

Safety of pre-cultivated sandfish (*Holothuria scabra*) as pharmaceutical raw material in terms of heavy metal (Hg, Cd, Pb and As) concentration

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Abstract. Sandfish (*Holothuria scabra*) is a very commercial species compared to other types of sea cucumber; hence, stock in nature decreases as the exploitation increases. It is, thus, designated by IUCN as an endangered species, consequently, the species requires cultivation activities. However, currently, there are many anthropogenic activities that have the potential to contaminate the aquaculture sites for sandfish with hazardous and toxic materials such as heavy metals. This study was aimed at analyzing the safety of sandfish from heavy metals, in pre-cultivation activities. In this study, Hg, Cd, Pb and As were analyzed from water, sediment and sandfish samples of three pond locations, i.e., station 1 (near the outlet), station 2 (near the inlet), and station 3 (next to settlements). In addition to that, analysis was also carried out to observe water quality, bioconcentration factor (BCF), maximum weekly intake (MWI), and maximum tolerable intake (MTI). The results showed that the water quality still supports the life of the sandfish. Cd, Pb and As were not detected in the water, but there was dissolved Hg at a concentration of 0.004-0.009 mg L⁻¹. In sediments Hg was detected at the value of 0.006-0.019 mg kg⁻¹, Cd was only detected at station 1 (0.006 mg kg⁻¹). Sandfish meat was contaminated with Hg, Pb, and Cd as high as 0.10-0.33 mg kg⁻¹, 0.60-0.69 mg kg⁻¹ and 0.046-0.109 mg kg⁻¹, respectively. Bioconcentration factors of heavy metals in sandfish from water were 1.29-8.29 (low) for Hg, 51-121.49 (low-moderate) for Cd and 315.7-363.1 (moderate) for Pb. Bioconcentration factors of heavy metals in sandfish from sediment were 9.02-22.31 (low), 1.58-1.75 (low), and 122.4-140.8 (medium) in respect to Hg, Cd, and Pb. The amount of heavy metals in sandfish that are allowed to enter the body of adults is maximum per week (MWI) for adults weighing 50 kg, 55 kg and 60 kg for Hg were 0.2, 0.22, 0.24 mg week⁻¹, respectively; for Cd were 0.35, 0.385, 0.42 mg week⁻¹ respectively; for Pb were respectively 1.25, 1.38, 1.50 mg week⁻¹. The maximum weight limit of sandfish that can be consumed in one week (MTI) for adults weighing 50 kg, 55 kg and 60 kg, respectively, is 2.08, 2.29 and 2.5 kg week⁻¹. Considering the purpose of pharmaceutical and cosmetic raw materials for adults and the elderly, with an amount of less than 100 grams, the pre-cultivated sandfish are very safe for medicine and cosmetic raw materials.

Key Words: heavy metals, pharmaceuticals, pre-cultivation, safety, sandfish.

Introduction. Sandfish (*Holothuria scabra*) is one of the aquatic resource organisms that has high economic value, even the highest compared to other sandfish (Purcell 2014). The high economic value has an impact on the increase of exploitation. As a result, more than 70% of the world's sandfish have been exploited (Purcell et al 2013), which led to a decline in the stock of *H. scabra* in nature (Riani et al 2018). The decline in stocks of sandfish in nature does not only occur in Indonesia, but it also occurs in most countries in the Asia-Pacific region (Toral-Granda et al 2008). Therefore, the sandfish has been designated by the IUCN (International Union for Conservation of Nature) as an endangered animal (Hamel et al 2013). This condition has eventually determined the researches to conduct sandfish cultivation activities in artificial facilities.

Considering that sandfish are highly commercial organisms, aquaculture activities will be better if they will be carried out in the vicinity areas that are not far from sandfish habitat of origin. One of the locations known to be productively inhabited by sandfish is Lampung Province (KKP 2021). Therefore, the experiment of cultivating sandfish on the

coast of Bandar Lampung City, such as on Pasaran Island, is an option that should be considered.

Pasaran Island is located in Region of Bandar Lampung, therefore, not far from Pasaran Island there are economic activities such as port activities, shipbuilding, container industry, warehousing, coal storage, steel fabrication, chemical industry and a power plant. In addition to that, there are agricultural activities, settlements and other activities that have the potential to pollute the aquatic environment with hazardous and toxic materials. One of the B3 that will be generated from these activities and has the potential to contaminate the aquatic ecosystem on Pasaran Island is heavy metals. Heavy metals that enter aquatic ecosystems will undergo several processes such as dissolved, suspended, deposited, diluted, dispersed and absorbed in existing organisms, on organic materials and on various existing materials, as well as adsorbed on various components (Riani & Cordova 2016). Considering that heavy metals have a high specific gravity and tend to settle, the organisms affected by heavy metal pollution are basic biota such as sandfish (Ali et al 2019). Therefore, if the cultivation of sandfish is carried out on Pasaran Island, both water, sediment and sandfish have the potential to be contaminated with heavy metals.

Heavy metals that are categorized as hazardous and toxic materials, not only reduce water quality, but also can accumulate in the bodies of living organisms (Frazier 1979; Feraro et al 2004; Weber 2006; Riani 2010; Cordova 2011; Takarina et al 2013; Riani et al 2018; Vasseur et al 2021). There have been evidences that in the aquatic eukaryotic organisms, heavy metals may cause damage to various organs such as gills, liver or hepatopancreas, kidneys, spleen, heart and brain (Riani 2021), and impair reproduction (Riani 2011); therefore, those generally harm fish health (Zeitoun & Mehana 2014; Kolarova & Napiórkowski 2021). Heavy metals are also teratogenic which cause congenital defects in embryos (Gooding et al 2003; Feraro et al 2004; Horiguchi et al 2006; Lugowska 2007; Riani et al 2018). In addition, it will also cause genetic disorders and mutations in biota (Cordova 2016). Considering that these cultivated sandfish have been widely used as potential raw materials for cosmetics and pharmaceuticals, it is then necessary to analyze the heavy metals in sandfish cultivated on Pasaran Island. Unfortunately, research related to heavy metal content in cultivated sandfish on Pasaran Island has never been conducted.

Research on heavy metals in sandfish has been carried out, including the research conducted by Lestari (2015) on Hg and Pb in eggplant sandfish (*Phyllophyrus* sp.) on Kenjeran Beach, Surabaya. Research on the effect of heavy metals on Pasaran Island has not been conducted, but one by Safitri et al (2018), namely the content of Pb and Cu in green mussels. Therefore, in order to predict the potential for bioaccumulation of B3, especially heavy metals in sandfish, it is necessary to conduct a study on the safety of consumption of pre-cultivated sandfish on Pasaran Island.

Material and Method. This research was conducted from June 2019 to June 2020 in the coastal aquaculture ponds of Pasaran Island (Figure 1). Analysis of heavy metals in water was carried out at the Environmental Productivity Laboratory, Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, IPB. Analysis of heavy metals in sediment samples and sandfish samples were analyzed at the Nutrition and Dairy Laboratory, Faculty of Animal Science, IPB.

In this study, samples of water, sediment and sandfish were taken from three stations. The first two stations, named as station 1 and station 2, are respectively adjacent to outlet and inlet. Station 3 is located by the side of the pond, adjacent to the settlement. Specifically for water quality analysis, several physical and chemical parameters were measured. The physical parameters analyzed were turbidity, temperature, pH, salinity, total dissolved solid (TDS) and total suspended solid (TSS). Chemical parameters analyzed were dissolved oxygen (DO), total phosphate, ammonia, nitrate, nitrite, chemical oxygen demand (COD), biological oxygen demand (BOD₅), sulfide (H₂S), oil and fat, total phenol, detergent and heavy metals (Hg, Cd, Pb and As). Specifically for DO, pH and salinity parameters were carried out in situ, but other parameters were analyzed in the laboratory.



Figure 1. Map of the study area and sampling locations

In this study, apart from taking water samples, sediment samples were also taken which were then analyzed for heavy metal concentrations, namely Hg, Cd, Pb and As. In addition to this, heavy metal analysis (Hg, Cd, Pb and As) was also carried out on sandfish meat. Prior to heavy metal analyses on sediment samples and sandfish samples, the samples were prepared, destructed and read by using AAS (Atomic Absorption Spectrophotometer).

Data on water quality, sediment quality and heavy metal content in sandfish obtained in this study were analyzed descriptively. In addition, the bioconcentration factor (BCF) was also analyzed and it was calculated by comparing the concentration of similar heavy metals in sandfish with their concentrations in water and or in sediments (Potipat et al 2015):

$$BCF = C \text{ sandfish} / C \text{ media ambient}$$

where: C sandfish = heavy metal concentration in sandfish (ppm);

C media ambient = heavy metal concentration in water/sediment (ppm).

The BCF values obtained are classified based on Van Esch (1978):

- low accumulation: $BCF < 100$;
- medium accumulation: $100 < BCF < 1000$;
- high accumulation: $BCF > 1000$.

Furthermore, the limit of maximum weight of sandfish which contain certain heavy metals with a certain concentration and may be consumed within one week is analyzed. The limit of maximum weight is known as the number of sandfish that are still allowed to be consumed, known as the maximum tolerable intake (MTI), with reference to the threshold limit recommended by the World Health Organization (WHO) and Joint FAO/WHO Expert Committee on Food Additives (JEFCA) (2004). This MWI calculation uses the formula:

$$MWI \text{ (g)} = \text{body weight}^a) \times PTWI^b)$$

a) average body weight of Indonesian adult women (50, 55 and 60 kg);

b) PTWI (provisional tolerable weekly intake).

The maximum tolerance value for certain types of heavy metals per week (PTWI) is taken from JEFCA as shown in Table 1.

Table 1

The maximum tolerance value for various types of heavy metal consumption per week

No	Heavy metals	PTWI ($\mu\text{g kg}^{-1}$ body weight) per week
1	Cd	7 ^{a)}
2	Hg	4 ^{b)}
3	Pb	25 ^{c)}
4	As	15 ^{c)}

a) JEFCA in FAO/WHO (2004); b) JEFCA in FAO/WHO (2010); c) JEFCA in Turkmen et al (2008).

Furthermore, the maximum amount of sandfish weight that is tolerated for consumption within one week (MTI) is as follows:

$$\text{MTI} = \text{MWI} / \text{Ct}$$

where: MWI = maximum weekly intake (mg for Indonesian adults weighing 60 kg);

Ct = the concentration of certain specific heavy metals found in sandfish (mg kg^{-1}).

Results

Water quality. Pond water quality in terms of the average value of turbidity and TSS was best at station 1 compared to stations 2 and 3 (Table 2), but in terms of nutrients (total phosphate and nitrate) as well as oil and fat were highest at the outlet. This is presumably because the lower pond elevation towards the outlet allows organic matter downwards the outlet and accumulated at that location. In addition to this, the total phosphate and nitrate as well as oil and grease at all stations exceeded the specified quality standards. This is presumably because the pond is in a location adjacent to the main settlement on Pasaran Island that livelihoods are dominated by fishermen who not only catch fish but also process their catch, resulting in high protein waste. However, these conditions are not too disturbing for the life of sandfish, even specifically for nitrate and phosphate will actually support the growth of natural food of sandfish. In addition, oil and fat also relatively less disturb the life of sandfish because sandfish are benthic animals, while oils and fats that have small specific gravity will be on the surface of the water.

The other parameters were relatively comparable among the three stations; however, the pH value at station 3 was the lowest compared to other stations. The results of field observations showed that station 3 is adjacent to fishermen's settlements. On the other hand, the fishing communities on Pasaran Island do not really understand environmental problems, so there are still those who dispose food scraps and other easily biodegradable organic waste directly to station 3. This condition results in high decomposition of organic matter (producing CO_2), resulting in high organic matter in the location. This is thought to have caused the buffering system to be disrupted, which in turn resulted in a lower pH compared to other stations. The high organic matter from domestic activities at station 3 can also be seen from the higher BOD, ammonia and nitrite values as well as the lower DO concentration.

Table 2

Average water quality at the pre-cultivation site for sandfish

No	Parameter	Unit	Station 1	Station 2	Station 3	Quality standards*
<i>Physics</i>						
1	Turbidity	NTU	4.5	39	42	< 5
2	TSS	mg L^{-1}	8.5	74.0	89.0	20-80
3	TDS	mg L^{-1}	35930	36470	27870	-
4	Temperature	$^{\circ}\text{C}$	29	29	30	29-30
5	pH	-	8	7.9	7.5	7-8.5
6	Salinity	ppt	28	28	27	Natural

<i>Chemistry</i>						
1	DO	mg L ⁻¹	6.4	6,5	5.6	> 5
2	Total phosphate	mg L ⁻¹	0.446	0.297	0.214	0.015
3	Ammonia (NH ₃ -N)	mg L ⁻¹	0.065	0.048	0.073	0.3
4	Nitrate (NO ₃ -N)	mg L ⁻¹	0.764	0.464	0.343	0.008
5	Nitrite (NO ₂ -N)	mg L ⁻¹	0.028	0.036	0.043	-
6	COD	mg L ⁻¹	30.65	31.60	30.97	-
7	BOD ₅	mg L ⁻¹	2.7	2.4	3.2	20
8	Sulfide (H ₂ S)	mg L ⁻¹	< 0.001	< 0.001	< 0.001	0.01
9	Oil and fat	mg L ⁻¹	12.5	9.0	8.3	1
10	Total phenol	mg L ⁻¹	< 0.0005	< 0.0005	< 0.0005	0.002
11	Detergent	mg L ⁻¹	< 0.025	< 0.025	< 0.025	1

* Decree of The Minister of State for The Environment of The Republic of Indonesia No. 51//2004 (Appendix III - Concerning Quality Standard for Aquatic Biota).

Heavy metal concentration in water, sediment and sandfish. Almost all parameters of heavy metals dissolved in water, i.e., Cd, Pb and As were measily detected with values below the detection limit (Table 3). However, at all stations there was heavy metal Hg dissolved in water with an average concentration of between 0.004 to 0.009 mg L⁻¹. The concentration of Hg at all research stations exceeded the quality standard allowed by the Government of Indonesia for the needs of marine life. This needs to be very careful considering that mercury is one of the most dangerous environmental pollutants for environmental sustainability and if it penetrates the food chain, it will be bioaccumulative (Riani et al 2018; Mahmoudi et al 2020).

Table 3
Content of heavy metals dissolved in water

No	Heavy metals	Unit	Station 1	Station 2	Station 3	Quality standards*
1	Mercury (Hg)	mg L ⁻¹	0.009	0.004	0.008	0.001
2	Cadmium (Cd)	mg L ⁻¹	< 0.001	< 0.001	< 0.001	0.001
3	Lead (Pb)	mg L ⁻¹	< 0.002	< 0.002	< 0.002	0.008
4	Arsenic (As)	mg L ⁻¹	< 0.005	< 0.005	< 0.005	0.012

* Decree of The Minister of State for The Environment of The Republic of Indonesia No. 51//2004 (Appendix III - Concerning Quality Standard for Aquatic Biota).

Hg was also detected in sediments with a concentration range between 0.006 to 0.019 mg kg⁻¹. Besides Hg, Cd was also detected in the sediment at station 1 with an average concentration of 0.006 mg kg⁻¹, while at stations 2 and 3 the presence of Cd was detected below 0.005 mg kg⁻¹. In addition to that, heavy metals Pb and As were also detected below the detection limit of 0.002 mg kg⁻¹ and 0.005 mg kg⁻¹, respectively (Table 4).

Table 4
Heavy metal content in sediment

No	Heavy metals	Unit	Station 1	Station 2	Station 3	Quality standards*
1	Cadmium (Cd)	mg kg ⁻¹	0.006	< 0.005	< 0.005	
2	Mercury (Hg)	mg kg ⁻¹	0.015	0.019	0.006	
3	Lead (Pb)	mg kg ⁻¹	< 0.002	< 0.002	< 0.002	47.82
4	Arsenic (As)	mg kg ⁻¹	< 0.005	< 0.005	< 0.005	< 10

* Swedish Environmental Protection Agency (SEPA 2000) Environmental Quality Criteria (Classification of Very low concentrations).

Not to mention the water and sediment, heavy metals Cd, Hg and Pb were also detected in pre-cultivated sandfish as shown in Figures 2, 3, and 4. For heavy metal As, its presence was not detected in all sandfish meat samples. Cd at all stations was detected

in sandfish meat with concentrations between 0.046 to 0.109 mg kg⁻¹, with the highest concentration at station 2 at an average of 0.109 mg kg⁻¹.

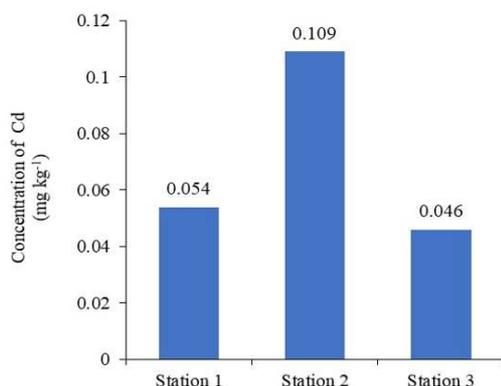


Figure 2. Heavy metal (Cd) concentration in pre-cultivated sandfish on Pasaran Island.

Heavy metal mercury (Hg) was detected in sandfish meat at all stations (Figure 2), with a concentration range between 0.10 and 0.33 mg kg⁻¹. As with heavy metal Cd, the highest concentration of heavy metal Hg also occurred in sandfish at station 2, at the concentration of 0.033 mg kg⁻¹.

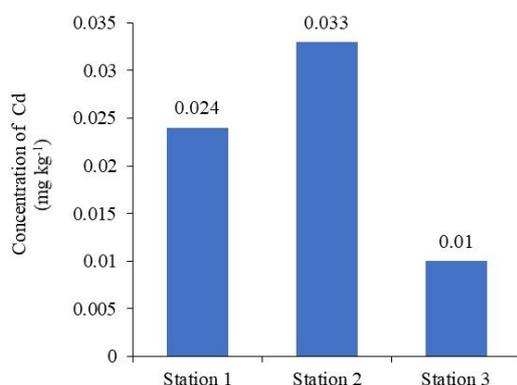


Figure 3. Heavy metal (Hg) concentration in pre-cultivated sandfish on Pasaran Island.

Heavy metal lead (Pb) was detected in sandfish flesh at all observation stations (Figure 3), with a concentration range between 0.60 and 0.69 mg kg⁻¹. In contrast to heavy metals Hg and Cd, the highest concentration of heavy metal Pb occurred in sandfish at station 1, at a concentration of 0.69 mg kg⁻¹.

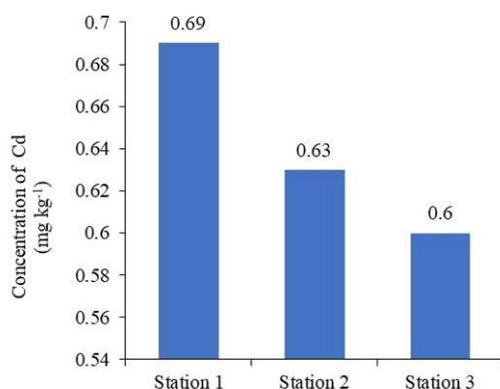


Figure 4. Heavy metal (Pb) concentration in pre-cultivated sandfish on Pasaran Island.

Bioconcentration of heavy metals in sandfish uptake from water. In this study, following the concentration of heavy metals in water, sediment and sandfish was recognized, BCF was then calculated. Heavy metal Cd dissolved in water was not detected; however, it does not imply that there was no Cd in the water, because there is evidence from the presence of Cd in sandfish. The undetectable Cd in water was thought to be due to the limitations of the AAS detection tool used. Therefore, in order to calculate the bioconcentration, it is assumed that the concentration of Cd in water from a concentration of less than 0.001 is assumed to be 0.0009 mg L⁻¹, so that the value of the BCF of Cd from water was 51.42-121.49 (Table 5). The highest BCF of Cd occurred at station 2, with BCF values between 100 and 1000, so that based on Van Esch (1978) classification, the Cd bioconcentration factor at station 2 was in the moderate accumulation category; however, the BCF values at stations 1 and 3 were categorized at low accumulation.

The BCF of heavy metal Hg in sandfish from water at the three stations was calculated at the ranges of 1.29 to 8.29 times. Based on Van Esch (1978) classification, BCF values of heavy metal Hg in all stations 1, 2 and 3 were in the category of low accumulation (Table 5).

The BCF of heavy metal Pb was between 315.7 and 363.1 times. According to the Van Esch (1978) classification, the bioconcentration factor at stations 1, 2 and 3 was in the category of moderate accumulation (Table 5). In contrast to other types of heavy metal, As was not detected neither in the water nor in the body of sandfish, therefore the BCF value of heavy metal As at all stations could not be calculated. This implied that there was no As contamination at the location of sandfish cultivation on Pasaran Island.

Table 5

Average bioconcentration factor of heavy metals in sandfish in water

<i>Heavy metal BCF value</i>	<i>Station 1</i>	<i>Station 2</i>	<i>Station 3</i>
Cd	60.12	121.49	51.42
Hg	2.65	8.29	1.29
Pb	363.1	331.5	315.7
As	-	-	-

Bioconcentration factors of heavy metals in sandfish uptake from sediment. BCF of heavy metals Cd, Hg, Pb and As between sediments and sandfish is presented in Table 6. The highest value of BCF for heavy metal Cd between sediments and sandfish is at station 2, i.e., 1.75. The highest BCF value for heavy metal Hg between sediment and sandfish also occurred at station 2, as high as 22.31. The highest BCF value for heavy metal Pb between sediment and sandfish occurred at station 1, as high as 140.8. However, as with the BCF between water and sandfish, the BCF between sediment and sandfish for heavy metal As also cannot be calculated because As was not detected in any of them or in other words because the pre-cultivated sandfish ponds are not polluted (even uncontaminated) by As.

Table 6

Factors of heavy metal bioconcentration in sandfish in sediments

<i>Heavy metal BCF value</i>	<i>Station 1</i>	<i>Station 2</i>	<i>Station 3</i>
Cd	1.58	1.75	1.72
Hg	9.02	22.31	9.5
Pb	140.8	128.5	122.4
As	-	-	-

As with the calculation of BCF of heavy metal Cd between water and sandfish, the BCF value of metal Cd between sediment and sandfish was also obtained from the assumption that the concentration of heavy metals at stations 2 and 3 (less than 0.005 mg kg⁻¹) was then assumed to be 0.0049 mg kg⁻¹. The results of BCF calculations for heavy metals Hg

and Cd in sediments with sandfish from three stations still show a low accumulation rate, which is less than 100. However, the results of BCF calculations for heavy metals Pb between sediment and sandfish from the three stations show a value of more than 100. This indicates that the level of accumulation of heavy metal Pb from the sediment to the body of the sandfish is in the medium category.

Maximum weekly intake (MWI) and maximum tolerable intake (MTI). Based on the heavy metal concentration found in sandfish, it can be determined the maximum amount of each heavy metal consumption per week that was still allowed to enter the human body, known as the maximum weekly intake (MWI), as well as the maximum weight limit of sandfish may consumed in one week or known as the maximum tolerable intake (MTI). Considering that drugs and cosmetics commonly made from sandfish are currently only intended for adults and seniors (elderly humans), the calculations are then carried out for adults and seniors. In Indonesia, adults are generally weighed between 50 kg and 60 kg, so the calculation of MWI and MTI for adults is assumed to be 50, 55 and 60 kg. The results of the MWI calculations can be seen in Table 7, and the results of the MTI calculations can be seen in Table 8.

Table 7

The amount of heavy metals in sandfish that are allowed to enter the body of adult is maximum per week (MWI)

No	Adult body weight (kg)	MWI (mg)			
		Cd	Hg	Pb	As
1	50	0.35	0.2	1.25	0.75
2	55	0.385	0.22	1.38	0.83
3	60	0.42	0.24	1.5	0.9

Based on the FAO/WHO (2004); FAO/WHO (2010); JECFA in Turkmen et al (2009), the result of calculation in Table 7 shows that among the heavy metals Cd, Hg, Pb and As, the most tolerated to enter the body is Pb of 1.25 mg kg⁻¹ for adult weighing 50 kg, while the Hg is the least tolerated with just 0.2 mg kg⁻¹ for adult weighing 50 kg. It means that Hg has the highest toxicity, while Pb has the lowest.

Table 8 presented that the maximum sandfish that can be consumed in one week by adult with 50 kg body weight is 2.08 kg. For person weighing 55 kg, they can consume 2.29 kg of sandfish, while the person weighing 60 kg can consume sandfish up to 2.5 kg per week. The MTI for As cannot be calculated because there was no heavy metal As contamination found in sandfish.

Table 8

Maximum weight limit of sandfish that can be consumed in one week (MTI) for adult

No	Body weight (kg)	MTI (kg week ⁻¹)			
		Cd	Hg	Pb	As
1	50	3.211	6.061	2.08	375
2	55	3.532	6.667	2.29	412.5
3	60	3.853	7.273	2.5	450

Discussion. The overall result of water quality analysis showed that almost all water quality parameters in pre-cultivation sites are sufficient to support the life of sandfish. However, the presence of dissolved Hg in the water was something to be aware of, especially at station 3 where the pH value was relatively lower than the others. Considering that if the pH is acidic, then various types of heavy metals that are already at the bottom of the waters and are static even though they can be dissolved in water, and will be relatively bioavailable, making it easy to contaminate the aquatic biota. Therefore, at the location of the cultivation of sandfish or any biota, the pH must be

maintained stable, because the lower the pH, the greater the solubility of the metal in it, and vice versa. This is in accordance with the opinion of Berlin et al (2015) who said that one of the determinants of metal solubility is a low pH.

All sandfish in this pre-cultivation activity have been contaminated with Cd. However, at stations I and III all sandfish analyzed for heavy metal concentrations of Cd, were still below the BPOM RI (2018) quality standard of 0.1 mg kg^{-1} . At station 2, Cd concentration had exceeded the quality standard, so it should take into account seriously, considering Cd can cause chronic effects on health (Garçon et al 2007 in Cabral et al 2015), causing damage to cell macromolecules, and lipids, especially polyunsaturated fats, and proteins (Dai et al 2010; Jomova & Valko 2011; Matovic et al 2013). Cd and/or Pb also cause kidney damage which is often associated with excess reactive oxygen species (ROS) (Xu et al 2008; Whittaker et al 2011; Lee et al 2012) and failure of the proximal tubule to reabsorb protein (Garçon et al. 2007 in Cabral et al 2015). Those are also carcinogenic (Joseph 2009), causing DNA damage (Filipic 2012; Vincent-Hubert et al 2014). Those two heavy metals were also reported to cause defects in the embryo (Riani et al 2018) and various other problems.

The average concentration of Hg in sandfish meat collected from the three stations is still below the quality standard set by BPOM RI (2018) of 0.5 mg kg^{-1} . However, as Hg has the highest toxicity, this certainly needs serious consideration, because Hg can cause digestive disorders and kidney damage in the form of tubular dysfunction, with tubular necrosis in severe cases (Berlin et al 2015). In addition to endangering the excretory system (kidneys), Hg will also disrupt the nervous system (Langford & Ferner in Al-Saleh et al 2014); cause oxidative stress, DNA damage, damage on liver and on various other vital organs (Ung et al 2010); cause congenital defects in birth (Bergeron et al 2011; Al-Sabbak et al 2012; Hansen & Harris 2013; Riani 2018), disturbances in various physiological processes (Amachree et al 2014). Hg can be transferred from the mother to the ovum (Guirlet et al 2008 in Hopkins 2012) thus causing lethal and sublethal effects on infant development, as well as various other problems.

The average concentration of Pb in sandfish meat from the three stations was all still below the quality standard set by BPOM RI (2018), which was 1.5 mg kg^{-1} . The Pb is also toxic and can cause various consequences such as disrupting the hemoglobin synthesis system, damaging to the nervous system, urinary system, gastrointestinal system, cardiovascular system, endocrine system, and it is carcinogenic (Ferraro et al 2004). Pb also may cause the occurrence of disability in children (Osman et al 2007; Riani et al 2018); occurrence of reproductive disorders (Ferraro 2004; Riani 2011) such as in ovum and sperm (Gopalakrishnan et al 2008); direct damage to DNA that affects chromatin stabilization (Bonacker et al 2005 in Ramakritinan et al 2014), as well as various other problems. Considering that the sandfish are consumed by humans, the presence of Pb in the flesh of the sandfish also needs serious attention. In contrast to heavy metals Cd, Hg and Pb, heavy metal As was not detected in all sandfish meat at all observation stations.

The detection of heavy metals Cd, Hg and Pb, dissolved in pond water column, and/or in pond sediments and/or in sandfish meat at the three stations, is thought to have originated from industrial activities around Pasaran Island such as shipbuilding industry, public port activities and from the coal port in the Panjang area which is quite close to Pasaran Island. In addition, the presence of heavy metals, especially Hg at the pre-cultivation site, and the detection of Cd in sediments at station 1 and the detection of Hg, Cd and Pb in sandfish are thought to have come from the Way Belau River water flow, from fishing boat traffic activities and it is also possible to detect inputs from domestic waste as well as from other anthropogenic activities.

PLTU activities which are located close to Pasaran Island, especially PLTU Tarahan which is approximately 19 km away from Pasaran Island and located on a relatively similar coastline, and PLTU Sebelang which is approximately 27 km away from Pasaran Island also have the potential as a donor of heavy metals into the environment. This is in accordance with the USEPA (1995) statement that there are various types of heavy metals in coal ash; while in fly ash, the abundant metal elements are Cd and Pb. Further,

in coal ash there are elements that are only detected in the gas phase, especially Hg. According to Pirrone et al (2010) and Sprovieri et al (2010), after emission occurs, some elements such as elemental Hg (Hg(0)) can be transported long distances before oxidation and removal by particulate matter and gas-phase dry deposition or flushing with precipitation occurs. Considering that the presence of Hg (0) in the atmosphere can last several months up to one year, it can also be transported and stored to remote locations such as the Arctic and Antarctic (Ebinghaus et al 2002; Durnford et al 2010; Drisscoll et al 2013). However, reactive gas Hg (RGM) and particulates ionic bound Hg (Hg (II)) have a shorter residence time in the atmosphere than Hg (0) (within hours to days), so it is generally stored locally or regionally. Therefore, Hg that enters the ecosystem is mostly in the form of Hg (II), whereas most of the CH₃Hg is actually produced within the ecosystem itself (Drisscoll et al 2013), so that the Hg contribution from outside can increase its toxicity after the Hg enters an ecosystem.

Based on the calculation of the bioconcentration factor of heavy metals Cd, Hg, Pb and As in sandfish both from water and sediments, it shows that heavy metal Pb has the ability to accumulate moderately. Therefore, Pb pollution, (or even limited to contamination though) in the sediment as well as in the water should receive serious attention. Considering that the ability to accumulate in the body of sandfish is in the moderate category, Pb will tend to increase continuously in the body of sandfish, over time.

In this study, it was seen that dissolved Pb in pre-cultivated ponds and in sediments were still below the detection limit, but the concentrations in the body of sandfish at all stations had bioconcentration above 100. This shows that although the concentration of Pb in the water and sediment is so low, the cautious is still needed in this situation. This is thought to occur because the sandfish in its life requires mineral elements, especially calcium (Ca) in large quantities. It is suspected that there are quite a lot of receptors in the body of sandfish, and among these receptors, it is suspected that there are many receptors for Pb. Another assumption is that the properties between Ca and Pb are almost similar, so that Pb under certain conditions is able to substitute for Ca. This results in receptors not recognizing and/or not being able to distinguish Pb from Ca, resulting in the binding of Pb by receptors that should carry Ca (Florea et al 2013; Leonas et al 2016). However, this assumption certainly needs scientific proof.

Based on the data above, it can also be seen that the bioconcentration from water is much higher than from sediment, that is the bioconcentration from water is almost three times that from sediment. Penetration of heavy metal Pb into the body of the sandfish is not only sourced from water but also from the sediment that enters the body of the sandfish through feeding habits. This happens because the sandfish are benthic animals that eat detritus from the sediment so that what is contained in it will also enter the body of the sandfish. This can be clearly seen from the results of the observations we conducted on its digestive tract which showed a lot of sand in it. This is in line with the research of Chiarelli & Roccheri (2014) that most marine invertebrates accumulate heavy metals mainly from sea water and/or from food. Furthermore, it is said that molluscs, crustaceans and other marine invertebrates are known as biota accumulating high levels of heavy metals in their body tissues, whereas heavy metals Hg, Cd and Pb are non-essential heavy metals that are toxic to living organisms even at low concentrations (Chiarelli & Roccheri 2014).

Considering that these pre-cultivated sandfish are not for consumption purposes, but for the purpose of being used as a source of raw materials for pharmaceuticals (drugs) and cosmetics, which are very few in number, and generally in one week the average need is less than 100 grams, then the amount is still far below the safe limit that is allowed to be consumed every week. Therefore, the pre-cultivated sandfish are categorized as very safe to be used as raw materials for medicines and cosmetics for the needs of adults and the elderly.

Conclusions. Location of pre-cultivated ponds adjacent to port activities, steam power plants and industries is prone to contamination by heavy metals Hg, Cd and Pb. Heavy metals in water and sediment which presence is not detected, does not certainly imply

that sandfish are safe from heavy metal contamination. For whole consumption purposes, pre-cultivated sandfish on Pasaran Island, Lampung, must be restrained in number; adults weighing 50 kg, 55 kg and 60 kg are allowed to consume sandfish 2.08, 2.29 and 2.5 kg week⁻¹, respectively. For the purposes of pharmaceutical and cosmetic raw materials for adult and the elderly, pre-cultivated sandfish are still considered very safe.

Conflict of interest. The authors declare that there is no conflict of interest.

References

- Al-Sabbak M., Sadik Ali S., Savabi O., Savabi G., Dastgiri S., Savabieasfahani M., 2012 Metal contamination and the epidemic of congenital birth defects in Iraqi cities. *Bulletin of Environmental Contamination and Toxicology* 89(5):937-944.
- Al-Saleh I., Al-Rouqi R., Obsum C. A., Shinwari N., Mashhour A., Billedo G., Al-Sarraj Y., Rabbah A., 2014 Mercury (Hg) and oxidative stress status in healthy mothers and its effect on birth anthropometric measures. *International Journal of Hygiene and Environmental Health* 217(4-5):567-585.
- Ali H., Khan E., Ilahi I., 2019 Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of Chemistry* 2019:6730305.
- Amachree D., Moody A. J., Handy R. D., 2014 Comparison of intermittent and continuous exposures to inorganic mercury in the mussel, *Mytilus edulis*: accumulation and sub-lethal physiological effects. *Ecotoxicology and Environmental Safety* 109:133-142.
- Bergeron C. M., Hopkins W. A., Bodinof C. M., Budischak S. A., Wada H., Unrine J. M., 2011 Counterbalancing effects of maternal mercury exposure during different stages of early ontogeny in American toads. *Science of the Total Environment* 409(22):4746-4752.
- Berlin M., Zalups R. K., Fowler B. A., 2015 Mercury. In: *Handbook on the toxicology of metals*. 4th edition. Volume II. Nordberg G. F., Fowler B. A., Nordberg M. (eds), Academic Press, pp. 1013-1075.
- BPOM-RI (National Agency of Drug and Food Control of Indonesia), 2018 PP No 5/2018 concerning to maximum limit of heavy metal contamination in processed food.
- Cabral M., Toure A., Garçon G., Diop C., Bouhsina S., Dewaele D., Cazier F., Courcot D., Tall-Dia A., Shirali P., Diouf A., Fall M., Verdin A., 2015 Effects of environmental cadmium and lead exposure on adults neighboring a discharge: evidences of adverse health effects. *Environmental Pollution* 206:247-255.
- Chiarelli R., Roccheri M. C., 2014 Marine invertebrates as bioindicators of heavy metal pollution. *Open Journal of Metal* 4(4):93-106.
- Cordova M. R., 2011 [Heavy metals bioaccumulation and green mussels (*Perna viridis*) malformation in Jakarta bay waters]. MSc thesis, Graduate School of Bogor Agricultural University, pp. 73-77. [in Indonesian]
- Cordova M. R., 2016 [Mechanism of genetic disorders and mutations in bivalves affected by lead heavy metal]. *Jurnal Oseana* 41(3):27-34. [in Indonesian]
- Dai W., Fu L., Du H., Liu H., Xu Z., 2010 Effects of montmorillonite on Pb accumulation, oxidative stress, and DNA damage in tilapia (*Oreochromis niloticus*) exposed to dietary Pb. *Biological Trace Element Research* 136(1):71-78.
- Decree of The Minister of State for The Environment of The Republic of Indonesia No. 51/2004 – Appendix III [Quality Standard for Aquatic Biota]. pp. 1497-1498. [in Indonesian]
- Driscoll C. T., Mason R. P., Chan H. M., Jacob D. J., Pirrone N., 2013 Mercury as a global pollutant: sources, pathways, and effects. *Environmental Science and Technology* 47(10):4967-4983.
- Durnford D., Dastoor A., Figueras-Nieto D., Ryjkov A., 2010 Long range transport of mercury to the Arctic and across Canada. *Atmospheric Chemistry and Physics* 10(13):6063-6086.

- Ebinghaus R., Kock H. H., Temme C., Einax J. W., Löwe A. G., Richter A., Burrows J. P., Schroeder W. H., 2002 Antarctic springtime depletion of atmospheric mercury. *Environmental Science and Technology* 36(6):1238-1244.
- FAO/WHO (Food Agricultural Organization / World Health Organization), 2004 Summary of evaluations performed by the joint FAO/WHO expert committee on food additive (JECFA 1956-2003). ILSI Press International Life Sciences Institute, Washington, pp. 1-18.
- FAO/WHO (Food Agricultural Organization / World Health Organization), 2010 Summary report of the seventy-third meeting of joint FAO/WHO expert committee on food additive (JECFA/73/SC). pp. 1-17.
- Feraro M. V. M., Fenocchio A. S., Mantovani M. S., de Oliveira Ribeiro C. A., Cestari M. M., 2004 Mutagenic effect of tributyltin and inorganic lead (Pb II) on fish *H. malabaricus* as evaluated using the comet assay and the piscine micronucleus and chromosome aberration tests. *Genetics and Molecular Biology* 27(1):103-107.
- Filipic M., 2012 Mechanisms of cadmium induced genomic instability. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 733(1-2):69-77.
- Florea A. M., Taban J., Varghese E., Alost B. T., Moreno S., Busselberg D., 2013 Lead (Pb²⁺) neurotoxicity: ion-mimicry with calcium (Ca²⁺) impairs synaptic transmission. A review with animated illustrations of the pre- and post-synaptic effects of lead. *Journal of Local and Global Health Science* 2013:4.
- Frazier J. M., 1979 Bioaccumulation of cadmium in marine organisms. *Environmental Health Perspectives* 28:75-79.
- Gooding M. P., Wilson V. S., Folmar L. C., Marcovich D. T., LeBlanc G. A., 2003 The biocides tributyltin reduces the accumulation of testosterone as fatty acid esters in the mud snail (*Ilyanassa obsoleta*). *Environmental Health Perspectives* 111(4):426-430.
- Gopalakrishnan S., Thilagam H., Raja P. V., 2008 Comparison of heavy metal toxicity in life stages (spermiotoxicity, egg toxicity, embryotoxicity and larval toxicity) of *Hydroides elegans*. *Chemosphere* 71(3):515-528.
- Hamel J. F., Mercier A., Conand C., Purcell S., Toral-Granda T. G., Gamboa R., 2013 *Holothuria scabra*. The IUCN Red List of Threatened Species 2013: e.T180257A1606648.
- Hansen J. M., Harris C., 2013 Redox control of teratogenesis. *Reproductive Toxicology* 35:165-179.
- Hopkins B. C., 2012 Mercury bioaccumulation and adverse reproductive effects in snapping turtles inhabiting a historically contaminated river. MSc Thesis, Faculty of the Virginia Polytechnic Institute and State University, 89 pp.
- Horiguchi T., Kojima M., Hamada F., Kajikawa A., Shiraishi H., Morita M., Shimizu M., 2006 Impact of tributyltin and triphenyltin on ivory shell (*Babylonia japonica*) populations. *Environmental Health Perspectives* 114(1):13-19.
- Jomova K., Valko M., 2011 Advances in metal-induced oxidative stress and human disease. *Toxicology* 283:65-87.
- Joseph P., 2009 Mechanisms of cadmium carcinogenesis. *Toxicology and Applied Pharmacology* 238(3):272-279.
- Kementerian Kelautan dan Perikanan (KKP), 2021 [Fishery production statistic by province]. Available at: <https://statistik.kkp.go.id/home.php>. Accessed: July, 2021. [in Indonesian]
- Kolarova N., Napiórkowski P., 2021 Trace elements in aquatic environment. Origin, distribution, assessment and toxicity effect for the aquatic biota. *Ecology and Hydrobiology* DOI: 10.1016/j.ecohyd.2021.02.002. (in press)
- Lee J. C., Son Y. O., Pratheeshkumar P., Shi X., 2012 Oxidative stress and metal carcinogenesis. *Free Radical Biology and Medicine* 53(4):742-757.
- Leonas R., Noor Z., Rasyid H. N., Madjid T. H., 2016. Effect of Lead Nanoparticles Inhalation on Bone Calcium Sensing Receptor, Hydroxyapatite Crystal and Receptor Activator of Nuclear Factor-Kappa B in Rats. *Acta Informatica Medica* 24(5):343-346.

- Lestari L. I. D., 2015 [Sex determination of sandfish *Paracaudina australis* (Semper, 1868) in Madura strait]. BSc thesis, Fakultas Sains dan Teknologi, Universitas Airlangga, 79 pp. [in Indonesian]
- Lugowska K., 2007 The effect of cadmium and cadmium/copper mixture during the embryonic development on deformation of common carp larvae. *Journal of Ichthyology* 2:46-60.
- Mahmoudi N., Jafari A. J., Moradi Y., Esrafil A., 2020 The mercury level in hair and breast milk of lactating mothers in Iran: a systematic review and meta-analysis. *Journal of Environmental Health Science & Engineering* 18(1):355-366.
- Matovic V., Dukic-Cosic D., Buha A., Bulat Z., 2013 Route, dose and duration of exposure to cadmium-relevance to oxidative stress induction. In: Peroxidases: biochemical characteristics, functions and potential applications. Bogaert L., Coppens N. (eds), Nova Science Publishers Inc., pp. 159-175.
- Osman A. G. M., Wuertz S., Mekkiy I. A., Exner H. J., Kirschbaum F., 2007 Lead induced malformations in embryos of the African catfish *Clarias gariepinus* (Burchell, 1822). *Environmental Toxicology* 22(4):375-389.
- Pirrone N., Cinnirella S., Feng X., Finkelman R. B., Friedli H. R., Leaner J., Mason R., Mukherjee A. B., Stracher G. B., Streets D. G., Telmer K., 2010 Global mercury emissions to the atmosphere from anthropogenic and natural sources. *Atmospheric Chemistry and Physics* 10(13):5951-5964.
- Potipat J., Tangkrockolan N., Helander H. F., 2015 Bioconcentration factor (BCF) and depuration of heavy metals of oysters (*Saccostrea cucullata*) and mussels (*Perna viridis*) in the river basins of coastal area of Nagarajan Nagarani, Arumugam Kuppusamy Kumaraguru, Velmurugan Janaki Devi Chantburi Province. *Environment Asia* 8(2):118-128.
- Purcell S. W., 2014 Value, market preferences and trade of beche-de-mer from Pacific island sea cucumbers. *PLoS ONE* 9(4):e95075.
- Purcell S. W., Mercier A., Conand C., Hamel J. F., Toral-Granda M. V., Lovatelli A., Uthicke S., 2013 Sea cucumber fisheries: global analysis of stocks, management measures and drivers of overfishing. *Fish and Fisheries* 14(1):34-59.
- Ramakritinan C. M., Babu M. Y., Palanikumar L., Muneeswaran T., Kumaraguru A. K., 2014 Cytogenetic effects of chosen heavy metals to marine mussel, *Modiolus philippinarum* L. under acute stress. *International Journal of Marine Science* 4(52): 1-9.
- Riani E., 2010 [Mercury (Hg) contamination in the body organ of common ponyfish (*Leiognathus equulus*) in Ancol waters, Jakarta Bay]. *Jurnal Teknologi Lingkungan* 11(2):313-322. [in Indonesian]
- Riani E., 2011 [Reproductive disorder due to heavy metal contamination in green mussels (*Perna viridis*) cultured in Muara Kamal waters, Jakarta Bay]. *Jurnal Moluska Indonesia* 2(2):67-74. [in Indonesian]
- Riani E., 2021 Menguak sisi ekotoksikologis dampak kompleksitas kegiatan antropogenik di DAS Citarum dan pencemaran di Sungai Citarum dan Teluk Jakarta, serta opsi pengelolaannya. In: *Teknologi pengelolaan dan pelestarian sumberdaya alam dalam perspektif pembangunan berkelanjutan*. IPB Press, Bogor, Indonesia, 199 pp.
- Riani E., Cordova C., 2016 Pengantar ilmu lingkungan. UT Press, Tangerang, Indonesia, 310 pp. [in Indonesian]
- Riani E., Cordova M. R., Arifin Z., 2018 Heavy metal pollution and its relation to the malformation of green mussels cultured in Muara Kamal waters, Jakarta Bay, Indonesia. *Marine Pollution Bulletin* 133:664-670.
- Safitri S. S., Efendi E., Yudha I. G., 2018 Pencemaran Pb dan Cu pada kerang hijau di Pulau Pasaran, Lampung. *Jurnal Pengelolaan Perairan* 1(2):10-18. [in Indonesian]
- SEPA (Swedish Environmental Protection Agency), 2000 Environmental quality criteria. Coasts and seas. Report 5052, pp. 51-75.
- Sprovieri F., Pirrone N., Ebinghaus R., Kock H., Dommergue A., 2010 A review of worldwide atmospheric mercury measurements. *Atmospheric Chemistry and Physics* 10(17):8245-8265.

- Takarina N. D., Bengen D. G., Sanusi H. S., Riani E., 2013 Geochemical fractionation of copper (Cu), lead (Pb), and zinc (Zn) in sediment and their correlations with concentrations in bivalve mollusc *Anadara indica* from coastal area of Banten Province, Indonesia. *International Journal of Marine Science* 3(30):238-243.
- Toral-Granda V., Lovatelli A., Vasconcellos M., 2008 Sea cucumbers. A global review of fisheries and trade. *FAO Fisheries and Aquaculture Technical Paper No. 516*, FAO, Rome, 317 pp.
- Turkmen M., Turkmen A., Tepe Y., 2008 Metal contamination in five fish species from Black, Marmara, Aegean, and Mediterranean Seas, Turkey. *Journal of the Chilean Chemical Society* 53(1):1435-1439.
- Ung C. Y., Lam S. H., Hlaing M. M., Winata C. L., Korzh S., Mathavan S., Gong Z., 2010 Mercury-induced hepatotoxicity in zebrafish: *in vivo* mechanistic insights from transcriptome analysis, phenotype anchoring and targeted gene expression validation. *BMC Genomics* 11(1):212.
- USEPA (United States Environmental Protection Agency), 1995 Compilation of air pollution emission factor. AP-42 fifth edition, Volume I. Stationary point and area source. Chapter 1: External combustion sources. 13 pp.
- Van Esch G. J., 1978 Aquatic pollutant and their potential ecological effects. In: *Aquatic pollution: transformation and biological effects*. Hutzinger O., van Lelyved I. H., Zoeteman B. C. J. (eds), Pergamon Press, pp. 1-12.
- Vasseur P., Masfarau J. F., Blaise C., 2021 Ecotoxicology, revisiting its pioneers. *Environmental Science and Pollution Research International* 28(4):3852-3857.
- Vincent-Hubert F., Chatel A., Gourlay-France C., 2014 Metallothionein mRNA induction is correlated with the decrease of DNA strand breaks in cadmium exposed zebra mussels. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 766:10-15.
- Weber N., 2006 Dose-dependent effects of developmental mercury exposure on C-start escape responses of larval zebrafish *Danio rerio*. *Journal of Fish Biology* 69(1):75-94.
- Whittaker M. H., Wang G., Chen X. Q., Lipsky M., Smith D., Gwiazda R., Fowler B. A., 2011 Exposure to Pb, Cd, and As mixtures potentiates the production of oxidative stress precursors: 30-day, 90-day, and 180-day drinking water studies in rats. *Toxicology and Applied Pharmacology* 254(2):154-166.
- Xu J., Lian L. J., Wu C., Wang X. F., Fu W. Y., Xu L. H., 2008 Lead induces oxidative stress, DNA damage and alteration of p53, Bax and Bcl-2 expressions in mice. *Food and Chemical Toxicology* 46(5):1488-1494.
- Zeitoun M. M., Mehana E. E., 2014 Impact of water pollution with heavy metals on fish health: overview and updates. *Global Veterinaria* 12(2):219-231.

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