BIOACCUMULATION OF COPPER AND LEAD BY BIVALVE *Meretrix lyrata* CULTURED IN WATER – SEDIMENT ENVIRONMENT

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TÓM TẮT

NGHIÊN CỨU KHẢ NĂNG TÍCH LŨY SINH HỌC ĐỒNG, CHÌ CỦA NGHÊU (*Meretrix lyrata*) ĐƯỢC NUÔI TRONG MÔI TRƯỜNG CHỨA NƯỚC VÀ TRẦM TÍCH

Nghiên cứu thực hiện nuôi các thể nghêu Meretrix lyrata (M.lyrata) trong môi trường có chứa đồng (Cu) và chì (Pb) ở các nồng độ khác nhau trong 28 ngày nhằm đánh giá khả năng tích lũy sinh học của các kim loại này vào cơ thể nghêu. Môi trường nuôi nghêu được chuẩn bị bằng cách hòa tan kim loại vào pha nước của các bể nuôi (chứa nước biển, trầm tích và nghêu được lấy tại vùng cửa sông Tiền thuộc xã Tân Thành, huyện Gò Công Đông, tỉnh Tiền Giang) với các nồng độ ban đầu: 30 µg Cu/L và 50 µg Pb/L (viết tắt là 30Cu-50Pb), 60Cu-150Pb, 100Cu-300Pb và 200Cu-600Pb. Sau 2 ngày phơi nhiễm, nồng độ kim loại trong nước đã suy giảm một cách nhanh chóng. So với nồng độ ban đầu, nồng độ Cu, Pb chỉ còn lại tương ứng là 10% và 1%. Phần lớn kim loại khi hòa tan vào nước đã phân tán vào chất rắn lơ lửng, trầm tích và bị hấp thu bởi nghêu. Phân tích tương quan cho thấy có tương quan tuyến tính (với hệ số tương quan R = 0,73 – 0,99) giữa lượng kim loại trong nghêu (y) và nồng độ kim loại thêm vào lúc bắt đầu thí nghiệm (x). Bên cạnh đó, kết quả còn cho thấy có tương quan tuyến tính (R = 0,78 – 0,98) giữa y và thời gian phơi nhiễm (x). Tốc độ tích lũy sinh học (rate of metal accumulation – RMA) của nghêu đối với Cu, Pb trong 28 ngày phơi nhiễm tương ứng là 5 – 12 ng/g/ngày và 0,8 – 1,7 ng/g/ngày. Nghiên cứu còn cho thấy có tương quan tuyến tính giữa RMA và nồng độ kim loại thêm vào ban đầu (R = 0.94 – 0.98).

1. INTRODUCTION

Bivalve is one of the preferred foods with large amounts in Vietnam and around the world. However, due to toxic metals capable of bioaccumulation in bivalve via food chain, they could be harm to consumers. Many researches shown that bivalve could be used as bio-indicator for pollution of toxic metals in surrounding environment (water, sediment) [1]. For that reason, accumulation of the toxic metals in bivalve species is one of the problems that have been paid to attention from researchers for years [2, 3]. Copper (Cu) and lead (Pb) are two metals among the toxic metals of environmental concerns, and they are commonly found in environmental samples (water, sediment and biological).

In Vietnam, many bivalve species have

been cultured in large scale in estuary areas, of which there is the estuary area at Tan Thanh commune, Go Cong Dong district, Tien Giang province located at South Vietnam (Fig. 1). This area is where Tien river - a tributary of the Mekong river meets the sea. For years, this estuary area has been accepted to be one of the focal areas culturing clam *M.lyrata* in South Vietnam with average yield of 20,000 tons per year for domestic consumers. Culture cycles of the *M.lyrata* – a filter feeder living at bottom - ranged from 8 to 10 months. So far, there are not many studies on Cu and Pb accumulation by the *M.lyrata* cultured at the area yet.

Researches on toxic metal exposure and bio-accumulation were carried out for bivalves cultured in sea or fresh water environment with different dissolved metal levels [4-6]. Other studies were implemented in water-sediment medium with various metal levels, in which the sediment was saturated with metals prior to bivalve culture [7-9]. When metals released from natural and artificial sources enter water environment, a part of them would come into sediment (due to prepcipitation/co-precipitation, absorption, ion exchange, complexing). In practice, it takes a long time to reach to saturation with metals in surface sediment. For that reason, experiments on exposure of living-at-bottom bivalves to different metal levels might be conducted in water-sediment medium, in which it is unnecessary to make sediment saturated with metal.

This study deals with the metal bioaccumulation by clam *M.lyrata* cultured in water-sediment medium contaminated with different contents of dissolved metals (Cu and Pb), in order to examine the use of the *M.lyrata* as bio-indicator for the metal pollution in water environment.

2. MATERIALS AND METHODS

2.1. Instrument and chemicals

Microwave Multiwave 3000 (Anton Paar) accompanied with Teflon vessels was used for bivalve sample digestion. Inductively coupled plasma mass spectrometry/ICP-MS (model 7700x, Agilent) was used to analyze the metals (Cu and Pb) in water and *M.lyrata* samples (the metal analysis conducted in Institute of Public Health in Ho Chi Minh city, Vietnam). Standard solutions of 1000 ppm Cu^{II}, Pb^{II} (nitrate salts) were the pure grades used for AAS and ICP-MS analysis (Accu Standard). Chemicals used for bivalve sample digestion were HNO_3 65%, HCl 36.5%, H_2O_2 30% (AR grade, Merck). Clean water used for preparing chemicals, rinsing and washing glasswares… was de-ionized and distilled water of 18 $M\Omega.cm^{-1}$ conductivity (water purification system, Easy pure, Fisher Scientific).

2.2. Experimental design

Prior to metal exposure, bivalves were acclimatized in water taken from the estuary area for 3 days. Groups of 30 clams *M.lyrata* (of 3 to 4 cm shell length) were selected from the area in order to minimize effects caused by size differences. The estuary water, which was settled overnight and decanted to remove solids and wet surface sediment (0 – 10 cm) were used for exposure experiments. Each group of 30 clams *M.lyrata* was exposured for 28 days to one of a number of metal concentrations in 30 L estuary water of 7.5 pH and 15‰ salinity at $25^oC \pm 2^oC$ under a 12:12 h light:dark regime in acid-washed perspex tank (40 cm length × 50 cm width × 30 cm height) containing 7 cm thick sediment of 15% sand. The

volume ratio of water/sediment was 2:1. Density of the clams in each tank was equal to the one cultured at farming areas at the estuary area (130 – 150 individuals/m^2). Initial concentrations of dissolveld metals in the experimental tanks were prepared as follows: control level (2.1 ± 0.4 µg/L Cu and < 0.2 – 0.5 µg/L Pb; these obtained from the analysis of 14 water samples collected from the estuary area in 3 sampling sessions, June to October 2015); level M1 - 30 µg/L Cu and 50 µg/L Pb (abbreviated to M1-30-50); M2-60-150; M3-100-300 and M4-200-600. These metal levels were selected basing on the allowable maximum concentrations of Cu and Pb according to Vietnam technical regulations on sea water quality QCVN10-MT:2015/BTNMT. Water phase in tanks was aerated continuously and lightly. The clams were not fed during the experiments.

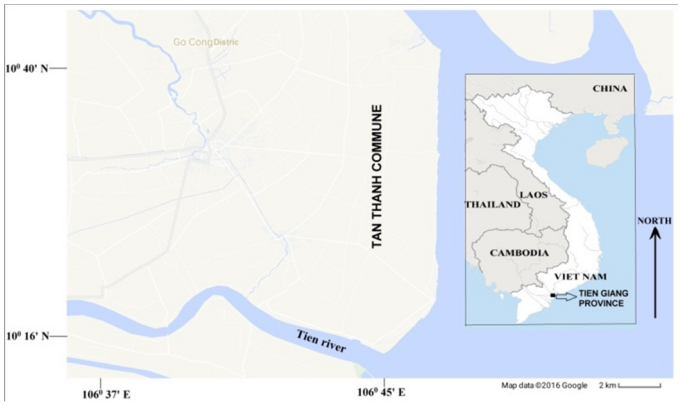

Figure 1. Map showing Tien River and the estuary area at Tan Thanh commune, Tien Giang province

Each experiment on exposure of clam *M.lyrata* to a level of Cu and Pb was repeatedly conducted in the same two tanks.

Dissolved metal levels were monitored periodically (after 1, 2, 7, 14, 21 and 28 days of exposure). Metal contents in clam body tissues were also determined periodically (7, 14, 21, 28 days of exposure). 50 mL water sample and five clams were collected from each experimental tank for metal measurement.

2.3. Analysis method

Analysis of the metals (Cu, Pb) in water samples: Water samples filtered through 0.45 µm nylon fiber membrane was acidified to pH = 2 with 65% HNO_3 (2 mL/1 L sample) prior to metal measurement. The samples were analyzed by ICP – MS method for Cu and Pb (according to Standard Methods for the Examination of Water and Wastewater [10]) with 3 replicates per sample (n = 3). Blank sample prepared from clean water was also analyzed by the same way.

Analysis of the metals in clam samples: Body tissues separated from shells (by using a titanium knife to reduce contamination) were washed with clean water and then homogenized by GM-200 grinder (Retsch). The tissue samples (300 – 500 mg each) were analyzed for Cu and Pb according to method 4.7 of FDA – EAM (Food and Drug Administration – Elemental Analysis Manual) [11].

2.4. Assessment of metal bio-accumulation by bivalve

The rate of metal accumulation (RMA) by bivalve *M.lyrata* was calculated [12]:

RMA (ng/g per day) = $(C_{end} - C_{control})/D$.

Where, C_{end} (ng/g wet weight) is metal level in bivalve body tissue during given exposure time; $C_{control}$ (ng/g wet weight) is metal level in bivalve body tissue in control experiment (595 ng/g Cu and 21 ng/g Pb; these obtained from the analysis of 14 *M.lyrata* samples collected from the estuary area in 3 sampling sessions, June to October 2015); D (day) is number of exposure days.

3. RESULTS AND DISCUSSION

3.1. Quality control of analysis method

Quality control of ICP-MS method was verified via analyzing a standard reference material SRM-2976 (mussel tissue freeze-dried, certified by National Institute of Standards and Technology/NIST, USA; valid until 31 January 2018) for Cu and Pb. For water analyis, quality control of the method was verified by analysis of Cu and Pb in a spiked sample selected randomly from the estuary area. The results obtained in Table 1 shown that the analysis methods gained good repeatability [13]. Also, the analysis method had good trueness with recovery in the range of 98 – 106% for the metal levels in the water sample [14].

Table 1. Results of quality control of the analysis methods (*)

	The estuary water sample selected randomly				Sample SRM – 2976	
Metal	C_o (µg/L)	C_1 (µg/L)	C_2 (µg/L)	Recovery (%)	x ± ε (ng/g wet weight)	Content found (ng/g wet weight)
Cu	5.0	3.2 (RSD = 4.0%;n = 3)	8.5 (RSD = 4.7%; n = 3)	106	4020 ± 330	4219 (RSD = 6%; n = 3)
Pb	5.0	0.5 (RSD = 8.3%; n = 3)	5.4 (RSD = 6.0%; n = 3)	98	1190 ± 180	1292 (RSD = 5%; n = 3)

(*) C_o: Metal concentration added to the water sample; C_1: Metal concentration in the water sample; C_2: Metal concentration found in the spiked sample; x ± ε: Certified values ± confidence limit 95%; Limit of detection (LOD) for Cu and Pb ranged from 0.2 to 0.3 µg/L.

3.2. Dissolved metal contents and metal bio-accumulation by clam *M.lyrata*

i) Dissolved metal concentrations in experimental tanks:

Although there was an increase in initial concentrations of dissolved metals, dissolved metal levels in tanks decreased rapidly (Table 2): Dissolved Cu and Pb concentrations decreased by 91 – 99% and 99.6%, respectively (compared with their initial concentrations); Dissolved Pb levels decreased more rapidly than Cu (Pb level below 0.2 µg/L just after one day exposure). For all experiments, a small amount of the metals distributed at dissolved forms, rest part of the metals distributed on suspended solids and mainly at sediment due to physio-chemical processes occurred simultaneously: metal adsorption, precipitation/co-precipitation, coagulation, ion exchange, complexation...

Table 2. Concentrations of dissolved metals for different initial levels of dissolved metals and exposure times(*)

Exposure time (day)	Concentrations of dissolved Cu and Pb							
	M1-30-50		M2-60-100		M3-100-300		M4-200-600	
	Cu(µg/L)	Pb(µg/L)	Cu (µg/L)	Pb(µg/L)	Cu (µg/L)	Pb(µg/L)	Cu (µg/L)	Pb(µg/L)
0	30	50	60	100	100	300	200	600
1	4.8 ± 1.8	< 0.2	4.4 ± 1.3	< 0.2	4.1 ± 0.6	< 0.2	4.9 ± 1.6	< 0.2
2	3.0 ± 0.3	< 0.2	3.7 ± 0.7	< 0.2	3.8 ± 0.3	< 0.2	4.1 ± 0.1	< 0.2
7	3.8 ± 1.2	< 0.2	3.7 ± 1.2	< 0.2	3.6 ± 0.5	< 0.2	4.4 ± 0.3	< 0.2
14	2.9 ± 1.1	< 0.2	3.3 ± 0.9	< 0.2	3.2 ± 1.1	< 0.2	2.4 ± 0.4	< 0.2
21	3.1 ± 1.0	< 0.2	3.0 ± 0.9	< 0.2	2.9 ± 0.1	< 0.2	3.3 ± 1.4	< 0.2
28	2.8 ± 0.1	< 0.2	2.0 ± 0.1	< 0.2	3.1 ± 0.1	< 0.2	2.9 ± 0.1	< 0.2

(*) Results in the table are mean ± standard deviation with n = 2 (2 experimental tanks replicated)

ii) Metal bio-accumulation by the M.lyrata:

- For all exposure times, linear correlations bewteen the metal contents in the clam body tissues (y) and initial concentrations of dissolved metals (x) with correlation coefficient (R) = 0.73 – 0.99, except the case for Pb during 7 days of exposure (R = 0.034);

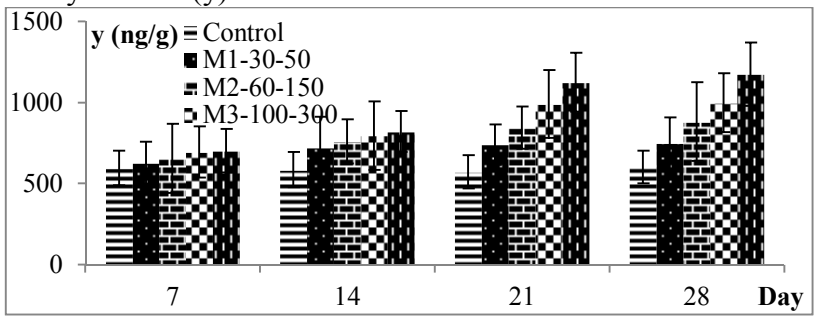

Figure 2. Variation in Cu average contents (n = 2) in clam M.lyrata via exposure days

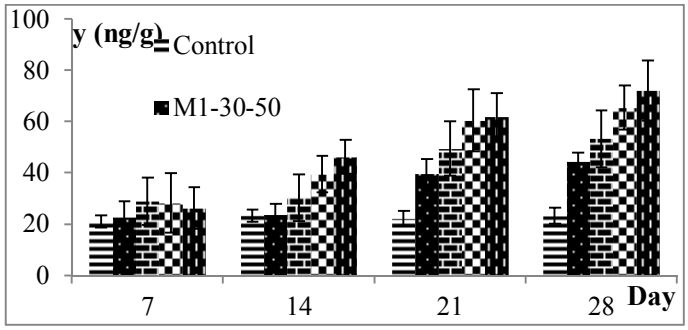

Figure 3. Variation in Pb average contents (n = 2) in clam M.lyrata via exposure days

- For all initial concentrations of dissolved metals, the metal levels in the clam body tissues (y) increased with exposure times (x) with R = 0.78 - 0.98 (Table 3). However, after 21 days of exposure, Cu bio-accumulation by clams *M.lyrata* reduced or nearly reached to plain. Cu accumulated in the clams much more than Pb: during 28 days of exposure to the highest initial levels of dissolved metals (level M4 - 200 µg/L Cu and 600 µg/L Pb), Cu and Pb levels accumulated in the clams were 1175 ng/g and 72 ng/g, respectively.

- The obtained results shown that the metals absorbed by the *M.lyrata* derived from both dissolved and suspended solid parts. Due to remarkable part of the metals present as labile (or bioavailable) forms, they were easily accumulated by *M.lyrata*. That, the metal contents in the *M.lyrata* body tissue increased with increases in initial concentration of dissolved metals and exposure times, allowed to confirm that the *M.lyrata* could be used as bio-indicator for the metal pollution in aquatic environment.

Table 3. Linear correlation between the metal contents in the M.lyrata and initial concentration of dissolved metals, and exposure time

Factor		Cu			Pb		
		Equation	R	p	Equation	R	p
Correlation between the metal content in the *M.lyrata* (y) and initial level of dissolved metals (x) during different exposure days	7 days	y = 0.99x + 596(*)	0.999	0.01	y = 0.002x + 25.9	0.034	0.81
	14 days	y = 1.99x + 621(*)	0.845	0.07	y = 0.038x + 24.8	0.915	0.04
	21 days	y = 2.19x + 711	0.933	0.03	y = 0.035x + 43.5	0.731	0.14
	28 days	y = 2.38x + 719	0.955	0.02	y = 0.046x + 46.6	0.881	0.06
Correlation between y and exposure time (x) for different initial level sof dissolved metals	M1-30-50	y = 5.54x + 613	0.784	0.11	y = 1.16x + 12.2	0.894	0.05
	M2-60-100	y = 10.9x + 594	0.963	0.02	y = 1.32x + 17.4	0.882	0.06
	M3-100-300	y = 15.9x + 593	0.908	0.05	y = 1.89x + 15.2	0.953	0.02
	M4-200-600	y = 24.6x + 525	0.930	0.03	y = 2.19x + 13.3	0.980	0.009

(*) For these equations, initial concentrations of dissolved Cu (x) = 30, 60 and 100 µg/L, due to at x = 200 µg/L and above, y nearly reached to plain.

3.3. Rate of metal accumulation (RMA) by *M.lyrata*

The results obtained in Table 4 shown that: i) Increase in initial levels of dissolved metals led to increase in RMAs for Cu and Pb; For all initial concentrations of dissolved metals and during 28 days of exposure, RMAs for Cu were greater 7 – 12 times than that for Pb ; ii) There was strong linear correlation between the RMAs (y) and initial concentrations of dissolved metals (x) with R = 0.94 ($p < 0.05$). This once again proved that *M.lyrata* could be used as bio-indicator for Cu and Pb pollution in aquatic environment.

Table 4. Rate of the metal accumulation by the M.lyrata during 28 days of exposure(*)

No	Initial levels of dissolved metals (µg/L)	Cu		Pb	
		RMA (ng/g per day)	Equation	RMA (ng/g per day)	Equation
1	M1-30-50	5 ± 2	y = 0.089x + 3.8 (R = 0.982; p = 0.018)	0.8 ± 0.2	y = 0.002x + 0.8 (R = 0.943; p = 0.056)
2	M2-60-100	10 ± 1		1.1 ± 0.0	
3	M3-100-300	14 ± 3		1.5 ± 0.2	
4	M4-200-600	21 ± 4		1.7 ± 0.4	

(*) RMA data in the table are mean ± standard deviation with n =2 (2 experimental tanksreplicated); y: RMA; x: initial levels of dissolved metals.

4. CONCLUSION

The higher initial level of dissolved metals and the longer exposure time, the higher metal bio-accumulation by the *M.lyrata*. For all initial concentrations of dissolved metals and during 28 days of exposure, Cu bio-accumulation by the *M.lyrata* was higher than Pb one. The *M.lyrata* could be used as bio-indicator for the metal pollution in aquatic environment.

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