Vietnam Journal of Science and Technology ***58*** *(4) (2020) 493-504*

doi:10.15625/2525-2518/58/4/14473

BIOACCUMULATION OF MERCURY IN CLAMS *MERETRIX LYRATA* (SOWERBY, 1825) CULTURED AT THE BACH DANG ESTUARY: A RECOMMENDATION FOR SAFE DAILY DOSAGE CONSUMPTION OF CLAMS IN VIET NAM

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Received: 18 October 2019; Accepted for publication: 4 May 2020

Abstract. This study assessed the level of total mercury (THg) and methylmercury (MeHg) in seawater and clams *Meretrix lyrata* (Sowerby, 1825) during all four seasons (July 2010 to May 2011) at the Dong Bai intertidal area in the Bach Dang estuary. The results showed that THg and MeHg concentrations of the water were under the allowable limit. THg and MeHg concentration for clams ranged from 12.5 - 87.9 ng/g dw and 1.1 - 24.6 ng/g dw, respectively. The highest bioaccumulation factor (BAF) of *Meretrix lyrata* was 303.2 for THg and 165,000 for MeHg. The recommended safe dosage for daily clam consumption depends on the season. Over an one-year period, mercury contamination in clams harvested in the Bach Dang estuary ranged from 1.4 to 38.1 g/kg bw. It is not recommended to consume too many clams in March or April.

Keywords: Meretrix lyrata, accepted daily intake, bioaccumulation factor (BAF), methylmercury, total mercury.

Classifictaion numbers: 3.3.2, 3.6.1, 3.6.2.

1. INTRODUCTION

The Bach Dang estuary is a typical tropical climate estuarine zone with a, high biodiversity and abundant aquatic resources. The estuary has a high biodiversity of marine species that are harvested all year. In recent years, with the rapid industrialization and advanced urbanization in Hai Phong city, residential and industrial wastewater from social-economic activities, such as aquaculture, agriculture, high population density and big seaports with busy shipping, has been discharged into the estuary without treatment. Consequently, water quality of the estuary has been impacted and caused deterioration of the estuary. To date, several studies on determining the levels of contaminants in various ecosystems of Hai Phong (Viet Nam) have been conducted on estuaries such as the Cam estuary [1] and the Bach Dang estuary [2]. One of the most toxic substances is mercury because of its high aquatic toxicity and high potential for bioaccumulation in benthic species.

In 1953, mercury pollution reached a dangerous level when some people began experiencing paralysis or contracted Minamata disease [3]. The methylmercury (MeHg) contamination in the daily diet of local residents resulted in Minamata disease in Japan [3]. It has been demonstrated that after exposure for 2 days, approximately 95 % of the MeHg was absorbed into the organs of the fish and could exist in the fish tissues from 70 - 90 days. Most likely, mercury had entered the food chain from lower tropic level species (plankton) and was transferred to higher tropic level species (small and large fish). It was demonstrated that mercury could be easily assimilated by aquatic organisms, especially benthic species, such as the clams *Meretrix lusoria* [4, 5] and *Scapharca subcrenata, Mactra veneriformis and Ruditapes philippinarum* [6]. Therefore, MeHg can enter the human body through eating these fish and mollusks. If humans consume more than 0.1 µg/kg/day of MeHg, they can get Minamata disease, which results from losing control of the central nervous systems-CNS [7].

In the coastal environment, bivalve species have been used for studying environmental pollution because they have a sedentary lifestyle and filter feed. Previous studies showed that the clam *Meretrix* sp. was bioindicator because they live among the benthos and are able to accumulate the toxins [8 - 11]. However, in Viet Nam, mercury pollution has been traditionally documented in terms of quality and quantity in the environment, but mercury bioaccumulation in benthic species is unknown. According to Le Xuan Sinh *et al*. [12], *Meretrix lyrata* is one of the most popular cultured species in the Bach Dang estuary because of its relatively high economic value for the local population. Therefore, the goal of this study was to assess the mercury bioaccumulation in *Meretrix lyrata* cultured in the Bach Dang estuary. The potential health risk associated with ingesting mercury and MeHg in clams raised in aquaculture was evaluated to estimate human Hg and MeHg dietary intakes for the local people living around the Bach Dang estuary.

2. METERIALS AND METHODS

Sea water and hard clams *Meretrix lyrata* (Sowerby, 1825) of commercial size were collected 10 times (T1-T10) at the intertidal area in Dong Bai, Cat Hai, Hai Phong, Viet Nam (20°48'833"N & 106°53'508" E – Fig. 1) from July 27th, 2010, to May 6th, 2011 (Table 1). Dong Bai intertidal is also the largest place of clam farming in Bach Dang estuary because it has favorable natural conditions and composition of sediment is suitable for culturing clam.

Table 1. Collection of hard clam samples from 27 July 2010 to 6 May 2011.

Time	Label	Time	Label
27 July, 2010	T1	5 January, 2011	T6
29 August, 2010	T2	27 January, 2011	T7
27 September, 2010	T3	4 March, 2011	T8
30 October, 2010	T4	8 April, 2011	T9
4 December, 2010	T5	6 May, 2011	T10

The experiment area was conducted in the Dong Bai intertidal area with three replicates (about 4 m^2 for each replicate) (Fig. 2). This area has the highest water level of 2.5 - 3.2 m and the lowest water level of 0.5 - 1 m. Nylon mesh with mesh 10 × 10 mm was buried under a layer of sand about 30 cm to prevent the mesh from being turned on because of the strong waves and to ensure that clam could not escape from the experimental sites. Clams were cultured from size of 400 - 500 individual/kg in May 2010. Samples of clam and sediment were collected from July 2010 until May 2011 in order to analyze the bioaccumulation of mercury in clams.

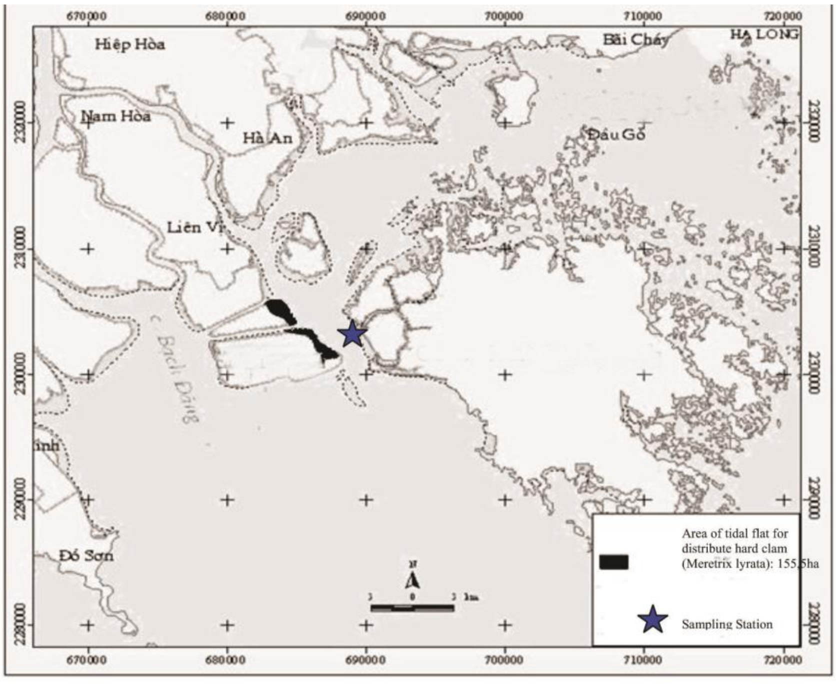

Figure 1. Map of intertidal area for culturing clam in Dong Bai commune, Cat Hai district, Hai Phong.

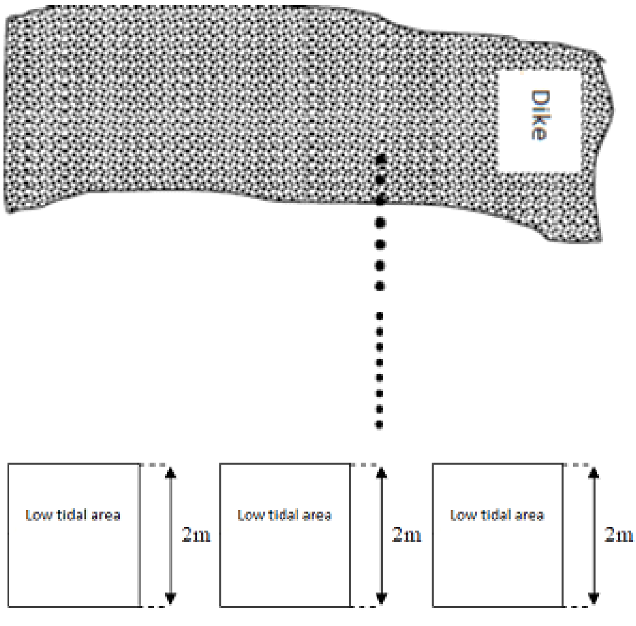

Figure 2. Sampling sites in the Dong Bai intertidal, Bach Dang estuary.

The data were validated from April 2012 to 2017. Clam age at the samples collected from April 2012 to 2017 was similar to that at T9 (clams at 14 months old – the period of harvesting). This is a data from the national monitoring station in Hon Dau area (Do Son) in the Bach Dang estuary, the sampling location of *Meretrix lyrata* clam coincides with the sampling position in Fig. 2.

Total mercury (THg) load in the water was analyzed by the EPA method 1631e [13]. This method is based on the steam atom absorption method associated with cold-evaporation techniques using reducing agents ($SnCl_2$). The accuracy of the analytical method was validated by the analysis of standard reference materials as follow: Canadian MESS-3 sediment samples were determined to be 0.091 µg/g ± 0.009. The results of measuring MESS-3 samples in the laboratory (n = 4) were 0.101 µg/g ± 0.012. Water samples were adjusted to pH 2.3 and then hydrated by HVG-1. Then, Hg hydride was passed through quartz cells mounted on atomic absorption spectroscopy (AAS) burners and measured by AAS instrument. A coefficient of variation (CV%) was applied to test the reliability and found to be less than 5% for all experiments.

The THg content in clam tissue was analyzed using the EPA1631e method [13]. Each clam was separated into stomach and muscle tissue. Clam muscle tissue was ground to homogenize the sample and stored in a specialized freeze for 48 hours. The sample was dried and ground and stored in sealed zipper bags at 4°C until analyzed. An 1 gram sample of the dried tissue was weighed and placed in a Teflon bomb (170°C and 10 atm). Then, 5 mL of HNO_3 PA and 2 mL of H_2O_2 were added to the sample in the bomb and mixed well. The solution samples were dried in the oven at 140°C for at least 3 hours. During drying, the color of the solutions was checked to determine completion of the decomposition of the samples. The samples were decomposed completely if the samples were clear or yellowish-brown in color. After decomposition, the samples were filtered and water added to achieve a final volume of 50 mL. The filtered solutions were analyzed using AAS [14].

Analytical procedures were performed at the laboratories of Shizuoka University and Kumamoto University, Minamata province, Japan. The principle of this process is based on the physiochemical characteristics of MeHg compounds with anionic bonding. MeHg cysteine is soluble in water and insoluble in benzene, whereas MeHg chloride is insoluble in water but dissolves in organic solvents. Those physical and chemical differences were used by Wessto to isolate and clean samples before analysis. MeHg was then analyzed using gas chromatography (EPA method 1630) with an electronic capture detector (ECD) [15].

Accuracy evaluation: To assess the accuracy of the method, we use the following types of standard samples. The Canadian MESS-3 sediment standard sample has a defined content of 0.091 µg/g ± 0.009. The measurement of MESS-3 sample at the Laboratory of Institute of Marine Resources and Environment (Viet Nam) (n = 4) is 0.11 µg/g ± 0.012 in which the analytical error compared to the standard sample is 108%. This findings shows the method of inorganic mercury meets requirements for analysis of environmental samples.

Biological Accumulation Factor (BAF) is the ratio of substance concentration in tissues of living organisms to the concentration of the substance in the environmental water [16].

$$\mathbf{BAF} = \frac{Ct}{Cw}$$

in which: BAF is bioaccumulation factor; Ct is the pollutant concentration in biological tissues (mg/kg dry tissue); and Cw is the pollutant concentration in water (mg/L ≈ mg/kg).

Acceptable Daily Intake (ADI) is a measure of the amount of a specific substance (originally applied to food additives or the residues of a veterinary medicine or pesticide) in food or drinking water that can be ingested on daily basis over a lifetime without an appreciable health risk. The calculation according to the ASEAN-Canada CPMS-II (1999) guidelines [17] is as follows:

$$\mathbf{ACD} = \frac{\mathbf{ADI}}{(\mathbf{BAF}) \times (\mathbf{EC})}$$

in which: ACD is the amount of consumption hard clam per day (kg hard clam tissue/kg body weight); ADI is Acceptable Daily Intake (µg/kg); BAF is bioaccumulation factor; and EC is environmental standard (QCVN 10:2015/BTMNT) for aquaculture water (µg/L).

Statistical Analysis: Data were analyzed by Excel software to determine the correlations and relationships of the results.

3. RESULTS AND DISCUSSION

Hard clam and water samples were collected from 27th July, 2010, to 6th May, 2011, to analyze THg and MeHg concentrations (Table 2).

Table 2. THg and MeHg concentration in water at Dong Bai zone (Cat Hai district) from 6/2010 to 5/2011.

Time	MeHg (ng/l)	THg (µg/l)
T 1 (27/6) (n = 5)	0.13 ± 0.01	0.44 ± 0.15
T 2 (29/8) (n = 5)	0.16 ± 0.03	0.52 ± 0.09
T 3 (27/9) (n = 5)	0.12 ± 0.02	0.23 ± 0.05
T 4 (30/10) (n = 5)	0.18 ± 0.03	0.36 ± 0.15
T 5 (4/12) (n = 5)	0.16 ± 0.02	0.31 ± 0.14
T 6 (5/1) (n = 5)	0.15 ± 0.01	0.30 ± 0.09
T 7 (27/1) (n = 5)	0.18 ± 0.03	0.35 ± 0.11
T 8 (4/3) (n = 5)	0.13 ± 0.03	0.25 ± 0.08
T 9 (8/4) (n = 5)	0.14 ± 0.02	0.28 ± 0.10
T 10 (6/5) (n = 5)	0.23 ± 0.02	0.77 ± 0.15
Average	**0.16**	**0.42**

THg concentrations ranged of 0.25 - 0.77 µg/L in all water samples. The results showed that the THg concentrations in the rainy season (June, July, August and the following May) were higher than that in the dry season (from September to April) (Fig. 3). Meanwhile, the trend of MeHg was not as pronounced as the trend of THg. MeHg concentrationsin water samples ranged in 0.12 - 0.23 ng/L and were lower in comparison with THg concentrations. Typically, in natural surface waters, freshwater and marine, concentrations of THg range from < 1 to 20 ng/L, while concentrations of MeHg are usually less than 1 ng/L. Thus, MeHg represents less than 10% of total Hg found in surface waters [18].

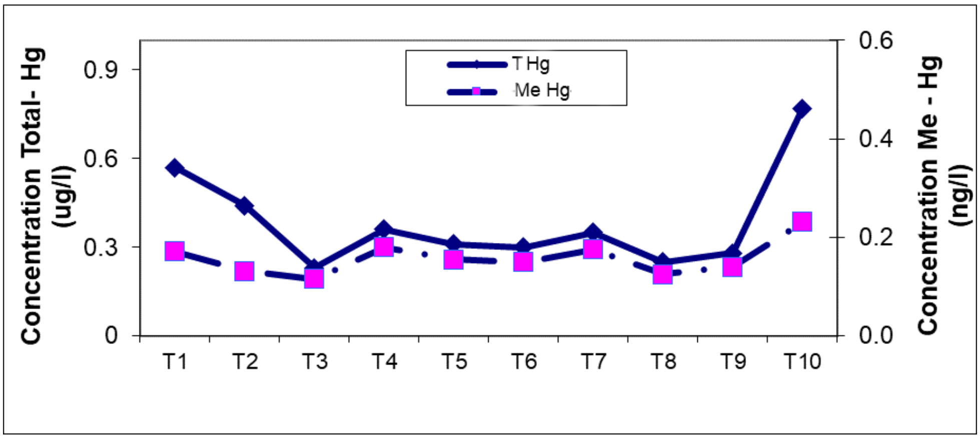

Figure 3. MeHg and THg concentrations in the water at Dong Bai zone (Cat Hai district) during the sampling period.

Table 3 shows the concentration of two mercury forms in the *Meretrix lyrata* meat tissue from 2012 to 2017 which expresses the mercury concentration ranged from 78.1 to 84.8 ng/g dw and the methyl mercury concentration ranged from 19.8 to 24.1 ng/g dw. This presents that the concentration of the two mercury forms in the range of concentrations detected in clam tissue is accounted on the observed data series in 2010-2011.

Table 3. Mercury concentration collected at Do Son monitoring station in the period of April through years 2012 – 2017.

No.	**The period collecting samples at Hon Dau monitoring station, Do Son**	**Mercury in hard clam tissue**	
		THg (ng/g dw)	**MeHg** (ng/g dw)
1	April, 2012 (n = 5)	84.8 ± 0.04	19.8 ± 0.03
2	April, 2013 (n = 5)	78.1 ± 0.05	22.1 ± 0.04
3	April, 2014 (n = 5)	80.4 ± 0.03	20.2 ± 0.02
4	April, 2015 (n = 5)	82.1 ± 0.05	24.1 ± 0.04
5	April, 2016 (n = 5)	81.1 ± 0.04	23.1 ± 0.03
6	April, 2017 (n = 5)	82.8 ± 0.04	23.9 ± 0.04

The concentrations of THg and MeHg accumulated in hard clam tissue are shown in Fig. 4. The THg in hard clam tissue ranged from 12.5 to 87.9 ng/g dw for the period sampled.

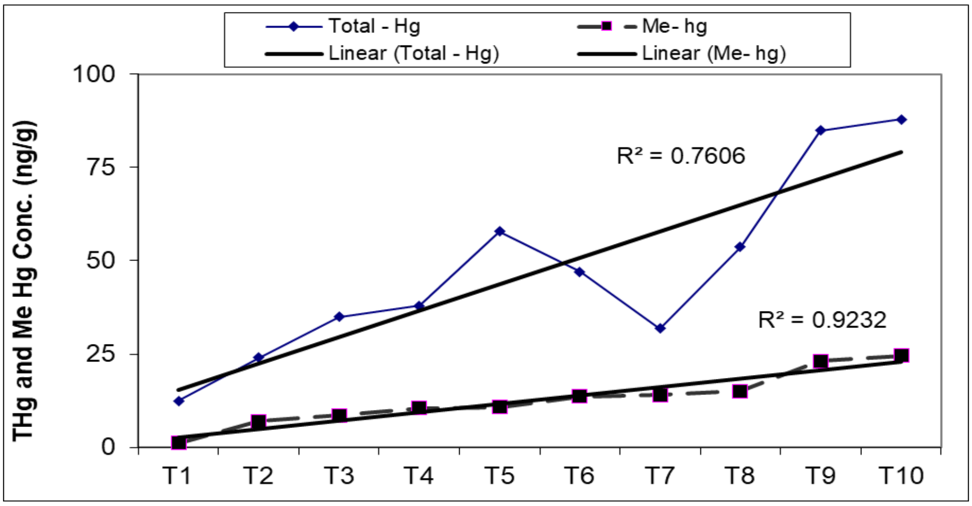

Figure 4. THg and MeHg concentrations in clam tissue during the sampling period.

Results from this study were lower than in study of Otchere *et al*. [19] who reported that THg concentration for both mussels and oysters ranged from 40 to 840 ng/g dw. However, it is consistent with study of Kehrig *et al.* [20] who reported that the THg concentration in bivalve species (mussel, oyster and clam) in Guanabara and Sepetiba Bays ranged from 1 ng/g dw to 83 ng/g dw. It was found that the concentrations of THg were lowest in January 2011 (T7), at 31.8 ng/g dw. The decrease of THg in hard clam tissue is probably due to the elimination process being more effective than the accumulation process [21]. In January, temperature was approximately 15 °C, and the wave and flow regime in the clam aquaculture area is strong because it is affected by the northeast monsoons [22]. Therefore, the clam spent energy to bury themselves in the sand in order to cope with harsh weather [23]. In addition, the fat in clam could be reduced due to the lack of nutrients in January. According to the research of Le Xuan Sinh [24], the lipid concentration in clam tissues decreased in January. The lower concentrations of mercury in clams in the wet season were due to primary production and dilution effects [19]. The lipid reduction could result in reduced accumulation of mercury in fat tissue [21]. However, the THg concentration in clam tissue increased to 53.7 ng/g dw in March 2011 (T8) and reached 87.9 ng/g dw in May 2011 (T10). Most likely, nutrients are abundant for clam aquaculture in the spring. Thus, the accumulation of mercury by clams could increase in the spring. Further studies should be implemented to understand the mechanism of mercury accumulation during different seasons of the year.

MeHg is the most toxic form of mercury and toxicity to aquatic organism increases if it moves up the food chain [25]. It has been proven in 2007 by Korea Food and Drug Administration that MeHg has high fat solubility and is toxic to the central nervous system, which has a high fat content. As it is easily dissolved in fat and quickly absorbed in the digestive tract, MeHg showed 17 to 35 times faster absorption than inorganic mercury [26]. In general, more than 90 % of the mercury in fish is found as MeHg, but concentrations of MeHg can vary considerably among species [27]. In this study, the accumulated MeHg concentrations in hard clam tissue tended to increase during the sampling period. The concentrations of MeHg in clam tissue were from 1.1 ng/g dw in July 2010 to 24.6 ng/g dw in May 2011. The analytical results of clam samples in the same month age in T9 period collected in April from 2012 to 2017 showed that the accumulation level was within the range of the above data series (Table 3).

The results also showed that the average proportion of MeHg accounted for 26 % of the total amount of mercury in clams. This finding is consistent with the study of some bivalve species in a Rio de Janeiro estuary in Brazil [28]. Those authors reported that there was a significant difference in the MeHg/THg ratio in different aquatic organisms: MeHg percentages found in the carnivorous fish were higher (98 %) than that in the detritivorous fish (54 %) and the mollusks (33 %). Otchere [17] also indicated that the MeHg/THg ratios varied from 30 to 80 %, depending on the season, location, species and the type of lagoon.

Compared to the previous study on kind of mercury formed in environment and mercury accumulation mechanism of Meretrix lyrata hard clam [30, 31], the results of this paper compute the BAF accumulation coefficient and recommendation for safe dosage for daily consumption of hard clams depending upon regulations for Acceptable Daily Intake. The bioaccumulation factor (BAF) observed relating to the THg and MeHg accumulation by clams collected intertidallyat Dong Bai increased over time during the period of study. The bioaccumulation factor of THg ranged from 20.5 to 303.2 and from 6111 to 165000 for MeHg (Table 4). The highest BAF values were reported in April 2011 (T9), with 303.2 for THg and 165000 for MeHg. It is probable that the clams at the Bach Dang estuary reached commercial size and had high proportions of fat at that time. Previous study reported that one of the factors that can affect the BAF is the total shell length, which is proportional to age [20]. However, the BAF for THg of clam *Meretrix lyrata* in this study was not substantially different from the study of cockles, *Anadara granosa,* in the study (VAST06.07/11-12) [29]. Further work needs to be completed to define the actual BAF values of THg and MeHg for the Bach Dang estuary in different seasons of the year.

Table 4. THg and MeHg bioaccumulation factors (BAFs) ([L/kg].

Sampling period	BAF of Σ-Hg	BAF of Me-Hg
T1	20.5	6111
T2	46.2	43750
T3	152.2	70833
T4	105.3	58333
T5	186.8	67500
T6	157.0	91333
T7	90.9	78333
T8	214.8	116153
T9	303.2	165000
T10	114.2	106957

A recommendation for safe dosage for daily consumption of hard clams depends upon regulations for Acceptable Daily Intake, environmental standards for aquaculture water (EC) and the bioaccumulation factor (BAF) for each country/region. The ADI of hard clams, *Meretrix lyrata*, for THg and MeHg in Viet Nam, the European Union (EFSA) and the FAO/WHO is presented in Table 5. According to the JECFA [32, 33], the ADI for mercury in Viet Nam has been higher (0.714 µg/kg body weight) than that in European (EFSA) and FAO/WHO guidelines (0.571 µg/kg), while the environment standard concentrations of mercury in water and the bioaccumulation factors were similar for clam culture, at 1 µg/L and 139.3, respectively. As a consequence, the safe dosage per day for daily consumption of mercury in Viet Nam was recommended to be higher than in the EFSA and FAO/WHO recommendations.

With regard to MeHg, the values for ADI from the Viet Nam National Regulation and Joint FAO/WHO Expert Committee on Food Additives were 0.229 µg/kg body weight; it was 0.186 µg/kg body weight in the EFSA. Thus, calculated daily values for safe dosage of hard clam consumption based on Viet Nam National technical regulations and FAO/WHO Committee were 0.0028 kg tissue of clam per kg body weight. Meanwhile, EFSA was more stricter with a recommendation for safe dosage of MeHg in hard clams, with 0.0023 kg hard clam tissue (2.3 grams) per kg body weight per day.

Table 5. Recommendations of safe dosage/day for THg and MeHg from different organizations [32, 33].

Organization	ADI		EC		BAF		Safe dosage per day	
	µg/kg bw		µg/L		L/kg		kg tissue/kg bw	
	THg	MeHg	THg	MeHg	THg	MeHg	THg	MeHg
Viet Nam[1]	0.714	0.229	1	1	139.3	81.557	0.0051	0.0028
Europe[2]	0.571	0.186	1	1	139.3	81.557	0.0041	0.0023
FAO/WHO[3]	0.571	0.229	1	1	139.3	81.557	0.0041	0.0028

[1]*Viet Nam National technical regulation on the limits of heavy metals contamination in food*.
[2]*European food safety authority*.
[3]*Joint FAO/WHO Expert Committee on Food Additives (JECFA).*

Table 6. Estimated safe dosage for daily consumption of clams cultured at the Bach Dang estuary.

Sampling time	THg (kg tissue/kg bw)	MeHg (kg tissue/kg bw)
T1	0.035	0.0375
T2	0.015	0.0051
T3	0.005	0.0031
T4	0.007	0.0039
T5	0.004	0.0033
T6	0.005	0.0025
T7	0.008	0.0029
T8	**0.003**	**0.0019**
T9	**0.002**	**0.0014**
T10	0.006	0.0021

Table 6 presents the estimated safe dosage for daily clam tissue consumption, for clams cultured in the intertidal zone of Dong Bai, Bach Dang estuary. These figures were calculated based on the bioaccumulation factor from this study and the ADI and EC in Viet Nam. The results showed that the safe dosage varied for different seasons of the year. The highest safe amount of hard clam consumption per day was found to be in July and August (summer season).

In contrast, it is not recommended to consume too many clams per day from September to April (from autumn to spring seasons) because the high bioaccumulation factor for clams occurred during that period. Specifically, people should limit consumption of hard clams in the spring (March and April) because the safe dosage was found to be very low, at only 0.002 kg clam tissue/kg bw.

4. CONCLUSIONS

In this study, total mercury (THg) and methylmercury (MeHg) levels found in environmental water and *Meretrix lyrata* clams in the intertidal zone of Dong Bai were not considered high. In hard clams, the accumulation of mercury and MeHg was found to result in higher concentrations during the summer and lower concentrations during the winter. Based on the acceptable daily intake (ADI) factors, environmental standard (EC) values of Viet Nam, and the bioaccumulation factors of THg and MeHg as determined in this study, the estimated safe dosage for daily hard clam consumption has been calculated in Bach Dang estuary for the first time and was found to vary seasonally throughout the year. The results suggest that the local people should consume daily less than 0.0023 kg clam tissue/kg body weight in the spring season and 0.03 kg clam tissue/kg body weight in the summer.

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