

## INVESTIGATION OF HEAVY METAL POLLUTION ON CULTURED CLAMS AND OYSTERS IN VANDON - QUANGNINH, NORTH VIETNAM

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### TÓM TẮT

## KHẢO SÁT Ô NHIỄM KIM LOẠI NẶNG TRONG CÁC LOÀI NGAO VÀ HÀU NUÔI TẠI VÂN ĐỒN - QUẢNG NINH, MIỀN BẮC VIỆT NAM

Nghiên cứu này nhằm khảo sát ô nhiễm kim loại nặng (As, Cd, Hg, and Pb) trong hai loài động vật hai mảnh vỏ ngao trắng (*Meretrix lyrata*) and loài hàu (*Saccostrea glomerata*) được nuôi ở Vân Đồn - Quảng Ninh. Các kết quả minh chứng rằng các loài khác nhau tích lũy kim loại ở các mức khác nhau. Các nồng độ kim loại nặng nghiên cứu được theo thứ tự là  $As > Hg > Cd > Pb$  đối với loài ngao, và  $As > Cd > Hg > Pb$  đối với loài hàu đã được phát hiện. Nhìn chung, các mẫu hàu và ngao bị ô nhiễm nhiều nhất với nồng độ As trong 4 kim loại nặng đánh giá và nồng độ As trung bình trong 3 vị trí lấy mẫu là  $3.72 \pm 1.89$  và  $1.56 \pm 0.41$  mg/kg w.w trong hàu và ngao nuôi tại Vân Đồn - Quảng Ninh.

**Từ khóa:** kim loại nặng, ngao, hàu, tích tụ, Vân Đồn – Quảng Ninh.

### 1. INTRODUCTION

Naturally, potentially harmful heavy metals and metalloids could be present in all environments [1]. All heavy metals could become toxic to humans at certain concentrations, but the accumulation at even small amount of some metals such as arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb), could be a potential threat to wildlife in interact with these elements [2–4]. Since humans are now at the top trophic level in numerous habitats, by bioaccumulating through the food chain, these metals maybe eventually transferred to us at risky concentrations.

Bivalves are globally widespread benthic species that mostly inhabit coastal and estuarine

regions [5]. For examples, oysters and clams as sessile organisms in the water-sediment interface are continuously and directly exposed to aquatic pollutants from seawater and sediment [6]. Since oysters have high nutritional and economic values, consuming these mollusks with high levels of heavy metals is dangerous and could pose a risk to human health [7]. In addition, it is nearly impossible to degrade heavy metals into harmless metabolites through biological ways so it is of particular significance to known of their concentrations in tissues of bivalve species [4].

In Vietnam, most of the polluted sources in the coastal area originated from untreated or poorly treated wastewater from urban cities. The

wastewater may come through the sewer system, thus eventually ending up in the oceans. Particularly, the coastal zone of Quang Ninh province is one of the important sea areas with a main aquaculture purpose. However, they are facing the possibility of being polluted by many factors, including heavy metals. On the other hand, data on heavy metal contents in bivalves in Vietnam in general and Quang Ninh province, in particular, are still quite scarce and inconsistent. Typical studies [8] also reported the high accumulation of As and lower contents of Cd and Pb in mollusk species inhabited along the Northern coastal region. Other studies [9] using oysters *Saccostrea glomerata* inhabited along the Haiphong- Halong coastal regions also announced the particularly high level of heavy metals found in the oyster samples.

Therefore, investigation of the bioaccumulation of heavy metals in two commercial benthic bivalve species: the oyster *Saccostrea glomerata* and the white clam *Meretrix lyrata* collected from the cultured areas of Van Don - Quang Ninh province was studied. The concentrations of four heavy metals (As, Cd, Hg, and Pb) in the soft tissues of these bivalves were studied to achieve the following objectives: 1) determination of heavy metals in tissues of the two studied species; 2) assessment of the risk level of heavy metals in the bivalves' tissues to human health.

## 2. MATERIALS AND METHODS

### 2.1. Sampling sites

Situated in Bai Tu Long Bay, Van Don District belongs to Quang Ninh province which is in the Northeast region of Vietnam.

Van Don District is one of the important tourist centers of Quang Ninh province and it is also a general economic zone including both a high-quality island tourism center and an international trade hub. Moreover, Van Don is also developed with aquacultures of bivalves.

The samples of white clams *Meretrix lyrata* and the oyster *Saccostrea glomerata* were collected from three cultured sites (Oy 1, Oy 2 and Oy 3 for oysters in the coastal areas of Van Don District, Quang Ninh province in May 2021

(Figure 1) with geographical coordinate points (X, Y) on the Table 1. At each site, 30 individuals of each species were collected along the cultured areas. Afterward, the organism samples were stored in an iced boxes and transferred to the laboratory for analysis.

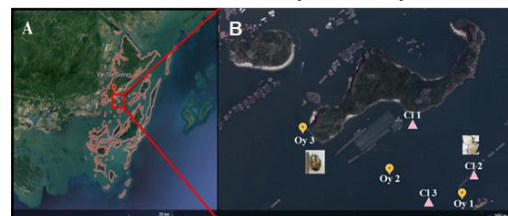


Figure 1. A) Van Don sampling area, B) sampling sites for oysters and clams.

Table 1. Geographical coordinate points (X, Y) of the sampling sites for oysters and clams

Oyster sampling sites		Clam sampling sites	
Oy 1	21.05056, 107.43747	Cl 1	21.05333, 107.43546
Oy 2	21.05151, 107.43444	Cl 2	21.05139, 107.43772
Oy 3	21.0531, 107.43083	Cl 3	21.05021, 107.43635

### 2.2. Analytical methods

The sample preparing methods were according to Dang et al. [10]. Following samples collection, the mud and other debris attached on the shells surface were thoroughly removed with a stainless-steel brush and the samples were then defrosted prior to analysis. Thirty individuals of each species with similar size were sampled. The shell lengths and weights of each individual were measured and recorded carefully. The bivalves were dissected, and their tissues were collected, weighed, and reported. For each species, the soft tissues of one individual were homogenized as a single sample to analyse. Five homogenized samples were analyzed for each species of oyster and clam at each sampling site.

Standard solutions, blank and replicate samples were prepared and used in analysis. The tissues samples of the heavy metals were treated by HNO<sub>3</sub> (Merck, Germany) in Teflon bombs. Heavy metals such as As, Cd, Hg, and Pb in the

tissues of the oysters and clams were determined using the coupled plasma mass spectrometry [11]. Briefly, heavy metals (As, Cd, Hg, and Pb) in the bivalve tissues were analyzed using ICP-MS 7900, Agilent Technologies, Inc., USA.

All reagents used during the analysis were of analytical grade and de-ionized water was used throughout the study. All the plastics and glassware were washed in nitric acid for 15 min and rinsed with deionized water before use. All samples were triplicated in analysis method.

Analyzing the significance of the variation in heavy metals concentrations in 3 sampling sites

for oysters and clams was performed by using STATISTICA v.7. Afterward, two-way ANOVA was used to check for the significant difference of the heavy metal concentration in two bivalves in terms of species (oyster and clam) in sampling sites of Van Don - Quang Ninh province.

### 3. RESULTS AND DISCUSSION

#### 3.1. Heavy metal concentrations in the oysters and clams

Heavy metal concentrations in the oysters and clams from sampling sites were showed on the table 2.

Table 2. Concentrations of heavy metals in the oysters and clams from different sampling sites.

Species	Sampling sites	Length (cm)	Heavy metals (mg/kg wet weight)			
			As	Cd	Hg	Pb
Oyster	Oy 1	8.96 ± 1.14	1.69 ± 0.38	0.48 ± 0.13	0.11 ± 0.07	0.12 ± 0.02
	Oy 2	9.48 ± 1.70	1.42 ± 0.14	0.47 ± 0.14	0.18 ± 0.11	0.12 ± 0.07
	Oy 3	9.10 ± 1.36	1.58 ± 0.62	0.41 ± 0.10	0.22 ± 0.05	0.11 ± 0.06
Clam	Cl 1	6.16 ± 1.37	3.50 ± 0.93	0.14 ± 0.04	0.44 ± 0.06	0.07 ± 0.05
	Cl 2	5.44 ± 0.28	3.14 ± 1.65	0.16 ± 0.09	0.44 ± 0.11	0.15 ± 0.12
	Cl 3	5.40 ± 0.14	4.53 ± 2.75	0.14 ± 0.08	0.03 ± 0.02	0.10 ± 0.02

As described in Table 2 and Figure 2, the mean levels (n = 30, 5 samples per site with 3 sampling sites of oyster and clam species) were presented. Four heavy metals in edible tissues of two target bivalves collected from three sampling sites in Van Don district, Vietnam were determined in the order of As > Cd > Hg > Pb concentrations for oysters and As > Hg > Cd > Pb concentrations for clams. Those results showed that the accumulation of metals in clams and oysters was different with each heavy metal detected. Moreover, there was a significant variation of heavy metal accumulation between two species. For instance, the highest concentrations of As and Hg were found in clams (7.83 and 0.53 mg/kg w.w, respectively) while oysters contained the highest concentration of Cd and Pb (0.68 and 0.19 mg/kg w.w, respectively).

The concentration of As in two bivalves of clams and oysters ranged from 1.01 to 7.83 mg/kg w.w., with an average level of 1.56 ±

0.41 mg/kg w.w for oysters and 3.72 ± 1.89 mg/kg w.w.

Among three sampling sites, oysters were the most polluted in site 1, with As concentration of 1.69 ± 0.38 mg/kg w.w.

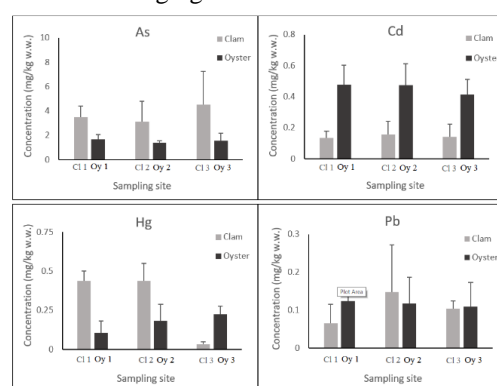


Figure 2. The concentration of heavy metals in bivalve tissue (mean ± SD, mg/kg w.w.) in sampling sites in Van Don district

Besides, the highest level of As concentration in clams was 4.53 ± 2.75 mg/kg w.w found in site 3 and average level of 3.72 ± 1.89 mg/kg w.w.

Comparing two species, clams had significantly higher concentration of As accumulated than oysters ( $3.72 \pm 1.89 > 1.56 \pm 0.41$  mg/kg w.w.,  $p < 0.05$ , ANOVA).

Compared with As, Cd accumulated less in all sampling sites. Comparing two species, there were no significant differences between the average level of Cd accumulated in both bivalves, though oysters tended to accumulate higher concentration of Cd than clams with the average level of  $0.46 \pm 0.12$  and  $0.14 \pm 0.07$  mg/kg w.w, respectively. In detail, for oysters, the highest concentration of Cd was reported in site 2 ( $0.68$  mg/kg w.w.) while the highest average Cd concentration was found in site 1 with concentration of  $0.48 \pm 0.13$  mg/kg w.w. However, clams had lower Cd accumulation, as the maximum Cd concentration found in clam tissues was only  $0.16 \pm 0.09$  mg/kg w.w. and the highest concentration was  $0.25$  mg/kg w.w in site 2. Since Cd is a highly toxic metal to humans and has a long biological half-life, thus this element is considered as being suitable for bioaccumulation [11, 12]

The accumulation of Hg varied between sampling sites and species. Hg values ranged between  $0.01 - 0.30$  mg/kg w.w. for oysters and between  $0.01 - 0.53$  mg/kg w.w. for clams. Moreover, Hg concentration reported in clams was significantly higher than that in oysters ( $0.30 \pm 0.21 > 0.17 \pm 0.09$ , ANOVA,  $p < 0.05$ ). Among oyster samples, the highest concentration of Hg was found in site 3 ( $0.22 \pm 0.05$  mg/kg w.w.) and the lowest one was recorded in site 1 ( $0.11 \pm 0.07$  mg/kg w.w.). As for clams, those sampled in sites 1 and 2 seemed to be under the most affected by Hg as they were higher than the other site ( $0.44 \pm 0.06$ ,  $0.44 \pm 0.11$ , and  $0.32 \pm 0.02$  mg/kg w.w. for sites 1, 2 and 3 respectively). Additionally, there was a significant difference between Hg concentration found in samples of site 2 and 3 ( $p < 0.05$ , ANOVA). Hg concentration is both a toxicant to humans and a non-essential element for aquatic organisms, thus more attention should be considered even at low concentrations due to

its rapid biomagnification in fishes and other seafood [13–15].

Compared with three previous heavy metals, Pb concentration seemed to be the one of the least accumulated in mollusk tissues in all sampling sites. For the oysters, they were accumulated Pb more in site 1 and site 2 ( $0.12 \pm 0.02$  and  $0.12 \pm 0.07$  mg/kg w.w, respectively) compared to  $0.11 \pm 0.06$  mg/kg w.w in site 3. A similar trend also happened with clams, Pb concentration was found the most popular in in site 2 ( $0.15 \pm 0.12$  mg/kg w.w), lower Pb concentration was reported in site 3 ( $0.10 \pm 0.02$  mg/kg w.w.) and the lowest Pb level was calculated in site 1 ( $0.07 \pm 0.05$  mg/kg w.w).

The variation of heavy metal concentration accumulated in individuals of the same species between different sampling sites could be due to numerous factors. The less concentration of heavy metals in bivalves could be attributed to appropriate recycling of water by ocean waters since they are in Bai Tu Long Bay. Hence, they are under the affected of the total flow which is in the south-west, and south-southeast direction with a speed of  $50$  to  $80$  cm/s [17], less anthropogenic activities such as mining, industrial activities, discharge of urban wastes, etc. Not only that, since some of the metals could occur in bioavailable forms, they could be accumulated by bivalves [18]. Lying in the second trophic level in the aquatic ecosystem, bivalves such as oysters have long been known to accumulate both essential and nonessential trace elements in aquatic ecosystems [19]. Their sedentary way of life combined with their ability to accumulate a wide range of pollutants in proportion to the degree of environmental contamination [20] is one of the main causes for a high concentration of heavy metals.

Among four heavy metals analyzed, As had the highest concentration accumulated in bivalves sampled. It is a typical metal contaminant in aquatic environment due to its ability to occur in either organic or inorganic form as a metalloid element [5]. Furthermore, the source of As could come from the metallurgy (e.g., copper and aluminium) and mining activities

[5, 10]. Not only that, many studies also agreed that As is a characteristic metal contaminant in various aquatic environments and is most efficiently bioaccumulated by bivalves species [5, 21, 22].

In Table 3, heavy metal concentrations measured based on wet weight in mollusk, crustaceans, and fish in this study are compared with the concentrations reported in other

literature. Data from the literature showed that heavy metal concentrations in those mollusks varied largely depending on species and sampling areas (Table 3). In general, as for oysters *S. glomerata*, the concentrations of As, Cd, Hg, and Pb in our study were lower than those reported in other studies. However, as for clams *M. lyrata*, it did not follow the same trend.

Table 3. Comparison of heavy metal concentrations (mg/kg, w.w.) in oysters (*Saccostrea glomerata*) and clams (*Meretrix lyrata*) collected from other areas.

Species	Region	As	Cd	Hg	Pb	References
<i>Saccostrea glomerata</i>	Van Don – Quang Ninh province, Vietnam	1.56± 0.41	0.46 ± 0.12	0.17 ± 0.09	0.12 ± 0.05	This study
	Phu Long beach, Hai Phong coast, Vietnam	28.58 ±2.44	4.58 ± 0.86	-	1.23 ± 0.09	[8]
<i>Saccostrea glomerata</i>	Quan Muc beach, Hai Phong coast, Vietnam	11.98 ±1.02	5.85 ± 1.49	-	1.36 ± 0.62	[8]
<i>Meretrix lyrata</i>	Van Don - Quang Ninh province, Vietnam	3.72 ± 1.89	0.14 ± 0.07	0.3 ± 0.21	0.11 ± 0.08	This study
<i>Meretrix lyrata</i>	Can Gio Coastline in Ho Chi Minh City, Vietnam	0.05 ±0.01	0.09 ± 0.01	-	0.21 ±0.03	[23]
	Phu Long beach, Hai Phong coast, Vietnam	10.65 ± 2.65	0.78 ± 0.25	-	1.31 ± 0.53	[8]
<i>Meretrix lyrata</i>	Quan Muc beach, Hai Phong coast, Vietnam	11,54 ± 4,71	1.15 ± 0.24	-	1.08 ± 0.71	
<i>Meretrix lyrata</i>	East Java Coast, Indonesia	-	0.67 ± 0.07	0.05 ± 0.01	0.94 ± 0.28	[24]

### 3.2. Risk assessment of heavy metal level in bivalve tissues of oysters and clams

As described in Table 4, many regulations were established to limit the heavy metal concentrations in bivalves in Vietnam and international organizations. According to different organizations and countries, there can be differences between limits. For instance, Table 4 showed that the European Commission (EC) in the Commission of the European

Communities (EU) suggested a guideline level of 1 mg/ kg for Pb and Cd in bivalves, while Vietnam's National technical regulation on the limits of heavy metals contamination in food (QCVN 8-2:2011/BYT) set a more lenient limit of 1.5 and 2 mg/kg, respectively. However, certain limitations are similar between different organizations and countries. For example, the limit of Hg concentration in bivalves is 0.5 mg/kg proposed by both EU regulation and

Vietnam's national technical regulation. Regarding the concentration of metals accumulated in oysters and clams, among all evaluated metals, the concentrations of three metals in the bivalves (Cd: 0.14 - 0.46, Hg: 0.17 - 0.30, Pb: 0.11-0.12 mg/kg.w.w) fell within the safe and permissible limits of both the national or international guidelines (Cd: 1 - 2, Hg: 0.5 - 1.0, Pb: 1.0 - 1.5 mg/kg.w.w).

*Table 4. Risk assessments of the heavy metals in clams and oysters collected from Van Don district in terms of relative permissible limits set by Vietnam and international organizations*

Heavy metal	Concentrations in		Safety guidelines (mg/kg w.w.)
	wet weight (mg/kg)		
	Oyster	Clam	
As	1.56	3.72	-
Cd	0.46	0.14	1 <sup>a,c</sup> , 2 <sup>b</sup>
Hg	0.17	0.30	0.5 <sup>a,b,c</sup>
Pb	0.12	0.11	1 <sup>c</sup> , 1.5 <sup>a,b</sup>

<sup>a</sup> Ministry of Health of Vietnam, Regulation 46/2007/QĐ-BYT [25]

<sup>b</sup> Ministry of Health of Vietnam, National technical regulation on the limits of heavy metal contamination in food, QCVN 8-2:2011/BYT [26]

<sup>c</sup> The Commission of the European Regulation (EC), No 466/2001 [27]

#### 4. CONCLUSION

This study provided a detailed and updating report about the heavy metal concentrations (As, Cd, Hg, and Pb) accumulated in clam *Meretrix lyrata* and oyster *Saccostrea glomerata* cultured in Van Don - Quang Ninh province. Our study showed that though both species were exposed to similar habitat conditions, clams and oysters accumulated heavy metals of As, Cd, Hg, and Pb at a different level. It was revealed that among four metals analyzed, bivalves accumulated As the most with concentration ranged from  $3.14 \pm 1.65$  to  $4.53 \pm 2.75$  and  $1.42 \pm 0.14$  to  $1.69 \pm 0.38$  for clams and oysters, respectively. These results of this study have contributed to the overall data about heavy metal pollution accumulated in clams and oysters cultured in the coastal area of Van Don - Quang Ninh province

and preliminarily assess about risk level in permissible limits of the As, Cd, Hg, and Pb in clams and oysters when using them as sea foods.

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