Metals Cd-Pb-Cu-Zn in Green Mussel and Health Risk Analysis in Semarang

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Heavy Metals (Cd, Pb, Cu, Zn) in Green Mussel (*Perna viridis*) and Health Risk Analysis on Residents of Semarang Coastal Waters, Central Java, Indonesia

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Abstract: Increasing environmental metal concentrations are usually attributed to the impact of urbanisation. This study emphasises on the metal contamination in green mussel (*Perna viridis*) from the coastal urban area. The field survey was carried out to evaluate the concentration of metals, i.e., Cd, Pb, Cu and Zn in green mussel captured from Semarang coastal waters, Central Java, Indonesia. Green mussels are the important species that are consumed by the local people as a source of animal protein. Therefore, keeping the mussels away from a wide range of contaminants, including heavy metals, has become an essential factor for people's health. The result of the study demonstrated that concentrations of Cd, Pb, Cu and Zn in soft tissues of *P. viridis* at all stations ranged from 0.013 to 0.042 mg/kg (average from 0.022±0.007 to 0.033±0.005 mg/kg) for Cd, 0.324–2.765 mg/kg (average from 0.406±0.059 to 2.268±0.293 mg/kg) for Pb, 0.621–2.760 mg/kg (average from 1.094±0.353 to 2.294±0.274 mg/kg) for Cu, and 7.886–31.115 mg/kg (average from 10.722±1.781 to 23.434±5.271 mg/kg) for Zn. Health risk analysis through the calculation of the HQ and HI index demonstrated that all the metal-contaminated green mussels had deleterious health risks to children living in the Semarang coastal areas. The HQ value <1 occurred in adults who consumed Cd-, Cu- and Zn-contaminated green mussels. A particular case occurred in Pb-contaminated green mussels, which showed the HQ values > 1 in the all over study sites threatening health risks to the children and adult inhabitants. The HI values > 1, which indicated that consumption of *P. viridis* at all study areas has a high health risk. Risk management efforts must be taken by reducing the rate of *P. viridis* consumption until the safe limit and decreasing metals concentration incorporated in green mussels with the depuration method as a reasonable way to protect people's health from heavy metals toxicity.

Keyword: Green mussel, *Perna viridis*, heavy metal, pollution, Hazard Quotient, Hazard Index.

Introduction

The North coast of Java is a very dense demographic region, resulting from the development of various intensive activities such as industries, agriculture, fisheries, transportation and settlements. Semarang city is in the middle of the north coast of Java, and administratively is the capital of Central Java. This situation is putting significant pressure on environmental changes and pollution. One of the critical areas of global concern is the increasing levels of pollution in urban areas, and it is one of the significant pressures on coastal and marine environments, especially heavy metal pollution (UNEP, 2017). Heavy metal waste requires

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special attention, given its characteristics cannot be degraded by microorganisms; therefore, its availability in the environment can harm marine flora and fauna.

Seafood products (such as mussels) contaminated by heavy metals and consumed by humans are a transfer of of heavy metals from the lower food chain towards the higher one. The present study enhances the knowledge of the metal content of Cd, Pb, Cu and Zn in *P. viridis* captured from Semarang coastal waters. Some authors indicated that heavy metal contaminants contained in marine mollusks are often used to assess the quality of seawaters. Study of metal residues in bivalves at several places has been realised, i.e., *Mytilus galloprovincialis* (Seguin et al., 2016); *Mytilus edulis* (Liu and Wang, 2015); *Perna viridis* (Liu and Wang, 2015); *Pecten maximus* (Saavedra et al., 2008).

In Indonesia, some studies of heavy metals have been performed along the northern coast of Java, i.e., heavy metals content in fish from north coast of Java (Wulandari et al., 2009), Hg in edible fish collected from Gresik coastal waters, Indonesia (Soegianto et al., 2010), heavy metals (Cd, Pb, Cu, Zn) residues in bivalves captured from northern coast of Central Java (Yulianto et al., 2019). Heavy metals (Cd, Pb, Zn, Hg, Cu, and Cr) are also reported in the blood mussels, *Anadara granosa* (Soegianto et al., 2020). These studies showed an increase in bivalve's contaminated with heavy metals on the northern coast of Java.

Considering green mussels is a source of protein and widely consumed by Indonesian people, are bought and sold in abundant and low prices; therefore, it is necessary to monitor green mussels contaminated with heavy metals and health risk assessments in the interest of people's health in Semarang coastal areas.

Materials and Methods

Sampling Location

The sampling location was at Semarang coastal waters spreading in 5 stations (Figure 1).

Mussel Samples

Green mussels (*Perna viridis* Linnaeus 1758) were collected from five stations, from December 2016 until December 2017. Green mussels were caught directly by hand, as they can stick to the hard material. Samples were kept in a coolbox at a temperature retained at about 4°C before being transported to the laboratory. The number of mussel samples for each station was between 100 and 250 mussels. The sample stations and its coordinate are shown in Figure 1 and Table 1.

Metals Analyses

Samples of the soft tissues were analysed for heavy metal content such as Cd, Pb, Cu and Zn. Solid samples (soft tissues) were washed, dried and mashed with

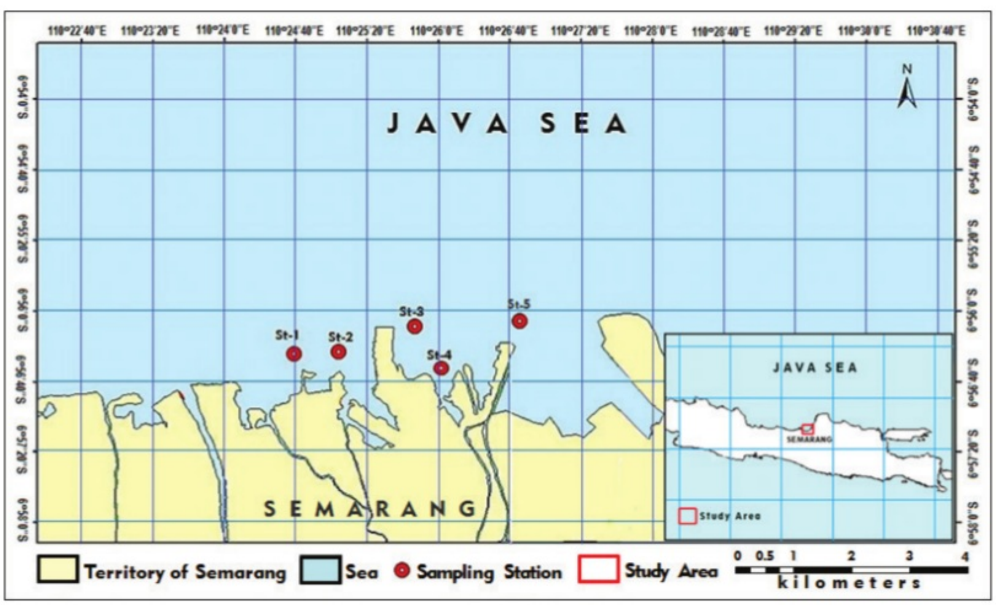

Figure 1: Research stations at Semarang coastal waters, Central Java, Indonesia.

Table 1: Concentration of metals (Cd, Pb, Cu, Zn) (range and average±SD) in soft tissue of Green Mussel (*Perna viridis*) captured from Port Tanjung Emas Semarang (PTES) and Outlet of Indonesia Power Plant Semarang (OIPPS). Values are representation from the average of metal content from two stations of PTES and three stations of OIPPS

Location	*Metal*	*Sampling Date*	*Range (mg/kg)*	*Metal in P. viridis (mg/kg)*	*MAL*
Port of Tanjung Emas Semarang (PTES)	Cd	November 2017	0.025-0.039	0.0334±0.0052	0.10 mg/kg[1]
St-1: - 6°56′24.6″; 110°24′35.8″		December 2017	0.027-0.038	0.0311±0.0041	1.0 mg/kg[2]
St-2: - 6°56′28.9″; 110°24′58.2″	Cu	December 2016	0.893-1.788	1.398±0.383	20 mg/kg[3]
		February 2017	1.194-2.760	2.294±0.274	
	Pb	December 2016	1.924-2.670	2.278±0.150	0.20 mg/kg[1]
		February 2017	1.878-2.765	2.268±0.293	1.50 mg/kg[2]
	Zn	December 2016	7.886-12.878	10.722±1.781	100 mg/kg[1]
		February 2017	11.415-15.342	14.388±1.964	
Outlet of Indonesia Power Plant Semarang (IPPS)	Cd	November 2017	0.014-0.042	0.02501±0.0099	0.10 mg/kg[1]
St-3: - 6°56′21.2″; 110°25′39.4″		December 2017	0.013-0.034	0.0215±0.0069	1.0 mg/kg[2]
St-4: - 6°56′29.5″; 110°26′10.5″	Pb	December 2016	0.324-0.534	0.406±0.059	0.20 mg/kg[1]
St-5: - 6°56′14.5″; 110°26′44.8″		February 2017	0.537-0.759	0.603±0.066	1.50 mg/kg[2]
	Cu	December 2016	0.621-1.744	1.094±0.353	20 mg/kg[3]
		February 2017	1.635-2.631	2.007±0.269	
	Zn	December 2016	12.976-22.638	17.778±4.271	100 mg/kg[1]
		February 2017	14.851-31.115	23.434±5.271	

1. The Indonesia National Agency of Drug and Food Control (BPOM) No 23/2017
2. Indonesia National Standard (SNI) No 7387/2009
3. The Indonesia National Agency of Drug and Food Control No: 03725/B/SK/VII/1989

mortar. Then the results were sifted with a mesh size of 100 mm and homogenised. The result was weighed as much as 0.5 g and put into the Teflon bomb digester, moistened with splashes of aquatrides and 1 ml of concentrated HNO_3 was added to it. The Teflon bomb digester was sealed and put into the furnace and heated at 150° C for 4 hours. Then, the sample was poured into a beaker and heated with the addition of aquatrides repeatedly. Once cold, the results were put into a 10 ml volumetric flask, and the sample was ready for metal analysis by using atomic absorption spectrophotometer (AAS).

Environmental Health Risk Analysis

To analyse the health risk of habitant of Semarang coastal waters concerning green mussel consumption, interview was conducted of 400 people in the study area using an opinion poll. The questionnaire contained inquiries about daily intake, frequency of exposure, age, body weight and resident's ways of consuming mussels.

Health risk analysis was performed by application formulas from USEPA to obtain Chronic Daily Intake (CDI), Hazard Quotient (HQ), and Hazard Index (HI) value. Non-carcinogenic health risks caused by intake of Cd, Pb, Cu and Zn through the consumption of green mussels are assessed based on HQ value. The methodology for determining HQ is described in detail by US EPA (2005). HQ is the ratio of the potential intake of a toxicant and the level at which no adverse effects are expected and calculated using formula (1).

$$HQ = \frac{CID}{RfD} \quad (1)$$

HQ < 1 indicates that no adverse health effects are expected as a result of exposure. HQ > 1 indicates the potential for adverse effects or non-carcinogenic health risk increases. Reference Dose (RfD) is an estimate of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (US EPA-IRIS, 1993). RfD for metals in food: Cd = 0.001, Cu = 0.04, Zn = 0.30 (US EPA-IRIS, 2015), and Pb = 0.035 (Song et al., 2015); CDI is the amount of a chemical a person can be exposed to on a daily basis over an extended period (usually a lifetime) without suffering deleterious effects (mg/kg/day) (US EPA-IRIS, 1993), calculated using equation (2) based on US EPA (1989, 1991):

$$\text{CDI} = \frac{C \times IR \times Ef \times ED}{Bw \times AT} \quad (2)$$

C is heavy metal concentration (mg/kg). IR = intake rate or the number of mussels consumed per day (mg/day). Ef = exposure frequency, the number of days the respondent consumes mussels in one year (days/year). ED = exposure duration or duration of the respondent is consuming mussels (30 years for an adult and 6 years for children). BW is body weight (kg), and AT is the average time (30 years × 365 days/year for non-carcinogenic effects) (days) (Ministry of Health of the Republic of Indonesia, 2012). HI is the sum of two or more hazard quotients for multiple substances and/or multiple exposure pathways (caused by Cd, Pb, Cu, and Zn) was calculated using equation (3):

$$HI = HQ_{CD} + HQ_{Pb} + HQ_{Cu} + HQ_{Zn} \quad (3)$$

HI > 1 is indicating the need to perform risk management by reducing daily intake. From equation (1) and (2), equation (4) can be derived to calculate a Safe Daily Intake.

$$\text{Safe Daily Intake} = \frac{Red \times BW \times AT}{C \times Ef \times ED} \quad (4)$$

Results and Discussion

The Concentration of Cd, Pb, Cu, Zn in the Soft Tissue of Green Mussel *P. viridis* in Semarang Coastal Waters

The average values of Cd, Cu and Zn in green mussels were still lower than the maximum acceptable limit (MAL) set by the Indonesian authorities (The Indonesia National Agency of Drug and Food Control No 03725/B/SK/VII/1989; The Indonesia National Agency of Drug and Food Control No 23/2017; and Indonesia National Standard - SNI No 7387/2009). Except for Pb content in green mussels at all locations indicated exceeding MAL (Table 1). High Pb concentrations are evident in the association of high levels with urban areas near cities with many human activities like industry, transportation, and there is a strong statistical correlation between human population density and Pb concentrations in oysters and mussels (NOAA, 1998). PTES has a higher Pb in seawater because it is a passenger/trading port. Ship fuel contains Pb as an anti-knocking agent (Parekh et al., 2002). Fuel combustion residues are suspected of penetrating and polluting marine seawater.

Health Risk Analysis and Safe Consumption of Green Mussel

The daily intake rate of mussels was 8.90 mg/day (for children) and 7.30 mg/day (for adults). The average body weight of children was 31.13 kg and 59.40 kg for adults. Ef to mussels was 53 days/year (for children) and 28 days/year (for adults). Furthermore, risk criteria can be evaluated based on the HQ index.

The HQ values for consumption Cd-, Pb-, Cu- and Zn-contaminated green mussels were > 1 for children at all study locations (Table 2), which means the non-carcinogenic health risks are threatening. Whereas, the adults will not be at threatened health risks (HQ < 1) due to intake of Cd, Cu, and Zn through green mussel consumption.

A different evident arose for Pb intake through consumption of green mussels, HQ values> 1 in all study locations, where both for children and adults will experience some form of dangerous health effects (Table 2). Similar results occur in a health risk analysis due to Pb intake through the consumption of green mussels in Jakarta Bay, where the HQ value > 1 for all study stations ranges from 9,104 to 12,929 (for children) and 1,951 to 2,771 (for adults) (Simbolon, 2018). Soegianto et al. (2020) found an HQ index > 1 for metals (Cd, Pb, Zn, Hg, Cu, and Cr) contained in blood mussels from East Java coastal waters, Indonesia, which indicated the causes for non-carcinogenic threats in consumers.

The present study demonstrated that health risks were more significant in children caused by the high consumption rate of heavy metals-contaminated green mussels. Their HQ showed values > 1 for all metals and locations. Significant health risks in children due to metals exposure via green mussels consumption should be focussed upon as children are more sensitive to toxicants than adults. Compared to adults, immune systems in children are less developed and feeding intake of children is more onerous than an adult with respect to per kilogram of body weight. If contaminated food (harmful substances) is consumed by children, it would lead to intake of larger toxicants (Hill, 1997).

HI > 1 indicated that consumption of *P. viridis* by Semarang coastal residents led to high health risk (Table 3). It was necessary to perform risk management for all study sites. Risk management effort may be realised by reducing the consumption rate of *P. viridis* until the safe limit using equation (4). By calculation, the safe consumption rate of *P. viridis* for children and adults was obtained, i.e., 0.3294 and 1.1897 g/day, respectively (for residents living in PTES), and 1.244 and 4.4944 g/day (for residents living in OIPPS) (Table 2). Depuration technology is needed to reduce the levels of heavy metals in mussels before being marketed in order to protect public health.

Table 2: CDI (mg/kg BW/day), HQ, safe concentration in mussel (mg/kg), and safe consumption (g/day) of green mussel *Perna viridis* contain metals (Cd, Cu, Pb, and Zn) for people in Semarang coastal waters, Central Java, Indonesia

Metal	*Location*	*Group*	*CDI (mg/kg/day)*	*RfD*	*HQ*	*Safe concentration (mg/kg)*	*Safe consumption (g/day)*
Cd	PTES	Children	0.00028	0.0010	1.3866	0.0241	6.4187
		Adult	0.00031	0.0010	0.3149	0.1061	23.1833
	OIPPS	Children	0.00021	0.0010	1.0378	0.0241	8.5754
		Adult	0.00024	0.0010	0.2357	0.1061	30.9729
Pb	PTES	Children	0.01891	0.0035	27.0196	0.0843	0.3294
		Adult	0.02148	0.0035	6.1360	0.3713	1.1897
	OIPPS	Children	0.00501	0.0035	7.1523	0.0843	1.2444
		Adult	0.00568	0.0035	1.6242	0.3713	4.4944
Cu	PTES	Children	0.01905	0.0400	2.3808	0.9635	3.7382
		Adult	0.02163	0.0400	0.5407	4.2429	13.5017
	OIPPS	Children	0.01666	0.0400	2.0830	0.9635	4.2728
		Adult	0.01892	0.0400	0.4730	4.2429	15.4324
Zn	PTES	Children	0.11946	0.3000	1.9910	7.2265	4.4701
		Adult	0.13564	0.3000	0.4521	31.8214	16.1452
	OIPPS	Children	0.19457	0.3000	3.2428	7.2265	2.7445
		Adult	0.22093	0.3000	0.7364	31.8214	9.9128

Table 3: HI value = Total HQ value from all metals

Location		*HQ-Pb*	*HQ-Cu*	*HQ-Zn*	*HQ-Cd*	*HI*
PTES (Port Tanjung Emas Semarang)	Children	5.40	0.48	0.40	0.28	6.56
	Adult	6.14	0.54	0.45	0.31	7.44
OIPPS (Outlet Indonesia Power Plant Semarang)	Children	1.43	0.42	0.65	0.21	2.70
	Adult	1.62	0.47	0.74	0.24	3.07

Conclusion

The green mussels' *P. viridis* captured from Semarang coastal waters were contaminated with heavy metals (Cd, Pb, Cu, Zn) in their soft tissues. Their concentrations have not exceeded the maximum acceptable limits (MAL) yet, except for Pb, they have surpassed the value of MAL.

Health risk analysis showed that the HQ values after consumption of all metals (Cd, Pb, Cu and Zn) contaminated green mussels were > 1 in children, which means that these metals are potentially hazardous to children's health. However, it does not threaten the adult's health since the HQ < 1. Exclusion occurred in Pb content in green mussels; the HQ > 1 for all study areas, which means children and adults, will be vulnerable to deleterious health risk.

The HI values for all metals > 1 indicated that consumption of *P. viridis* in all study areas has a high health risk. It is necessary to carry out risk management efforts by reducing the rate of *P. viridis* consumption until the safety measures are adapted.

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