TRƯỜNG ĐẠI HỌC SÀI GÒN
TẠP CHÍ KHOA HỌC
ĐẠI HỌC SÀI GÒN
ISSN 1859 - 3208
Số 80 (02/2022)

SAIGON UNIVERSITY
SCIENTIFIC JOURNAL
OF SAIGON UNIVERSITY
No. 80 (02/2022)

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BIOACCUMULATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN EDIBLE MARINE CLAM *MERETRIX* SP.: A LABORATORY STUDY

Tích lũy các Hyđrôcacbon thơm đa vòng (PAHs) trong nghêu biển Meretrix sp. ở điều kiện phòng thí nghiệm

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ABSTRACT

This study investigated the bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) in the edible saltwater clam *Meretrix sp.* under laboratory conditions. The clams were collected from the Can Gio coastal area and transported alive in an icebox to the laboratory on the same day. Before the exposure experiment, the tested clams were kept in artificial seawater under laboratory conditions for 20 days. Then clams were exposed to oil-suspended particulate matter aggregates (OSA) containing PAHs for 21 days of accumulation and 10 days of depuration periods. The results showed that PAHs quickly accumulated into the tested animal and were detected in all samples, with a total concentration of 15 parent PAHs in clam ranging from 223.72 to 640.04 ng/g wet weight (ww) in the accumulation experiment and from 74.51 to 214.96 ng/g ww in the elimination experiment. PAHs containing 5-6 benzene rings were mainly detected in the clam. The PAH isomer ratios in the clam *Meretrix* sp. *indicated that* the PAHs accumulated in the tested animal were original from the fuel burn process, suggesting that anthropogenic activities could contribute to many PAHs possible accumulation in aquatic life organisms.

Keywords: *aquatic organisms, accumulation of PAHs, depuration, marine bivalves*

TÓM TẮT

Trong nghiên cứu này, chúng tôi đã khảo sát sự tích luỹ sinh học của các hydrocacbon thơm đa vòng (PAHs) trong nghêu biển *Meretrix* sp. ở điều kiện phòng thí nghiệm. Nghêu được thu từ khu vực ven biển Cần Giờ và mang về phòng thí nghiệm ngay trong ngày. Trước khi thử nghiệm phơi nhiễm, nghêu được duy trì trong điều kiện phòng thí nghiệm để thích nghi trong 20 ngày. Sau đó, nghêu được phơi nhiễm với vật chất dạng hạt lơ lửng chứa dầu thô (OSA) có chứa PAHs trong 21 ngày cho tích lũy, sau đó nghêu được chuyển sang môi trường không có PAHs 10 ngày cho đào thải. Kết quả cho thấy PAH nhanh chóng tích luỹ vào nghêu và được phát hiện ở tất cả các mẫu với tổng hàm lượng của 15 PAH trong nghêu dao động từ 223,72 đến 640,04 ng/g trọng lượng ướt (ww) trong thí nghiệm tích lũy và từ 74,51 đến 214,96 ng/g ww trong pha đào thải. PAH được phát hiện trong nghêu chủ yếu là loại chứa 5-6 vòng benzen. Tỷ lệ đồng phân PAH trong nghêu *Meretrix* sp. cho thấy PAHs trong nghêu có nguồn gốc từ quá trình đốt cháy nhiên liệu. Nghiêu cứu cho thấy các hoạt động của con người như đốt than, xăng, dầu, khí đốt có thể giải phóng một lượng lớn PAHs và có thể tích tụ trong các sinh vật dưới nước.

Từ khoá: *sinh vật nước, tích luỹ PAHs, đào thải, nhuyễn thể hai mảnh vỏ biển*

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Introduction

Human activities have introduced into the aquatic environment various inorganic and organic pollutants [1], [2]. Among the main organic pollutants present in The coastal and marine environments, polycyclic aromatic hydrocarbons (PAHs) are one of the major environmental risk factors for human health [3]. PAHs is A group of semi-volatile chemical compounds with at least two fused benzene rings with different structures [2]. Up to now, Over 200 PAH compounds have been reported in the environment, many of which are toxic with carcinogenic, mutagenic, and teratogenic activities. 16 PAHs structures are being listed as priority pollutants by the US Environmental Protection Agency [2], [4]. Many of them such as benzo[a]anthracene (BaA), chrysene (Chr), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), benzo[b]fluoranthene (BbF), indeno[1,2,3-cd]pyrene (In(1,2,3-cd)P), dibenzo[a,h]anthracene (DahA), and benzo[g,h,i]perylene (BP) are potentially carcinogenic to humans [5], [6].

Although PAHs can come naturally from coal and gasoline, the largest sources of PAHs are anthropogenic activities as coal, oil, gas, wood, garbage, and tobacco are burned [4], [7]. The marine traffic and oil spill accidents also contribute a large amount of PAHs to the marine environment. Under the aquatic conditions, PAHs can widespread in the water columns and are rapidly adsorbed by suspended particulate matter due to their hydrophobic properties [4]. Then these comtaminants can be settled in benthic environment where they can bioaccumulate by several benthic organisms. The biaccumulation of PAHs in benthic organisms eventually affects human health due to consume seafood products [2], [3]. Previous studies have reported high PAH concentrations in aquatic organisms [8], [9]. The maximum PAH concentration (6.9 µg/g DW) has been reported in the oyster *Crassostrea gigas*, collected from San Francisco Bay [10]. Therefore, to reduce the ecological risk of PAH, the investigation on PAHs accumulation in aquatic organisms in important.

Saltwater clam such as *Meretrix* sp. is one of the largest clam families in Vietnam. They are filter feeders and less moving on the beds of the sea [11]. Therefore, they are seriously affected by the presence of toxic substances in the marine ecosystem. They are commonly used as food sources for humans, thus, the investigation of PAHs in this clam is more important. In the present study, we investigate PAHs accumulation in the edible saltwater clam Meretrix sp. under laboratory conditions. The clams were exposed to oil-suspended particulate matter aggregates (OSA) to model shallow, subtidal experiments. This study aims to (1) determine the bioaccumulation of PAHs into soft tissues of *Meretrix* sp. chronically exposed to OSA, (2) examine the specific patterns of PAHs accumulated in the test animal, and then examinate the sources of PAHs in the environment.

Materials and methods

Collection and maintenance of clam

The marine clams *Meretrix* sp. (Figure 1) having the same size (3.5 ± 0.5 mm shell length) were collected at Can Gio coastal area and kept in ice box to transport to the laboratory. To remove the pollutants from clam tissues, all clams were kept in 4 aquariums (60 × 50 × 40 cm) containing 40

L of artificial seawater with 5-cm sand layer as a substrate for 20 days. All aquariums were aerated 24/24h by 3 aerators under a lighting system with 2 fluorescent lamps of 20 W/60 cm at a light cycle of 12 hours/12 hours. The clams were fed every 2-3 days with a mixture of diatom *Thalassiosira* sp. and *Chaetoceros* sp. at the concentration of about 1×10^3 cells/mL.

Preparation of oil-suspended particulate matter aggregates

The OSAs were prepared according to the methods previously described by Isobe et al. (2007) [12] with some modifications. In brief, 0.6 g of crude oil, 0.2 g of ground and sieved sediment ($\Phi < 20$ μm), and 1 mL n-hexane were diluted in 1 L (1000 g) of artificial seawater in the dark. The sieved sediments were enriched withthe crude oil. The mixture was shaken (at 200 rpm) at room temperature (25 ± 1 °C) for 24h. Then, the OSAs were allowed to stabilize for about 5 min and used for the exposure experiment.

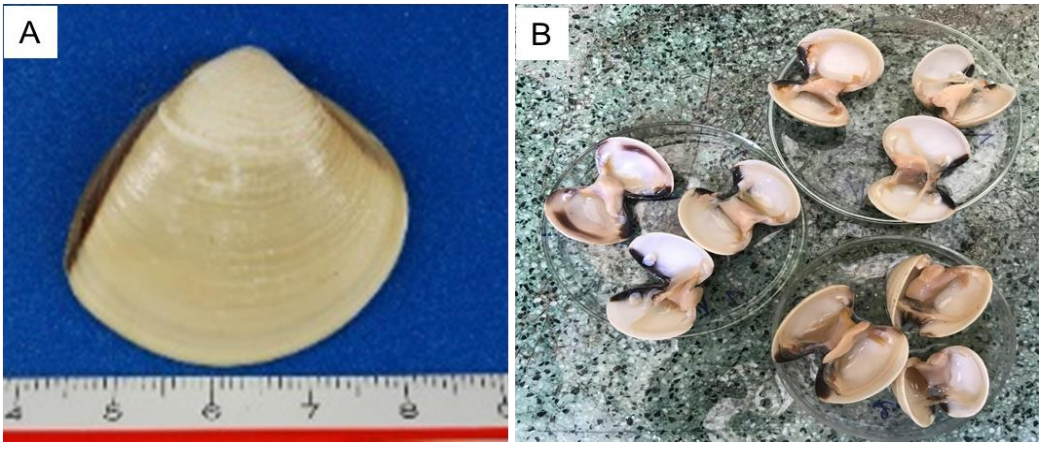

Figure 1. Photos of edible saltwater clam *Meretrix* sp. with *w*hole external shell (A) and the inner soft tissues (B).

Exposure experiment

The experiment consists of 6 tanks whose diameter × height is 20 × 17 cm. Clam is kept at a density of 10 individuals per tank, at a temperature of 27 ± 2°C, aerated 24/24h using 3 aerators. During the accumulation period (from day 0 to day 21 of the experiment), the clams were exposed to the OSAs containing PAHs at a concentration of 600 mL of OSAs/40 mL of artificial seawater. Then, the clams were transported to the artificial seawater without OSAs for the next 10 days for the elimination period. Clams were fed every two days with a mixture of the diatom *Thalassiosira* sp. and *Chaetoceros* sp. a concentration of 1×10^3 cells/mL during the experiment. 3 individuals of clam were collected on days 0, 7, 14, 21 for accumulation and on days 23, 27, 30 for elimination.

PAHs extraction and analysis

The content of PAHs in the soft clam tissues was extracted and analyzed according to the method previously reported by Pham et al. (2020) [8]. Briefly, the soft tissues of three clams were homogenized and kept overnight in a

Teflon vial containing 12 mL concentrated HCl and 20 mL chloroform. After centrifugation, the supernatant was collected and washed two times with 10 mL MQ water each. Then 2 mL of iso-octane were added to the sample and concentrated to about 1 ml by using evaporation. The samples were then redissolved with 1 mL pentane and passed throught a glass columns (1 cm i.d., 30 cm length) packed with 4 g of silica gel, 4 g of alumina and 3 g of Na_2SO_4 on the top. Then, the PAHs fraction were collected in 60 ml n:pentane/dichloromethane (8:2, v/v). The elution was added with 1 mL DMSO and dried to 100 µL. Samples were then redissolved in 500 µL of n-hexane for HPLC analysis.

The PAHs fractions were analyzed using a HPLC instrument equipped with a Dionex Ultimate 3000 Fluorescence detector. The system was operated by the Chromeleon chromatography management software (Dionex, Germany). 20 µL of each sample was passed through the eclipse PAH column (3.0 × 250 mm, 5 µm, Agilent, CA, USA) maintained at 25°C. ACN (solvent A) and Milli-Q water (solvent B) were used as mobile phase. The elution gradient was 45%(A)/55%(B) initially, 100%(A) at 17–28.5 minutes, and 45%(A)/55%(B) at 29.5–31.0 minutes. The excitation wavelength program and retention time shown in Table 1 were used to indentified the PAHs.

Table 1. The name, abbreviation, excitation wavelength and retention time of PAHs

No	Name	Abbreviations	Wavelength (nm)	Retention time (min)
1	Naphthalene	Nap	270/327	0-10.3
2	Acenaphthene	Acc	250/364	12.5-13.2
3	Fluorene	Flu	250/364	12.5-13.2
4	Phenanthrene	Phe	250/400	13.2-14.2
5	Anthracene	Ant	237/447	14.2-15.2
6	Fluoranthene	Flt	270/390	15.2-17.0
7	Pyrene	Pyr	265/388	17.0-19.2
8	Benz[a]anthracene	BaA	280/410	19.2-23.8
9	Chrysene	Chr	280/410	19.2-23.8
10	Benzo(b)fluoranthene	BbF	280/400	23.8-27.5
11	Benzo[k]fluoranthene	BkF	280/400	23.8-27.5
12	Benzo[a]pyrene	BaP	280/400	23.8-27.5
13	Benzo[g,h,i]perylene	BgP	293/500	27.5-31.0
14	Dibenzo[a,h]anthracene	dBA	293/500	27.5-31.0
5	Indeno[1,2,3-cd]pyrene	InP	293/500	27.5-31.0

Qualitative analysis of PAHs was based on the obtained spectrum of peaks and retention time values. 15 PAH standards (listed in Table 1) purchased from Sigma-Aldrich were used as standards. The PAHs concentration in each sample was calculated based on the fluorescence data (peak area measurement) by using an external calibration curve method.

Data analysis

One-way analysis of variance (ANOVA) tests were used to examinate the statistical differences of PAH concentration. All data were log(x+1) transformed to get a normal distribution. *p*-value less than 0.05 was consider statistical significance.

Results and discussion

PAHs concentration in clam Meretrix sp.

The concentrations of total PAHs and individual congeners measured in saltwater clam *Meretrix* sp. are shown in Table 2. PAHs quickly accumulated in the tested animal and were found in all samples. The total concentration of all PAH congeners in clam ranging from 223.72 to 640.04 ng/g wet weight (ww) in the accumulation experiment and from 74.51 to 214.96 ng/g ww in the elimination experiment. The dominant individual PAH variants in clam were BaP, BbF, BaA, dBA, BkF, Flt, Acc, Pyr, Chr and Phe. Compared with a number of studies around the world which have shown similar results on typical PAHs pollution as in *Perna viridis* mussel from the Pacific Ocean (21-1093 ng/g dw [10]; at *Mytilus galloprovincialis* from Osaka Bay, Japan (87.3-361 ng/g dw [11]); in the coast of Vietnam, the mussel *Perna viridis* (34-110 ng/g dw, [12]); The result of this study was higher than that in the Mediterranean coast of *Mytilus* with PAHs concentration (0.21-8.95 ng/g ww [13]). The highest concentration of PAHs (4670 ng/g dw [14]) was detected in Brachidontes mussel collected on the coast of Egypt, Indian Ocean, approximately 7 times higher than the study. Pham et al [8] reported the PAH concentrations in oysters (*Crassostrea* sp.) and gastropods (*Cymatium* sp.) collected from the Can Gio mangrove forest, Viet Nam ranged from 3.3 to 64.5 ng/g wet weight (WW), and from 4.8 to 23.8 ng WW, respectively. The concentrations of different heavy metals, including Mn, Fe, Co, Ni, Cu, Zn, As, Se, Cd, Hg and Pb up to thousand mg/kg DW have also been detected in various organs of the hard clam *Meretrix lyrata* collected from this area [15]. Therefore, it is important to implement a monitoring program for PAHs and trace metals to better manage these pollutants in the Can Gio coastal wetland.

Table 2. PAH concentrations (ng/g ww) accumulated in saltwater clam *Meretrix* sp.

	The accumulation experiment					The elimination experiment		
Days	0	1	7	14	21	24	27	30
Nap	UDL	0.1521	0.0385	UDL	UDL	UDL	UDL	UDL
Acc	UDL	5.0636	11.0182	4.4798	5.448	UDL	UDL	UDL
Flu	UDL	0.0047	UDL	UDL	UDL	UDL	UDL	UDL
Phe	UDL	1.1108	2.4185	0.1532	0.0173	UDL	UDL	UDL
Ant	UDL	0.0803	0.3251	0.0205	UDL	UDL	0.0980	UDL
Flt	UDL	3.3043	12.9743	2.3088	UDL	UDL	UDL	UDL
Pyr	UDL	1.3651	10.4239	0.67323	0.7679	0.4967	0.3266	0.0857
BaA	UDL	10.2209	40.5801	2.4029	1.6735	UDL	UDL	UDL
Chr	UDL	3.8306	9.7891	2.7050	3.2752	1.4655	0.3127	0.0751
BbF	UDL	27.3198	116.511	41.9557	37.8846	30.3629	54.2365	37.536
BkF	UDL	6.2831	11.5441	5.6572	5.0119	4.3389	3.30470	1.8227
BaP	UDL	203.783	397.578	275.105	157.374	168.853	77.1144	32.907
dBA	UDL	16.9768	26.8352	13.3612	12.2627	9.4444	UDL	2.0847
BgP	UDL	UDL	UDL	UDL	UDL	UDL	UDL	UDL
InP	UDL	UDL	UDL	UDL	UDL	UDL	UDL	UDL
ΣPAHs	**0**	**279.4953**	**640.0376**	**348.8231**	**223.7168**	**214.9622**	**135.3931**	**74.5121**

UDL: under detection limits

In addition to the ability to accumulate when clams are directly exposed to PAHs for 21 days, the study also carried out a 10 day elimination experiment by taking clams after 21 days of accumulation and transferring them to a clean environment to evaluate the depuration ability of the clam. The study results showed that PAHs concentration significantly declined during the depuration period (from 223.72 ng/g ww at the end of the accumulation period to 74.51 ng/g ww, decreased more than 3 times after elimination. These results showed that they doubted the clam ability in different environments.

Composition and source of PAHs

The composition profiles of PAHs accumulated in the clam *Meretrix* sp. was shown in Figure 2. PAHs containing 5-6 benzene rings were dominant (with concentrations ranging from 212.53 to 552.47 ng/g ww), accounting for about 86.32-96.35% in the accumulation phase. In the elimination phase, the PAHs concentration ranged from 74.35 ng/g ww to 213.01 ng/g ww, with PAHs 5-6 rounds accounted for more than 99%, ranging from 99.08 to 99.78% in average. The

content of 2-3 benzene rings PAHs in the accumulation experiment ranged from 4.65 to 13.80 ng/g ww on average, accounting for only 1.33-2.29% of the total 15 PAHs. For the elimination experiment, PAHs containing 2-3 benzene rings were even lower and detected only on day 7 with a concentration of 0.09 ng/g ww, accounting for 0.07%. PAHs containing 4 benzene rings ranged from 5.71 to 73.77 ng/g ww, accounting for about 2.55-11.53% in the accumulation phase and from 0.22 to 0.91% in the elimination phase. Our results showed that clam accumulated mainly PAHs with molecular structure from 5-6 ring benzene and the ability to eliminate these PAHs 5-6 rings of benzene is lower than PAH with 2-3 rings. This can happen because PAHs with higher molecular weight have lower water solubility, but they better absorb into matter particles and store in the bottom sediment, then accumulate in aquatic organisms [13].

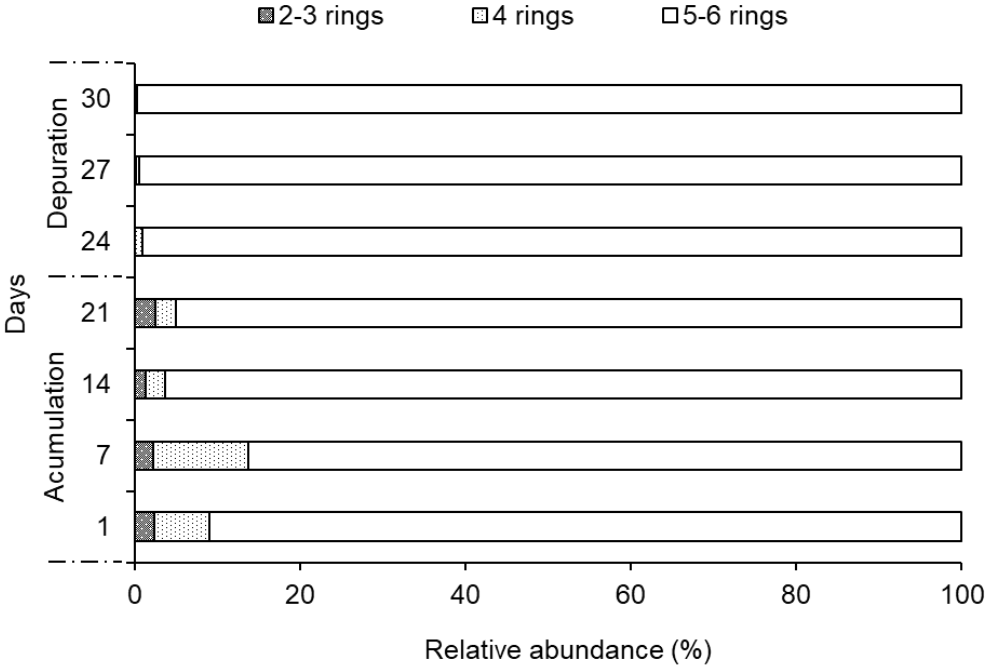

Figure 2. The composition of PAHs in saltwater clam *Meretrix sp.*

Contrasting with our observation, Barhoumi et al [19] reported the PAHs in mussel (*Mytilus galloprovincialis*) and eel (*Anguilla anguilla*) from Bizerte lagoon, Tunisia contained mainly of PAHs with 2-3 benzene rings. The differences in species, environmental composition, and accumulation capacity may cause differences in the accumulation of PAHs composition in biological tissues.

The PAHs isomer ratio in saltwater clam *Meretrix* sp. was shown in Figure 3. Petroleum and petrogenic activities could generate different molecular compositions of PAHs. Identification of PAH sources is important to control their input and assign responsibility for remedial activities [20], [21]. The isomer ratios of PAHs

composition were commonly used to identify PAHs from pyrogenic and petrogenic origins [9], [22], [23]. In this study, we used the ratios of Flt/Pyr and LMW PAHs/HMW PAHs to examine the potential sources of PAHs (Figure 3). Khim et al (1999) [24] reported that if the ratio of Flt/Pyr > 1, the source of PAHs pollution comes from the combustion processes (petroleum) and if this ratio <1, the pollution origin is characterized by the rock formation (petrogenic). The second selected rate is to evaluate the origin of PAHs according to Bihari et al. (2007) [25] based on the ratio of PAHs LMW/HMW> 1, the source of PAHs pollution from rock formations (petrogenic), if <1, the source of PAHs pollution from the combustion (petroleum). The results of PAHs origin of this study, presented in Figure 3 show that the ratio of Flt/Pyr compounds at 21 days of experiment ranged from 1.26 to 6.35 on average, characterizing the origin of the fuel burn. Our results agree with the use of crude oil or OSAs in the exposure experiment.

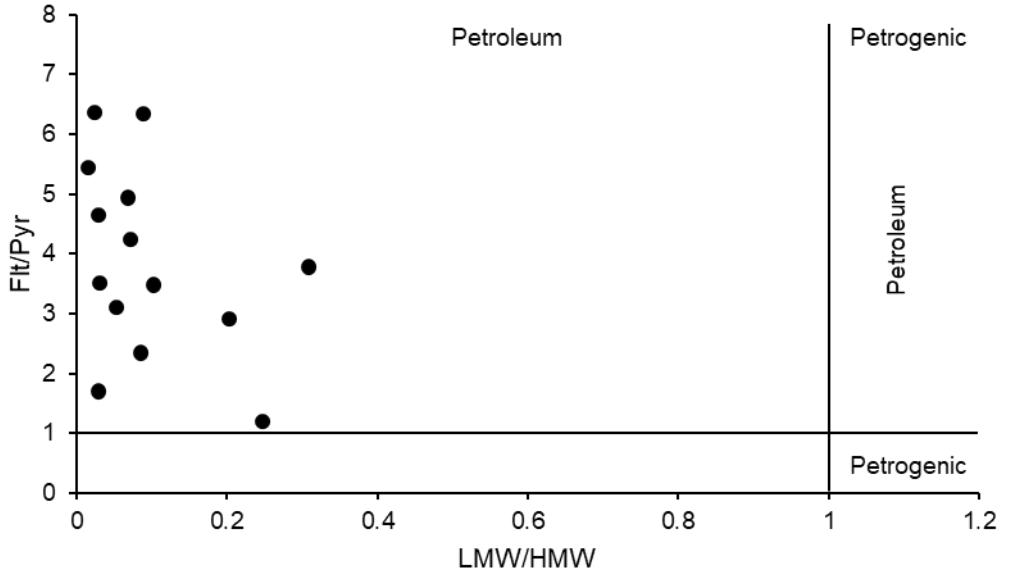

Figure 3. The PAH isomer ratio in saltwater clam *Meretrix* sp.

Conclusions

Our results confirmed the bioaccumulation of PAHs from crude oil in the saltwater clam *Meretrix* sp. PAHs quickly accumulated into the clam tissues and were detected in all samples, especially during exposure. The elimination PAHs indicated that PAHs were removed from the clam tissues when the clam was kept in the clear water but still detected PAHs after 10 days of elimination. PAHs containing 5-6 benzene rings were dominant in the clam. The original PAHs in the tested animal were mainly from the fuel burn process, suggesting that anthropogenic activities may contribute to a large amount of PAHs and possible accumulation in aquatic organisms.

Acknowledgments

This research was funded by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under Grant number 105.08-2016.06.

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Ngày nhận bài: 28/12/2020 Biên tập xong: 15/02/2022 Duyệt đăng: 20/02/2022