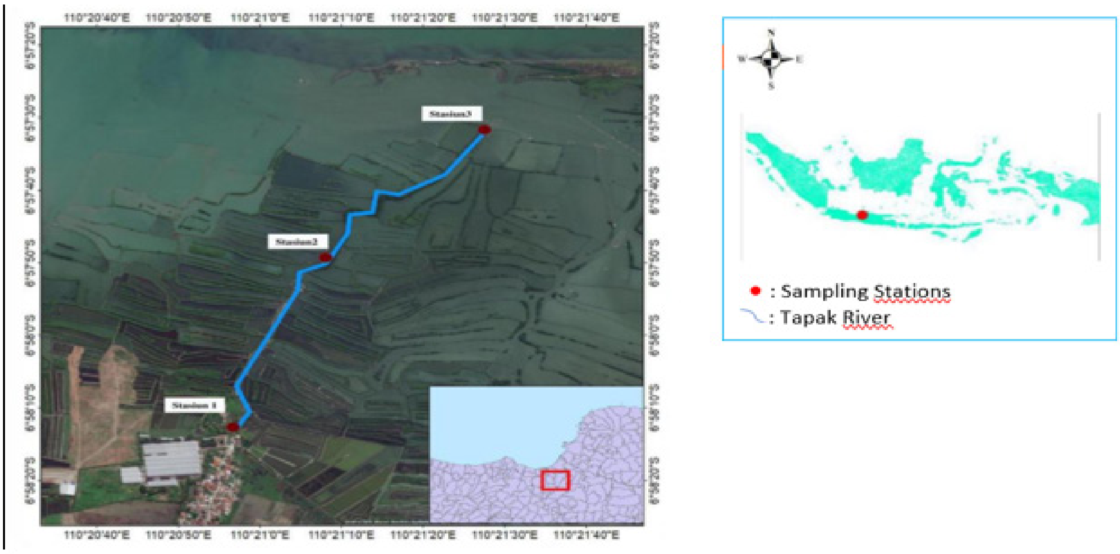

Figure 1 Sampling station locations.

Figure 2 Mangrove oysters after being separated from mangrove roots

added HNO_3 as much as 30 mL gradually until the sample dissolves. Stirring was done with a magnetic stirrer. Then the solution was cooled for 15 minutes, then added H_2O_2 as much as 10 mL until the solution becomes clear. Next heat slowly until it reached a temperature of 100°C until the solution is clear. Once clear, the solution was cooled, then transferred into a 50 mL measuring flask and diluted using 1% HNO_3. Next, the solution was filtered using Whatman filter paper 180 µm. The solution was then transferred to the cuvette for analysis of the metal content in the meat with AAS according to SNI 7387:2009 for Pb and Cd with wavelengths for Cd 228.8 nm and 283.3 nm for Pb.

The threshold for health risks from consuming oysters containing Pb and Cd was expressed as the MTI. According to Hidayah *et al.* (2014), the formula to calculate the MTI was as follows:

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

where:
MWI: the maximum weekly intake (g);
Ct: heavy metal content in the soft tissues of mangrove oyster (mg kg^{-1})

The MWI was determined with the following formula (Cahyani *et al.*, 2016):

$$MTI(g) = Bodyweight \times PTWI$$

where:
body weight equals 50 kg for Indonesian adults and 15 kg for children;
PTWI: the provisional tolerable weekly intake for metal Cd and Pb of 25 µg kg^{-1} body weight $week^{-1}$ (INS 2009: EFSA 2011).

Data Analysis

The concentration of Cd and Pb in the soft tissue of mangrove oysters was compared between stations, to find out whether there was a difference in the concentration of the two metals between stations. Comparisons were also made for the MTI values obtained. Comparison was carried out with a one-way analysis of variance using MINITAB. Before the analysis of variance, a data normality test was carried out, to determine whether the data spreads normally, so that it was qualified using parametric test statistics. If the data did not spread normally, a variance analysis was performed with parametric test statistics.

RESULTS AND DISCUSSIONS

The metal concentrations of Cd and Pb in oyster meat that were obtained through the AAS analysis are shown in Figures 2 and 3.

The concentration of Cd in oyster soft tissue ranged from 0.68 to 1.83 mg kg^{-1}. With an average range of 0.77±0.31 mg kg^{-1} (station 2) to 1.47±0.53 mg kg^{-1} (station 1). The lowest Cd

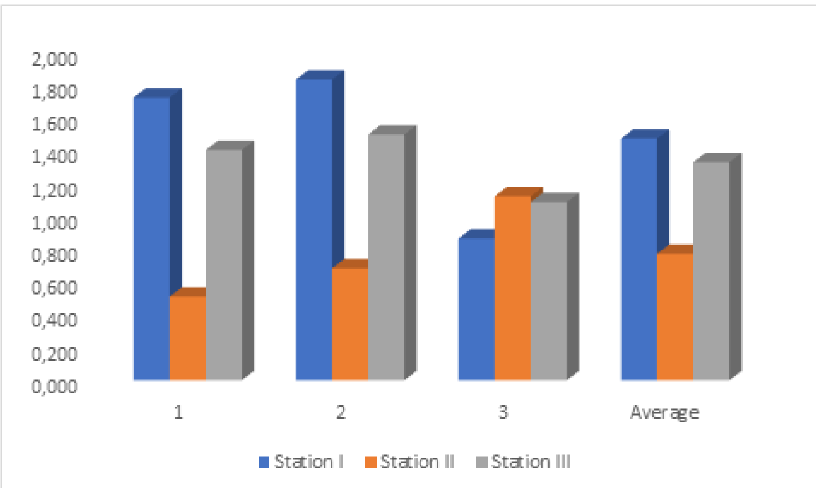

Figure 2 The concentrations of Cd (mg kg^{-1}) in oyster soft tissue

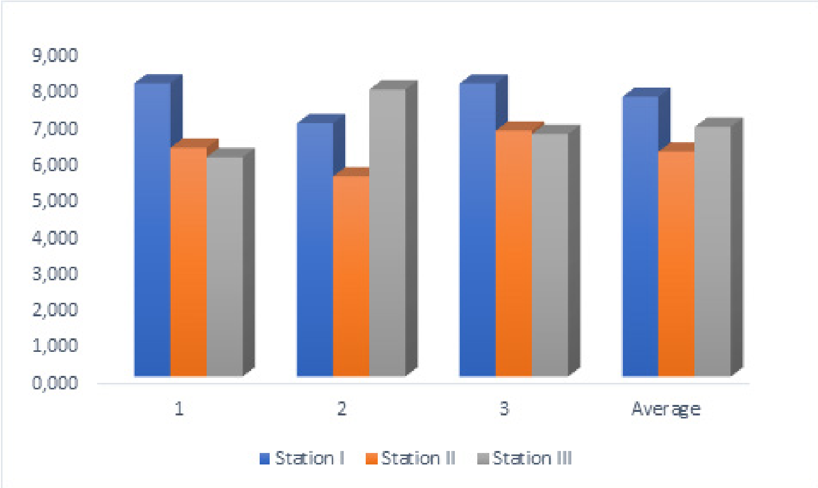

Figure 3 The concentrations of Pb (mg kg^{-1}) in oyster soft tissue.

concentrations were found in station 2 during session 1, and the highest at station 1 during session 2. The highest Cd concentrations were found at station 1 and the lowest at station 2. Analysis of variance (ANOVA) showed that Cd concentrations in mangrove oyster meat between stations did not differ markedly.

The concentration of Pb in oyster soft tissue ranged from 5.47 to 8.02 mg kg^{-1}. The highest Pb concentrations were found in station 1 during session 1, and the lowest at station 2 during session 2. The lowest Pb concentrations were found at station 2 (6.15±0.63 mg kg^{-1}) and the highest at station 1 (7.65±0.63 mg kg^{-1}). ANOVA showed that Pb concentrations in mangrove oyster meat between stations did not differ markedly.

The MWI and MTI of oyster meat from Tapak River were determined based on the concentration of Cd and Pb in the soft tissue of the oysters. The results are presented in Tables 1 and 2.

Table 1 shows that the lowest MTI for Cd metal-contaminated oyster meat is 0.95±0.43 mg week^{-1} and the highest MTI is 1.80±0.67 mg week^{-1}. The results of ANOVA showed that the MTI Cd values in adults between stations did not differ markedly.

Table 1 MWI and MTI of Cd metal-contaminated oyster meat for Indonesian adults

Station	Test	Concentration Cd (mg kg^{-1})	PTWI (µg kg^{-1})	MWI (µg week^{-1})	MTI (mg week^{-1})	Maximum limit of Cd in meat (mg Cd kg^{-1})*
I	Average	1.47	25	1.250	0.95	1.0
	SD	0.53	0		0.43	
II	Average	0.77	25	0	1.80	
	SD	0.31	0	1.250	0.67	
III	Average	1.33	25	0	0.96	
	SD	0.22	0	1.250	0.17	
Lowest MTI					0.95±0.43	
Highest MTI					1.80±0.67	

Note: * Based on Indonesian National Standard (SNI) No. 7387:2009

Table 2 MWI and MTI of Pb metal-contaminated oyster meat for Indonesian adults

Station	Test	Concentration Pb (mg kg^{-1})	PTWI (μg kg^{-1})	MWI (μg week^{-1})	MTI (mg week^{-1})	Maximum limit of Pb in meat (mg Pb kg^{-1})*
I	Average	7.65	25	1.250	0.16	1.5
	SD	0.63	0		0.01	
II	Average	6.15	25	0	0.20	
	SD	0.63	0	1.250	0.02	
III	Average	6.82	25	0	0.19	
	SD	0.94	0	1.250	0.02	
Lowest MTI					0.16±0.01	
Highest MTI					0.20±0.02	

Note: * Based on Indonesian National Standard (SNI) No. 7387:2009

Table 2 showed that the lowest MTI for Pb metal-contaminated oyster meat is 0.16±0.01 mg week^{-1} and the highest MTI is 0.20±0.02 mg week^{-1}. The results of the analysis of variance (ANOVA) showed that the MTI Pb value in adults between stations did not differ markedly.

The maximum consumption of mussel meat per week was calculated based on the MTI and the assumed body weight of the average adult weighing 50 kg (Cahyani *et al*., 2016) and the PTWI for Cd and Pb of 25 g kg^{-1} body weight week^{-1} (INS 2009: EFSA 2011). Results based on MTI calculations for adults assumed a bodyweight of 50 kg. The MTI value for Cd is 0.95–1.80 kg week^{-1} and for Pb is 0.16–0.20 kg week^{-1}.

The concentration of Cd contained in oyster meat caught in Tapak River, most of them have exceeded the limit of Cd metal concentration allowed by the Indonesian National Standard (INS) No. 7387:2009 stipulates that bivalves, mollusks, and sea cucumbers for human consumption should not exceed 1 mg Cd kg-1 and 1.5 mg Pb kg^{-1}. The provisions of the Food and Drug Supervisory Agency of the Republic of Indonesia (FDSA-RI) through the Regulation of the Head of the Food and Drug Supervisory Agency of the Republic of Indonesia Number HK.00.06.1.52.4011 concerning the Determination of Maximum Limits of Microbial and Chemical Contamination in Food stipulates the maximum limits for Cd and Pb metals in meat. Shellfish (bivalves) and processed sea cucumbers should not exceed 1 mg Cd kg^{-1} meat and 1.5 mg Pb kg^{-1} meat. FAO (2003) established that the metal content of Cd and Pb in bivalve and molluscs meat is a maximum of 1 mg/kg.

Pb is absorbed into our blood, bones, teeth, and soft tissues (liver, kidneys, lungs, brain, spleen, muscles, and heart (ATSDR, 2021). The results of the study Latorre *et al*. (2002) supports the hypothesis that release of bone lead stores increases during menopause and constitutes an internal source of exposure possibly associated with health effects in women in menopause transition. Post-menopausal women have higher blood-Pb levels than pre-menopausal women (Potula & Kaye, 2006).

Itai-Itai disease in humans is caused by the consumption of rice contaminated with Cd (Arakelyan, 2021). Cd-metal intake from food ranges from 8 to 25 g per day (Egan *et al*., 2007; Larsen *et al*., 2002; Llobet *et al*., 2003; Olsson *et al*., 2002; Ysart *et al*., 2000) but in Japan, the intake of Cd metal is higher (Nordberg *et al*., 2007). Cd is efficiently stored in the human body, where it accumulates throughout life. Cd is mainly toxic to the kidneys, especially the proximal tubular cells, the main site of accumulation. Cd can also cause bone demineralization, either through direct bone damage or indirectly as a result of renal dysfunction. In the industry in the Tapak River area, excessive exposure to Cd can impair lung function and increase the risk of lung cancer. The study by Bernard (2008), demonstrated a consistent relationship between various renal and bone biomarkers and urinary Cd excretion used to assess body Cd weights. Cd-induced renal damage is characterized by dysfunction of proximal tubular reabsorption. The earliest manifestation of tubular toxicity is increased excretion of low molecular weight proteins (LMW), such as 2-microglobulin (β2-M) and 1-microglobulin (α1-M), also called HC protein, and retinol-binding protein (RBP). Urinary excretion of cytolytic markers such as the lysosomal enzyme N-acetyl-glucosaminidase (NAG) is also increased (Jarup & Akesson, 2009).

Reciprocal exposure from time to time may make a person feel: stomach ache, forgetfulness, depression, annoyance, irritability, and nausea/sickness. It also increases the risk for high blood pressure, heart disease, kidney disease, and decreased fertility(CDC-NIOSH 2021). The American Department of Health and Human Services (DHHS), Environmental Protection Agency (EPA), and the International Agency for Research on Cancer (IARC) have determined that lead can cause cancer in humans (ATSDR 2020).

The adverse effects of Cd and Pb on health require that oysters contaminated with Cd and Pb are consumed with caution, and must comply with the MTI of these metals. This study showed that the MTI for Cd is 0.52–2.47 mg week^{-1} and 0.15–0.22 mg week^{-1} for Pb. These values are lower than the MTI Cd and Pb in green shellfish (*Perna viridis*) originating from aquaculture crops in the waters of Semarang Bay (Haeruddin *et al*., 2020) a safe consumption limit for human health for blood clams from Tambak Mulyo and Bedono. Shellfish contaminated with Cd from Tambak Mulyo had an MTI of 0.001 g week^{-1} for adults and 0,003 g week^{-1} for children, and shellfish from Bedono had an MTI of 0.02 g week^{-1} for adults and 0.06 g week^{-1} for children. Shellfish contaminated with Pb from Tambak Mulyo had an MTI of 0.001 g week^{-1} for adults and 0.0004 g week^{-1} for children. Mussels from Bedono had an MTI limit of 0.003 g week^{-1} for adults and 0.001 g week^{-1} for children.

The MTI of elements found in meat, aquatic biota, and other organisms for human consumption, is determined by several factors, namely the bodyweight of the consumer, the PTWI standard, and the concentration of the element (in this case metals). This study and the research of Haeruddin *et al.*, (2021) assumed the same average adult weight, namely 50 kg, and the same PTWI based on INS (2009) and EFSA (2011), which set PTWI for Cd and Pb at 25 g kg^{-1} of bodyweight. The Cd and Pb concentrations in oyster meat from Tapak River were higher than that in green mussels from the waters around Tambak Mulyo and Bedono, Semarang Bay, so the MTI of green mussels was higher than the MTI of oysters in this study. Likewise in this study, the concentration of Pb in oyster meat was higher than that of Cd, so the MTI of Cd was higher than the MTI of Pb.

CONCLUSION

Oysters caught in the Tapak River, Semarang City, contain Cd and Pb metals. However, they are still safe for consumption as long as the MTI of oysters of 0.95±0.43 mg week^{-1} for Cd and 0.16 0.01 mg week^{-1} for Pb is maintained.

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