Bioaccumulation of Lead in White Shrimp (*Litopenaeus vannamei*) and Tiger shrimp (*Penaeus monodon*)

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***Abstract*—**The lead concentrations in muscles and carapace of white shrimp *(Litopenaeus vannamei)* fromPaliyan village, Temon district, Kulon Progo county, Yogyakarta Special Province in Indonesia and those of tiger shrimp (*Penaeus monodon*) bought in a grocery store in Yogyakarta were determined and compared to assses bioaccumulation and food safety.Ten samples of 40-day-old white shrimp composites (n=8), ten samples of 60-day-old White shrimp composites (n =5), ten samples of 80 day-old white shrimp composites (n=5), and ten samples of tiger shrimp composites (n=2) were collected and analyzed for Pb concentrations using Atomic Absorption Spectrophotometer. The mean concentration of Pb in 40-day-old white shrimps were below the detection limit of 0.05 µg/g d.w. No significant differences was found between the mean concentrations of Pb in muscles of 60-day-old (1.71 ± 0.74) and those of 80-day-old (1.84 ± 0.30 µg/g d.w.) white shrimps. However, significant differences ($p < 0.05$) were found between the mean concentrations of Pb in carapace of 60-day-old (8.22 ± 3.35) and those of 80-day-old (4.55 ± 1.03 µg/g d.w.) white shrimps. The mean of lead concentrations in shrimps in this study (0.25 – 0.41 µg/g w.w.) were lower than the recommended limit (0.50 µg/g w.w.). Similarly, the average daily intake of Pb through shrimp consumption was 0.39-13.39 µg/person/day and was relatively lower than the recommended value of 12,86 µg/person/day. Based on the results, it can be concluded that the shrimps analyzed in this study were safe for human consumption.

***Keywords*—** *bioaccumulation, lead, white shrimp, tiger shrimp*.

I. Introduction

White shrimp (*Litopenaeus vannamei*) is a widely cultured shrimp in many parts of Indonesia, especially in shrimp farming in the southern coast of Java [1].Agriculture, industry, domestic, and naturalsources produce polluted water containing lead (Pb) that enters shrimp aquaculture and other water bodies and accumulates in shrimp tissues [2], [3]. A study comparing nutrition contents (amino acids, fatty acids, and mineral) and also some heavy metals including Cd and Cu of between white shrimp and tiger shrimp was done by [4]. However, Pb was not included as one of the chemicals investigated. The purposes of this study wereto assess current lead concentrations and accumulated amounts in white shrimps in southern coast of Yogyakarta Special Province, to compare the results with the lead concentrations and amount in the tiger shrimp (*Penaeusmonodon*), and use the results to assess human exposure of Pb through shrimp consumption.

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II. Materials And Methods

White shrimps were collected by shrimp nets in June, 2015 from three shrimp ponds in Paliyan village, Temon, Kulon Progo County, Yogyakarta, Indonesia. Ten shrimp samples were collected each for shrimps aged 40, 60, and 80 days. Each sample of 40-day-old shrimps(WS_{40}) consisted of eight individuals while each sample of 60-day-old shrimps (WS_{60}) and 80 day- old-shrimp (WS_{80}) consisted of five individuals. Tiger shrimps (TS) were bought from a grocery store in Yogyakarta city and analyzed as ten composite samples of two shrimps. The shrimps were washed by distilled water, separated into muscle and carapace, and weighted to obtain wet weight. In the next step, all muscles and carapace were dried in oven at 105^0C for 24 hours, and the tissues were reweighted to get dry weight.Composites were done in order to obtain adequate sample weight (2 g dry weight sample).

Two grams of dry weight shrimp samples were extracted by a mixture of 18 mL HCl and 6 mL HNO_3 (3:1). This extraction step was repeated once by using the same volume of acid solutions. The digested sample was subsequently filtered through Whatman filter paper and made up to 10 mL using deionized water. The concentrations of lead were determined by Atomic Absorption Spectrophotometer (AAS, Perkin Elmer 3110) at Chemistry Laboratory of Gadjah Mada University. The lead concentrations were expressed in unit of µg/g dry weight (d.w.). Six water samples (100 mL each) were extracted by using HNO_3, filtered and analyzed with AAS and the lead concentrations were determined in unit of µg/mL.

III. Results And Discussion

The concentrations and amount of lead in shrimp's tissues are provided in TABLE I. In this study, amount is defined as

$$A_i = W_i \times C_i \quad (1)$$

where A_i is the amount of lead in an organ (muscle or carapace) in µg, W_i is the organweight (g) and C_i is the concentration of lead in the organ (µg/g).

TABLE I: CONCENTRATIONS AND AMOUNT OF LEAD IN SHRIMP TISSUES

Parameter	Symbol	Unit	40-days-old white shrimp (WS_{40})	60-days-old white shrimp (WS_{60})	80-days-old white shrimp (WS_{80})	tiger shrimp (TS)
Muscle dry weight	-	g	0.41 ± 0.10	0.97 ± 0.12	3.77 ± 0.93	3.74 ± 0.91
Carapace dry weight	-	G	0.41 ± 0.12	0.67 ± 0.08	1.24 ± 0.18	5.45 ± 1.06
Total dry weight	-	G	0.82 ± 0.19	1.64 ± 0.14	5.01 ± 1.03	9.18 ± 1.93
Conc. in muscle(d.w.)	C_m	μg/g	0.03	1.71 ± 0.74	1.84 ± 0.30	1.10 ± 0.57
Conc. in carapace (d.w.)	C_c	μg/g	0.03	8.22 ± 3.35	4.55 ± 1.03	1.49 ± 0.61
Amount in muscle	A_m	μg	0.01	1.67 ± 0.71	6.95 ± 2.01	4.18 ± 3.09
Amount in carapace	A_c	μg	0.01	5.52 ± 2.40	5.60 ± 1.26	8.35 ± 4.21
Total amount	A_t	μg	0.02	7.19 ± 2.58	12.55 ± 2.42	12.53 ± 6.36

The moisture contents of white shrimp and tiger shrimp in this study were 76.37 ± 2.00 and 78.39 ± 1.80 (% w.w.), respectively. The values are similar to those reported in [4] as 77.21 ± 0.18 and 80.47 ± 0.26, respectively. The concentrations of Pb in muscles and carapaces of all of WS_{40} were below the limit of detection (0.05 μg/g). The approach in this study is to set those values as one-half of the detection limit as described in [5] or 0.03 μg/g.

The average lead concentration in white shrimp in this study (1.71 ± 0.74 μg/g d.w) was higher than those reported for *Acetes* shrimp in [6]as 0.59 ± 0.13 μg/g d.w. Lead concentrations were higher in the carapace than in the muscle, in all age groups of shrimps (Table I). The ratios between the lead concentrations in carapace to those in muscle for WS_{60}, WS_{80} and TS were 4.81, 2.47, and 1.35, respectively. Those ratios are in accordance to the same ratios (0.71 - 5.43), as in [7]. The lead concentrations in carapace may be influenced by molting processes such as reported for fiddler crabs *Ucapugnax*by [8]thatmolting reduces the body burden.

Relationships between Pb concentrations and amounts in WS_{60} and WS_{80} were tested by paired t-test using SPSS v.17.0 [9]. Statistically significant differences were found between Pb concentrations in muscle of WS_{80} and TS, and also between Pb concentrations in carapace of WS_{80} and TS. There was also significant difference between Pb amount in muscle of WS_{80} and TS. However, no significant differences were observed between total Pb amount in WS_{80} and TS (Table II).

The average concentration of lead in water (C_w) of shrimp ponds in this study was 15.7 ± 18.5 $\mu g.L^{-1}$ (n = 6). Three from six samples contained Pb below the detection limit of 10 μg/L and determined as 5 μg/L. The average lead concentrations in the water was equivalent to chronic value of Pb on Mysid shrimp 25 $\mu g.L^{-1}$ as reported in [10]. The bioconcentration factor (BCF) can be determined as

$$BCF = \frac{C_m}{C_w} \quad (2)$$

where C_m is the average lead concentration in the muscle of WS_{80}. By using C_m 1.84 μg/g or 1.840 μg/kg and C_w15.7 μg/L, the BCF in this study could be determined as 117 L/kg. This BCF value is lower than the mean of BCF as reported by [11] as 410, based on 14 studies. In order to estimate human exposure to Pb through shrimp consumption, a test and a survey were performed. In the test, 20 participants were asked to consume WS_{80} which were

TABLE II: T-TEST ANALYSES BETWEEN TWO GROUPS OF CONCENTRATIONS AND ACCUMULATED AMOUNT OF LEAD

Parameter	Groups	t value	P
Concentrations of Lead	C_m (WS_{60})vsC_m (WS_{80})	-0.52	non significant
	C_m (WS_{80})vs C_m (TS)	4.03	< 0.01
	C_c (WS_{60})vs C_c (WS_{80})	3.31	< 0.01
	C_c (WS_{80})vs C_c (TS)	8.59	< 0.01
Accumulated amount of Lead	A_m(WS_{60})vs A_m(WS_{80})	-7.84	<0.01
	A_m (WS_{80}) vs A_m (TS)	-2.38	< 0.05
	A_c (WS_{60}) vs A_c (WS_{80})	-0.10	non significant
	A_c (WS_{80}) vs A_c (TS)	1.98	non significant
	A_t (WS_{80}) vs A_t (TS)	0.01	non significant

previouslycooked and weighted. The consumed amount was determined by subtracting the initial weight with the final weight. The average consumed amount of WS_{80} was 6.5 g or 58.0 ± 11.2 % of the initial w.w. In the survey, 77 respondents were asked using questionnaire to choose one of several descriptors as listed in Table III to know the probabilities of consumption frequency and shrimp number per serving as well as part(s) to be consumed.

The probability of the number of shrimp consumers in this study was 0.94 (Table III) and this value was comparable to 0.98reported for Asian and Pacific Islander [12].Five from a total of 77 participants(P = 0.06) in this study did not consume shrimps as they are vegetarians or allergic to shrimps. In this study, the probabilities to consume shrimp muscle only and whole body were 0.63 and 0.11 (Table III) and were relatively comparable to those reported in [12] as 0.78 and 0.21, respectively. This study found that a probability to consume muscle and some parts of carapace was 0.26 (Table III).The weighted-average of consumption frequency ($\overline{CF}$) is calculated as

$$\overline{CF} = \frac{\sum P_i F_i}{\sum P_i} \quad (3)$$

where $P_i$is the probability that a certain CF will occur and F_i is the number of consumption frequency per month (1, 2, 3, or 4). The weighted-average of CF in this study was 1.88 times per month, and this value is lower than to that reported in [12]as 41 times per year or 3.42 times per month.It can be suggested that the people in a developed country may consume shrimp more often than those in a developing country. The weighted-average of shrimp number per serving ($\overline{SN}$) is calculated as

$$\overline{SN} = \frac{\sum P_i N_i}{\sum P_i} \quad (4)$$

where $P_i$is the probability that a certain number of shrimps will be consumed and N is the number of shrimps in a serving menu (2, 4, 6, or 8). The weighted-average of number of shrimps per serving in this study was 4.54 shrimps/ serving. Multiplying this value by 10.1 g/shrimp (average wet weight of WS_{80}), resulted in 45.86 g of shrimp per serving.

TABLE III: PROBABILITIES OF SHRIMP CONSUMPTION SCENARIOS

Parameter	Symbol	Descriptor	Probability (P_i)
Shrimp consumer type	-	eating shrimp	0.94
		not eating shrimp	0.06
Part(s) to be consumed	-	muscle	0.63
		complete muscle and carapace	0.11
		muscle and parts of carapace	0.26
Consumption frequency	CF	once per month	0.51
		twice per month	0.25
		thrice per month	0.11
		four times per month	0.13
Shrimp number per serving	SN	two shrimps	0.22
		four shrimps	0.47
		six shrimps	0.13
		eight shrimps	0.18

In the next step, the value of 45.86 g was further multiplied by 0.47 shrimp consumption/week (from 1.88 consumption/month divided by 4) to get 21.55 g/wk, which is approximately 8.19% of the total consumption of fresh fish and shrimps by Indonesian (263 g/wk.) [13].

Three scenarios of human exposure (HE) to Pb through fish consumption are determined as

$$HE_m = CF \times SN \times A_m \quad (5)$$

$$HE_p = CF \times SN \times 0.58 \times A_t \quad (6)$$

$$HE_w = CF \times SN \times A_t \quad (7)$$

where HE_m, HE_p, and HE_w are human exposures to lead through consumptions of muscles, partial body, and whole body, respectively.

The adjusted average daily intake (ADI) of Pb through crustacean consumption for Asian population is 12.86 µg/person/day. The value is derived from European daily intake of 15 µg/person/day [14] and converted by multiplying with 0.86 which is the ratioof average Asian body weight (60 kg) to average European body weight (70 kg).Three scenarios as expressed in (5), (6), and (7) were shown in Table IV and Fig. I.

The human exposure levels to lead vary notably among the scenarios (Fig. I). The human exposures in low and weighted-average consumption scenarios were below than the adjusted ADI while the exposure in high scenario (13.39 and 13.07 µg/person/day) were slightly higher than the ADI (Table IV, Fig.I). The amounts of Pb in WS_{80}in high and low consumption scenarios were 12.95 and 6.95 µg, respectively, so the ratio between the two amount could be determined as 1.80. The situation was very much different when comparing the human exposure to Pb inWS_{80} in high consumption scenario (13.39 µg/person/day) to that in low consumption scenario (0.46 µg/person/day) (Table IV). The ratio of the Pb exposure between the two exposure scenario was 29.1 which was much more relevant to be used in risk assessment, rather than the use of the ratio between two amounts of Pb only (1.80).

TABLE IV: ESTIMATION OF HUMAN EXPOSURES TO LEAD THROUGH SHRIMP CONSUMPTION

Parameter	Unit	Shrimp	Shrimp Consumption Patterns: Low Consumption	Weighted- Average Consumption	High Consumption
Consumption Frequency (CF)	consumption per month	Both	1	1.88	4
Part(s) Consumed	-	Both	muscle	muscle, parts of carapace	whole body
Shrimp Number (SN)	shrimp per serving	Both	2	4.54	8
Amount of Pbin shrimp	µg/shrimp	WS_{80}	6.95	7.28	12.55
		TS	5.60	7.27	12.53
Human Exposure (HE) to Pb	µg/person/month	WS_{80}	13.90	62.14	401.60
		TS	11.20	62.05	400.96
Human Exposure (HE) to Pb	µg/person/day	WS_{80}	0.46	2.07	13.39
		TS	0.39	2.07	13.07

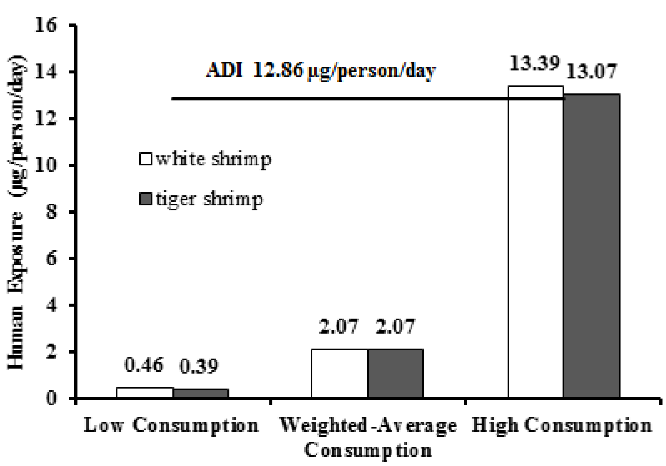

Fig. 1. Human exposure scenarios to Pb through shrimp consumption

The range of lead concentrations in white and tiger shrimps in this study was 0.25 – 0.41 µg/g (w.w.). These values are higher compared to those generally found in routine monitoring programs 0.10 – 0.24µg/g (w.w.)[15] which indicates that shrimps in Paliyan village as well as those in Temon district should be included in regular monitoring programs. However, the lead concentrations were below the maximum recommended limit based on the Indonesia National Standard of 0.50 µg/g (w.w.) [15].The human exposure to Pb through shrimp consumption in this study (0.39-13.39 µg/person/day) was mostly lower than the adjusted ADI of 12,86 µg/person/day except for values close to high consumption scenario. Therefore, the shrimps analyzed in this study were safe for human consumption. However, people should refrain from consuming whole body shrimps.

IV. Conclusion

Lead concentrations in white shrimps from aquaculture facilities and tiger shrimps brought from grocery store were evaluated in this study. The lead concentrations were lower than the maximum recommended limit and the shrimps were safe for human consumption. This study provided current information of lead pollution in aquaculture in southern part of Yogyakarta and analyzed relationship between amounts of lead accumulated in shrimp tissues with human health exposure. Integrated and periodical monitoring programs of lead concentrations in shrimp tissues at various development stages as well as those in pond waterwould provide more complete and detail information for aquaculture management and aquatic ecological assessment in the southern part of Yogyakarta Special Province.

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