KORESPONDENSI PAPER C1 dan C2

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Korespondensi untuk artikel publikasi C1 dan C2

Judul:

Artikel ke-1 (C1): "Health Risk Analysis of Cd, Pb, and Hg in Blood Mussel (*Anadara granosa*) on Wedung Demak Residents "

Artikel ke-2 (C2): "Heavy Metals (Cd, Pb, Cu, Zn) in Green Mussel (*Perna viridis*) and Health Risk Analysis on Residents of Semarang Coastal Waters, Central Java, Indonesia"

Jurnal : ***Asian Journal of Water and Environmental Pollution (AJWEP)***

No	Aktivitas	Tanggal	Keterangan	Halaman
1	Komunikasi lewat Email Corresponding Author (Prof. Agoes Soegianto) dengan Editor (Mr. Raj D. Mirchandani)	2 Februari 2020	Penjelasan pengiriman (submit) manuscript secara kolektif ke Jurnal AJWEP	4
2	Attachment 1 dari komunikasi email no. 1	2 Februari 2020	List of selected papers from Icobiodiv for publication in Asian Journal of Water, Environment and Pollution 2020	5
3	Attachment 1 (lanjutan): Manuscript pada daftar di List No 05 (Artikel C1)	2 Februari 2020	Artikel C1: "Health Risk Analysis of Cd, Pb, and Hg in Blood Mussel (*Anadara granosa*) on Wedung Demak Residents "	7
4	Attachment 1 (lanjutan): Manuscript pada daftar di List No 13 (Artikel C2)	2 Februari 2020	"Heavy Metals (Cd, Pb, Cu, Zn) in Green Mussel (*Perna viridis*) and Health Risk Analysis on Residents of Semarang Coastal Waters, Central Java, Indonesia"	13
5	Komunikasi lewat Email dari Editor ke Corresponding Author	3 Februari 2020	Mengenai konfirmasi penerimaan 15 manuscript oleh Editor dan permintaan dari Editor untuk koreksi format artikel menjadi 1 kolom dan 2 spasi	19
6	Komunikasi lewat Email Corresponding Author dengan Editor	3 Februari 2020	Mengenai pengiriman kembali manuscript yang telah direvisi formatnya menjadi 1 kolom dengan 2 spasi	20

7	Komunikasi lewat Email dari Editor ke Corresponding Author	4 Februari 2020	Jawaban Editor atas pengiriman manuscript yang telah dibuat format 1 kolom 2 spasi oleh Corresponding author	20
8	Attachment 4:	3 Februari 2020	Artikel No 5 (C1) direvisi menjadi format 1 (satu) kolom dan 2 (dua) spasi	21
9	Attachment 5:	3 Februari 2020	Artikel No 13 (C2) direvisi menjadi format 1 (satu) kolom dan 2 (dua) spasi	33
10	Komunikasi Corresponding author (Prof. Agoes Soegianto) dengan Author (Dr. Bambang Yulianto)	6 April 2020	Mengenai koreksi manuscript dari reviewer AJWEP disampaikan kepada Author (Dr. Bambang Yulianto) oleh Corresponding Author (Prof. Agoes Soegianto)	46
11	Komunikasi Email dari Author kepada Corresponding author	6 April 2020	Jawaban Author (Dr. Bambang Yulianto) kepada Corresponding author mengenai kesiapan merevisi koreksi dari reviewer pada manuscript secepat mungkin	47
			Hasil Review Manuscript No. 05 (Artikel C1) dari reviewer AJWEP	48
			Hasil Review Manuscript No. 13 (Artikel C2) dari reviewer AJWEP	59
12	Komunikasi lewat Whatsapp dari Author kepada Corresponding author	8 April 2020	Komunikasi lewat Whatsapp dari Author kepada Corresponding author mengenai pengiriman kembali manuscript/artikel ke-1 (C1) dan artikel ke-2 (C2) yang telah direvisi	72
			Corresponding author telah menjawab Author dengan akan segera memproses ke Editor	72
			Hasil Revisi manuscript no 05 (Artikel C1)	73
			Hasil Revisi manuscript no 13 (Artikel C2)	84
13	Komunikasi Corresponding author dengan Editor AJWEP	4 Juni 2020	Update informasi tentang kapan menerima proof artikel	97
14	Komunikasi Corresponding author dengan Editor AJWEP	5 Juni 2020	Jawaban Editor atas pertanyaan kapan proof diterima Corresponding author? Manuscript sedang diambil untuk diterbitkan dalam edisi Juli 2020. Proof Paper akan dikirimkan kepada Anda sekitar pertengahan Juni.	97
15	Komunikasi email Editor ke Corresponding author :	5 Juni 2020	Ucapan terima kasih atas update informasi dari Editor	98

16	Komunikasi email Editor ke Corresponding author :	9 Juli 2020	Komunikasi Editor ke Corresponding author : update informasi dari Editor mengenai tahapan copy-editing dari artikel untuk dipublikasikan pada issue sekarang dari jurnal AJWEP	99
			Hasil Copy-editing dan Final Review dari Editor untuk Artikel C1	100
			Hasil Copy-editing dan Final Review dari Editor untuk Artikel C2	106
17	Komunikasi lewat WA dari Corresponding author ke Author	25 Juli 2020	Komunikasi Corresponding Author dengan Author mengenai informasi proof final artikel di AJWEP	114
			Proof Final Artikel C1	115
			Proof Final Artikel C2	121

1. Komunikasi lewat Email Corresponding Author (Prof. Agoes Soegianto) dengan Editor

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From: **Agoes Soegianto** <agoes_soegianto@fst.unair.ac.id>
Date: Sun, Feb 2, 2020 at 9:07 AM
Subject: Selected papers from ICOBIODIV for publication in AJWEP
To: Capital Books <capitalb@capital-publishing.com>

Dear Mr. Raj D Mirchandani,
I send these papers again through my other email address since my email has not been received by your email.
Please find attached the selected papers from Icobiodiv to be published in AJWEP.
Please contact me if you have any inquiries.
Thank you very much for your kind cooperation.

Best regards,

Prof. Agoes Soegianto
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2. Attachment 1: adalah Daftar Judul Artikel-artikel Dikirim untuk Publikasi di Asian Journal of Water, Environment and Pollution 2020

List of selected papers from Icobiodiv for publication in Asian Journal of Water, Environment and Pollution 2020

No	Title	Author
1	The Feasibility of Algae Treatment Treating Fecal Sludge Wastewater at Surabaya, Indonesia	Aulia Ulfah Farahdiba, Euis Nurul Hidayah, Djuni Wulan Zara, and Nguyen Thi Thuy Linh
2	Effect of salinity on osmoregulation and histopatology in gills of tilapia (Oreochromis niloticus)	Kiki Syaputri Handayani, Agoes Soegianto and Ching-Fong Chang
3	Effect of Mercury on Growth of Several Microalgae	Sulastri Arsad, Siti Nur Kholifah, Estuningdyah Prabawati, Luthfiana Aprilianita Sari, Miftahul Khair Kadim, and Yuni Kilawati
4	Evaluation of Radioactivity in Surabaya Coastal Estuary Ecosystem with Spectrometry αβϒ	Siswanto, Agus Taftazani, and Dedy Prasetyo
5	Health Risk Analysis of Cd, Pb, and Hg in Blood Mussel (Anadara granosa) from Demak, Central Java, Indonesia	Bambang Yulianto, Wahyu Andre Wijaya, Wilis Ari Setyati, Sunaryo, Adi Santosa and Trisnadi W. C. Putranto
6	Visualization of the Microbial Community and Elemental Mapping of Anadara Granosa Media Used in a Slow Sand Filter Using a SEM-EDS	Ni'matuzahroh, Nurina Fitriani, Eddy Setiadi Soedjono, Eko Prasetyo Kuncoro, Radin Maya Saphira Radin Mohamed, and Timothy Tjahja Nugraha O'Marga
7	Consortium of Marsilea crenata and Ludwigia adscendens for Linear Alkylbenzene Sulfonate Detergent Phytoremediator	F. Rachmadiarti, M.T. Asri, A. Bashri, Yuliani, and I. A. Pratiwi
8	Microalgae Skeletonema costatum for Cd and Cu Remediation	Dwi Candra Pratiwi, Niken Pratiwi, Defri Yona, Respati Dwi Sasmita and Intan Ayu Pratiwi
9	Effects of Cd, Zn and Cd+Zn Combination on osmoregulation of Tilapia (Oreochromis niloticus)	Trisnadi Widyaleksono Catur Putranto, Dewi Shinta, Mochammad Affandi and Agoes Soegianto
10	Ability of Mangrove Fungi in Biodegradation of Hexadecane	Nengah Dwianita Kuswytasari , Riva Ariny Elhaque, Alfia R Kurniawati, Nur Hidayatul Alami, Enny Zulaika ,Maya Shovitri, Ni Nyoman Tri Puspaningsih and Ni'matuzahroh

11	Supplement Feed to Recovery Sperm Quality and Total Bacteria of Freshwater Fish after Acute Cadmium Exposure	Alfiah Hayati, Farah Annisa Nurbani, Meirizka Amira, Windy Seftiarini1, Aken Puti Wanguyun, and Bayyinatul Muchtaromah
12	Effect of Media on Constructed Wetlands Performance with Equisetum hyemale	Febri Eko Wahyudianto, Muhammad Fauzul Imron, Nur Indradewi Oktavitri, Salsabilla Choirun Nisa' AlFikry, Lintang Tubagus Rahmatullah and Danar Arifka Rahman
13	Heavy Metals (Cd, Pb, Cu, Zn) in Green Mussel (Perna viridis) and Health Risk Analysis on Residents of Semarang Coastal Waters, Central Java, Indonesia	Bambang Yulianto, Ocky Karna Radjasa, Agoes Soegianto
14	Characterization and Lipase Production of Micrococcus Sp. L69 Isolated from Palm Oil-Contaminated Soil	Sri Sumarsih, Fatimah, Sofijan Hadi, Ragil Tri Adhiningsih and Fakhrudin Eka Prasetyo
15	Effect of Water Quality on Community Structure of Bivalve at Segoro Tambak Estuary, Sidoarjo, East Java, Indonesia	Widya Wahyu Hutami, Luthfiana Aprilianita Sari, Endang Dewi Masithah, Adriana Monica Sahidu and Kustiawan Tri Pursetyo

3. Attachment 1 (lanjutan): Manuscript No 05 (Artikel C1)

Health Risk Analysis of Cd, Pb, and Hg in Blood Mussel (*Anadara granosa*) from Demak, Central Java, Indonesia

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Abstract: Wedung waters, Demak, Central Java Indonesia, is a famous location that produces blood mussel, *Anadara granosa*. Anthropogenic activities can lead to contamination of heavy metals such as Pb, Cd, and Hg to the living environment of *A. granosa*. This study was to analyze heavy metals content in the soft tissue of *A.granosa* and health risks arising to Wedung residents from consuming the mussels. Heavy metals were analyzed using *atomic absorption spectrometry* (AAS). The result showed that Cd and Pb content were found in *A. granosa* soft tissue in the range of 0.56 - 0.70 mg/kg for Cd, 0.05 - 0.10 mg/kg for Pb and Hg was not detected. A health risk analysis showed that the HQ value for Pb from *A. granosa* intake was 0.0 <1, so that it was not at risk. While for Cd, the value reached 26.5 (HQ > 1). Thus, there was a noticeable health risk for the residents from consuming the mussel. The safe limit in consuming *A. granosa* was 0.33 g/day for children and 1.2 g/day for adults.

Key words: Cd, Pb, Hg, health risk analysis

Introduction

Blood mussel (*Anadara granosa*) is one of main types of shellfish and becomes an important source of protein in Southeast Asian countries (Yunus et al., 2014). *Anadara granosa* has an affordable price and can be developed as a source of protein and minerals needs by residents (Lindawaty et al., 2016). In 2015, shellfish production in Indonesia reached 59,613 tons and 85.26% of that was *A. granosa* (Ministry of Maritime Affairs and Fisheries, 2015).

A. granosa is a type of bivalve that contributes to the commercial fisheries in the Wedung district, the Demak Regency (Brotohadikusumo, 1994). Activities of Wedung residents, who are mostly farmers, fishermen, and housewives, could lead to the entrance of heavy metals of Cd, Pb, and Hg to Wedung waters. Agricultural activities such as the use of pesticides and fertilizers also the runoff from these activities contributed to the increase of heavy metals content in water and sediment (Ansari et al., 2004; Soegianto et al., 2010; Farejiya & Dikhsit, 2016). Garcia et al. (1996) stated that fertilizers and pesticides used in agriculture contained Cd and Pb. Household activities and burning of fossil fuels caused environmental pollution of mercury (Hg) that eventually settled into water or land (EPA, 2017). Port activities such as painting could spread Cd. Cd is a dangerous heavy metal since it can increase the risk of blood vessel disorders (Tuner, 2010). The consumption of blood mussels by the people of Wedung and its surroundings is a way of exposure to various heavy metals contained in the mussels. The level of shellfish consumption, the concentration of heavy metals in shellfish, as well as the duration of exposure, are determinants of health risk hazards.

This study aimed to analyze contamination of heavy metals Cd, Pb, and Hg in *A. granosa* and their impact on the health risk of Wedung residents.

Materilas and Methods

Sampling Location

Samples of *A. granosa* were collected from four stations that were chosen using *a purposive sampling* method. The criteria used to determine the stations were they have a significant population of *A. granosa* placed near to the mainland that was considered as the source of contamination. The coordinates of the four stations were as follows:

1) Station A was located at 6°44'39.20"S - 110°33'4.37"E
2) Station B was located at 6°44'57.29"S - 110°33'17.02"E
3) Station C was located at 6°46'1.84"S - 110°32'21.60"E, dan
4) Station D was located at 6°47'14.36"S - 110°33'25.79"E.

The four locations can be seen in Figure 1.

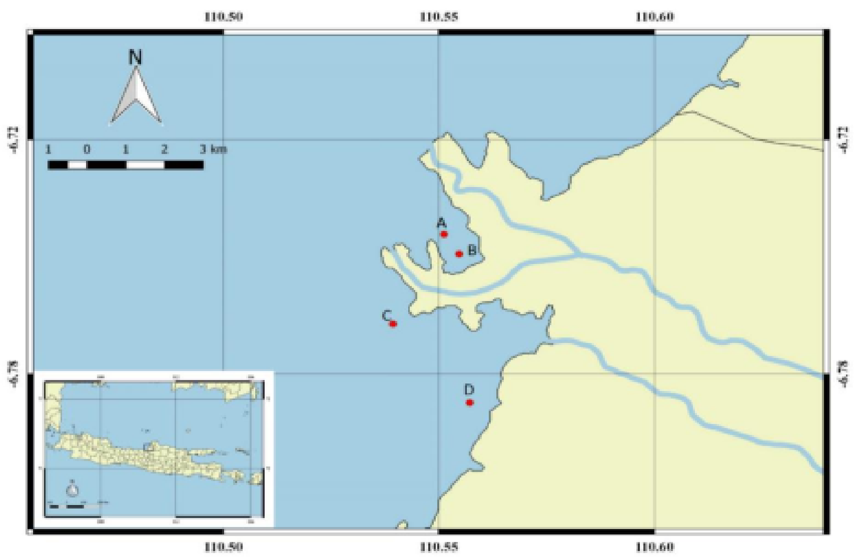

Figure 1: Sampling Location

Anadara granosa Collection

A group of samples of *A. granosa* with a total of 100 was collected from each station in February and March 2019. Thus, the total number of samples collected was 400. It stored in *polyethylene* plastics and put in an iced *coolbox* to keep its maximum temperature of 4°C during transportation. Before getting analyzed, the samples were stored at a temperature below -20ºC (Kumar *et al.,* 2011).

Heavy metals analysis

An analysis of Cd and Pb content in *A. granosa* was carried according to SNI 2354.5: 2011. Five grams of samples were destroyed under temperature that was increased gradually by 100°C every 30 minutes until reaching a temperature of 450 °C, in 18 hours. Then, 1 ml of 65% HNO_3 was added to the sample. It then got evaporated on a hot plate at 100°C until dry. The sample was again destroyed under the temperature of 450°C for 3 hours. Next, 5 ml of 6 M HCl was added to the sample and got evaporated on a hot plate at 100°C until dry. Ten milliliters of 0.1 M HNO_3 were then added to the dried sample, and it was put in a measuring flask 50 ml. Further, 0.1 M HNO_3 solution was added to the sample until reaching the mark. Analysis of heavy metals was continued by reading the sample solution using AAS with a wavelength of 228.8 mm for Cd and 283.3 mm for Pb.

An analysis Hg content was carried out according to SNI 2354.6: 2016. Five grams of sample was added with 3 - 5 boiling stones and 10 - 20 mg V_2O_5. Ten ml HNO_3 65% and 10 ml H_2SO_4 95 - 97% were then added to the sample, respectively. The sample was then heated until it turned to a yellowish-brown solution. Rinse the sample with 15 ml of deionized water. Add two drops of 30% H_2O_2 through the top end of the cooler, then rinse. The solution was then transferred to a 100 ml measuring flask and then set with deionized water. The solution was read using AAS with a wavelength of 253.7 nm. R-value for all heavy metal test is set to > 0.995.

Collection of Consumption Data

To measure the health risk of Wedung residents in correlation with *A. granosa* consumption, interviews were conducted to 400 person in Wedung using a prepared questionaire. The questionaire contained questions about daily intake, frequency of exposure, age, weight dan resident's ways in consuming mussels.

Health Risk Analysis

Non-carcinogenic health risk caused by Cd, Pb, and Hg faced by the residents who consume blood mussels was estimated by HQ value according to EPA A.S. using formula (1).

$$HQ = \frac{CDI}{RfD} \text{ ....(1)}$$

CDI (*Chronic* Daily Intake) was calculated using equation (2) based on (U.S. EPA 1989, 1991):

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

Where: C is heavy metal concentration; IR is intake rate or the weight of blood mussels consumed per day; Ef is exposure frequency; ED is exposure duration (6 years for kid and 30 years for adult); BW is bodyweight; and AT is average time (6 years x 365 days for kid and 30 years x 365 days for adult).

HQ > 1 in indicating a significant non-carcinogenic health risk. Hazard index (HI) caused by Cd, Pb dan Hg were calculated using equation (3) :

$$HI = HQ_{Cd} + HQ_{Pb} + HQ_{Hg} \text{.........(3)}$$

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.

$$\text{A Safe Daily Intake} = \frac{Rfd \times BW \times AT}{C \times EF \times ED} \text{.....(4)}$$

Result and Discusion

Heavy Metals in *A. granosa*

Cd and Pb were found in the soft tissue of *A. granosa* in the range of 0.56 – 0.70 mg/kg for - Cd and 0.05 - 0.10 mg/kg for Pb, while Hg was not detected in *A. granosa*. Those heavy metals spread into waters through agricultural activities such as the use of fertilizers, pesticides dan herbicides. Garcia *et al.* (1996) stated that fertilizers and pesticides used in agricultural activities contained Cd and Pb. It was supported by Lias *et al.* (2013) that stated that pesticides and herbicides are containing Pb and arsenic that polluted the ocean, and both were accumulated in sediments and bivalves. Fishing boat fuel contains Pb as an anti-knocking material (Parekh *et al.*, 2002). The residue of fuel-burning was suspected of entering and contaminating Wedung waters.

The test result on the three heavy metals was showed in Table 1, while the hazard identification in the form of the average of Cd and Pb were shown in Table 2.

The concentration of Cd and Pb in *A. granosa* was under the maximum limit issued according to SNI (Indonesia National Standard) No 7387/2009 and European Council Regulation 2006. Thus, *A. granosa* was still safe to be consumed.

Table 1: Concentration of Cd, Pb, and Hg in *A. granosa* captured from Wedung Coastal Waters, Demak, Indonesia

Content	Heavy metal content on soft tissue ($mg.kg^{-1}$)				Month	Standard **
	A	B	C	D		
Cd	0.64 ±0.18	0.64 ±0.20	0.60 ± 0.02	0.66 ± 0.08	Feb	1.00[1), 2)]
Pb	0.08 ±0.03	0.08 ±0.02	0.05 ±0.00	0.10 ±0.05		1.50[1), 2)]
Hg	Nd	Nd	Nd	Nd		1.00[1), 2)]
Cd	0.62 ±0.05	0.56 ±0.01	0.70 ±0.06	0.66 ±0.13	March	1.00[1), 2)]
Pb	0.09 ±0.03	0.09 ±0.03	0.08 ±0.04	0.06 ±0.01		1.50[1), 2)]
Hg	Nd	Nd	Nd	Nd		1.00[1), 2)]

[1)] SNI 7387: 2009; and [2)] European Council (2006); Nd=Not Detected

Table 2: Hazard identification in form of the average of Cd and Pb

Source	Media Potential Agent	Average Concentration of Hazard Agent ($mg.kg^{-1}$)	
		Cd	Pb
Heavy metal Contamination on waters	*Anadara granosa*	0.64	0.08

Table 3: Sampling of the average consumption and frequency of exposure *A. granosa* on Wedung Resident in 2019 (N = 400 persons)

Group of Age and Profession	Number of Sampel	Average of Consumption g/ day	Average of Age	Average of Weight (kg)	Frequency of Exposure *A. granosa* in one year
Children	**31.00**	**8.96**	**10.84**	**31.13**	**52.84**
Toddler	4.00	3.72	3.50	12.75	39.00
Student	27.00	9.74	11.93	33.85	54.89
Adult	**369.00**	**7.30**	**39.35**	**59.40**	**27.87**
House wife	98.00	5.17	40.78	57.17	22.67
Fisherman	76.00	9.38	44.41	62.00	32.61
Trader	22.00	7.17	36.41	61.73	41.09
Student	23.00	6.23	19.91	47.48	17.52
Village Officials	31.00	5.54	41.52	63.16	28.39
Salt Farmers	23.00	12.73	45.39	61.30	48.61
Farmer	18.00	17.54	45.89	57.44	29.83
Civil Servant	2.00	4.14	32.50	60.00	18.00
Private employee	35.00	4.79	35.97	59.40	20.60
Others	41.00	5.31	34.34	62.27	24.02
Grand Total	**400.00**	**7.43**	**37.14**	**57.21**	**29.81**

Health Risk Analysis

The analysis result on collected questionaries showed that the intake rate of *A. granosa* was 8.90 g/day for children and 7.30 g/day for adults. The annual exposure frequency for children was 53 times a year, while adults were 28 times a year. The average body weight of children in Wedung was 31.13 kg, while adults averaged in 59.40 kg. The complete data of *A.granosa* exposure to the residents in 2019 can be seen in Table 3.

Using equation (2), the CDI value for Cd were 0.026 mg/kg bw/day for children and 0.006 mg/kg bw/day for adults. While for Pb, the CDI value were 33E7 mg/kg bw/day for children and were 75E7 mg/kg bw/day for adults.

HQ analysis was performed using equation (1). The RfD value for Cd was 0.001 mg/kg-day (EPA 2018), while Pb was 0.0035 mg/kg-day (Khan *et al.*, 2008). From this equation, the HQ value of Cd for children and adults was 26.5 (> 1). The HQ value of Pb was 0.9 for children and 0.2 for adults. Similar results were also obtained by Soegianto et al. (2020), where the HQ index for Cd contained in blood mussels from the coastal waters of East Java, Indonesia was > 1, which indicates that these metals have the potential to cause non-carcinogenic for consumers.

The hazard index (HI) of Cd and Pb were 27.4 for children and 26.7 for adults. These HI values were > 1, indicated that consumption of *A. granosa* by Wedung residents has a high health risk.

From the HQ analysis, it was found that Cd content in blood mussel consumed by the residents had a risk to health. Thus, it was necessary to perform risk management. Risk management can be realized by reducing the rate of intake of *A. granosa* into the safe limit using equation (4). By using this equation, the safe consumption of *A. granosa* was 0.33 g/day for children and 1.2 g/day for adults.

Cadmium is a very toxic element. It naturally occurs in the soil; it is also spread to the environment through human activities and can eventually enter the human body through the food chain. Food is the primary access to Cd exposure in humans (Hosseini *et al.,* 2015). Exposure to cadmium could risk human health. The impact of cadmium has been reported to be the cause of "Itai-itai" disease since 1960 (Pan *et al.,* 1960). Cadmium is also carcinogenic on the prostate (Tallaa *et al.,* 2007).

Conclusion

Hg content was not detected in the soft tissue of *A. granosa* collected from Wedung waters. While, Cd and Pb content were detected in the soft tissue of *A. granosa* with an average value of 0.64 mg/kg for Cd and 0.08 mg/kg for Pb, respectively. The HQ value of Pb from *A. granosa* was < 1. Thus, it does not risk the resident's health. On the other hand, the HQ value of Cd reached 26.5 (HQ > 1). Therefore, it can risk the resident's health. The safe intake of *A. granosa* would be 0.33 g/day for children and 1.2 g/day for adults.

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4. Attachment 1 (lanjutan): Manuscript No. 13 (Artikel C2)

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 urbanization. This study emphasized on 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 consumed by the local people as a source of animal protein. Therefore, keeping the mussels from a wide range of contaminants, including heavy metals, has become an essential factor in 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 – 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 of the metals contaminated green mussels had deleterious health risks to children living in the Semarang coastal areas. The HQ value <1 occurred for adults consumed Cd-, Cu-, and Zn-contaminated green mussels. A particular case occurred in Pb-contaminating green mussels, which showed the HQ values > 1 in allover 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 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 consumed by humans are a transfer form of heavy metals from the lower food chain towards the higher one. This 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 realized, 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 realized 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) in the blood mussels, *Anadara granosa* (Soegianto et al., 2020). These studies showed an increase in the bivalve's contaminated heavy metals on the northern coast of Java.

Considering green mussels is a source of protein and widely consumed by Indonesian people, abundant and low prices; therefore, it is necessary to carry out monitoring green mussels contaminated 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 due to their lives stick to the hard material. Samples were kept in a coolbox at a temperature retained at about 4° C before the transport to the laboratory. The number of mussel samples for each station was between 100 to 250 mussels. The sample stations and its coordinate are shown in Figure 1 and Table 1.

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

Metals Analyses

Samples of the soft tissues were analyzed for heavy metal content Cd, Pb, Cu, and Zn. Solid samples (soft tissues) were washed, dried, and mashed with mortar. Then the results were sifted with a mesh size of 100 mm and homogenized. The result was weighed as much as 0.5 g and put into the Teflon bomb digester, moistened with splashes of aquatrides, then added 1 ml of concentrated HNO_3. 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 AAS.

Environmental Health Risk Analysis

To analyze the health risk of habitant of Semarang coastal waters concerning green mussel consumption interview was conducted to 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 CDI (Chronic Daily Intake), HQ (Hazard Quotient), and HI (Hazard Index) 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).

$$HQ = \frac{CDI}{RfD} \text{ .... (1)}$$

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. RfD (Reference Dose) 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 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):

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

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) (years). BW is body weight (kg), and AT is the average time (30 years x 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, dan Zn) was calculated using equation (3):

$$HI = HQ_{Cd} + HQ_{Pb} + HQ_{Cu} + HQ_{Zn} \ldots (3).$$

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.

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

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 Indonesia 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 exceeded the MAL (Table 1). High Pb concentrations are evident in the association of high levels with urban areas near cities with many human activities as like as 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-, Zn-contaminated green mussels were > 1 for children at all study locations (Table 2), which means the non-carcinogenic health risks will threaten. Contrary, the adults will not be 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, both for children and adults, which will cause 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 potential causes non-carcinogenic for consumers.

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) **St-1:** - 6°56'24.6"; 110°24'35.8" **St-2:** - 6°56'28.9"; 110°24'58.2"	Cd	November 2017	0.025 - 0.039	0.0334±0.0052	0.10 mg/kg[1)] 1.0 mg/kg[2)]
		December 2017	0.027 - 0.038	0.0311±0.0041	
	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)]; 1.50 mg/kg [2)]
		February 2017	1.878-2.765	2.268±0.293	
	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) **St-3:** - 6°56'21.2"; 110°25'39.4" **St-4:** - 6°56'29.5"; 110°26'10.5" **St-5:** - 6°56'14.5"; 110°26'44.8"	Cd	November 2017	0.014-0.042	0.02501±0.0099	0.10 mg/kg[1)] 1.0 mg/kg[2)]
		December 2017	0.013-0.034	0.0215±0.0069	
	Pb	December 2016	0.324-0.534	0.406±0.059	0.20 mg/kg [1)]; 1.50 mg/kg [2)]
		February 2017	0.537-0.759	0.603±0.066	
	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

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

HI > 1 indicated that consumption of *P. viridis* in Semarang coastal residents has a high health risk (Table 3). It was necessary to perform risk management for all study sites. Risk management effort may be realized 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). Reduce metals contained in mussels also a reasonable way to protect public health by the use of depuration technology for bivalves landed from fishing vessels before to be marketed.

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	Children	5.40	0.48	0.40	0.28	6.56
Semarang)	Adult	6.14	0.54	0.45	0.31	7.44
OIPPS (Outlet Indonesia	Children	1.43	0.42	0.65	0.21	2.70
Power Plant Semarang)	Adult	1.62	0.47	0.74	0.24	3.07

Conclusion

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

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

The HI values for all metals > 1, which indicated that consumption of *P. viridis* at 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 safe limit.

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5. Komunikasi Editor dengan Corresponding Author mengenai konfirmasi penerimaan 15 manuscript dan koreksi format artikel

Prof. Agoes Soegianto
Dept. Biology, Airlangga University
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INDONESIA

From: Capital Books <capitalb@capital-publishing.com>
Date: Mon, Feb 3, 2020 at 6:42 PM
Subject: RE: Selected papers from ICOBIODIV for publication in AJWEP
To: Agoes Soegianto <agoes_soegianto@fst.unair.ac.id>
Cc: Capital Books <capitalb@capital-publishing.com>

Dear Prof. Agoes Soegianto,

We thank you for your email of yesterday and confirm receipt of all the papers in good order. We have a small request – please send us all the 15 papers in double spacing and single column as in the current format copy-editing becomes very difficult for us.

We will publish your papers in our July, 2020 issue i.e. Volume 17 Number 3.

Look forward receiving the corrected papers at the earliest.

Regards,

Raj D Mirchandani

6. **Komunikasi Corresponding Author dengan Editor mengenai revisi manuscript menjadi 1 kolom dengan 2 spasi**

From: Agoes Soegianto [mailto:agoes_soegianto@fst.unair.ac.id]
Sent: 03 February 2020 19:06
To: Capital Books
Subject: Re: Selected papers from ICOBIODIV for publication in AJWEP

Dear Mr. Raj D Mirchandani

•••

Please find attached 15 selected papers in double spacing and single column.
Thank you very much for considering these papers in AJWEP vol 17 no 3 2020.

With our best regards,

Prof. Agoes Soegianto
Dept. Biology, Airlangga University
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INDONESIA

7. **Jawaban Editor atas pengiriman manuscript yang telah dibuat format 1 kolom 2 spasi oleh Corresponding author**

Prof. Agoes Soegianto
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INDONESIA

On Tue, Feb 4, 2020 at 4:47 PM Capital Books <capitalb@capital-publishing.com> wrote:

> Dear Prof. Agoes Soegianto,
>
> We confirm receipt of your email attaching therein the 15 papers for the July issue of AJWEP.
>
> We will get back to you in case of any query etc while copy-editing.
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> Regards,
>
> Raj D Mirchandani

Lampiran Artikel ke-1 (C1) dan Artikel ke-2 (C2) yang telah direvisi menjadi format 2 spasi dan 1 kolom ditampilkan halaman berikut.

8. Attachment 4: Artikel No 5 (C1) direvisi menjadi format 1 (satu) kolom dan 2 (dua) spasi

Health Risk Analysis of Cd, Pb, and Hg in Blood Mussel (*Anadara granosa*) from Demak, Central Java, Indonesia

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Abstract: Wedung waters, Demak, Central Java Indonesia, is a famous location that produces blood mussel, *Anadara granosa*. Anthropogenic activities can lead to contamination of heavy metals such as Pb, Cd, and Hg to the living environment of *A. granosa*. This study was to analyze heavy metals content in the soft tissue of *A.granosa* and health risks arising to Wedung residents from consuming the mussels. Heavy metals were analyzed using *atomic absorption spectrometry* (AAS). The result showed that Cd and Pb content were found in *A. granosa* soft tissue in the range of 0.56 - 0.70 mg/kg for Cd, 0.05 - 0.10 mg/kg for Pb and Hg was not detected. A health risk analysis showed that the HQ value for Pb from *A. granosa* intake was 0.0 <1, so that it was not at risk. While for Cd, the value reached 26.5 (HQ > 1). Thus, there was a noticeable health risk for the residents from consuming the mussel. The safe limit in consuming *A. granosa* was 0.33 g/day for children and 1.2 g/day for adults.

Key words: Cd, Pb, Hg, health risk analysis

Introduction

Blood mussel (*Anadara granosa*) is one of main types of shellfish and becomes an important source of protein in Southeast Asian countries (Yunus et al., 2014). *Anadara granosa* has an affordable price and can be developed as a source of protein and minerals needs by residents (Lindawaty et al., 2016). In 2015, shellfish production in Indonesia reached 59,613 tons and 85.26% of that was *A. granosa* (Ministry of Maritime Affairs and Fisheries, 2015).

A. granosa is a type of bivalve that contributes to the commercial fisheries in the Wedung district, the Demak Regency (Brotohadikusumo, 1994). Activities of Wedung residents, who are mostly farmers, fishermen, and housewives, could lead to the entrance of heavy metals of Cd, Pb, and Hg to Wedung waters. Agricultural activities such as the use of pesticides and fertilizers also the runoff from these activities contributed to the increase of heavy metals content in water and sediment (Ansari et al., 2004; Soegianto et al., 2010; Farejiya & Dikhsit, 2016). Garcia et al. (1996) stated that fertilizers and pesticides used in agriculture contained Cd and Pb. Household activities and burning of fossil fuels caused environmental pollution of mercury (Hg) that eventually settled into water or land (EPA, 2017). Port activities such as painting could spread Cd. Cd is a dangerous heavy metal since it can increase the risk of blood vessel disorders (Tuner, 2010). The consumption of blood mussels by the people of Wedung and its surroundings is a way of exposure to various heavy metals contained in the mussels. The level of shellfish consumption, the concentration of heavy metals in shellfish, as well as the duration of exposure, are determinants of health risk hazards.

This study aimed to analyze contamination of heavy metals Cd, Pb, and Hg in *A. granosa* and their impact on the health risk of Wedung residents.

Materials and Methods

Sampling Location

Samples of *A. granosa* were collected from four stations that were chosen using *a purposive sampling* method. The criteria used to determine the stations were they have a significant population of *A. granosa* placed near to the mainland that was considered as the source of contamination. The coordinates of the four stations were as follows:

1) Station A was geographically located at 6°44'39.20"S - 110°33'4.37"E

2) Station B was geographically located at 6°44'57.29"S - 110°33'17.02"E

3) Station C was geographically located at 6°46'1.84"S - 110°32'21.60"E, dan

4) Station D was geographically located at 6°47'14.36"S - 110°33'25.79"E.

The four locations can be seen in Figure 1.

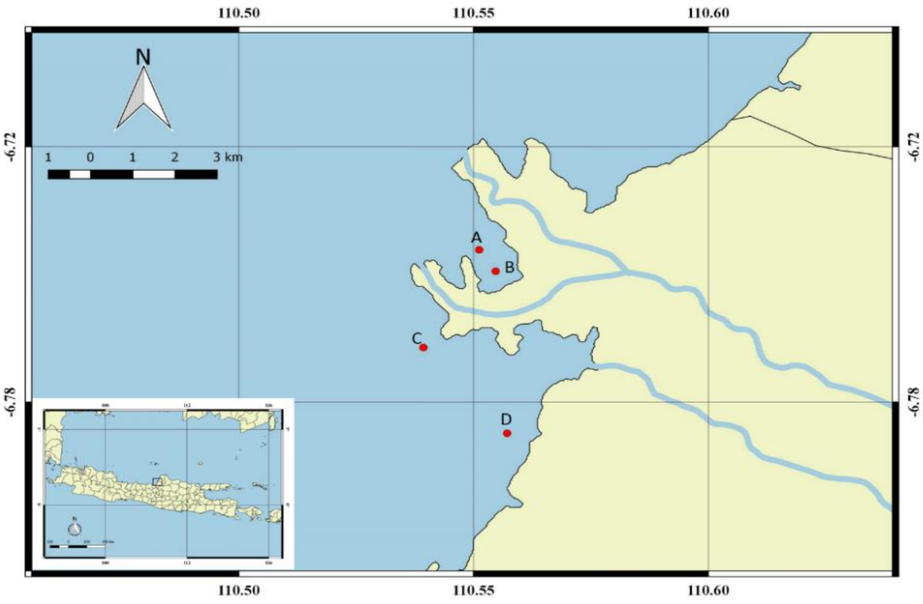

Figure 1: Sampling Location

Anadara granosa Collection

A group of samples of *A. granosa* with a total of 100 was collected from each station in February and March 2019. Thus, the total number of samples collected was 400. It stored in *polyethylene* plastics and put in an iced *coolbox* to keep its maximum temperature of 4°C during transportation. Before getting analyzed, the samples were stored at a temperature below -20ºC (Kumar *et al.*, 2011).

Heavy metals analysis

An analysis of Cd and Pb content in *A. granosa* was carried according to SNI 2354.5: 2011. Five grams of samples were destroyed under temperature that was increased gradually by 100°C every 30 minutes until reaching a temperature of 450 °C, in 18 hours. Then, 1 ml of 65% HNO_3 was added to the sample. It then got evaporated on a hot plate at 100°C until dry. The sample was again destroyed under the temperature of 450°C for 3 hours. Next, 5 ml of 6 M HCl was added to the sample and got evaporated on a hot plate at 100°C until dry. Ten milliliters of 0.1 M HNO_3 were then added to the dried sample, and it was put in a measuring flask 50 ml. Further, 0.1 M HNO_3 solution was added to the sample until reaching the mark. Analysis of heavy metals was continued by reading the sample solution using AAS with a wavelength of 228.8 mm for Cd and 283.3 mm for Pb.

An analysis Hg content was carried out according to SNI 2354.6: 2016. Five grams of sample was added with 3 - 5 boiling stones and 10 - 20 mg V_2O_5. Ten ml HNO_3 65% and 10 ml H_2SO_4 95 - 97% were then added to the sample, respectively. The sample was then heated until it turned to a yellowish-brown solution. Rinse the sample with 15 ml of deionized water. Add two drops of 30% H_2O_2 through the top end of the cooler, then rinse. The solution was then transferred to a 100 ml measuring flask and then set with deionized water. The solution was read using AAS with a wavelength of 253.7 nm. R-value for all heavy metal test is set to > 0.995.

Collection of Consumption Data

To measure the health risk of Wedung residents in correlation with *A. granosa* consumption, interviews were conducted to 400 person in Wedung using a prepared questionaire. The questionaire contained questions about daily intake, frequency of exposure, age, weight dan resident's ways in consuming mussels.

Health Risk Analysis

Non-carcinogenic health risk caused by Cd, Pb, and Hg faced by the residents who consume blood mussels was estimated by HQ value according to EPA A.S. using formula (1).

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

CDI (*Chronic Daily Intake*) was calculated using equation (2) based on (U.S. EPA 1989, 1991):

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

Where: C is heavy metal concentration; IR is intake rate or the weight of blood mussels consumed per day; Ef is exposure frequency; ED is exposure duration (6 years for kid and 30 years for adult); BW is bodyweight; and AT is average time (6 years x 365 days for kid and 30 years x 365 days for adult).

HQ > 1 in indicating a significant non-carcinogenic health risk. Hazard index (HI) caused by Cd, Pb dan Hg were calculated using equation (3) :

$$HI = HQ_{Cd} + HQ_{Pb} + HQ_{Hg} \quad \text{.........(3)}$$

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.

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

Result and Discusion

Heavy Metals in *A. granosa*

Cd and Pb were found in the soft tissue of *A. granosa* in the range of 0.56 – 0.70 mg/kg for Cd and 0.05 - 0.10 mg/kg for Pb, while Hg was not detected in *A. granosa*. Those heavy metals spread into waters through agricultural activities such as the use of fertilizers, pesticides dan herbicides. Garcia *et al.* (1996) stated that fertilizers and pesticides used in agricultural activities contained Cd and Pb. It was supported by Lias *et al*. (2013) that stated that pesticides and herbicides are containing Pb and arsenic that polluted the ocean, and both were accumulated in sediments and bivalves. Fishing boat fuel contains Pb as an anti-knocking material (Parekh *et al.*, 2002). The residue of fuel-burning was suspected of entering and contaminating Wedung waters.

The test result on the three heavy metals was showed in Table 1, while the hazard identification in the form of the average of Cd and Pb were shown in Table 2.

The concentration of Cd and Pb in *A. granosa* was under the maximum limit issued according to SNI (Indonesia National Standard) No 7387/2009 and European Council Regulation 2006. Thus, *A. granosa* was still safe to be consumed.

Table 1: Concentration of Cd, Pb, and Hg in *A. granosa* captured from Wedung Coastal Waters, Demak, Indonesia

Content	Heavy metal content on soft tissue ($mg.kg^{-1}$)				Month	Standard **
	A	B	C	D		
Cd	0.64 ±0.18	0.64 ±0.20	0.60 ± 0.02	0.66 ± 0.08	Feb	1.00[1), 2)]
Pb	0.08 ±0.03	0.08 ±0.02	0.05 ±0.00	0.10 ±0.05		1.50[1), 2)]
Hg	Nd	Nd	Nd	Nd		1.00[1), 2)]
Cd	0.62 ±0.05	0.56 ±0.01	0.70 ±0.06	0.66 ±0.13	March	1.00[1), 2)]

Pb	0.09 ±0.03	0.09 ±0.03	0.08 ±0.04	0.06 ±0.01		1.50[1), 2)]
Hg	Nd	Nd	Nd	Nd		1.00[1), 2)]

[1)] SNI 7387: 2009; and [2)] European Council (2006); Nd=Not Detected

Table 2: Hazard identification in form of the average of Cd and Pb

Source	Media Potential Agent	Average Concentration of Hazard Agent (mg.kg^{-1})	
		Cd	Pb
Heavy metal Contamination on waters	*Anadara granosa*	0.64	0.08

Table 3: Sampling of the average consumption and frequency of exposure *A. granosa* on Wedung Resident in 2019 (N = 400 persons)

Group of Age and Profession	Number of Sampel	Average of Consumption g/day	Average of Age	Average of Weight (kg)	Frequency of Exposure *A. granosa* in one year
Children	**31.00**	**8.96**	**10.84**	**31.13**	**52.84**
Toddler	4.00	3.72	3.50	12.75	39.00
Student	27.00	9.74	11.93	33.85	54.89
Adult	**369.00**	**7.30**	**39.35**	**59.40**	**27.87**

House wife	98.00	5.17	40.78	57.17	22.67
Fisherman	76.00	9.38	44.41	62.00	32.61
Trader	22.00	7.17	36.41	61.73	41.09
Student	23.00	6.23	19.91	47.48	17.52
Village Officials	31.00	5.54	41.52	63.16	28.39
Salt Farmers	23.00	12.73	45.39	61.30	48.61
Farmer	18.00	17.54	45.89	57.44	29.83
Civil Servant	2.00	4.14	32.50	60.00	18.00
Private employee	35.00	4.79	35.97	59.40	20.60
Others	41.00	5.31	34.34	62.27	24.02
Grand Total	**400.00**	**7.43**	**37.14**	**57.21**	**29.81**

Health Risk Analysis

The analysis result on collected questionaries showed that the intake rate of *A. granosa* was 8.90 g/day for children and 7.30 g/day for adults. The annual exposure frequency for children was 53 times a year, while adults were 28 times a year. The average body weight of children in Wedung was 31.13 kg, while adults averaged in 59.40 kg. The complete data of *A.granosa* exposure to the residents in 2019 can be seen in Table 3.

Using equation (2), the CDI value for Cd were 0.026 mg/kg bw/day for children and 0.006 mg/kg bw/day for adults. While for Pb, the CDI value were 33E7 mg/kg bw/day for children and were 75E7 mg/kg bw/day for adults.

HQ analysis was performed using equation (1). The RfD value for Cd was 0.001 mg/kg-day (EPA 2018), while Pb was 0.0035 mg/kg-day (Khan *et al.*, 2008). From this equation, the HQ value of Cd for children and adults was 26.5 (> 1). The HQ value of Pb was 0.9 for children and 0.2 for adults. Similar results were also obtained by Soegianto et al. (2020), where the HQ index for Cd contained in blood mussels from the coastal waters of East Java, Indonesia was > 1, which indicates that these metals have the potential to cause non-carcinogenic for consumers.

The hazard index (HI) of Cd and Pb were 27.4 for children and 26.7 for adults. These HI values were > 1, indicated that consumption of *A. granosa* by Wedung residents has a high health risk.

From the HQ analysis, it was found that Cd content in blood mussel consumed by the residents had a risk to health. Thus, it was necessary to perform risk management. Risk management can be realized by reducing the rate of intake of *A. granosa* into the safe limit using equation (4). By using this equation, the safe consumption of *A. granosa* was 0.33 g/day for children and 1.2 g/day for adults.

Cadmium is a very toxic element. It naturally occurs in the soil; it is also spread to the environment through human activities and can eventually enter the human body through the food chain. Food is the primary access to Cd exposure in humans (Hosseini *et al.*, 2015). Exposure to cadmium could risk human health. The impact of cadmium has been reported to be the cause of "Itai-itai" disease since 1960 (Pan *et al.*, 1960). Cadmium is also carcinogenic on the prostate (Tallaa *et al.*, 2007).

Conclusion

Hg content was not detected in the soft tissue of *A. granosa* collected from Wedung waters. While, Cd and Pb content were detected in the soft tissue of *A. granosa* with an average value of 0.64 mg/kg for Cd and 0.08 mg/kg for Pb, respectively. The HQ value of Pb from *A. granosa* was < 1. Thus, it does not risk the resident's health. On the other hand, the HQ value of Cd reached 26.5 (HQ > 1). Therefore, it can risk the resident's health. The safe intake of *A. granosa* would be 0.33 g/day for children and 1.2 g/day for adults.

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9. Attachment 5: Artikel No 13 (C2) direvisi menjadi format 1 (satu) kolom dan 2 (dua) spasi

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 urbanization. This study emphasized on 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 consumed by the local people as a source of animal protein. Therefore, keeping the mussels from a wide range of contaminants, including heavy metals, has become an essential factor in 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 – 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 of the metals contaminated green mussels had deleterious health risks to children living in the Semarang coastal areas. The HQ value <1 occurred for adults consumed Cd-, Cu-, and Zn-contaminated green mussels. A particular case occurred in Pb-contaminating green mussels, which showed the HQ values > 1 in allover 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 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 consumed by humans are a transfer form of heavy metals from the lower food chain towards the higher one. This 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 realized, 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 realized 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) in the blood mussels, *Anadara granosa* (Soegianto et al., 2020). These studies showed an increase in the bivalve's contaminated heavy metals on the northern coast of Java.

Considering green mussels is a source of protein and widely consumed by Indonesian people, abundant and low prices; therefore, it is necessary to carry out monitoring green mussels contaminated 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 due to their lives stick to the hard material. Samples were kept in a coolbox at a temperature retained at about 4° C before the transport to the laboratory. The number of mussel samples for each station was between 100 to 250 mussels. The sample stations and its coordinate are shown in Figure 1 and Table 1.

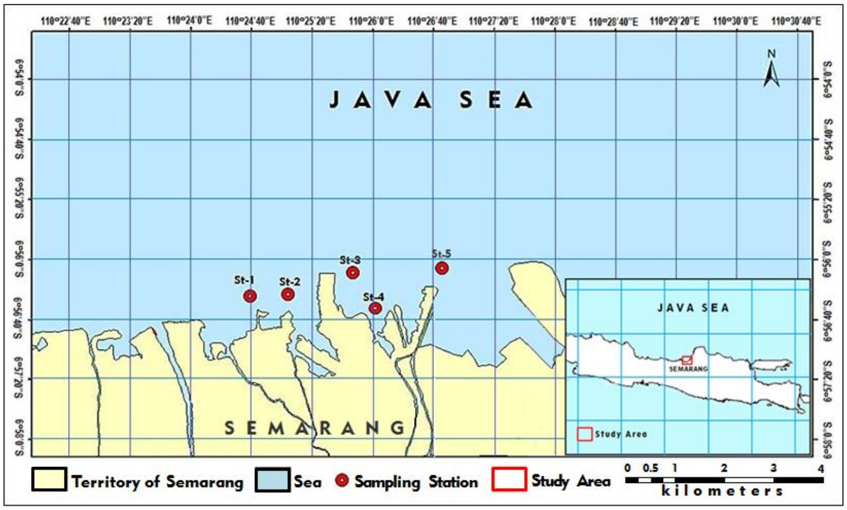

Figure 1: Research stations at Semarang coastal waters, Central Java, Indonesia

Metals Analyses

Samples of the soft tissues were analyzed for heavy metal content Cd, Pb, Cu, and Zn. Solid samples (soft tissues) were washed, dried, and mashed with mortar. Then the results were sifted with a mesh size of 100 mm and homogenized. The result was weighed as much as 0.5 g and put into the Teflon bomb digester, moistened with splashes of aquatrides, then added 1 ml of concentrated HNO_3. 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 AAS.

Environmental Health Risk Analysis

To analyze the health risk of habitant of Semarang coastal waters concerning green mussel consumption interview was conducted to 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 CDI (Chronic Daily Intake), HQ (Hazard Quotient), and HI (Hazard Index) 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).

$$HQ = \frac{CDI}{RfD} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(1)$$

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. RfD (Reference Dose) 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 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):

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

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) (years). BW is body weight (kg), and AT is the average time (30 years x 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, dan Zn) was calculated using equation (3):

$$HI = HQ_{Cd} + HQ_{Pb} + HQ_{Cu} + HQ_{Zn} \ldots(3).$$

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.

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

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 Indonesia 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 exceeded the MAL (Table 1). High Pb concentrations are evident in the association of high levels with urban areas near cities with many human activities as like as 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-, Zn-contaminated green mussels were > 1 for children at all study locations (Table 2), which means the non-carcinogenic health risks will threaten. Contrary, the adults will not be 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, both for children and adults, which will cause 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 potential causes non-carcinogenic for consumers.

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	Cd	November 2017	0.025 - 0.039	0.0334±0.0052	0.10 mg/kg[1)]
Emas Semarang		December 2017	0.027 - 0.038	0.0311±0.0041	1.0 mg/kg[2)]
(PTES)	Cu	December 2016	0.893-1.788	1.398±0.383	20 mg/kg[3)]
St-1: - 6°56'24.6";		February 2017	1.194-2.760	2.294±0.274	
110°24'35.8"	Pb	December 2016	1.924-2.670	2.278±0.150	0.20 mg/kg[1)];
St-2: - 6°56'28.9";		February 2017	1.878-2.765	2.268±0.293	1.50 mg/kg[2)]
110°24'58.2"	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) **St-3:** - 6°56'21.2"; 110°25'39.4" **St-4:** - 6°56'29.5"; 110°26'10.5" **St-5:** - 6°56'14.5"; 110°26'44.8"	Cd	November 2017	0.014-0.042	0.02501±0.0099	0.10 mg/kg[1]
		December 2017	0.013-0.034	0.0215±0.0069	1.0 mg/kg[2]
	Pb	December 2016	0.324-0.534	0.406±0.059	0.20 mg/kg[1];
		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

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

HI > 1 indicated that consumption of *P. viridis* in Semarang coastal residents has a high health risk (Table 3). It was necessary to perform risk management for all study sites. Risk management effort may be realized 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). Reduce metals contained in mussels also a reasonable way to protect public health by the use of depuration technology for bivalves landed from fishing vessels before to be marketed.

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 the green mussels *P. viridis* captured from Semarang coastal waters were contaminated the heavy metals (Cd, Pb, Cu, Zn) in their soft tissues. Their concentrations have not exceeded the Maximum Acceptable Limits (MAL) yet, except for Pb has surpassed the value of MAL.

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

The HI values for all metals > 1, which indicated that consumption of *P. viridis* at 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 safe limit.

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Health Risk Analysis of Cd, Pb, and Hg in Blood Mussel (*Anadara granosa*) from Demak, Central Java, Indonesia

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Abstract: Wedung waters, Demak, Central Java Indonesia, is a famous location that produces blood mussel, Anadara granosa. Anthropogenic activities can lead to contamination of heavy metals such as Pb, Cd, and Hg to the living environment of *A. granosa*. This study was to analyze heavy metals content in the soft tissue of *A.granosa* and health risks arising to Wedung residents from consuming the mussels. Heavy metals ~~are~~ analyzed using *atomic absorption spectrometry* (AAS). The result showed that Cd and Pb content were found in *A. granosa* soft tissue in the range of 0.56 - 0.70 mg/kg for Cd, 0.05 - 0.10 mg/kg for Pb and Hg was not detected. A health risk analysis showed that the HQ value for Pb from *A. granosa* intake was 0.0 <1, so that it was not at risk. While for Cd, the value reached 26.5 (HQ > 1). Thus, there was a noticeable health risk for the residents from consuming the mussel. The safe limit in consuming *A. granosa* was 0.33 g/day for children and 1.2 g/day for adults.

Key words: Cd, Pb, Hg, health risk analysis

Introduction

Blood mussel (*Anadara granosa*) is one of main types of shellfish and becomes an important source of protein in Southeast Asian countries (Yunus et al., 2014). *Anadara granosa* has an affordable price and can be developed as a source of protein and minerals needs by residents (Lindawaty et al., 2016). In 2015, shellfish production in Indonesia reached 59,613 tons and 85.26% of that was *A. granosa* (Ministry of Maritime Affairs and Fisheries, 2015).

A. granosa is a type of bivalve that contributes to the commercial fisheries in the Wedung district, the Demak Regency (Brotohadikusumo, 1994). Activities of Wedung residents, who are mostly farmers, fishermen, and housewives, could lead to the entrance of heavy metals of Cd, Pb, and Hg to Wedung waters. Agricultural activities such as the use of pesticides and fertilizers also the runoff from these activities contributed to the increase of heavy metals content in water and sediment (Ansari et al., 2004; Soegianto et al., 2010; Farejiya & Dikhsit, 2016). Garcia et al. (1986) stated that fertilizers and pesticides used in agriculture contained Cd and Pb. Household activities and burning of fossil fuels caused environmental pollution of mercury (Hg) that eventually settled into water or land (EPA, 2017). Port activities such as painting could spread Cd. Cd is a dangerous heavy metal since it can increase the risk of blood vessel disorders (Tuner, 2010). The consumption of blood mussels by the people of Wedung and its surroundings is a way of exposure to various heavy metals contained in the mussels. The level of shellfish consumption, the concentration of heavy metals in shellfish, as well as the duration of exposure, are determinants of health risk hazards.

This study aimed to analyze contamination of heavy metals Cd, Pb, and Hg in *A. granosa* and their impact on the health risk of Wedung residents.

Materilas and Methods

Sampling Location

Samples of *A. granosa* were collected from four stations that were chosen using *a purposive sampling* method. The criteria used to determine the stations were they have a significant population of *A. granosa* placed near to the mainland that was considered as the source of contamination. The coordinates of the four stations were as follows:

1) Station A was geographically located at 6°44'39.20"S - 110°33'4.37"E

2) Station B was geographically located at 6°44'57.29"S - 110°33'17.02"E

3) Station C was geographically located at 6°46'1.84"S - 110°32'21.60"E, dan

4) Station D was geographically located at 6°47'14.36"S - 110°33'25.79"E.

The four locations can be seen in Figure 1

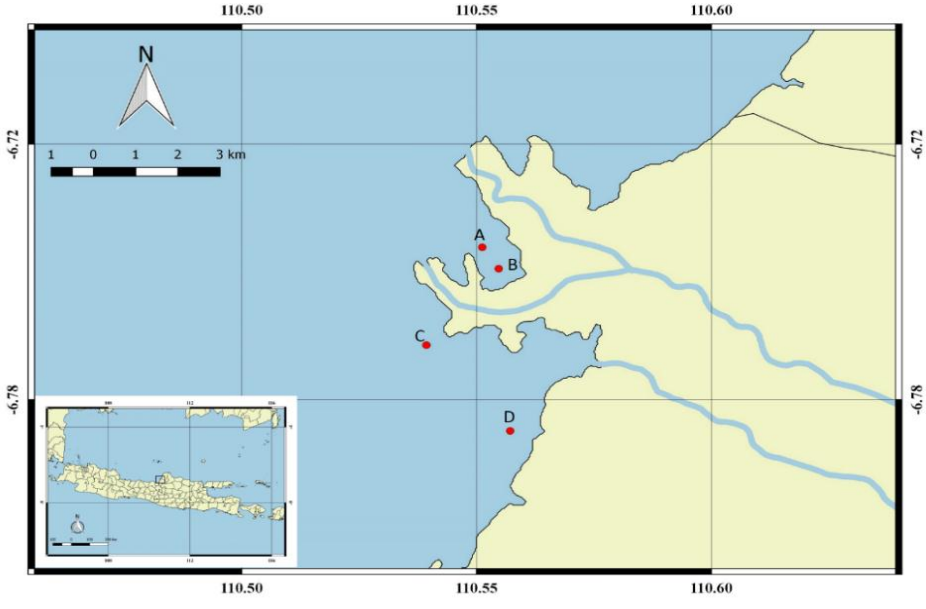

Figure 1: Sampling Location

***Anadara granosa* Collection**

A group of samples of *A.granosa* with a total of 100 was collected from each station in February and March 2019. Thus, the total number of samples collected was 400. It stored in *polyethylene* plastics and put in an iced *coolbox* to keep its maximum temperature of ~~4°C~~ during transportation. Before getting analyzed, the samples were stored at a temperature below -20°C (Kumar *et al.*, 2011).

Heavy metals analysis

An analysis of Cd and Pb content in *A. granosa* was carried according to SNI 2354.5: 2011. Five grams of samples were destroyed under temperature that was increased gradually by 100°C every 30 minutes until reaching a temperature of 450 °C, in 18 hours. Then, 1 ml of 65% HNO_3 was added to the sample. It then got evaporated on a hot plate at 100°C until dry. The sample was again destroyed under the temperature of 450°C for 3 hours. Next, 5 ml of 6 M HCl was added to the sample and ~~got~~ evaporated on a hot plate at 100°C until dry. Ten milliliters of 0.1 M HNO_3 were then added to the dried sample, and it was put in a measuring flask 50 ml. Further, 0.1 M HNO_3 solution was added to the sample until reaching the mark. Analysis of heavy metals was continued by reading the sample solution using AAS with a wavelength of 228.8 mm for Cd and 283.3 mm for Pb.

An analysis Hg content was carried out according to SNI 2354.6: 2016. Five grams of sample was added with 3 - 5 boiling stones and 10 - 20 mg V_2O_5. Ten ml HNO_3 65% and 10 ml H_2SO_4 95 - 97% were then added to the sample, respectively. The sample was then heated until it turned to a yellowish-brown solution. Rinse the sample with 15 ml of deionized water. Add two drops of 30% H_2O_2 through the top end of the cooler, then rinse. The solution was then transferred to a 100 ml measuring flask and then set with deionized water. The solution was read using AAS with a wavelength of 253.7 nm. R-value for all heavy metal test is set to > 0.995.

Collection of Consumption Data

To measure the health risk of Wedung residents in correlation with *A. granosa* consumption, interviews were conducted to 400 person in Wedung using a prepared questionaire. The questionaire contained questions about daily intake, frequency of exposure, age, weight dan resident ways ~~in~~ consuming mussels.

Health Risk Analysis

Non-carcinogenic health risk caused by Cd, Pb, and Hg faced by the residents who consume blood mussels was estimated by HQ value according to EPA A.S. using formula (1).

$$HQ = CDI/RfD \quad \text{.................(1)}$$

CDI (*Chronic* Daily Intake) was calculated using equation (2) based on (U.S. EPA 1989, 1991):

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

Where: C is heavy metal concentration; IR is intake rate or the weight of blood mussels consumed per day; Ef is exposure frequency; ED is exposure duration (6 years for kid and 30 years for adult); BW is bodyweight; and AT is average time (6 years x 365 days for kid and 30 years x 365 days for adult).

HQ > 1 in indicating a significant non-carcinogenic health risk. Hazard index (HI) caused by Cd, Pb dan Hg were calculated using equation (3) :

$$HI = HQ_{Cd} + HQ_{Pb} + HQ_{Hg} \ldots\ldots\ldots(3)$$

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.

$$\text{A Safe Daily Intake} = \frac{RfD \times BW \times AT}{C \times EF \times ED} \ldots\ldots\ldots\ldots.(4)$$

Result and Discusion

Heavy Metals in *A. granosa*

Cd and Pb were found in the soft tissue of *A. granosa* in the range of 0.62 – 0.70 mg/kg for Cd and 0.05 - 0.10 mg/kg for Pb, while Hg was not detected in *A. granosa*. Those heavy metals spread into waters through agricultural activities such as the use of fertilizers, pesticides dan herbicides. Garcia et al. (1996) stated that fertilizers and pesticides used in agricultural activities contained Cd and Pb. It was supported by Lias *et al*. (2013) that stated that pesticides and herbicides are containing Pb and arsenic that polluted the ocean, and both were accumulated in sediments and bivalves. Fishing boat fuel contains Pb as an anti-knocking material (Parekh *et al.*, 2002). The residue of fuel-burning was suspected of entering and contaminating Wedung waters.

The test result on the three heavy metals was showed in Table 1, while the hazard identification in the form of the average of Cd and Pb were shown in Table 2.

The concentration of Cd and Pb in *A. granosa* was under the maximum limit issued according to SNI (Indonesia National Standard) No 7387/2009 and European Council Regulation 2006. Thus, *A. granosa* was still safe to be consumed.

Table 1: Concentration of Cd, Pb, and Hg in *A. granosa* captured from Wedung Coastal Waters, Demak, Indonesia

Content	Heavy metal content on soft tissue (mg.kg^{-1})				Month	Standard **
	A	B	C	D		
Cd	0.64 ±0.18	0.64 ±0.20	0.60 ± 0.02	0.66 ± 0.08		1.00[1), 2)]
Pb	0.08 ±0.03	0.08 ±0.02	0.05 ±0.00	0.10 ±0.05	Feb	1.50[1), 2)]
Hg	Nd	Nd	Nd	Nd		1.00[1), 2)]
Cd	0.62 ±0.05	0.56 ±0.01	0.70 ±0.06	0.66 ±0.13		1.00[1), 2)]
Pb	0.09 ±0.03	0.09 ±0.03	0.08 ±0.04	0.06 ±0.01	March	1.50[1), 2)]
Hg	Nd	Nd	Nd	Nd		1.00[1), 2)]

[1)] SNI 7387: 2009; and [2),] European Council (2006); Nd=Not Detected

Table 2: Hazard identification in form of the average of Cd and Pb

Source	Media Potential Agent	Average Concentration of Hazard Agent (mg.kg^{-1})	
		Cd	Pb
Heavy metal Contamination on waters	*Anadara granosa*	0.64	0.08

Table 3: Sampling of the average consumption and frequency of exposure *A. granosa* on Wedung Resident in 2019 (N = 400 persons)

Group of Age and Profession	Number of Sampel	Average of Consumption g/ day	Average of Age	Average of Weight (kg)	Frequency of Exposure *A. granosa* in one year
Children	**31.00**	**8.96**	**10.84**	**31.13**	**52.84**
Toddler	4.00	3.72	3.50	12.75	39.00
Student	27.00	9.74	11.93	33.85	54.89
Adult	**369.00**	**7.30**	**39.35**	**59.40**	**27.87**
House wife	98.00	5.17	40.78	57.17	22.67
Fisherman	76.00	9.38	44.41	62.00	32.61
Trader	22.00	7.17	36.41	61.73	41.09
Student	23.00	6.23	19.91	47.48	17.52
Village Officials	31.00	5.54	41.52	63.16	28.39
Salt Farmers	23.00	12.73	45.39	61.30	48.61
Farmer	18.00	17.54	45.89	57.44	29.83
Civil Servant	2.00	4.14	32.50	60.00	18.00
Private employee	35.00	4.79	35.97	59.40	20.60
Others	41.00	5.31	34.34	62.27	24.02
Grand Total	**400.00**	**7.43**	**37.14**	**57.21**	**29.81**

Health Risk Analysis

The analysis result on collected questionaries showed that the intake rate of *A. granosa* was 8.90 g/day for children and 7.30 g/day for adults. The annual exposure frequency for children was 53 times a year, while adults were 28 times a year. The average body weight of children in Wedung was 31.13 kg, while adults averaged in 59.40 kg. The complete data of *A.granosa* exposure to the residents in 2019 can be seen in Table 3.

Using equation (2), the CDI value for Cd were 0.026 mg/kg bw/day for children and 0.006 mg/kg bw/day for adults. While for Pb, the CDI value were 33E7 mg/kg bw/day for children and were 75E7 mg/kg bw/day for adults.

HQ analysis was performed using equation (1). The RfD value for Cd was 0.001 mg/kg-day (EPA 2018), while Pb was 0.0035 mg/kg-day (Khan *et al.*, 2008). From this equation, the HQ value of Cd for children and adults was 26.5 (> 1). The HQ value of Pb was 0.9 for children and 0.2 for adults. Similar results were also obtained by Soegianto et al. (2020), where the HQ index for Cd contained in blood mussels from the coastal waters of East Java, Indonesia was > 1, which indicates that these metals have the potential to cause non-carcinogenic for consumers.

The hazard index (HI) of Cd and Pb were 27.4 for children and 26.7 for adults. These HI values were > 1, indicated that consumption of *A. granosa* by Wedung residents has a high health risk.

From the HQ analysis, it was found that Cd content in blood mussel consumed by the residents had a risk to health. Thus, it was necessary to perform risk management. Risk management can be realized by reducing the rate of intake of *A. granosa* into the safe limit using equation (4). By using this equation, the safe consumption of *A. granosa* was 0.33 g/day for children and 1.2 g/day for adults.

Cadmium is a very toxic element. It naturally occurs in the soil; it is also spread to the environment through human activities and can eventually enter the human body through the food chain. Food is the primary access to Cd exposure in humans (Hosseini *et al.*, 2015). Exposure to cadmium could risk human health. The impact of cadmium has been reported to be the cause of "Itai-itai" disease since 1960 (Pan *et al.*, 1960). Cadmium is also carcinogenic on the prostate (Tallaa *et al.*, 2007).

Conclusion

Hg content was not detected in the soft tissue of *A. granosa* collected from Wedung waters. While, Cd and Pb content were detected in the soft tissue of *A. granosa* with an average value of 0.64 mg/kg for Cd and 0.08 mg/kg for Pb, respectively. The HQ value of Pb from *A. granosa* was < 1. Thus, it does not risk the resident's health. On the other hand, the HQ value of Cd reached 26.5 (HQ > 1). Therefore, it can risk the resident's health. The safe intake of *A. granosa* would be 0.33 g/day for children and 1.2 g/day for adults.

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Heavy Metals (Cd, Pb, Cu, Zn) in Green Mussel (*Perna viridis*) and Health Risk Analysis on Residentsof 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^{-1}) for Pb, 0.621–2.760 mg.kg^{-1} (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 dan 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-contaminatedgreen 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 environmentalchanges and pollution. One of the critical areas of globalconcern 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 require special attention, given its characteristics cannot be degraded by microorganisms; therefore, its availabilityin the environment can harm marine flora and fauna.

Seafood products (such as mussels) contaminatedby heavy metals and consumed by humans are atransfer of heavy metals from the lower food chaintowards the higher one. The present study enhances theknowledge 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 assessthe quality of seawaters. Study of metal residues inbivalves at several places has been realised, i.e., *Mytilus galloprovincialis* (Seguin et al., 2016); *Mytilus edulis* (Liu and Wang, 2015); *Perna viridis* (Liu andWang, 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 fromGresik coastal waters, Indonesia (Soegianto et al., 2010),heavy metals (Cd, Pb, Cu, Zn) residues in bivalvescaptured from northern coast of Central Java (Yuliantoet al., 2019). Heavy metals (Cd, Pb, Zn, Hg, Cu, andCr) are also reported in the blood mussels, *Anadaragranosa* (Soegianto et al., 2020). These studies showedan increase in bivalve's contaminated with heavy metals on the northern coast of Java.

Considering green mussel 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 withheavy metals and health risk assessments in the interestof 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 byhand, 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 stationsand its coordinate are shown in Figure 1 dan Table 1.

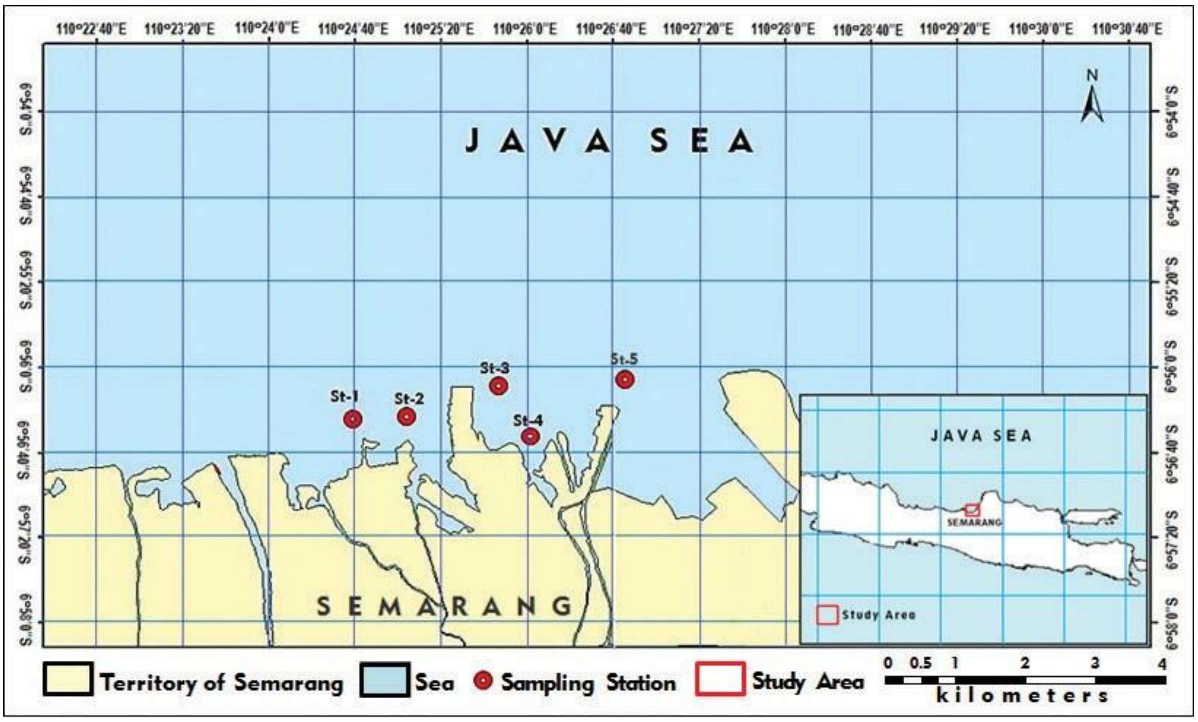

Figure 1: Research stations at Semarang coastal waters, Central Java, Indonesia.

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 mortar. Then the results were sifted with a mesh sizeof 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 heatedat 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 mlvolumetric 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).

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

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 anappreciable 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):

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

C is heavy metal concentration (mg/kg). IR = intakerate 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 6years 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):

$$HI = HQ_{Cd} + HQ_{Pb} + HQ_{Cu} + HQ_{Zn} \ldots\ldots.(3)$$

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.

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

Results and Discussion

The Concentration of Cd, Pb, Cu, Zn in the SoftTissue 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 ingreen 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 inoysters and mussels (NOAA, 1998). PTES has a higherPb in seawater because it is a passenger/trading port. Ship fuel contains Pb as an anti-knocking agent (Parekhet al., 2002). Fuel combustion residues are suspected of penetrating and polluting marine seawater.

Health Risk Analysis and Safe Consumption ofGreen 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 $days.year^{-1}$ (for children) and 28 $days.year^{-1}$ (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 willexperience some form of dangerous health effects (Table2). 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 potential causes for non-carcinogenic threats in consumers.

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	Cd	November 2017	0.025-0.039	0.0334±0.0052	0.10 mg/kg[1]
Semarang (PTES)		December 2017	0.027-0.038	0.0311±0.0041	1.0 mg/kg[2]
St-1: - 6°56′24.6″;	Cu	December 2016	0.893-1.788	1.398±0.383	20 mg/kg[3]
110°24′35.8″		February 2017	1.194-2.760	2.294±0.274	
St-2: - 6°56′28.9″;	Pb	December 2016	1.924-2.670	2.278±0.150	0.20 mg/kg[1]
110°24′58.2″					1.50 mg/kg[2]
		February 2017	1.878-2.765	2.268±0.293	
	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	Cd	November 2017	0.014-0.042	0.02501±0.0099	0.10 mg/kg[1]

PowerPlant Semarang		December 2017	0.013-0.034	0.0215±0.0069	1.0 mg/kg[2]
(IPPS)	Pb	December 2016	0.324-0.534	0.406±0.059	0.20 mg/kg[1]
St-3: - 6°56′21.2″;		February 2017	0.537-0.759	0.603±0.066	1.50 mg/kg[2]
110°25′39.4″	Cu	December 2016	0.621-1.744	1.094±0.353	20 mg/kg[3]
St-4: - 6°56′29.5″;		February 2017	1.635-2.631	2.007±0.269	
110°26′10.5″	Zn	December 2016	12.976-22.638	17.778±4.271	100 mg/kg[1]
St-5: - 6°56′14.5″;		February 2017	14.851-31.115	23.434±5.271	
110°26′44.8″					

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

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 dueto metals exposure via green mussels consumption should be focussed upon as children are more sensitiveto 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 allstudy sites. Risk management effort may be realized 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 $g.day^{-1}$, respectively (for residents living in PTES), and 1.244 and 4.4944 $g.day^{-1}$ (for residents living in OIPPS) (Table 2). Depuration technology is needed to reduce the levelsof 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*	*concentration (mg/kg)*	*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 occurredin Pb content in green mussels; the HQ > 1 for allstudy 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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Hasil Revisi manuscript no 05 artikel (C1) oleh Author

Health Risk Analysis of Cd, Pb, and Hg in Blood Mussel (*Anadara granosa*) from Demak, Central Java, Indonesia

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Abstract: Wedung waters, Demak, Central Java Indonesia, is a famous location that produces blood mussel, *Anadara granosa*. Anthropogenic activities can lead to contamination of heavy metals such as Pb, Cd, and Hg to the living environment of *A. granosa*. This study was to analyze heavy metals content in the soft tissue of *A. granosa* and health risks arising to Wedung residents from consuming the mussels. Heavy metals were analyzed using *atomic absorption spectrometry* (AAS). The result showed that Cd and Pb content were found in *A. granosa* soft tissue in the range of 0.56 - 0.70 mg/kg for Cd, 0.05 - 0.10 mg/kg for Pb and Hg was not detected. A health risk analysis showed that the HQ value for Pb from *A. granosa* intake was 0.0 < 1, so that it was not at risk. While for Cd, the value reached 26.5 (HQ > 1). Thus, there was a noticeable health risk for the residents from consuming the mussel. The safe limit in consuming *A. granosa* was 0.33 g/day for children and 1.2 g/day for adults.

Key words: Cd, Pb, Hg, health risk analysis

Introduction

Blood mussel (*Anadara granosa*) is one of main types of shellfish and becomes an important source of protein in Southeast Asian countries (Yunus et al., 2014). *Anadara granosa* has an affordable price and can be developed as a source of protein and minerals needs by residents (Lindawaty et al., 2016). In 2015, shellfish production in Indonesia reached 59,613 tons and 85.26% of that was *A. granosa* (Ministry of Maritime Affairs and Fisheries, 2015).

A. granosa is a type of bivalve that contributes to the commercial fisheries in the Wedung district, the Demak Regency (Brotohadikusumo, 1994). Activities of Wedung residents, who are mostly farmers, fishermen, and housewives, could lead to the entrance of heavy metals of Cd, Pb, and Hg to Wedung waters. Agricultural activities such as the use of pesticides and fertilizers and also the runoff from these activities contributed to the increase of heavy metals content in water and sediment (Ansari et al., 2004; Soegianto et al., 2010; Farejiya & Dikhsit, 2016). Garcia et al. (1986) stated that fertilizers and pesticides used in agriculture contained Cd and Pb. Household activities and burning of fossil fuels caused environmental pollution of mercury (Hg) that eventually settled into water or land (EPA, 2017). Port activities such as painting could spread Cd. Cd is a dangerous heavy metal since it can increase the risk of blood vessel disorders (Tuner, 2010). The consumption of blood mussels by the people of Wedung and its surroundings is a way of exposure to various heavy metals contained in the mussels. The level of shellfish consumption, the concentration of heavy metals in shellfish, as well as the duration of exposure, are determinants of health risk hazards.

This study aimed to analyze contamination of heavy metals Cd, Pb, and Hg in *A. granosa* and their impact on the health risk of Wedung residents.

Materilas and Methods

Sampling Location

Samples of *A. granosa* were collected from four stations that were chosen using *a purposive sampling* method. The criteria used to determine the stations were they have a significant population of *A. granosa* placed near to the mainland that was considered as the source of contamination. The coordinates of the four stations were as follows:

1) Station A was located at 6°44'39.20"S - 110°33'4.37"E

2) Station B was located at 6°44'57.29"S - 110°33'17.02"E

3) Station C was located at 6°46'1.84"S - 110°32'21.60"E, dan

4) Station D was located at 6°47'14.36"S - 110°33'25.79"E.

The four locations can be seen in Figure 1

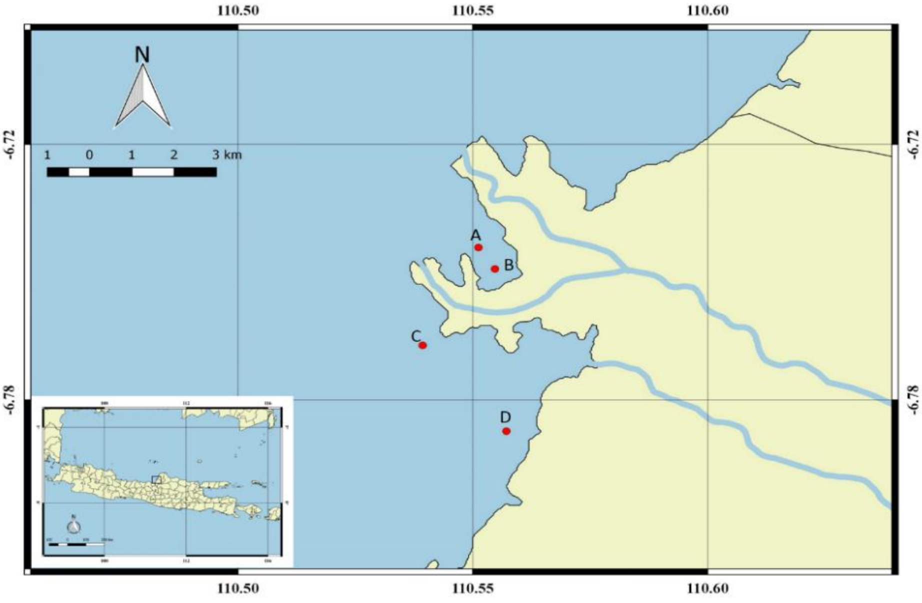

Figure 1: Sampling Location

Anadara granosa Collection

A group of samples of *A. granosa* with a total of 100 was collected from each station in February and March 2019. Thus, the total number of samples collected was 400. It stored in *polyethylene* plastics and put in an iced *coolbox* to keep its maximum temperature of 4°C during transportation. Before getting analyzed, the samples were stored at a temperature below -20ºC (Kumar et al., 2011).

Heavy metals analysis

An analysis of Cd and Pb content in *A. granosa* was carried out according to SNI 2354.5: 2011. Five grams of samples were destroyed under temperature that was increased gradually by 100°C every 30 minutes until reaching a temperature of 450 °C, in 18 hours. Then, 1 ml of 65% HNO_3 was added to the sample. It then got evaporated on a hot plate at 100°C until dry. The sample was again destroyed under the temperature of 450°C for 3 hours. Next, 5 ml of 6 M HCl was added to the sample and evaporated on a hot plate at 100°C until dry. Ten milliliters of 0.1 M HNO_3 were then added to the dried sample, and it was put in a measuring flask 50 ml. Further, 0.1 M HNO_3 solution was added to the sample until reaching the mark. Analysis of heavy metals was continued by reading the sample solution using AAS with a wavelength of 228.8 mm for Cd and 283.3 mm for Pb.

An analysis of Hg content was carried out according to SNI 2354.6: 2016. Five grams of sample was added with 3 - 5 boiling stones and 10 - 20 mg V_2O_5. Ten ml HNO_3 65% and 10 ml H_2SO_4 95 - 97% were then added to the sample, respectively. The sample was then heated until it turned to a yellowish-brown solution. Rinse the sample with 15 ml of deionized water. Add two drops of 30% H_2O_2 through the top end of the cooler, then rinse. The solution was then transferred to a 100 ml measuring flask and then set with deionized water. The solution was read using AAS with a wavelength of 253.7 nm. R-value for all heavy metal test is set to > 0.995.

Collection of Consumption Data

To measure the health risk of Wedung residents in correlation with *A. granosa* consumption, interviews were conducted with 400 person in Wedung using a prepared questionaire. The questionaire contained questions about daily intake, frequency of exposure, age, weight and resident's ways consuming mussels.

Health Risk Analysis

Non-carcinogenic health risk caused by Cd, Pb, and Hg faced by the residents who consume blood mussels was estimated by HQ value according to EPA A.S. using formula (1).

$$HQ = \frac{CDI}{RfD} \quad \text{................(1)}$$

CDI (*Chronic* Daily Intake) was calculated using equation (2) based on (U.S. EPA 1989, 1991):

$$CDI = \frac{C \ x \ IR \ x \ Ef \ x \ Ed}{Bw \ x \ AT} \quad ....(2)$$

Where: C is heavy metal concentration; IR is intake rate or the weight of blood mussels consumed per day; Ef is exposure frequency; ED is exposure duration (6 years for kid and 30 years for adult); BW is bodyweight; and AT is average time (6 years x 365 days for kid and 30 years x 365 days for adult).

HQ > 1 in indicating a significant non-carcinogenic health risk. Hazard index (HI) caused by Cd, Pb and Hg were calculated using equation (3) :

$$HI = HQ_{Cd} + HQ_{Pb} + HQ_{Hg} ....(3)$$

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.

$$\textbf{Safe Daily Intake} = \frac{RfD \ x \ Bw \ x \ AT}{C \ x \ EF \ x \ ED} \quad ....(4)$$

Result and Discussion

Heavy Metals in *A. granosa*

Cd and Pb were found in the soft tissue of *A. granosa* in the range of 0.56 – 0.70 mg/kg for Cd and 0.05 - 0.10 mg/kg for Pb, while Hg was not detected in *A. granosa*. Those heavy metals spread into waters through agricultural activities such as the use of fertilizers, pesticides and herbicides. Garcia et al. (1996) stated that fertilizers and pesticides used in agricultural activities contained Cd and Pb. It was supported by Lias *et al*. (2013) that stated that pesticides and herbicides are containing Pb and arsenic that polluted the ocean, and both were accumulated in sediments and bivalves. Fishing boat fuel contains Pb as an anti-knocking material (Parekh *et al*., 2002). The residue of fuel-burning was suspected of entering and contaminating Wedung waters.

The test result on the three heavy metals is shown in Table 1, while the hazard identification in the form of the average of Cd and Pb were shown in Table 2.

The concentration of Cd and Pb in *A. granosa* was under the maximum limit issued according to SNI (Indonesia National Standard) No 7387/2009 and European Council Regulation 2006. Thus, *A. granosa* was still safe to be consumed.

Table 1: Concentration of Cd, Pb, and Hg in *A. granosa* captured from Wedung Coastal Waters, Demak, Indonesia

Content	Heavy metal content on soft tissue (mg.kg^{-1})				Month	Standard **
	A	B	C	D		
Cd	0.64 ±0.18	0.64 ±0.20	0.60 ± 0.02	0.66 ± 0.08	Feb	1.00[1), 2)]
Pb	0.08 ±0.03	0.08 ±0.02	0.05 ±0.00	0.10 ±0.05		1.50[1), 2)]
Hg	Nd	Nd	Nd	Nd		1.00[1), 2)]
Cd	0.62 ±0.05	0.56 ±0.01	0.70 ±0.06	0.66 ±0.13	March	1.00[1), 2)]
Pb	0.09 ±0.03	0.09 ±0.03	0.08 ±0.04	0.06 ±0.01		1.50[1), 2)]
Hg	Nd	Nd	Nd	Nd		1.00[1), 2)]

[1)] SNI 7387: 2009; and [2),] European Council (2006); Nd=Not Detected

Table 2: Hazard identification in form of the average of Cd and Pb

Source	Media Potential Agent	Average Concentration of Hazard Agent (mg.kg^{-1})	
		Cd	Pb
Heavy metal Contamination on waters	*Anadara granosa*	0.64	0.08

Table 3: Sampling of the average consumption and frequency of exposure *A. granosa* on Wedung Resident in 2019 (N = 400 persons)

Group of Age and Profession	Number of Sample	Average of Consumption g/ day	Average of Age	Average of Weight (kg)	Frequency of Exposure *A. granosa* in one year
Children	**31.00**	**8.96**	**10.84**	**31.13**	**52.84**
Toddler	4.00	3.72	3.50	12.75	39.00
Student	27.00	9.74	11.93	33.85	54.89
Adult	**369.00**	**7.30**	**39.35**	**59.40**	**27.87**
House wife	98.00	5.17	40.78	57.17	22.67
Fisherman	76.00	9.38	44.41	62.00	32.61
Trader	22.00	7.17	36.41	61.73	41.09
Student	23.00	6.23	19.91	47.48	17.52
Village Officials	31.00	5.54	41.52	63.16	28.39
Salt Farmers	23.00	12.73	45.39	61.30	48.61
Farmer	18.00	17.54	45.89	57.44	29.83
Civil Servant	2.00	4.14	32.50	60.00	18.00
Private employee	35.00	4.79	35.97	59.40	20.60
Others	41.00	5.31	34.34	62.27	24.02
Grand Total	**400.00**	**7.43**	**37.14**	**57.21**	**29.81**

Health Risk Analysis

The analysis result on collected questionaries showed that the intake rate of *A. granosa* was 8.90 g/day for children and 7.30 g/day for adults. The annual exposure frequency for children was 53 times a year, while for adults were 28 times a year. The average body weight of children in Wedung was 31.13 kg, while adults averaged in 59.40 kg. The complete data of *A.granosa* exposure to the residents in 2019 can be seen in Table 3.

Using equation (2), the CDI value for Cd was 0.026 mg/kg bw/day for children and 0.006 mg/kg bw/day for adults. While for Pb, the CDI value was 33E7 mg/kg bw/day for children and were 75E7 mg/kg bw/day for adults.

HQ analysis was performed using equation (1). The RfD value for Cd was 0.001 mg/kg-day (EPA 2018), while Pb was 0.0035 mg/kg-day (Khan *et al.*, 2008). From this equation, the HQ value of Cd for children and adults was 26.5 (> 1). The HQ value of Pb was 0.9 for children and 0.2 for adults. Similar results were also obtained by Soegianto et al. (2020), where the HQ index for Cd contained in blood mussels from the coastal waters of East Java, Indonesia was > 1, which indicates that these metals have the potential to cause non-carcinogenic for consumers.

The hazard index (HI) of Cd and Pb were 27.4 for children and 26.7 for adults. These HI values were > 1, indicating that consumption of *A. granosa* by Wedung residents has a high health risk.

From the HQ analysis, it was found that Cd content in blood mussel consumed by the residents had a risk to health. Thus, it was necessary to perform risk management. Risk management can be realized by reducing the rate of intake of *A. granosa* into the safe limit using equation (4). By using this equation, the safe consumption of *A. granosa* was 0.33 g/day for children and 1.2 g/day for adults.

Cadmium is a very toxic element. It naturally occurs in the soil; it is also spread to the environment through human activities and can eventually enter the human body through the food chain. Food is the primary access to Cd exposure in humans (Hosseini *et al.*, 2015). Exposure to cadmium could risk human health. The impact of cadmium has been reported to be the cause of "Itai-itai" disease since 1960 (Pan *et al.*, 1960). Cadmium is also carcinogenic on the prostate (Tallaa *et al.*, 2007).

Conclusion

Hg content was not detected in the soft tissue of *A. granosa* collected from Wedung waters, while, Cd and Pb content were detected in the soft tissue of *A. granosa* with an average value of 0.64 mg/kg for Cd and 0.08 mg/kg for Pb, respectively. The HQ value of Pb from *A. granosa* was < 1. Thus, it does not risk the resident's health. On the other hand, the HQ value of Cd reached 26.5 (HQ > 1). Therefore, it can risk the resident's health. The safe intake of *A. granosa* would be 0.33 g/day for children and 1.2 g/day for adults.

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Hasil Revisi Manuscript No 13 (Artikel C2) oleh Author

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 emphasized 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 fro m 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 environmentalchanges and pollution. One of the critical areas of globalconcern is the increasing levels of pollution in urban areas, and it is one of the significant pressure on coastal and marine environments, especially heavy metal pollution (UNEP, 2017). Heavy metal waste require special attention, given its characteristics cannot be degraded by microorganisms; therefore, its availabilityin the environment can harm marine flora and fauna.

Seafood products (such as mussels) contaminatedby heavy metals and consumed by humans are atransfer of heavy metals from the lower food chaintowards the higher one. The present study enhances theknowledge 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 assessthe quality of seawaters. Study of metal residues inbivalves at several places has been realised, i.e., *Mytilus galloprovincialis* (Seguin et al., 2016); *Mytilus edulis* (Liu and Wang, 2015); *Perna viridis* (Liu andWang, 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 fromGresik coastal waters, Indonesia (Soegianto et al., 2010),heavy metals (Cd, Pb, Cu, Zn) residues in bivalvescaptured from northern coast of Central Java (Yuliantoet al., 2019). Heavy metals (Cd, Pb, Zn, Hg, Cu, andCr) are also reported in the blood mussels, *Anadaragranosa* (Soegianto et al., 2020). These studies showedan increase in bivalve's contaminated with heavy metals on the northern coast of Java.

Considering green mussel 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 withheavy metals and health risk assessments in the interestof 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 byhand, 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 stationsand its coordinate are shown in Figure 1 dan Table 1.

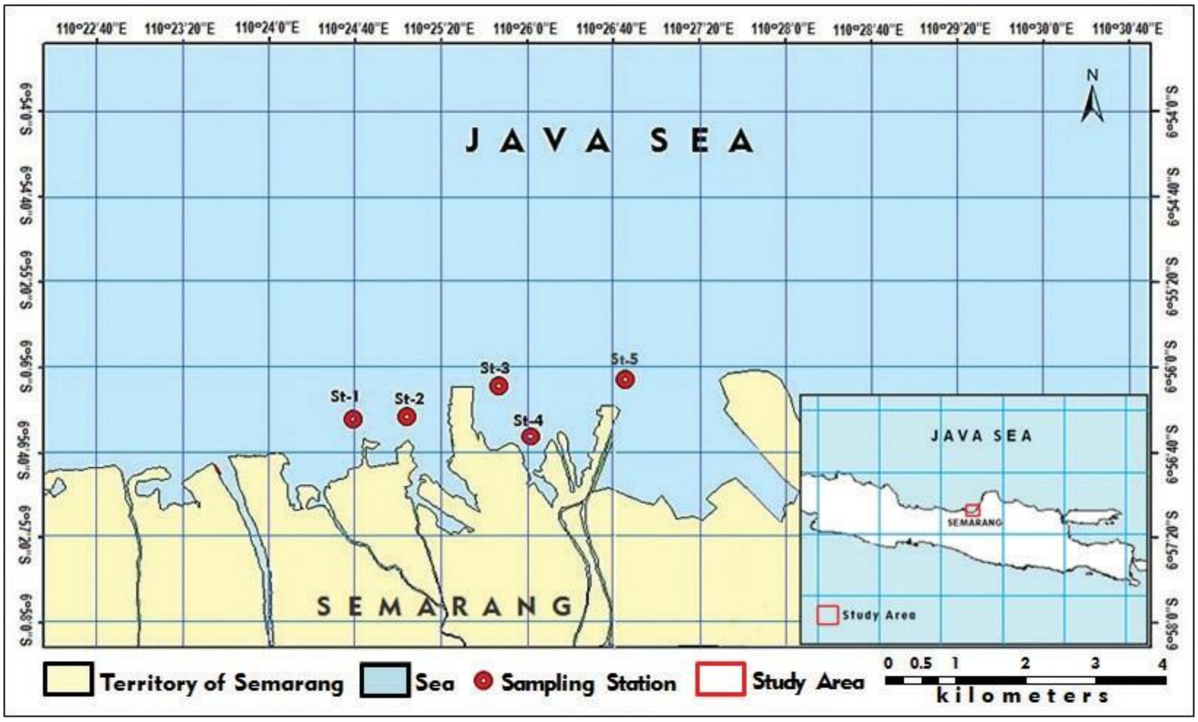

Figure 1: Research stations at Semarang coastal waters, Central Java, Indonesia.

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 mortar. Then the results were sifted with a mesh sizeof 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 heatedat 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 mlvolumetric 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).

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

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 anappreciable 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):

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

C is heavy metal concentration (mg/kg). IR = intakerate 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 6years 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):

$$HI = HQ_{Cd} + HQ_{Pb} + HQ_{Cu} + HQ_{Zn} \ldots\ldots(3)$$

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.

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

Results and Discussion

The Concentration of Cd, Pb, Cu, Zn in the SoftTissue 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 ingreen 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 inoysters and mussels (NOAA, 1998). PTES has a higherPb in seawater because it is a passenger/trading port. Ship fuel contains Pb as an anti-knocking agent (Parekhet al., 2002). Fuel combustion residues are suspected of penetrating and polluting marine seawater.

Health Risk Analysis and Safe Consumption ofGreen 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 $days.year^{-1}$ (for children) and 28 $days.year^{-1}$ (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 willexperience some form of dangerous health effects (Table2). 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 potential causes for non-carcinogenic threats in consumers.

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	Cd	November 2017	0.025-0.039	0.0334±0.0052	0.10 mg/kg[1]
Semarang (PTES)		December 2017	0.027-0.038	0.0311±0.0041	1.0 mg/kg[2]
St-1: - 6°56′24.6″;	Cu	December 2016	0.893-1.788	1.398±0.383	20 mg/kg[3]
110°24′35.8″		February 2017	1.194-2.760	2.294±0.274	
St-2: - 6°56′28.9″;	Pb	December 2016	1.924-2.670	2.278±0.150	0.20 mg/kg[1]
110°24′58.2″					1.50 mg/kg[2]
		February 2017	1.878-2.765	2.268±0.293	
	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	Cd	November 2017	0.014-0.042	0.02501±0.0099	0.10 mg/kg[1]

PowerPlant Semarang		December 2017	0.013-0.034	0.0215±0.0069	1.0 mg/kg[2]
(IPPS)	Pb	December 2016	0.324-0.534	0.406±0.059	0.20 mg/kg[1]
St-3: - 6°56′21.2″;		February 2017	0.537-0.759	0.603±0.066	1.50 mg/kg[2]
110°25′39.4″	Cu	December 2016	0.621-1.744	1.094±0.353	20 mg/kg[3]
St-4: - 6°56′29.5″;		February 2017	1.635-2.631	2.007±0.269	
110°26′10.5″	Zn	December 2016	12.976-22.638	17.778±4.271	100 mg/kg[1]
St-5: - 6°56′14.5″;		February 2017	14.851-31.115	23.434±5.271	
110°26′44.8″					

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

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 dueto metals exposure via green mussels consumption should be focussed upon as children are more sensitiveto 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 allstudy sites. Risk management effort may be realized by reducing the consumption rate of *P. viridis* untilthe 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^{-1}, respectively (for residents living in PTES), and 1.244 and 4.4944 g.day^{-1} (for residents living in OIPPS) (Table 2). Depuration technology is needed to reduce the levelsof 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*	*concentration (mg/kg)*	*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 occurredin Pb content in green mussels; the HQ > 1 for allstudy 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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13. Komunikasi Corresponding Author dengan Editor AJWEP tentang kapan menerima proof artikel

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From: Agoes Soegianto <agoes_soegianto@fst.unair.ac.id>
Date: Thu, Jun 4, 2020 at 11:16 PM
Subject: Re: Selected papers from ICOBIODIV for publication in AJWEP
To: Capital Books <capitalb@capital-publishing.com>

Dear Mr. Raj D Mirchandani
On behalf of all authors, we would like to obtain the progress of our papers are scheduled to be published in AJWEP Vol. 17, No. 3, 2020.
When will we receive the proofs of the papers?
Thank you in advance for your kind attention.
Best regards,

Prof. Agoes Soegianto
Dept. Biology, Airlangga University
Kampus C, Jl. Mulyorejo, Surabaya 60115
INDONESIA

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14. Komunikasi Editor ke Corresponding author : Jawaban Editor atas email dari Corresponding author

Prof. Agoes Soegianto
Dept. Biology, Airlangga University
Kampus C, Jl. Mulyorejo, Surabaya 60115
INDONESIA

From: Capital Books <capitalb@capital-publishing.com>
Date: Fri, Jun 5, 2020 at 2:24 PM
Subject: RE: Selected papers from ICOBIODIV for publication in AJWEP
To: Agoes Soegianto <agoes_soegianto@fst.unair.ac.id>
Cc: Capital Books <capitalb@capital-publishing.com>

Dear Prof. Agoes Soegianto,
Your papers are being taken up for publication in our July, 2020 issue.
The proofs of the papers will be sent to you around the middle of June.
Regards,
Raj D Mirchandani

From: Agoes Soegianto [mailto:agoes_soegianto@fst.unair.ac.id]
Sent: 04 June 2020 21:47

15. Komunikasi Corresponding author ke Editor: Ucapan terima kasih atas update informasi dari Editor

From: Agoes Soegianto [mailto:agoes_soegianto@fst.unair.ac.id]
Sent: 05 June 2020 13:04
To: Capital Books
Subject: Re: Selected papers from ICOBIODIV for publication in AJWEP

Thank you very much for the update.

•••

Prof. Agoes Soegianto
Dept. Biology, Airlangga University
Kampus C, Jl. Mulyorejo, Surabaya 60115
INDONESIA

16. Komunikasi Editor ke Corresponding author : update informasi dari Editor mengenai tahapan copy-editing dari artikel untuk dipublikasikan pada issue sekarang dari jurnal AJWEP

Prof. Agoes Soegianto
Dept. Biology, Airlangga University
Kampus C, Jl. Mulyorejo, Surabaya 60115
INDONESIA

From: **Capital Books** <capitalb@capital-publishing.com>
Date: Thu, Jul 9, 2020 at 3:31 AM
Subject: RE: Selected papers from ICOBIODIV for publication in AJWEP
To: Agoes Soegianto <agoes_soegianto@fst.unair.ac.id>
Cc: Capital Books <capitalb@capital-publishing.com>

Dear Prof. Agoes Soegianto,

We are working towards copy editing and typesetting of your 15 papers to be published in the current issue of our Journal.

By separate email attachments, we are attaching eight papers to begin with and the balance papers will be also forwarded in a day or two. You are requested to go through all the papers thoroughly and clear the queries marked to the authors therein. Please do not make any additions at this stage. There are some markings made by us to the typesetter and you need not worry about those. They will take care of it in the final pdf.

Please note that the DOI number is not the final one and we will be putting the final number in the final pdf.

The receiving date for all the papers would be February 2, 2020 and the revised and accepted date would be June 5, 2020.

Look forward receiving the corrected papers from you at the earliest.

The current issue has got slightly delayed in view of the pandemic situation.

Regards,

Raj D Mirchandani

Hasil copy-editing dari Editor untuk 2 (dua) manuscript adalah sebagai berikut

Copy-editing dan Final Review dari Editor untuk Artikel C1

Asian Journal of Water, Environment and Pollution, Vol. 17, No. 3 (2020), pp. 19-24.
DOI 10.3233/AJW200030

Health Risk Analysis of Cd, Pb and Hg in Blood Mussel (*Anadara granosa*) from Demak, Central Java, Indonesia

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Received ...; revised and accepted ....

Abstract: The famous location of Wedung waters, Demak, Central Java, Indonesia, produces blood mussel, *Anadara granosa*. Anthropogenic activities can lead to contamination of heavy metals such as Pb, Cd and Hg to the living environment of *A. granosa*. This study was done to analyse heavy metals content in the soft tissue of *A. granosa* and health risks arising to Wedung residents from consuming the mussels. Heavy metals were analysed using *atomic absorption spectrometry* (AAS). The result showed that Cd and Pb contents were found in *A. granosa* soft tissue in the range of 0.56 - 0.70 mg/kg for Cd, 0.05 - 0.10 mg/kg for Pb and Hg was not detected. A health risk analysis showed that the HQ value for Pb from *A. granosa* intake was 0.0 <1, so that it was not at risk; whereas for Cd, the value reached 26.5 (HQ > 1). Thus, there was a noticeable health risk for the residents after consuming the mussel. The safety limit in consuming *A. granosa* was 0.33 g/day for children and 1.2 g/day for adults.

Key words: Cd, Pb, Hg, health risk analysis.

Introduction

Blood mussel (*Anadara granosa*) is one of main types of shellfish and is an important source of protein in Southeast Asian countries (Yunus et al., 2014). *Anadara granosa* has an affordable price and can be developed as a source of protein and minerals consumed by the residents (Lindawaty et al., 2016). In 2015, shellfish production in Indonesia reached 59,613 tons and 85.26% of that was *A. granosa* (Ministry of Maritime Affairs and Fisheries, 2015).

A. granosa is a type of bivalve that contributes to the commercial fisheries in the Wedung district, the Demak Regency (Brotohadikusumo, 1994). Activities of Wedung residents, who are mostly farmers, fishermen and housewives, could lead to the entrance of heavy metals of Cd, Pb and Hg to Wedung waters. Agricultural activities such as the use of pesticides and fertilizers and also the runoff from these activities contributed to the increase of heavy metals content in water and sediment (Ansari et al., 2004; Farejiya and Dikhsit, 2016; Soegianto et al., 2010). Garcia et al. (1996) stated that fertilizers and pesticides used in agriculture contained Cd and Pb. Household activities and burning of fossil fuels caused environmental pollution of mercury (Hg) that eventually settled into water or land (USEPA, 2017). Port activities such as painting could spread Cd contamination. Cd is a dangerous heavy metal since it

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can increase the risk of blood vessel disorders (Tuner, 2010). The consumption of blood mussels by the people of Wedung and its surroundings is a way of exposure to various heavy metals contained in the mussels. The level of shellfish consumption, the concentration of heavy metals in shellfish, as well as the duration of exposure, are determinants of health risk hazards.

This study is aimed to analyse contamination of heavy metals Cd, Pb and Hg in *A. granosa* and their impact on the health risk of Wedung residents.

Materials and Methods

Sampling Location

Samples of *A. granosa* were collected from four stations that were chosen using *a purposive sampling* method. The criteria used to determine the stations were ~~are~~ they have a significant population of *A. granosa* placed near to the mainland that was considered as the source of contamination. The coordinates of the four stations were as follows:

(1) Station A was located at 6°44’39.20”S - 110°33’4.37”E
(2) Station B was located at 6°44’57.29”S - 110°33’17.02”E
(3) Station C was located at 6°46’1.84”S - 110°32’21.60”E, dan
(4) Station D was located at 6°47’14.36”S - 110°33’25.79”E.

The four locations can be seen in Figure 1.

Anadara granosa Collection

A group of samples of *A. granosa* with a total of 100 was collected from each station in February and March 2019. Thus, the total number of samples collected was 400. It was stored in *polyethylene* plastics and put in an iced *coolbox* to keep its maximum temperature of 4°C during transportation. Before getting analyzed, the samples were stored at a temperature below -20ºC (Kumar *et al.,* 2011).

Heavy Metals Analysis

An analysis of Cd and Pb content in *A. granosa* was carried according to SNI 2354.5: 2011. Five grams of samples were destroyed under temperature that was increased gradually by 100°C every 30 minutes until reaching a temperature of 450 °C in 18 hours. Then, 1 ml of 65% HNO_3 was added to the sample. It was evaporated on a hot plate at 100°C until dry. The sample was again destroyed under the temperature of 450°C for 3 hours. Next, 5 ml of 6 M HCl was added to the sample and evaporated on a hot plate at 100°C until dry. Ten milliliters of 0.1 M HNO_3 were then added to the dried sample, and it was put in a measuring flask

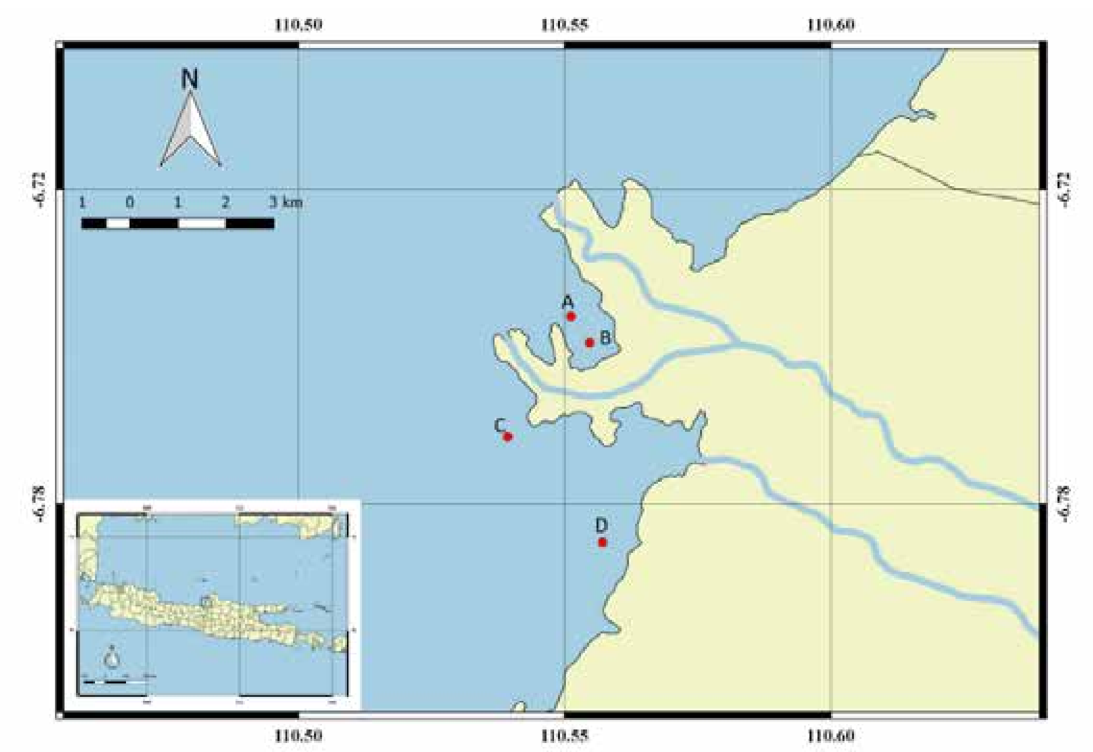

Figure 1: Sampling Location

50 ml. Further, 0.1 M HNO_3 solution was added to the sample until reaching the mark. Analysis of heavy metals was continued by reading the sample solution using AAS with a wavelength of 228.8 mm for Cd and 283.3 mm for Pb.

An analysis of Hg content was carried out according to SNI 2354.6: 2016. Five grams of sample was added with 3 - 5 boiling stones and 10 - 20 mg V_2O_5. Ten ml HNO_3 65% and 10 ml H_2SO_4 95 - 97% were then added to the sample, respectively. The sample was then heated until it turned to a yellowish-brown solution. Rinse the sample with 15 ml of deionized water. Add two drops of 30% H_2O_2 through the top end of the cooler, then rinse. The solution was then transferred to a 100 ml measuring flask and then set with deionized water. The solution was read using AAS with a wavelength of 253.7 nm. *R*-value for all heavy metal test is set to > 0.995.

Collection of Consumption Data

To measure the health risk of Wedung residents in correlation with *A. granosa* consumption, interviews were conducted for 400 people in Wedung using a prepared questionnaire. The questionnaire contained questions about daily intake, frequency of exposure, age and weight dan resident's ways in consuming mussels.

Health Risk Analysis

Non-carcinogenic health risk caused by Cd, Pb and Hg to the residents who consume blood mussels was estimated by HQ value according to EPA A.S. using formula (1).

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

Chronic *Daily Intake (CDI)* was calculated using equation (2) based on (U.S. EPA 1989, 1991):

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

where C is heavy metal concentration; IR is intake rate or the weight of blood mussels consumed per day; Ef is exposure frequency; ED is exposure duration (6 years for kid and 30 years for adult); ~~BW~~ is bodyweight; and AT is average time (6 years x 365 days for kid and 30 years x 365 days for adult).

HQ > 1 indicates a significant non-carcinogenic health risk. Hazard index (HI) caused by Cd, Pb dan Hg were calculated using equation (3) :

$$HI = HQ_{cd} + HQ_{pb} + HQ_{Hg} \quad (3)$$

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.

$$\text{A safe daily intake} = \frac{Rfd \times BW \times AT}{C \times EF \times ED} \quad (4)$$

Result and Discusion

Heavy Metals in *A. granosa*

Cd and Pb were found in the soft tissue of *A. granosa* in the range of 0.56 – 0.70 mg/kg for Cd and 0.05 - 0.10 mg/kg for Pb, while Hg was not detected in *A. granosa*. Those heavy metals spread into waters through agricultural activities such as the use of fertilizers, pesticides dan herbicides. Garcia *et al.* (1996) stated that fertilizers and pesticides used in agricultural activities contained Cd and Pb. It was supported by Lias ~~*et al.*~~ (2013) that stated that pesticides and herbicidescontaining Pb and arsenic polluted the ocean, and both were accumulated in sediments and bivalves. Fishing boat fuel contains Pb as an anti-knocking material (Parekh *et al.,* 2002). The residue of fuel-burning was suspected of entering and contaminating Wedung waters.

The test result on the three heavy metals is shown in Table 1, while the hazard identification in the form of the average of Cd and Pb are shown in Table 2.

The concentration of Cd and Pb in *A. granosa* was under the maximum limit issued according to SNI (Indonesia National Standard) No 7387/2009 and European Council Regulation 2006. Thus, *A. granosa* was still safe to be consumed.

Health Risk Analysis

The analysis result on collected questionnaires showed that the intake rate of *A. granosa* was 8.90 g/day for children and 7.30 g/day for adults. The annual exposure frequency for children was 53 times a year, while for adults were 28 times a year. The average body weight of children in Wedung was 31.13 kg, while adults averaged in 59.40 kg. The complete data of *A.granosa* exposure to the residents in 2019 can be seen in Table 3.

Using equation (2), the CDI value for Cd was 0.026 mg/kg bw/day for children and 0.006 mg/kg bw/day for adults. While for Pb, the CDI value was 33E7 mg/kg bw/day for children and 75E7 mg/kg bw/day for adults.

HQ analysis was performed using equation (1). The RfD value for Cd was 0.001 mg/kg-day (EPA 2018), while Pb was 0.0035 mg/kg-day (Khan *et al.,* 2008).

Table 1: Concentration of Cd, Pb, and Hg in *A. granosa* captured from Wedung Coastal Waters, Demak, Indonesia

Content	*Heavy metal content on soft tissue (mg.kg^{-1})*				*Month*	*Standard ***
	A	*B*	*C*	*D*		
Cd	0.64 ±0.18	0.64 ±0.20	0.60 ± 0.02	0.66 ± 0.08	Feb	1.00[1), 2)]
Pb	0.08 ±0.03	0.08 ±0.02	0.05 ±0.00	0.10 ±0.05		1.50[1), 2)]
Hg	Nd	Nd	Nd	Nd		1.00[1), 2)]
Cd	0.62 ±0.05	0.56 ±0.01	0.70 ±0.06	0.66 ±0.13	March	1.00[1), 2)]
Pb	0.09 ±0.03	0.09 ±0.03	0.08 ±0.04	0.06 ±0.01		1.50[1), 2)]
Hg	Nd	Nd	Nd	Nd		1.00[1), 2)]

(1) SNI 7387: 2009; and [(2),] European Council (2006); Nd =Not Detected

Table 2: Hazard identification in form of the average of Cd and Pb

Source	*Media potential agent*	*Average concentration of hazard Agent (mg.kg^{-1})*	
		Cd	*Pb*
Heavy metal Contamination on waters	Anadara granosa	0.64	0.08

Table 3: Sampling of the average consumption and frequency of exposure *A. granosa* on Wedung Resident in 2019 (N = 400 persons)

Group of Age and Profession	*Number of Sampel*	*Average of Consumption g/day*	*Average of Age*	*Average of Weight (kg)*	*Frequency of Exposure A. granosa in one year*
Children	**31.00**	**8.96**	**10.84**	31.13	52.84
Toddler	4.00	3.72	3.50	12.75	39.00
Student	27.00	9.74	11.93	33.85	54.89
Adult	**369.00**	**7.30**	**39.35**	**59.40**	**27.87**
House wife	98.00	5.17	40.78	57.17	22.67
Fisherman	76.00	9.38	44.41	62.00	32.61
Trader	22.00	7.17	36.41	61.73	41.09
Student	23.00	6.23	19.91	47.48	17.52
Village Officials	31.00	5.54	41.52	63.16	28.39
Salt Farmers	23.00	12.73	45.39	61.30	48.61
Farmer	18.00	17.54	45.89	57.44	29.83
Civil Servant	2.00	4.14	32.50	60.00	18.00
Private employee	35.00	4.79	35.97	59.40	20.60
Others	41.00	5.31	34.34	62.27	24.02
Grand Total	**400.00**	**7.43**	**37.14**	**57.21**	**29.81**

From this equation, the HQ value of Cd for children and adults was 26.5 (> 1). The HQ value of Pb was 0.9 for children and 0.2 for adults. Similar results were also obtained by Soegianto et al. (2020), where the HQ index for Cd contained in blood mussels from the coastal waters of East Java, Indonesia was > 1, which indicates that these metals have the potential to cause non-carcinogenic health risk to the consumers.

The hazard index (HI) of Cd and Pb were 27.4 for children and 26.7 for adults. These HI values were > 1, indicating that consumption of *A. granosa* by Wedung residents has a high health risk.

From the HQ analysis, it was found that Cd content in blood mussel consumed by the residents had a risk to health. Thus, it was necessary to perform risk management. Risk management can be realized by reducing the rate of intake of *A. granosa* into the safe limit using equation (4). By using this equation, the safe consumption of *A. granosa* was 0.33 g/day for children and 1.2 g/day for adults.

Cadmium is a very toxic element. It naturally occurs in the soil and spreads in the environment through human activities and can eventually enter the human body through the food chain. Food is the primary access to Cd exposure in humans (Hosseini *et al.,* 2015). Exposure to cadmium could risk human health. The impact of cadmium has been reported to be the cause of "Itai-itai" disease since 1960 (Pan *et al.,* 1960). Cadmium is also carcinogenic for the prostate gland (Tallaa *et al.,* 2007).

Conclusion

Hg content was not detected in the soft tissue of *A. granosa* collected from Wedung waters while Cd and Pb contents were detected in the soft tissue of *A. granosa,* with an average value of 0.64 mg/kg for Cd and 0.08 mg/kg for Pb, respectively. The HQ value of Pb from *A. granosa* was < 1. Thus, it does not risk the resident's health. On the other hand, the HQ value of Cd reached 26.5 (HQ > 1); therefore, it can risk the resident's health. The safe intake of *A. granosa* would be 0.33 g/day for children and 1.2 g/day for adults.

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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 urbanization. This study emphasized on 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 consumed by the local people as a source of animal protein. Therefore, keeping the mussels from a wide range of contaminants, including heavy metals, has become an essential factor in 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 – 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 of the metals contaminated green mussels had deleterious health risks to children living in the Semarang coastal areas. The HQ value <1 occurred for adults consumed Cd-, Cu-, and Zn-contaminated green mussels. A particular case occurred in Pb-contaminating green mussels, which showed the HQ values > 1 in allover 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

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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 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 consumed by humans are a transfer form of heavy metals from the lower food chain towards the higher one. This 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 realized, 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 realized 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) in the blood mussels, *Anadara granosa* (Soegianto et al., 2020). These studies showed an increase in the bivalve's contaminated heavy metals on the northern coast of Java.

Considering green mussels is a source of protein and widely consumed by Indonesian people, abundant and low prices; therefore, it is necessary to carry out monitoring green mussels contaminated 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 due to their lives stick to the hard material. Samples were kept in a coolbox at a temperature retained at about 4° C before the transport to the laboratory. The number of mussel samples for each station was between 100 to 250 mussels. The sample stations and its coordinate are shown in Figure 1 and Table 1.

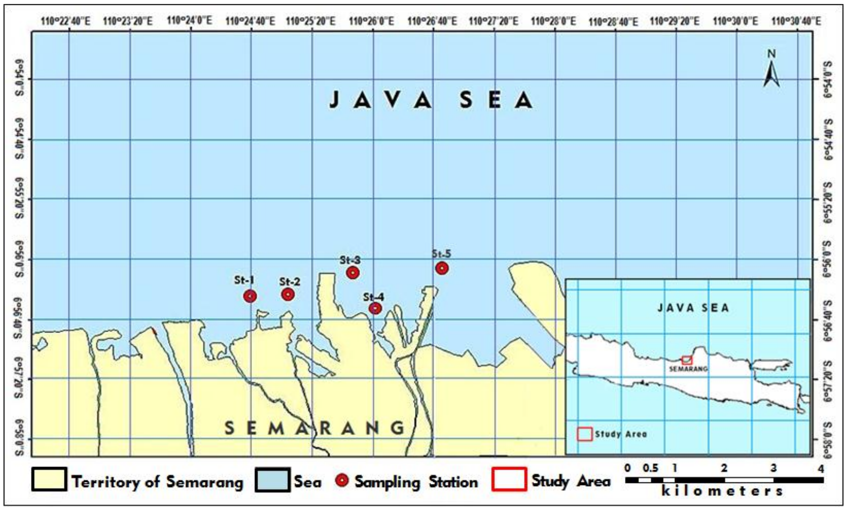

Figure 1: Research stations at Semarang coastal waters, Central Java, Indonesia

Metals Analyses

Samples of the soft tissues were analyzed for heavy metal content Cd, Pb, Cu, and Zn. Solid samples (soft tissues) were washed, dried, and mashed with mortar. Then the results were sifted with a mesh size of 100 mm and homogenized. The result was weighed as much as 0.5 g and put into the Teflon bomb digester, moistened with splashes of aquatrides, then added 1 ml of concentrated HNO_3. 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 AAS.

Environmental Health Risk Analysis

To analyze the health risk of habitant of Semarang coastal waters concerning green mussel consumption interview was conducted to 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 CDI (Chronic Daily Intake), HQ (Hazard Quotient), and HI (Hazard Index) 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).

$$HQ = \frac{CDI}{RfD} \text{ .... (1)}$$

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. RfD (Reference Dose) 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 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):

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

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 dan 6 years for children) (years). BW is body weight (kg), and AT is the average time (30 years x 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, dan Zn) was calculated using equation (3):

$$HI = HQ_{Cd} + HQ_{Pb} + HQ_{Cu} + HQ_{Zn} \ldots (3).$$

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

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

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 Indonesia 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 exceeded the MAL (Table 1). High Pb concentrations are evident in the association of high levels with urban areas near cities with many human activities as like as 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-, Zn-contaminated green mussels were > 1 for children at all study locations (Table 2), which means the non-carcinogenic health risks will threaten. Contrary, the adults will not be 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, both for children and adults, which will cause 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 potential causes non-carcinogenic for consumers.

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) **St-1:** - 6°56'24.6"; 110°24'35.8" **St-2:** - 6°56'28.9"; 110°24'58.2"	Cd	November 2017	0.025 - 0.039	0.0334±0.0052	0.10 mg/kg[1)] 1.0 mg/kg[2)]
		December 2017	0.027 - 0.038	0.0311±0.0041	
	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)]; 1.50 mg/kg [2)]
		February 2017	1.878-2.765	2.268±0.293	
	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) **St-3:** - 6°56'21.2"; 110°25'39.4" **St-4:** - 6°56'29.5"; 110°26'10.5" **St-5:** - 6°56'14.5"; 110°26'44.8"	Cd	November 2017	0.014-0.042	0.02501±0.0099	0.10 mg/kg[1)] 1.0 mg/kg[2)]
		December 2017	0.013-0.034	0.0215±0.0069	
	Pb	December 2016	0.324-0.534	0.406±0.059	0.20 mg/kg [1)]; 1.50 mg/kg [2)]
		February 2017	0.537-0.759	0.603±0.066	
	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

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

HI > 1 indicated that consumption of *P. viridis* in Semarang coastal residents has a high health risk (Table 3). It was necessary to perform risk management for all study sites. Risk management effort may be realized 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). Reduce metals contained in mussels also a reasonable way to protect public health by the use of depuration technology for bivalves landed from fishing vessels before to be marketed.

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 the green mussels *P. viridis* captured from Semarang coastal waters were contaminated the heavy metals (Cd, Pb, Cu, Zn) in their soft tissues. Their concentrations have not exceeded the Maximum Acceptable Limits (MAL) yet, except for Pb has surpassed the value of MAL.

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

The HI values for all metals > 1, which indicated that consumption of *P. viridis* at 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 safe limit.

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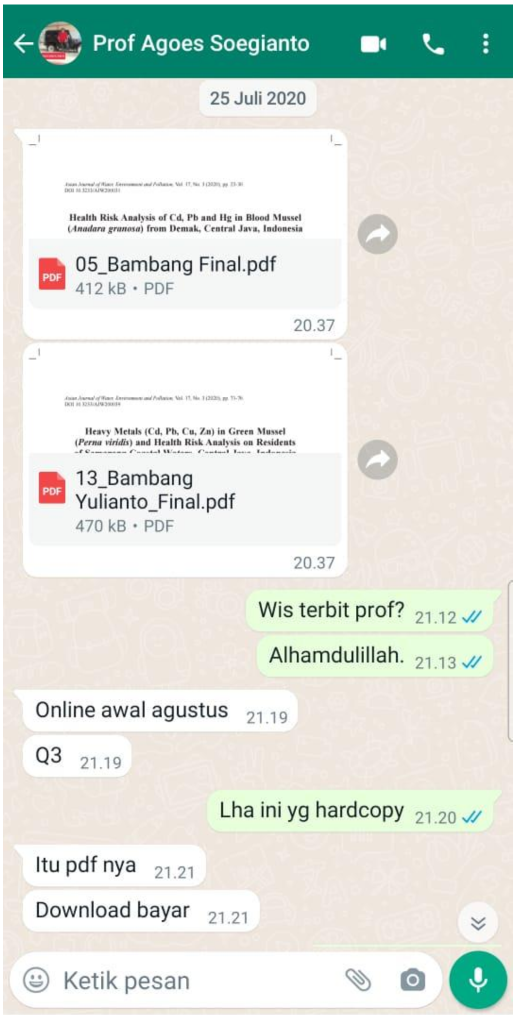

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Health Risk Analysis of Cd, Pb and Hg in Blood Mussel (*Anadara granosa*) from Demak, Central Java, Indonesia

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Abstract: The famous location of Wedung waters, Demak, Central Java, Indonesia, produces blood mussel, *Anadara granosa*. Anthropogenic activities can lead to contamination of heavy metals such as Pb, Cd and Hg to the living environment of *A. granosa*. This study was done to analyse heavy metals content in the soft tissue of *A. granosa* and health risks arising to Wedung residents from consuming the mussels. Heavy metals were analysed using *atomic absorption spectrometry* (AAS). The result showed that Cd and Pb contents were found in *A. granosa* soft tissue in the range of 0.56 - 0.70 mg/kg for Cd, 0.05 - 0.10 mg/kg for Pb and Hg was not detected. A health risk analysis showed that the HQ value for Pb from *A. granosa* intake was 0.0 <1, so that it was not at risk; whereas for Cd, the value reached 26.5 (HQ > 1). Thus, there was a noticeable health risk for the residents after consuming the mussel. The safety limit in consuming *A. granosa* was 0.33 g/day for children and 1.2 g/day for adults.

Key words: Cd, Pb, Hg, health risk analysis.

Introduction

Blood mussel (*Anadara granosa*) is one of main types of shellfish and is an important source of protein in Southeast Asian countries (Yunus et al., 2014). *Anadara granosa* has an affordable price and can be developed as a source of protein and minerals consumed by the residents (Lindawaty et al., 2016). In 2015, shellfish production in Indonesia reached 59,613 tons and 85.26% of that was *A. granosa* (Ministry of Maritime Affairs and Fisheries, 2015).

A. granosa is a type of bivalve that contributes to the commercial fisheries in the Wedung district, the Demak Regency (Brotohadikusumo, 1994). Activities of Wedung residents, who are mostly farmers, fishermen and housewives, could lead to the entrance of heavy metals of Cd, Pb and Hg to Wedung waters. Agricultural activities such as the use of pesticides and fertilizers and also the runoff from these activities contributed to the increase of heavy metals content in water and sediment (Ansari et al., 2004; Farejiya and Dikhsit, 2016; Soegianto et al., 2010). Garcia et al. (1996) stated that fertilizers and pesticides used in agriculture contained Cd and Pb. Household activities and burning of fossil fuels caused environmental pollution of mercury (Hg) that eventually settled into water or land (USEPA, 2017). Port activities such as painting could spread Cd contamination. Cd is a dangerous heavy metal since it

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can increase the risk of blood vessel disorders (Tuner, 2010). The consumption of blood mussels by the people of Wedung and its surroundings is a way of exposure to various heavy metals contained in the mussels. The level of shellfish consumption, the concentration of heavy metals in shellfish, as well as the duration of exposure, are determinants of health risk hazards.

This study is aimed to analyse contamination of heavy metals Cd, Pb and Hg in *A. granosa* and their impact on the health risk of Wedung residents.

Materials and Methods

Sampling Location

Samples of *A. granosa* were collected from four stations that were chosen using *a purposive sampling* method. The criteria used to determine the stations were they have a significant population of *A. granosa* placed near to the mainland that was considered as the source of contamination. The coordinates of the four stations were as follows:

(1) Station A was located at 6°44′39.20″S – 110°33′4.37″E

(2) Station B was located at 6°44′57.29″S – 110°33′17.02″E

(3) Station C was located at 6°46′1.84″S – 110°32′21.60″E, and

(4) Station D was located at 6°47’14.36″S – 110°33′25.79″E.

The four locations can be seen in Figure 1.

Anadara granosa Collection

A group of samples of *A. granosa* with a total of 100 was collected from each station in February and March 2019. Thus, the total number of samples collected was 400. It was stored in *polyethylene* plastics and put in an iced *coolbox* to keep its maximum temperature of 4°C during transportation. Before getting analyzed, the samples were stored at a temperature below -20ºC (Kumar et al., 2011).

Heavy Metals Analysis

An analysis of Cd and Pb content in *A. granosa* was carried according to SNI 2354.5: 2011. Five grams of samples were destroyed under temperature that was increased gradually by 100 °C every 30 minutes until reaching a temperature of 450 °C in 18 hours. Then, 1 ml of 65% HNO_3 was added to the sample. It was evaporated on a hot plate at 100 °C until dry. The sample was again destroyed under the temperature of 450 °C for 3 hours. Next, 5 ml of 6 M HCl was added to the sample and evaporated on a hot plate at 100 °C until dry. Ten milliliters of 0.1 M HNO_3 were then added to the dried sample, and it was put in a measuring flask

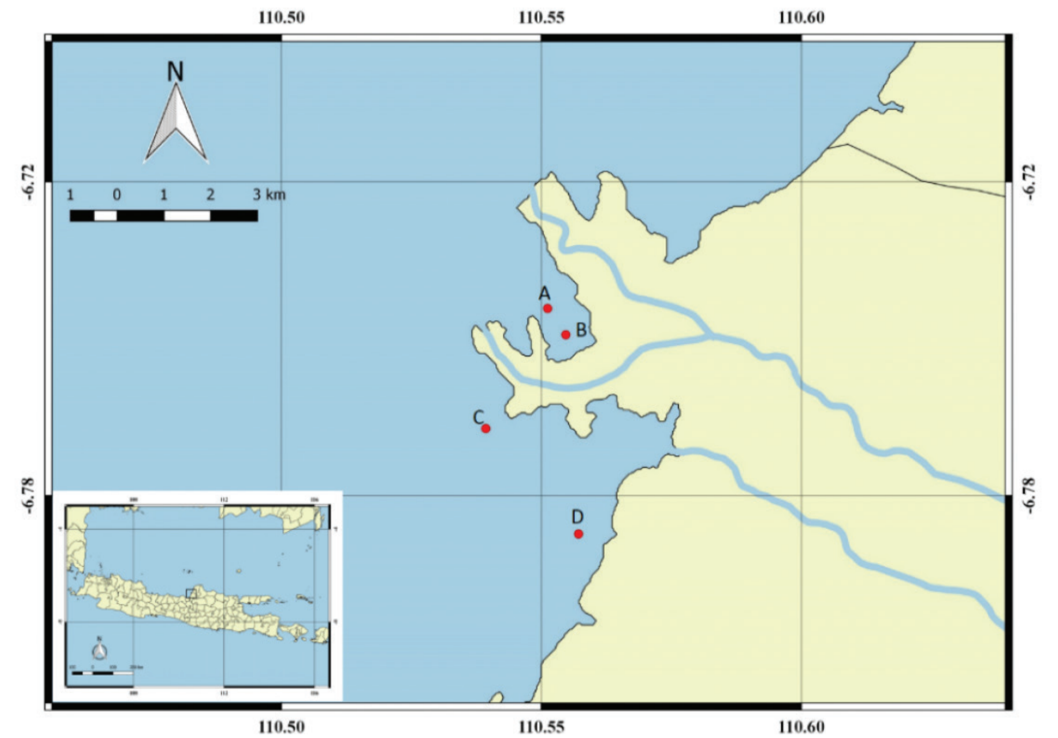

Figure 1: Sampling location.

of 50 ml. Further, 0.1 M HNO_3 solution was added to the sample until reaching the mark. Analysis of heavy metals was continued by reading the sample solution using AAS with a wavelength of 228.8 mm for Cd and 283.3 mm for Pb.

An analysis of Hg content was carried out according to SNI 2354.6: 2016. Five grams of sample was added with 3-5 boiling stones and 10-20 mg V_2O_5. Ten ml HNO_3 65% and 10 ml H_2SO_4 95-97% were then added to the sample, respectively. The sample was then heated until it turned to a yellowish-brown solution. Rinse the sample with 15 ml of deionized water. Add two drops of 30% H_2O_2 through the top end of the cooler, then rinse. The solution was then transferred to a 100 ml measuring flask and then set with deionized water. The solution was read using AAS with a wavelength of 253.7 nm. *R*-value for all heavy metal test is set to > 0.995.

Collection of Consumption Data

To measure the health risk of Wedung residents in correlation with *A. granosa* consumption, interviews were conducted for 400 people in Wedung using a prepared questionnaire. The questionnaire contained questions about daily intake, frequency of exposure, age and weight and resident's ways in consuming mussels.

Health Risk Analysis

Non-carcinogenic health risk caused by Cd, Pb and Hg to the residents who consume blood mussels was estimated by HQ value according to EPA A.S. using formula (1).

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

Chronic Daily Intake (CDI) was calculated using equation (2) based on (U.S. EPA 1989, 1991):

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

where C is heavy metal concentration; IR is intake rate or the weight of blood mussels consumed per day; Ef is exposure frequency; ED is exposure duration (6 years for kid and 30 years for adult); Bw is bodyweight; and AT is average time (6 years x 365 days for kid and 30 years x 365 days for adult).

HQ > 1 indicates a significant non-carcinogenic health risk. Hazard index (HI) caused by Cd, Pb and Hg were calculated using equation (3) :

$$HI = HQ_{cd} + HQ_{pb} + HQ_{Hg} \quad (3)$$

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.

$$\text{A safe daily intake} = \frac{Rfd \times BW \times AT}{C \times EF \times ED} \quad (4)$$

Result and Discusion

Heavy Metals in *A. granosa*

Cd and Pb were found in the soft tissue of *A. granosa* in the range of 0.56-0.70 mg/kg for Cd and 0.05-0.10 mg/kg for Pb, while Hg was not detected in *A. granosa*. Those heavy metals spread into waters through agricultural activities such as the use of fertilizers, pesticides and herbicides. Garcia et al. (1996) stated that fertilizers and pesticides used in agricultural activities contained Cd and Pb. It was supported by Lias et al. (2013) that stated that pesticides and herbicides containing Pb and arsenic polluted the ocean, and both were accumulated in sediments and bivalves. Fishing boat fuel contains Pb as an anti-knocking material (Parekh et al., 2002). The residue of fuel-burning was suspected of entering and contaminating Wedung waters.

The test result on the three heavy metals is shown in Table 1, while the hazard identification in the form of the average of Cd and Pb are shown in Table 2.

The concentration of Cd and Pb in *A. granosa* was under the maximum limit issued according to SNI (Indonesia National Standard) No 7387/2009 and European Council Regulation 2006. Thus, *A. granosa* was still safe to be consumed.

Health Risk Analysis

The analysis result on collected questionnaires showed that the intake rate of *A. granosa* was 8.90 g/day for children and 7.30 g/day for adults. The annual exposure frequency for children was 53 times a year, while for adults were 28 times a year. The average body weight of children in Wedung was 31.13 kg, while adults averaged in 59.40 kg. The complete data of *A.granosa* exposure to the residents in 2019 can be seen in Table 3.

Using equation (2), the CDI value for Cd was 0.026 mg/kg bw/day for children and 0.006 mg/kg bw/day for adults. While for Pb, the CDI value was 33E7 mg/kg bw/day for children and 75E7 mg/kg bw/day for adults.

HQ analysis was performed using equation (1). The RfD value for Cd was 0.001 mg/kg-day (EPA 2018), while Pb was 0.0035 mg/kg-day (Khan et al., 2008).

Table 1: Concentration of Cd, Pb, and Hg in *A. granosa* captured from Wedung Coastal Waters, Demak, Indonesia

Content	*Heavy metal content on soft tissue (mg.kg^{-1})*				*Month*	*Standard***
	A	*B*	*C*	*D*		
Cd	0.64 ± 0.18	0.64 ± 0.20	0.60 ± 0.02	0.66 ± 0.08	Feb	1.00[1,2]
Pb	0.08 ± 0.03	0.08 ± 0.02	0.05 ± 0.00	0.10 ± 0.05		1.50[1,2]
Hg	Nd	Nd	Nd	Nd		1.00[1,2]
Cd	0.62 ± 0.05	0.56 ± 0.01	0.70 ± 0.06	0.66 ± 0.13	March	1.00[1,2]
Pb	0.09 ± 0.03	0.09 ± 0.03	0.08 ± 0.04	0.06 ± 0.01		1.50[1,2]
Hg	Nd	Nd	Nd	Nd		1.00[11,2]

1. SNI 7387: 2009; and 2. European Council (2006); Nd = Not Detected

Table 2: Hazard identification in form of the average of Cd and Pb

Source	*Media potential agent*	*Average concentration of hazard Agent (mg.kg^{-1})*	
		Cd	*Pb*
Heavy metal contamination on waters	*Anadara granosa*	0.64	0.08

Table 3: Sampling of the average consumption and frequency of exposure *A. granosa* on Wedung Resident in 2019 (N = 400 persons)

Group of age and profession	*Number of sample*	*Average of consumption g/day*	*Average of age*	*Average of weight (kg)*	*Frequency of exposure A. granosa in one year*
Children	**31.00**	**8.96**	**10.84**	31.13	52.84
Toddler	4.00	3.72	3.50	12.75	39.00
Student	27.00	9.74	11.93	33.85	54.89
Adult	**369.00**	**7.30**	**39.35**	**59.40**	**27.87**
House wife	98.00	5.17	40.78	57.17	22.67
Fisherman	76.00	9.38	44.41	62.00	32.61
Trader	22.00	7.17	36.41	61.73	41.09
Student	23.00	6.23	19.91	47.48	17.52
Village Officials	31.00	5.54	41.52	63.16	28.39
Salt Farmers	23.00	12.73	45.39	61.30	48.61
Farmer	18.00	17.54	45.89	57.44	29.83
Civil Servant	2.00	4.14	32.50	60.00	18.00
Private employee	35.00	4.79	35.97	59.40	20.60
Others	41.00	5.31	34.34	62.27	24.02
Grand Total	**400.00**	**7.43**	**37.14**	**57.21**	**29.81**

From this equation, the HQ value of Cd for children and adults was 26.5 (> 1). The HQ value of Pb was 0.9 for children and 0.2 for adults. Similar results were also obtained by Soegianto et al. (2020), where the HQ index for Cd contained in blood mussels from the coastal waters of East Java, Indonesia was > 1, which indicates that these metals have the potential to cause non-carcinogenic health risk to the consumers.

The hazard index (HI) of Cd and Pb were 27.4 for children and 26.7 for adults. These HI values were > 1, indicating that consumption of *A. granosa* by Wedung residents has a high health risk.

From the HQ analysis, it was found that Cd content in blood mussel consumed by the residents had a risk to health. Thus, it was necessary to perform risk management. Risk management can be realized by reducing the rate of intake of *A. granosa* into the safe limit using equation (4). By using this equation, the safe consumption of *A. granosa* was 0.33 g/day for children and 1.2 g/day for adults.

Cadmium is a very toxic element. It naturally occurs in the soil and spreads in the environment through human activities and can eventually enter the human body through the food chain. Food is the primary access to Cd exposure in humans (Hosseini et al., 2015). Exposure to cadmium could risk human health. The impact of cadmium has been reported to be the cause of "Itai-itai" disease since 1960 (Pan et al., 1960). Cadmium is also carcinogenic for the prostate gland (Tallaa et al., 2007).

Conclusion

Hg content was not detected in the soft tissue of *A. granosa* collected from Wedung waters while Cd and Pb contents were detected in the soft tissue of *A. granosa,* with an average value of 0.64 mg/kg for Cd and 0.08 mg/kg for Pb, respectively. The HQ value of Pb from *A. granosa* was < 1. Thus, it does not risk the resident's health. On the other hand, the HQ value of Cd reached 26.5 (HQ > 1); therefore, it can risk the resident's health. The safe intake of *A. granosa* would be 0.33 g/day for children and 1.2 g/day for adults.

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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

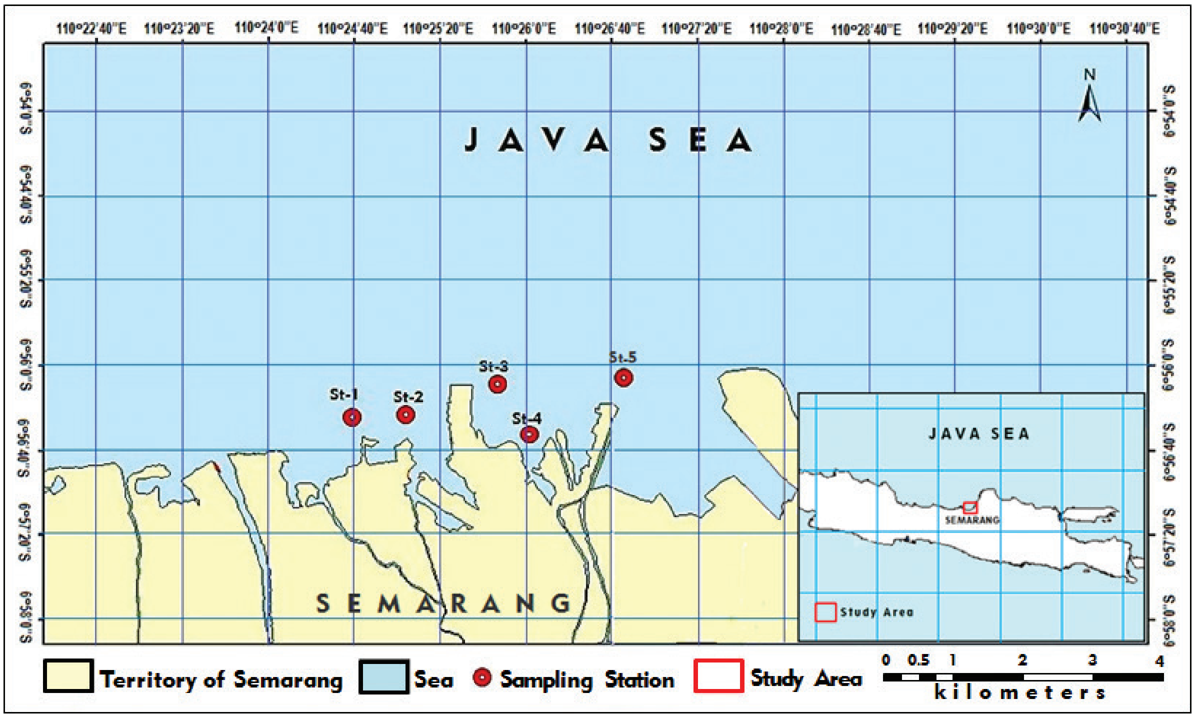

Figure 1: Research stations at Semarang coastal waters, Central Java, Indonesia.

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]
		December 2017	0.027-0.038	0.0311±0.0041	1.0 mg/kg[2]
St-1: - 6°56′24.6″; 110°24′35.8″	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	
St-2: - 6°56′28.9″; 110°24′58.2″	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]
		December 2017	0.013-0.034	0.0215±0.0069	1.0 mg/kg[2]
St-3: - 6°56′21.2″; 110°25′39.4″	Pb	December 2016	0.324-0.534	0.406±0.059	0.20 mg/kg[1]
		February 2017	0.537-0.759	0.603±0.066	1.50 mg/kg[2]
St-4: - 6°56′29.5″; 110°26′10.5″	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	
St-5: - 6°56′14.5″; 110°26′44.8″	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).

$$HQ = \frac{CID}{RfD} \tag{1}$$

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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