



# Health risk assessment of metal accumulated in marine bivalves from Semarang, Indonesia

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**Abstract.** Mussels are a kind of seafood commonly consumed in coastal area such as Semarang, Indonesia. Food safety standards in Indonesia are still adopting international regulation such as WHO, FAO and JECFA. The lack of research data and the information's concerning food processing safety are challenges that need to be overcome. This research aims to determine several parameter of human risk assessment of mussels for As, Pb, Cd, Cu, Se and Hg in Semarang. Samples were analyzed using Mercury Analyzer and ICP-OES. Those parameters are Mean Weekly Intake (MWI), Estimated Daily Intake (EDI), Hazard Quotient and Hazard Index. MWI is appointed by provisional tolerable weekly intake for 60-kg adult established by JECFA. EDI is referring to an average level of mussel consumption in Indonesia of 0.125 kg week<sup>-1</sup>. In comparison with maximum permissible limit (MPLs) of SNI, WHO, FAO and CODEX, Cd concentration in *Amusium pleuronectes* and *Tegillarca granosa* were higher than MPLs. In other fact, mean weekly intake for *A. pleuronectes* and *T. granosa* were 0.2 and 0.9 kg week<sup>-1</sup>. Estimated daily intake for *A. pleuronectes* and *T. granosa* is 0.53 and 1.06 mg kg day<sup>-1</sup>. Consumption of *A. pleuronectes* was noticed to promote higher health risk of As intoxication compared to other bivalves. According to several parameter of food safety, *A. pleuronectes* is most susceptible to health risk for human because of high hazard index. Alternative solution for this risk is to expand a simple guideline to consume bivalvia weekly based on MWI and EDI information.

**Key Words:** heavy metals, *Amusium pleuronectes*, *Tegillarca granosa*, *Perna viridis*, risk assessment.

**Introduction.** The transfer of metal compounds from the abiotic environment to aquatic organisms through the food chain is a concern because of biomagnification (Nfon et al 2009). Semarang, as one of the major coastal cities of Indonesia has a high vulnerability to metal contamination. Metals can enter into land through rivers due to human activities, industrialization and shipping activities. This waste can contain metal contaminants that are biodegradable, and persistent that endanger human health and the marine ecosystem (Javed & Usmani 2016).

Bivalves, as biomonitor agents, has the ability to accumulate metals from its environment and has the ability to reconstruct and monitor spatial and temporal changes since the 1970s (Degger et al 2016). Bivalves also comply the ideal requirements for monitoring contamination because it has a wide distribution, settles, can accumulate pollutants from low to high concentrations, resistant to pollutants, a commercial product and widely consumed in the world, and therefore poses a risk to human health (Yap et al 2016; Wang & Lu 2017). The significance of marine bivalves can be summarized by the following two aspects. First, many of these bivalves are commonly consumed by humans because of protein, essential fatty acids, vitamins and mineral content (Jovic & Stancovic 2014), and the shellfish farming has been an important industry in many countries especially Indonesia (Wang & Lu 2017). Secondly, bivalves are important in coastal and estuarine ecosystems as they control the primary productivity and act as prey food for predators. Although it has many important values, seafood can contain contaminants that are harmful to consumers (Wang & Lu 2017). Inorganic material such as As, Cd, Pb, Hg, Se, Cu, Zn and Fe), organic material (PCBs, dioxins and insecticides) and microbial threat associated seafood can threaten humans (Codex 2017).

Cases of degenerative and chronic diseases such as cancer are increasing throughout the world and one source of them can be from contaminated seafood, nowadays. The main source of metals exposure in humans is through the food web through seafood intake which can increase the level of daily or weekly consumption. Therefore, human health risk assessment is very important to protect human life. Research on this topic in Indonesia is still very limited. Several ways to minimize health issues from bivalve consumption which include protecting the bivalve natural habitat away from heavy metals pollution by strictly enforcing environmental laws and policies, specifying zones for bivalve fisheries, monitoring heavy metals concentration in bivalve on a regular basis and making depuration process a compulsory requirement in every seafood restaurant throughout the country (Denil et al 2017). Alternative point to protect consumer is to provide a simple guideline how to consume bivalves safely through limit consumption approach. Health risk analysis is required in order to determine the possibility of human health risk as a consequence of heavy metals accumulation following the consumption of bivalves.

The objective of the present study was to assess the health risk of metals accumulated in marine bivalves from Semarang, Indonesia. Five parameters used in human health risk assessment were considered, including the comparison of permissible limit with national and international standards, mean weekly consumption (MWI), estimated daily intake (EDI), target hazard quotient (THQ) and total hazard index (HI).

## Material and Method

**Materials.** Three species of marine bivalves (*Amusium pleuronectes*, *Tegillarca granosa* and *Perna viridis*) were collected from Tambak Bayan Market, Semarang on April 2016. Double distilled deionized water (DDDW) were used to clean all samples. All reagents used were of analytical-reagent grade. The solutions were prepared using ultra-pure water (Milli-Q). Full strength Nitric acid (65% HNO<sub>3</sub> Suprapur) (Merck) was used in wet digestion method for metals (non-Hg) analysis. L-Cysteine (Nacalai Tesque Inc., Japan) was used for Hg standard solution.

**Instrumentation.** The analyses were carried out with an Inductively Coupled Plasma Optical Emission Spectrometry/ICP OES (Thermo Fisher Scientific ICP OES 7400, USA) for non-Hg total metals (As, Pb, Cd, Cu and Se) and Direct Mercury Analyzer (NIC MA 3000, Japan) for total Hg. Morphometric analysis was carried out by using digital caliper, ruler and analytical balance (Sartorius BP 210 S, Germany). Samples preparation was carried out with a drying oven (Heraeus Instrument, Germany), petri dish, spatula, scissors, mortar and pestle.

**Sample collection.** All bivalves were cleaned and rinsed with water to remove attached particles and debris, stored in zip lock plastic bags, labeled and placed into an ice box and then freeze until analysis. In the laboratory, bivalves were cleaned, length-weight measured and dissected. Tissues were washed with demineralized water to clean any remaining sand and/or other particles, dehydrated in an oven (60°C during 24h) to constant dry weight and grinded into fine powder for heavy metals analysis. Separated bivalve samples were dried at 105°C; 24h for water content analysis (SNI-01-2354.2-2006).

**Metal analysis.** For mercury analysis, the triplicate dried samples were weighed (0.02–0.04 g) using Sartorius analytical balance. The dry samples were weighed in ceramic sample boat, then direct analyzed using Mercury Analyzer NIC MA-3000.

For the As, Se, Pb, Cd and Cu analysis, ten individuals from each species with same length and weight were selected. The triplicate of tissues were digested using 5 mL HNO<sub>3</sub> and 5 mL of DDDW which were transferred into 12 mL quartz tubes. The mixture was heated for 15 minutes at 185°C, hold for 30 minutes. After mineralization, the samples were diluted to 50 mL with high grade of water, Milli-Q, before analysis with ICP-

OES 7400 Thermo. Percentage of spike recovery as internal quality control is showed in Table 1.

Table 1

Percentage of spike recovery

	<i>As</i>	<i>Cd</i>	<i>Cu</i>	<i>Pb</i>	<i>Se</i>
Recovery (%)	97.85±3.31	84.89±6.99	89.57±11.39	83.34±3.81	94.09±5.32

**Health risk assessment.** All concentration data obtained in this study were converted to wet weight using water content calculations (Yap et al 2016). There are five parameters to determine human health risk assessment in this study. First parameter was to compare with national or international permissible limits. Second parameter was MWI. MWI is the maximum limit of consumption of food based on metal concentration. To calculate MWI, it is important to know the different PTWI values for each metal. PTWI shows the safe level of contaminants to be consumed without dangerous risks. PTWI for Cd, Cu, Hg is 0.0063, 3.50 and 0.004 mg week kg-bodyweight<sup>-1</sup> respectively (JECFA 2010). Thus, PTWI for adults with a weight of 60 kg is 0.42, 210 and 0.24 mg week<sup>-1</sup> for Cd, Cu and Hg, respectively and MWI values can be determined. The third parameter was EDI. The EDI is an estimated daily intake to determine the safe limits of daily consumption of damage without causing harmful health effects. According to (Jovic & Stankovic 2014), there are two categories of bivalve consumers, namely ALM, groups with bivalves consumption levels of 0.125 kg week<sup>-1</sup> or 17.86 g day<sup>-1</sup> and HLM, groups that have a consumption rate of 0.250 kg week<sup>-1</sup> or 35.71 g day<sup>-1</sup>. For the present study, the first category (ALM) was used based on the assumption that the Semarang community has a low consumption rate. EDI was calculated by equation:

$$EDI = (Mc \times \text{Consumption rate}) / \text{Body weight}$$

Where: Mc is the metal concentration in mussel (WW)  
Consumption rate - 17.86 g day<sup>-1</sup>, for ALM  
Body weight of 60 kg for adults

Fourth parameter is hazard quotient (HQ) of heavy metals for ALM in all mussel populations is to evaluate the non-carcinogenic risks that can arise from each metal to health. THQ is a ratio between estimated doses of metal exposure with ORD. THQ calculated by USEPA (2000):

$$THQ = (EF \times ED \times CR \times Mc) / (ORD \times ABW \times AET) \times 10^{-3}$$

Where: EF - exposure frequency (365 days year<sup>-1</sup>)  
ED - exposure duration (70 years), equivalent to the average lifetime  
CR - consumption rate (17.86 and 35.7 g day<sup>-1</sup> for ALM and HLM consumers, respectively)  
Mc - metal concentration in mussels (mg kg<sup>-1</sup> WW)  
ORD - oral reference dose (Cd 1; Cu 40; Pb 3.5; As 0.3; Se 5 and Hg 0.1 g)  
ABW - average body weight (60 kg for adults)  
AET - averaging exposure time for non-carcinogens (365 days year<sup>-1</sup> × ED)  
10<sup>-3</sup> unit conversion factor.

If the HQ is greater than 1, the metal exposure dose (estimated dose of metal exposure) is greater than the dose allowed by oral ORD, so it is assumed that it can lead to negative health effects on humans (Bogdanovic et al 2014).

The last parameter is total hazard index (HI), used to evaluate risks that can arise from a combination of several metals upon health. Jovic & Stankovic (2014) stated that HI is determined by total HQ value of each metal with the following formula:

$$HI = \sum_{i=1}^n (THQi)$$

Values of HI below 1.0 indicate no risk of adverse health effect and the greater the value, the greater the risk level of the heavy metal intoxication.

## Results and Discussion

**Metal concentration.** Metal concentrations in all mussel populations are given in Table 2. Cadmium concentration in *A. pleuronectes* (1.79 mg kg<sup>-1</sup> WW) had exceeded the permissible limit from national standard for Indonesia (SNI) and international standard from Codex (2017), but *P. viridis* (0.06 mg kg<sup>-1</sup> WW) and *A. granosa* (0.43 mg kg<sup>-1</sup> WW) were lower than the maximum permissible limit. There was different cadmium accumulation pattern among the three bivalves such as scallops, mussels and clam. Theoretically, shellfish, crustaceans, and fungi are natural accumulator for cadmium (Codex 2017). Cadmium concentrations in the tropical mussel species *P. viridis* was generally low (<2 mg kg<sup>-1</sup> WW). Yulianto et al (2019) reported that *A. pleuronectes* found in Tegal, Central Java also contained high Cd level (15.34 mg kg<sup>-1</sup> WW). There is a difference between the concentration of cadmium in mussel and oyster, where cadmium concentration in mussel was lower (1 ug g<sup>-1</sup> WW) than in oyster (2 ug g<sup>-1</sup> WW). This is mainly because of its lower dissolved uptake and dietary assimilation efficiency, and a higher efflux, which effectively removed Cd from the mussel body. The efflux rates in the mussels were 10 times lower than in oysters (Wang & Lu 2017). So, it is confirmed that *A. pleuronectes* scallops, has a highest Cd accumulation than others. Among the different bivalvia, scallops can be considered as hyperaccumulator of Cd. Mechanism leading to this phenomena were attributed by the very high dietary uptake and the low efflux rate (Pan & Wang 2008). High accumulation of Cd in mussels is not entirely caused by high concentration of Cd in water or sediment components, but can be significantly enhanced by other metals. Liu & Wang (2013) stated that Zn accumulation can facilitate the Cd uptake in oyster. Zn enhanced the Cd bioaccumulation, whereas Cu and Zn exposure both significantly enhanced the Hg bioaccumulation (Liu & Wang 2013, 2014). This phenomenon may be responsible for high cadmium accumulation in *A. pleuronectes* and *T. granosa*.

Copper concentration in scallops, *A. pleuronectes* (0.34 mg kg<sup>-1</sup> WW), clams, *T. granosa* (0.82 mg kg<sup>-1</sup> WW) and mussel, *P. viridis* (0.55 mg kg<sup>-1</sup> WW) were below the MPLs suggested by WHO (1982) (10 mg kg<sup>-1</sup> WW). Thus, there should be no noticeable Cu risk of mussel consumption. The present Cu ranges were comparatively lower than those reported from Malaysia (Denil et al 2017). In contrast to Cd, Cu concentration in scallops, *A. pleuronectes* was relatively low because of dietary assimilation, feeding rate and efflux rate (Wang & Lu 2017). The clams, *T. granosa* showed a low concentration of Cu because of a relatively high dietary assimilation and a high efflux, indicating that Cu was rapidly processed and eliminated by the clams (Pan & Wang 2009). Cu is an essential element of different enzymes for all organisms and it is important for the synthesis of haemoglobin. Marine mussels are an important source of Cu for human health because of the presence of hemocyanin which is a Cu-containing respiratory protein found in the blood of molluscs (Rivonker & Parukelar 1998). Nevertheless, the very high levels of Cu intake via the marine seafood consumption can cause adverse health problems such as liver and kidney damage, but it is not carcinogenic to humans and animals (Gorell et al 1997). Cu could be accumulated in Semarang waters because it receives inputs from river discharge and surface runoff from the surrounding areas.

Methylmercury is the most toxic form of mercury and is formed in aquatic environments. Methylmercury therefore is found mainly in aquatic organisms. It can accumulate in the food chain; the levels in large predatory fish species are therefore higher than in other species and fish is the predominant source of human exposure to methylmercury (Codex 2017).

Table 2

Mean concentration (mg kg<sup>-1</sup> WW) of metals in bivalve

No	Species	N	As	Cd	Cu	Pb	Se	Hg	Moisture content (%)
1	<i>A. pleuronectes</i> (scallops)	22	1.49±0.20	<b>1.79</b> ±0.36	0.34±0.10	0.18±0.06	0.66±1.04	0.01±0.00	83.13
2	<i>T. granosa</i> (clams)	27	0.48±0.11	0.43±0.09	0.82±0.21	0.97±1.03	0.5±0.10	0.03±0.01	85.48
3	<i>P. viridis</i> (mussels)	14	0.81±0.18	0.06±0.01	0.55±0.11	0.15±0.00	0.21±0.05	0.01±0.00	87.72
	Safe limit		-	1	10	-	-	0.5	-

Bolded value indicates exceed of the permissible limit.

Total Hg concentration in fish is often used as a measure of MeHg exposure, assuming that almost 100% Hg concluded in fish and seafood occurs in methyl mercury (Kuras et al 2017). Another reason for not to measure of methylmercury it is because it is an expensive high technology method and it is not always available, especially for developing countries. This was not always available, especially for developing countries. Methods for total mercury were reliable, widely available and less costly. There was no evidence that there were fish with high total mercury, but low methylmercury. In the present study, Hg concentration in *A. pleuronectes*, *T. granosa* and *P. viridis* had a range of 0.01-0.03 mg kg<sup>-1</sup> DW, which is still below the permissible limit from national standard in Indonesia (SNI) and international Codex.

Pb is a non-essential element and toxic metal with not known biological function, and with high neurotoxic and nephrotoxic influence (Garcia-Leston et al 2010). This metal can be accumulated at high level in the human body and may constitute a serious risk to public health (Jovic & Stancovic 2014). Pb concentrations in the oysters were generally <4mg g<sup>-1</sup> DW. The possible sources of Pb in Semarang water is from residues of fertilizers and herbicides and gasoline of fishing boats.

The possible sources of As in the environment could be originated from mineral weathering, arsenic-based pesticides and fertilizers as well as production of paints, dyes and soap. In case of Marudu bay, Malaysia, the leaching of fertilizers residue may promote the accumulation of As in the analyzed bivalves at the extent where it exceeds the permissible limit. Subsequently, presenting the risk of As intoxication to human consumers. High metal concentrations were mainly caused by the high ambient metal concentrations because of industrial effluent release and sewage discharge from inland (Wang & Lu 2017).

However, the concentration of heavy metals in bivalves is affected by numerous factors (Table 3). It was concluded that intrinsic factors such as species, age, size, metabolic system, spawning process and extrinsic factors such as type of metals, location and various environmental factors can affect the heavy metal content in bivalves (Denil et al 2017).

Table 3

Source of metal contamination in environment

<i>Metal</i>	<i>Source</i>	<i>References</i>
As	Weathering of natural mineral, production of paints, dyes and soaps, arsenical pesticides and fertilizers	Jaishankar et al (2014)
Cd	Production of Cu, Pb and Zn, production of alkaline batteries and electrode components, production of pigments, plastic stabilizers, fertilizers	Jaishankar et al (2014); Dinis & Fiúza (2011)
Cu	Waste from electroplating, iron and steel producers, and discarded copper products, overburdens from copper mines and mills	-
Pb	Production of batteries, smelting and metal plating process, exhaust from vehicles, pigment additives, gasoline, fertilizers and herbicide	Jaishankar et al (2014)
Se	Agriculture, combustion of coal and petroleum fuels, industries, natural source from environment	Fordyce (2007)
Hg	Agriculture, municipal wastewater discharges, mining, incineration, and discharges of industrial wastewater	Jaishankar et al (2014)

**Mean weekly intake.** Mean weekly intake would be a guidance for weekly human consumption for certain seafood. Theoretically, MWI is calculated for each species and each metal based on PTWI value, for example *A. pleuronectes* that contains 1.79 mg kg<sup>-1</sup> WW Cd would be consumed 0.22 kg week<sup>-1</sup> by a 60 kg adult. However, if we refer to Cu concentration (0.34 mg kg<sup>-1</sup>), the recommended weekly consumption limit is 660.07 kg (Table 4). Similarly, when we refer to the concentration of Hg (0.01 mg kg<sup>-1</sup>), consuming 28.5 kg week<sup>-1</sup> is still not a serious problem for health. For practical reasons, if we refer

to different metal concentrations in one species, it is recommended to consume according to the lowest limit which is 0.22 kg week<sup>-1</sup> for *A. pleuronectes*. Thus, the recommended consumption limit for *T. granosa* and *P. viridis* without a possible health risk is 0.92 kg week<sup>-1</sup> and 7.08 kg week<sup>-1</sup>, respectively.

Table 4  
Mean Weekly Intake (MWI) of mussels (kg) that could be consumed per week by a 60-kg adult

Species		As	Cd	Cu	Pb	Se	Hg
<i>Amusium pleuronectes</i>	Min	-	0.16	382.82	-	-	17.96
	Max	-	0.29	1,084.05	-	-	40.78
	Mean	-	0.22	660.07	-	-	28.50
	St. dev.	-	0.05	197.59	-	-	7.94
<i>Tegillarca granosa</i>	Min	-	0.66	168.84	-	-	6.17
	Max	-	1.28	405.45	-	-	13.02
	Mean	-	0.92	271.55	-	-	9.45
	St. dev.	-	0.20	67.03	-	-	2.51
<i>Perna viridis</i>	Min	-	5.11	276.99	-	-	22.65
	Max	-	10.59	556.92	-	-	42.61
	Mean	-	7.08	399.59	-	-	34.01
	St. dev.	-	1.91	83.84	-	-	5.86

Note: There are no PTWI value set by JECFA (2010) for As, Se and Pb.

**Estimated daily intake.** Estimated Daily Intake (EDI) would be a guidance for human consumption for certain food daily. Theoretically, it refers to Cd concentration only, the EDI (mg kg<sup>-1</sup> WW day<sup>-1</sup>) of *A. pleuronectes* for ALM consumers is 0.53, for *T. granosa* is 0.13 whether for *P. viridis* is 0.02, respectively. However, for practical reason, it must also consider another metal concentration, too. So, EDI suggestion for *A. pleuronectes* consumption was taken from lower limit of EDI (0.003 mg kg<sup>-1</sup>). Therefore suggestion for *A. pleuronectes* is 0.003 mg kg<sup>-1</sup> WW day<sup>-1</sup>, for *T. granosa* is 0.008 mg kg<sup>-1</sup> WW day and for *P. viridis* is 0.002 mg kg<sup>-1</sup> WW day<sup>-1</sup> (Table 5).

Table 5  
Estimated Daily Intake (EDI) (mg kg<sup>-1</sup> WW day<sup>-1</sup>) of heavy metals

Species		Cd	Cu	Pb	Se	Hg
<i>Amusium pleuronectes</i>	Min	0.38	0.06	0.03	0.07	0.002
	Max	0.70	0.16	0.09	1.07	0.004
	Mean	0.53	0.10	0.05	0.20	0.003
	SD	0.11	0.03	0.02	0.31	0.001
<i>Tegillarca granosa</i>	Min	0.09	0.15	0.07	0.11	0.005
	Max	0.17	0.37	0.51	0.20	0.012
	Mean	0.13	0.24	0.29	0.15	0.008
	SD	0.03	0.06	0.31	0.03	0.002
<i>Perna viridis</i>	Min	0.01	0.11	0.04	0.04	0.002
	Max	0.02	0.23	0.04	0.07	0.003
	Mean	0.02	0.16	0.04	0.06	0.002
	SD	0.00	0.03	-	0.01	0.000

**Hazard questions (HQ) and hazard index (HI).** Values of THQ and HI values in all populations for ALM are shown in Table 6, ranged from 0.04 to 1.47. THQ values of Pb, Se, Cd, Cu and Hg were found to be <1 except As. The HQ value for As were greater than 1 in *A. pleuronectes* indicates that there is a moderate risk associated with As intoxication. THQ <1 indicate no health risks effect on human regarding the Cu, Pb, Cd, Se and Hg concentration.

The cumulative effect of HQ of the respective heavy metals was indicated by the value of HI. It was found that the HI values for *A. pleuronectes* were greater than 1.0 because of main contribution of As. The HI value, in increasing order, of the respective bivalve begins with clams, *T. granosa* (0.80) followed by mussels, *P. viridis* (0.87), and scallops, *A. pleuronectes* (2.09). Arsenic measured in the present study is total arsenic and as it is well known that inorganic arsenic is harmful to organism (JECFA 2013; Jaishankar et al 2014). Therefore further study about speciation of As is needed.

Table 6

Hazard quotient (HQ) and hazard index (HI) of heavy metals for ALM consumers

	<i>As</i>	<i>Cd</i>	<i>Cu</i>	<i>Pb</i>	<i>Se</i>	<i>Hg</i>	<i>HI</i>
<i>A. pleuronectes</i>	<b>1.47</b>	0.53	0.00	0.02	0.04	0.03	2.09
<i>T. granosa</i>	0.48	0.13	0.01	0.08	0.03	0.08	0.80
<i>P. viridis</i>	0.80	0.02	0.00	0.01	0.01	0.02	0.87

Values of HQ greater than 1.0 highlighted in bold indicating presence of adverse health effect associated with respective heavy metal.

**Conclusions.** Metals accumulation among bivalves, were not entirely caused only by high concentration in compartment (water and sediment) but could be due to metal-metal interaction in affecting tissue metal accumulation. According to several parameter of food safety, *A. pleuronectes* is most susceptible for health risk of human because of Arsenic. A simple guidance on bivalves consumption is based on the MWI and EDI calculation. These two parameter monitors metal concentration in bivalves. For practical reasons, it is recommended to consume bivalves according to the lowest limit which is 0.22 kg week<sup>-1</sup> for *A. pleuronectes*, 0.92 kg week<sup>-1</sup> for *A. granosa* and 7.08 kg week<sup>-1</sup> for *P. viridis* without a possible health risk.

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**Authors contribution.** Rachma Puspitasari as main contributor responsible for on data analysis, laboratory analysis and drafted the manuscript. Suratno and Triyoni Purbonegoro analyzed the data, executed sampling and laboratory analysis.

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