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Bioaccumulation, Depuration of Heavy Metals (As, Cd, Pb) and Metabolism of These Metals in Body of Mussels (*Maretrix Lyrata*) During 20 Days in Artificial Media of Culture

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Abstract

The purpose of this work is to try to estimate for the first five days the extent of As, Pb, Cd bioaccumulation by *Meretrix Lyrata* mussels in artificial media of culture contaminated by heavy metals (As at 1.5 and 2.5 ppm, Pb at 1.5 and 2.5 ppm, Cd at 0.1, 0.5, 1 ppm) and that of release in clean water during the following 15 days. Quantitation was performed by ICP and AAS with Hydride System. Bioaccumulation increased with heavy metal concentrations in water and in the oder Pb > Cd > As. Release was also observed to increase with the amounts of absorbed metals. After 20 days of experiments, following figures of metal release were obtained (As~100%, Pb~68.9%, and Cd~39.65%). In the case of Cd contamination, no mussel survived after 10 days of experiments even at low Cd concentration of 0.1ppm. Residue of heavy metals in the body of mussels was metabolited to another chemical form. Inogranic Cd was metabolited to Cd-metallothionein and detected by LC/MS ESI(+). As was metabolited to monomethyl arsonic acid (MMA), dimethylarsinic acid (DMA) and detected by HPLC-UV-AAS-HG, and Pb was metabolited to photsphat hydroxyt lead [$Pb_5(OH)(PO_4)_3$]—which was detected by XRD.

Keywords

As, Cd, Pb, MMA, DMA, Cd7-MT, phosphate lead

1. Introduction

Mussels (*Meretrix lyrata*) live at warm sea of west Pacific Ocean from Taiwan to South Viet Nam. Mussels have ability of high bioaccumulation of heavy metals and contaminated organisms.

The bivalves are more popular for consume and export because they have some advantages such as, high protein, minerals, low cholesterol and lipid as compared to with crab, shrimp. Besides with these advantages, bivalves are proposed bio-indicator for water and sediment environment. Due to they have high ability of bioaccumulation and sensitivity to the changes in the environment. For sake of safety of health, this project will research bioaccumulation, release of heavy metals, and metabolism of metals when they accumulate in body of mussels.

2. Methods of Analysis

Equipment used for analysis:

Analysis of heavy metals: Cd, Pb by ICP Perking Elmer 5300, As by AAS-Hydride System

Analysis of each metabolite heavy metal: Cd-Metallothionein by LC/MS ESI(+), MMA, DMA by AAS-UV-HG, $[Pb_5(OH)(PO_4)_3]$ by XRD.

Samples

Mussels (*Meretrix lyrata*), water were collected at Can Thanh beach of province of Ho Chi Minh City and then brought to Thu Duc experimental farm. Mussels were cultured and exposed to heavy metals at different concentration (As, Pb at 0.5, 1.5 and 2.5 ppm, Cd at 0.1, 0.5, 1.0 ppm) for 5 days for accumulation and then contaminated water was replaced by clean sea water for next 15 days and release of heavy metals from the mussels was monitored.

Model of artificial culture of mussels:

Each tank was filled with sea water contaminated with heavy metals. All parameters such as pH, NO_3, NH_4, number of dead mussels were monitored every day. After designed, mussels samples were collected and brought to laboratory for analysis.

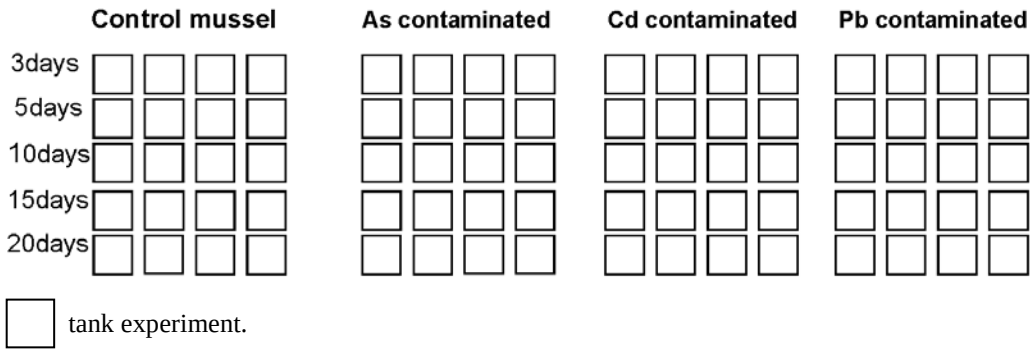

Figure 1. Model of artificial culture of mussels for each experiment.
As5+: BL 0.5-1.5-2.5mg/l; Pb2+: BL 0.5-1.5-2.5mg/l; Cd2+: BL 0.1-0.5-1.0mg/l.

3. Rusults and Discussion

3.1. Accumulation and depuration of trace metals: Cd, Pb, As in uncomplex form: $CdCl_2$, Pb $(CH_3COOH)_2$, AS_2O_3

Table 1. Accumulation of As, Cd, Pb in tissue of mussels exposed to different dissolved concentrations for 3 days and 5 days in artificial medium

Heavy metal in artificial media of culture water were contaminated with different concen-tration	**Control mussels** (mg/Kg)	**3 days** (mg/Kg)	**5 days** (mg/Kg)	**In comparison with control mussels (times)**
As 0.5 mg/L	1.01	1.02	1.01	the same control
As 1.5mg/L		1.55	1.57	1.5 more times
As 2.5mg/L		1.56	1.53	1.7
			1.79	
Pb 0.5mg/L	0.045	0.045	**0.046**	the same control
Pb 1.5mg/L		4.10	**9.97**	207.7
Pb 2.5mg/L		4.97	5.40	135
Cd 0.1mg/L	0.2	0.74	0.80	4.0
Cd 0.5mg/L		2.50	**3.20**	16.0
Cd 1.0mg/L		2.88	**3.48**	17.4
Cd 1.5mg/L		dead	dead	ND

After 3 and 5 days, mussels (*Meretrix lyrata*) were cultured in contaminated water. The results obtained in Table 1 show that the concentration of heavy metals strong impacts on ability of uptake and accumulation of heavy metals in

mussels, high concentration of metals, high accumulation of metals in mussels. Concentration at 0,5mg/L of Pb, As no change in comparison with control mussels. It this show that at very small concentration 0f As, Pb no strong impacts on mussels. But for Cd is strong impact to mussels even low concentration 0,1mg/kg. It shows that Mussels are very sensitive to metal Cd. All of three metals which accumulated in mussels were increased from first day to fifth day. But an increase is not proportion. In the case Pb content in mussels at Pb of 2.5 ppm is lower than Pb at 1.5ppm after 5 days mussels were exposed to Pb (5.4 ppm < 9.97 ppm). It explains when mussels reach maximum uptake of Pb, then mussels will depurate [7], therefore may be the maximum uptake of Pb reached by mussels after 3 days. The uptake characteristic of each heavy metal by mussels is different. For example, As and Pb have the same concentration (concentration of As, Pb in water is 1.5 ppm and 2.5 ppm) but accumulation is different: at 5^{th} day, content of As in mussels reached value 1.79 ppm and for Pb reached 9.97 ppm in mussel. Metal Cd is highly toxic for mussels. When mussels exposed with 1.5 ppm of Cd in water, all mussels were dead after one day. At small different concentration of Cd (0.1 ppm, 0.5 ppm, 1 ppm) mussel can live until 10^{th} day experiment and after that they also were dead. However, Cd content in body mussels were measured 3.48 ppm and 3.2 ppm on the 5^{th} day, for 1 ppm and 0.5 ppm respectively. At Cd 0.1ppm, content in mussel was very small 0.80 ppm. Bioaccumulation increased with heavy metal concentrations in water at 5^{th} day and in the order: Pb (9,97ppm) > Cd (3.48, 3.2ppm) > As (1.79 ppm).

Table 2. Residue of trace metals: Cd, Pb, As in mussel (*Meretrix lyrata*)

Metal in water	Control mussel (mg/kg)	Depuration of period				Average Depuration
		5^{th} day (mg/kg)	10^{th} day (mg/kg)	15^{th} day (mg/kg)	20^{th} day (mg/kg)	
As 1.5mg/L	1.0	1.57	1.05	1.02	1.02	**> 90 %**
As 2.5mg/L		1.79	1.42	1.02	1.02	
Pb 1.5mg/L	0.045	**9.97**	4.90	3.20	3.20	**68.9 %**
Pb 2.5mg/L		5.40	3.90	3.30	3.20	
Cd 0.1mg/L	0.2	0.80	0.83	Mussels were dead		**40%**
Cd 0.5mg/L		**3.20**	1.80			
Cd 1.0mg/L		**3.48**	2.10			

After five days, contaminated water was changed to clean water for study of depuration. After 10 days, heavy metals in body of mussels decreased. As compared to 5^{th} day the control mussels, As, Cd and Pb content decreased, although not by same degree. Results in table 2 show that:

Arsenic (As) contents in mussels continued to decrease during the remaining days. From day 15, mussels excreted more than 90% of As accumulated. An equilibrium between As bioaccumulation and excretion would be reached then. Mussels continue to live healthy after 20 days. For Pb, after five days, Pb content continued to decrease up to day 20^{th} at both concentrations. However, mussels did not excrete all of bio-accumulated Pb. By comparison with the control mussels, the amounts of retained Pb were substantial, almost the same at two concentrations. Mussels eliminated Pb by about 69%. Mussels continued to live healthy after 20 days. For Cd, after 5 days, the content decreased. Mussels eliminated Cd from body by about 40% and remaining content of Cd in body of mussels about 60%. By comparison with the control mussels, the amounts of retained Cd increased in the following order: 1 ppm Cd) > 0.5 ppm Cd) > 0.1 ppm Cd. At 0.1ppm Cd, no elimination of depuration Cd by mussels was detected. Cd is highly toxic to mussels so all mussels were dead after 10 days of experiment even at the lowest Cd concentration of 0.1 ppm. Control mussels continued to live healthily after 20 days of experiment.

The results show that when mussels were exposed to metals in contaminated water medium, mussels can uptake metals and accumulate these metals in their body. They can depurate metals from body, but Table 2 shows that mussel cannot eliminate all metals which they accumulate. We now ask the question: in what form do the metal ions remain in the mussels' body? For answer these questions, experiment continue to analyze metabolism of remaining metals in body of mussels.

3.2. Metabolism of remaining heavy metal in mussels

3.2.1. Arsenic metabolism

In many species, arsenic metabolism is characterized by two main reactions: First, reduction reactions of pentavalent to trivalent arsenic As(V) → As(III). Next oxidative methylation reaction in which trivalent forms of arsenic are methylated to form monomethylarsonic acid ($[(CH_3)AsO(OH)_2]$, dimethylarsinic acid ($[(CH_3)_2AsO(OH)]$ and trimethylarsenic acid $[(CH_3)_3AsO]$. Methylated arsenic forms are rapidly excreted from the body of mussel [1, 2]. Mussels sample after 3 days and 20 days were used for identification of MMA, DMA by HPLC—AAS-UV-HG equipment. Results ob-

tained are present in the Table 3 and Figures 2-5.

Table 3. Metabolism of As in the mussels for 3 days and 20 days in

N^0	Sample	As(III) (ppb)	DMA (ppb)	MMA (ppb)	Arsenobetain (ppb)	As(V) (ppb)
1	Standard solution	**25**	**25**	**25**	**25**	**25**
2	Control mussel	**ND**	**ND**	**ND**	**ND**	**ND**
3	Contaminated mussel in 3 days	**357**	**ND**	**ND**	**ND**	**32.5**
4	Contaminated mussel in 20 days	**8.7**	**7.2**	**6.1**	**ND**	**15.6**

ND: not detected

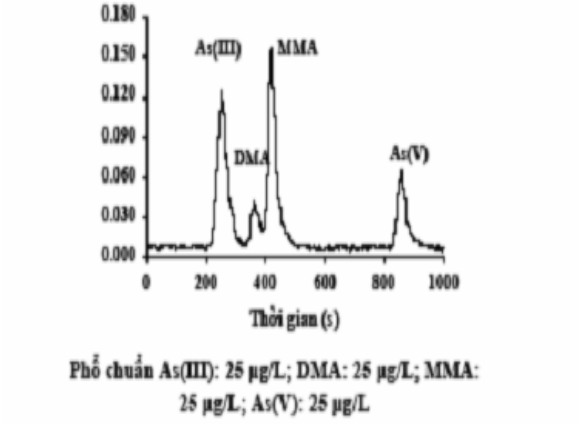

Figure 2. Standard chromatography.

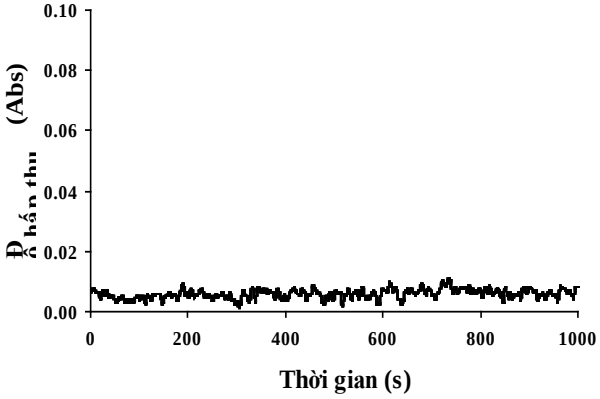

Figure 3. Control mussel.

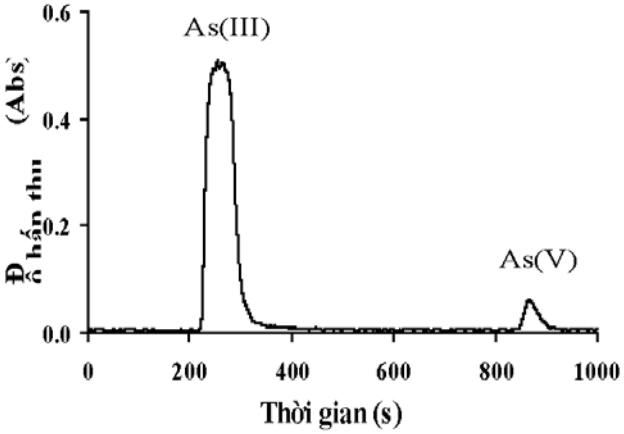

Figure 4. Contaminated mussel in 3 days.

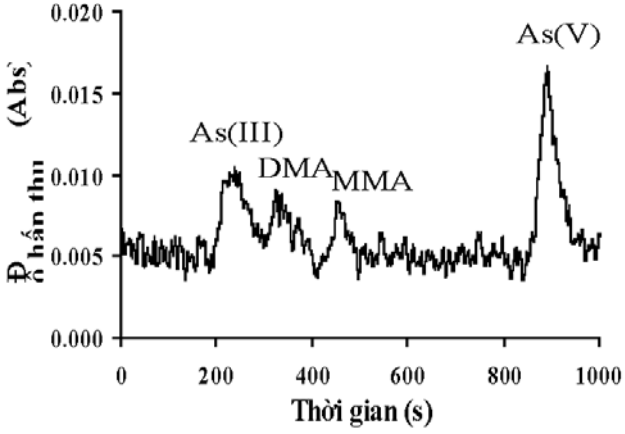

Figure 5. Contaminated mussel in 20 days.

Mussels exposed to As for 3 days and 20 days were treated by methanol solution and the extracts were saved identification of the arsenic forms: As(III), As(V), MMA, DMA and arsenobetain. Results presented in Table 3 shows that control mussels did not content any arsenic form. Mussels exposed to As for 3 days contain only As (III) and As(V). All arsenic forms appear in mussels expend to As for 20 days. In conclusion, there is the methylation of As in mussel body; so it can detoxificate and depurate toxic substrate to the environment

3.2.2. Metabolism of Cd

Metal Cd can bind to special small protein molecule (6000DA-10000DA) to become MT-metallothionein and complex of MT with metal that is Me-MT [4, 6] So Cd accumulated in body will transport to Cd_7-MT. In order to identify

Cd metabolism in mussel, LC/MS ion trap was used. Rabbit liver MT-1 standard from Sigma Aldrich was used. By SIM of LC/MS method on the Standard sample peaks of MT-1 and MT-2 were founds. Then extracts solution of mussel exposed Cd for 10 days were measured by LC/MS and were all peaks of MT-1, MT-2 found. Results are presented in Table 4 and Figure 6.

Table 4. Results measured of MT-1 of standard and mussel sample by LC/MS ion trap)

	Cd_7 MT-1 Rabbit liver			Cd_7 MT-1 – mussel samples	
MT-1	**M_w (Cd_7MT-1)**	**M_w [$5H^+$]**	**M_w [4H+]**	**Mw[(5H+)]**	**Mw[4H+)]**
α (a)	**6,917**	**1,384.4**	**1,730.2**	**1,384.1**	**1,730.1**
β	**6,949**	**1,390.8**	**1,738.2**	**1,390.7**	**1,738.0**
γ	**6,965**	**1,394.0**	**1,742.2**	**1,392.7**	**1,741.9**
δ	**6,988**	**1,398.6**	**1,748.0**	**1,399.1**	**1,747.7**
ε	**7,013**	**1,403.5**	**1,754.2**	**1,402.9**	**1,753.7**

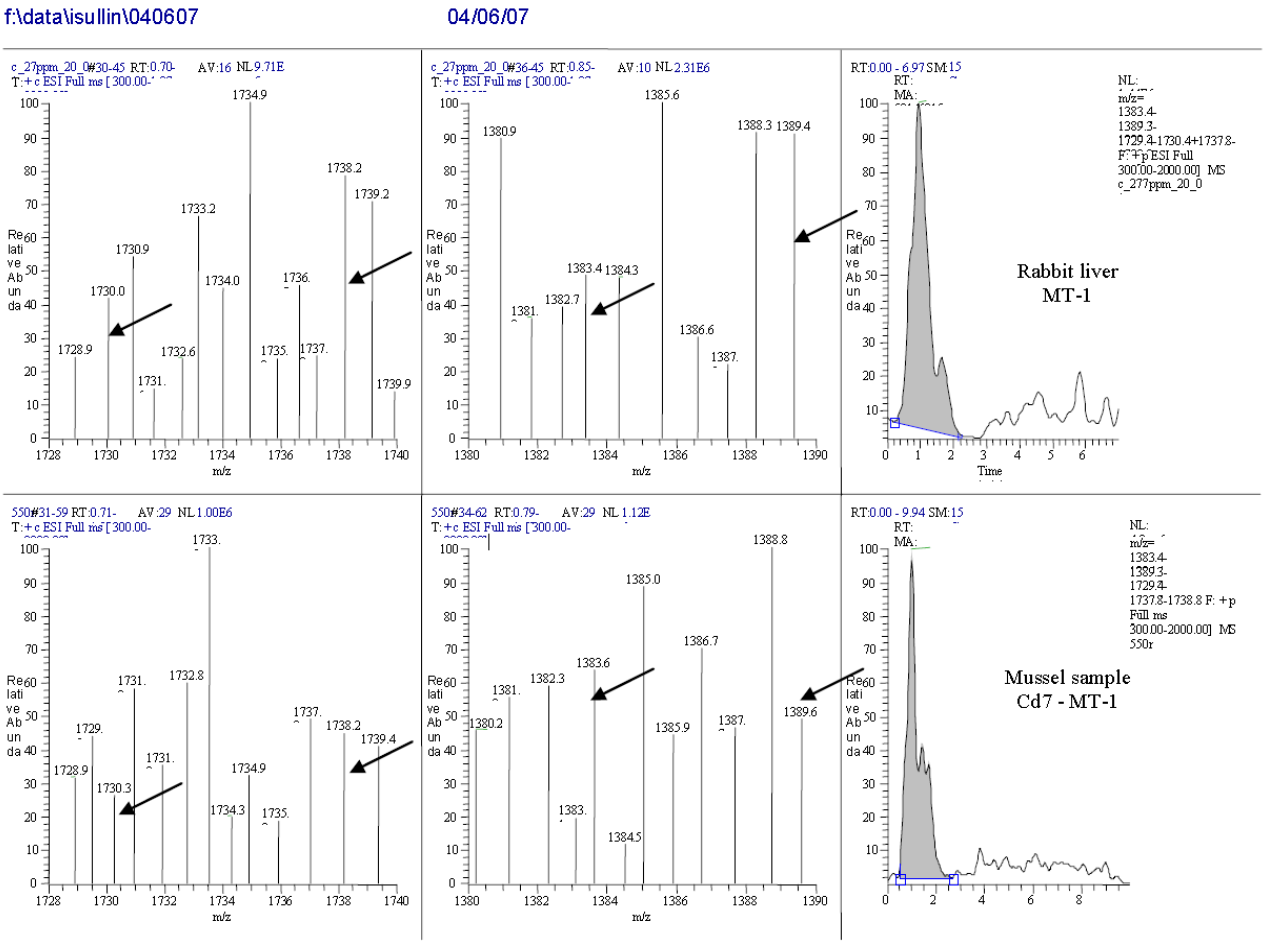

Figure 6. Chromatography of Standard MT-1 and mussel sample.

Like As, mussels metabolized Cd to metallothionein and complex Cd_7-MT and detoxificate by themselves. However, complex Cd-MT is depurated from mussel body not easily eliminated and it accumulates in body for a long time [4, 6]. Structure of Cd7-MT of mussel (Meretrix lyrata) is similar structure of Cd7-MT of Rabbit.

3.2.3. Metabolism of Pb

Remaining content of Pb in mussels body is 32%. It is known that when Pb accumulates in organism, it may be transported as lead phosphate and stored in kidney [3, 5]. Mussels of 20 days expose with Pb were for analysis. Mussels sample after burning at 500^0C, ash was obtained to identify the chemical forms of Pb by XRD equipment. Results are

presented in the Table 5, and Figure 7.

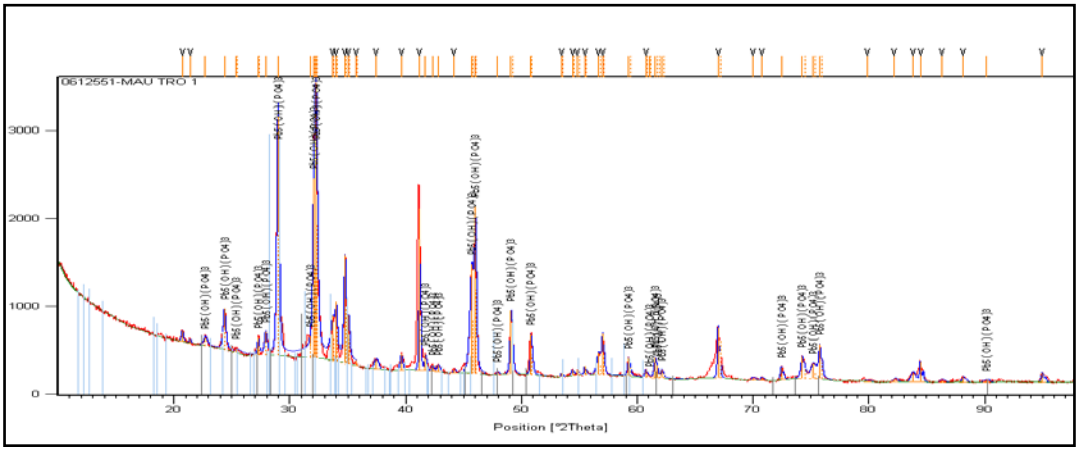

Figure 7. XRD chromatography of Pb metabolites in mussels.

Table 5. Chemical compounds of Pb in mussels sample in ash by XRD equipment

Chemical compounds	Chemical forms
Lead phosphate hydroxyt	**$Pb_5(O\ H)(PO_4)_3$**
Potasium chloride and sodium chloride	KCl, NaCl
Mixed metal phosphate	$Na_2CaMg(PO_4)_2$

4. Conclusions

Mussels (*Maretrix lyrata*) of Viet Nam were exposed to metal for 20 days in artificial culture media . The values show that:

Accumulation of heavy metals in body mussels depends on chemical properties and concentration of metals. Uptake of metals in mussels increases with concentration of metals in water (Pb > Cd > As) but not in proportion.

Depuration of metals depends on chemical properties of metals, but not on the concentration of metals in water. As was depurated about 90%, Cd 40% and Pb about 69%.

Modern analytical equipments were successfully applied for identification of metabolism of the remaining metals (As, Cd, Pb) in mussels (As by HPLC – UV- AAS – Hg, Cd by LC/MS – SIM and Pb by XRD).

Metabolisms of heavy metals :

As (V) reduction As(III) methylation MMA, DMA → fast depuration

Cd → metallothionein → Cd_7 – MT → slowly depuration

Pb → $Pb_5(O\ H)(PO_4)_3$ → storage in kidney → slowly depuration

Finally, because the mussels have better ability to accumulate and retain the toxic metals, As and Pb at high levels, carefully mussels should be treat for these toxic metals before consumption.

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