2011 2nd International Conference on Environmental Engineering and Applications
IPCBEE vol.17 (2011) © (2011) IACSIT Press, Singapore

Polycyclic aromatic hydrocarbons concentration in soft tissue of Ark clam (*Barbatia helblingii*) along Bushehr coasts (summer)

†[1]Alireza Safahieh, [1]Masoomeh Mahmoodi, [2]Yadollah Nikpour, [2]Kamal Ghanemi

[1]Department of Marine Biology, Faculty of Marine Science and Oceanograghy, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran

[2]Department of Marine Chemistry, Faculty of Science and Oceanograghy, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran

Abstract. In order to study polycyclic aromatic hydrocarbons concentration in Ark clam (*Barbatia helblingii*), sampling was performed in five stations along Bushehr coasts in north Persian Gulf. The soft tissues of the clams were digested in methanol and their PAHs content were extracted with hexane and measured by HPLC. Result showed that the total PAHs concentration in Rafael, Sheghab, Abshirinkon, Lian and Helyleh stations were 634.7, 476.7, 129.5, 452.5 and 415.0 ng g^{-1}(dw) respectively. Significant difference was observed between tPAHs concentration in different stations ($P<0.05$). The maximum and the minimum concentrations of PAHs was measured in clams collected from Rafael and Abshirinkon respectively. Among different PAHs compounds, 3 rings PAHs were the most abundant compounds in the clam's soft tissue. The mean concentration of tPAHs in Ark clam collected from Bushehr coast was 421.86 ng g^{-1}. Compared to previous studies, the PAHs content of the Ark clam in Bushehr was moderate to high.

Keywords: Ark clam (*B.helblingii*), PAHs, Bushehr shore line

1. Introduction

Crude oil and its derivatives are the major group of marine ecosystem contaminants which is widespread in the world. Two different types of hydrocarbons exist in crude oil, aliphatic and aromatic hydrocarbons, among them the second type have attracted more concerns due to its adverse effects on aquatic organisms. Polycyclic aromatic hydrocarbons constitute about 0.2 to 7% of the total hydrocarbons in the crude oil [1].Since these compounds have low vapor pressure and solubility in seawater they tend to be absorbed by suspended organic matter and finally deposit in the sediment [3]. These compounds are resistant against degradation and could be transferred along food chains and by this rout they even might appear as a threat for human health. The impairment in gametogenesis, sexuality, and growth [14], together with the increased risk of cancer and mutation are their major adverse effects of them on living organisms [11]. These compounds are released in to the marine environment through several pathways including oil exploitation and transportation, combustion of fuels, municipal and industrial wastes. Coastal waters of the Bushehr province one of the major locations for Iranian oil export is supposed to receive considerable amount of PAHs, however the amount of PAHs in biological samples is not well studied. The Ark clam (*Barbatia hellblingii*) is abundant and well distributed in the area providing a good candidate for PAHs biomonitoring. This study was carried out to investigate PAHs levels in *B.hellblingii* from Bushehr shore line.

2. Material and methods

† Corresponding author. Tel.: 09168331821; fax: +00986324234403.
E-mail address: safahieh@hotmail.com.

Clam samples were collected from five different stations along the Bushehr coasts including Rafael, Sheghab, Abshirinkon, Lian and Helyleh in August 2008 (Fig. 1). The Name and the major human activities of each station are presented in Table 1. About 30 Ark clams with the same size (35±5mm) were collected from each station. They were enveloped in aluminum foil, kept cold in Icebox, and transported to the laboratory.

In order to gain dried samples, the soft tissue of 5-6 individuals was homogenized and freeze-dried. Three pooled samples of clam tissues were analyzed for each station. About 1 ml decachlorobiphenyl (16 µ L^{-1}) as surrogate standard [18] was added to 5 g of each dried sample and PAHs content of the mixture was extracted by the method proposed by MOOPAM [12, 19].

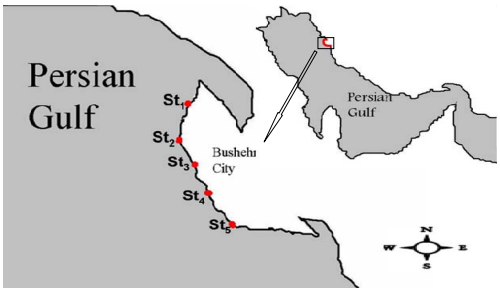

Fig. 1: The position of sampling stations.

Table 1: Description and geographical location of the sampling stations in the Bushehr coast.

Station	Position	Description
1-Rafael	28º 57' 45 /9"	landing port for fishing vessels
	50º 48 ' 43/2"	
2-Sheghab	28º 55' 37/7"	Building construction, residential area
	50º 48 ' 26/7"	
3-Abshrinkon	28º 54' 12/7"	urban dump
	50º 49 ' 9"	
4-Lian	28º 52' 20"	landing port for fishing vessels, small
	50º 50' 33/3"	industries
5-Helyleh	28º 50' 3/3"	landing port for fishing vessels, urban
	50º 52' 31/9"	dump

After the solvent was totally evaporated, 1ml acetonitrile was added to extracts [16] and they were injected to HPLC equipted with UV detector and C_{18} column. The standard solutions used for instrument calibration were PAHs calibration mix and decachlorobiphenyl. The obtained data were subjected to Shapiro-wilk normality test. The difference between tPAHs concentrations in different stations was compared by One Way ANOVA.

3. Results

Concentrations of PAHs in clam's soft tissue are shown in table 2.

Table 2: PAHs concentration in Ark clams' soft tissue from studied stations (mean ± standard deviation).

Compounds	Rafael	Sheghab	Abshirinkon	Lian	Helyleh

Naphthalene	ND	ND	ND	ND	ND
Acenaphtylene	59.7 ± 4.4	ND	39.4 ± 3.3	ND	10.8 ± 3.6
Acenaphthene	80.0 ± 5.7	55.8 ± 3.8	14.3 ± 1.9	41.4 ± 3.0	26.4 ± 1.3
Fluorine	40.2 ± 3.0	48.0 ± 3.2	3.6 ± 1.0	37.5 ± 3.6	2.8 ± 1.9
Phenanthrene	116.8 ± 7.0	94.0 ± 6.8	9.0 ± 1.6	109.5 ± 5.5	108.3 ± 3.9
Anthracene	55.5 ± 3.6	27.7 ± 2.4	1.9 ± 0.5	59.5 ± 5.2	5.0 ± 2.3
Fluoranthene	55.8 ± 3.0	38.4 ± 3.2	11.4 ± 2.4	33.2 ± 2.2	71.8 ± 4.0
Pyrene	148.7 ± 10.3	108.1 ± 8.0	17.2 ± 2.9	95.5 ± 5.9	37.3 ± 1.0
Benzo[a]anthracene	39.3 ± 3.3	53.0 ± 3.6	7.9 ± 1.9	3.3 ± 0.9	53.3 ± 1.5
Chrysene	ND	24.0 ± 2.1	7.5 ± 0.9	4.6 ± 1.0	ND
Benzo[b]fluoranthene	ND	ND	8.4 ± 0.7	ND	ND
Benzo[k]fluoranthene	32.7 ± 2.4	7.2 ± 0.6	8.9 ± 1.0	14.5 ± 1.5	ND
Benzo[a]pyrene	ND	21.3 ± 3.9	ND	53.4 ± 2.3	ND
Dibenzo[a,h]anthracene	ND	ND	ND	ND	ND
Benzo[ghi]perylene	ND	ND	ND	ND	ND
Indeno[1,2,3-cd]pyren	6.0 ± 0.6	ND	ND	ND	ND
tPAHs	634.7 ± 43.4	476.7 ± 37.7	129.5 ± 18.1	452.5 ± 33.1	415.0 ± 25.3

Internal standard recovery was 87%. ND: Not Detected.

According to the result tPAHs concentration in the soft tissue of clams were 634.7 ng g^{-1} in Rafael, 476.7 ng.g^{-1} in Sheghab, 129.5 ng.g^{-1} in Abshirinkon, 425.5n g.g^{-1} in Lian and 415.0 ng.g^{-1} in Helyleh. Significant difference was observed between tPAHs concentrations in various station ($P<0.05$). The concentrations of some PAHs compounds such as naphthalene, dibenzo[a,h]anthracene, benzo[ghi]perylene and indeno[1,2,3-cd] pyren were below the detection limits of the instrument some others such as pyrene and phenanthrene were found in high concentrations. The maximum tPAHs concentration was measured in samples taken from Rafael on the other hand the minimum tPAHs content was observed in clams collected from Abshirinkon. Phenanthrene and pyrene had considerable concentrations in Rafael, Sheghab and Lian. Compared with other compounds, Acenaphtylene in Abshirinkon, Phenanthrene and fluoranthene in Helyleh were found in higher concentrations. Three rings PAHs were the most abundant compounds, while 5+6rings PAHs were detected in lower concentrations (Fig. 2.). The mean concentration of 3rings, 4rings and 5+6rings PAHs in clams were 209.3, 172.9 and 30.5 ng g^{1} respectively.

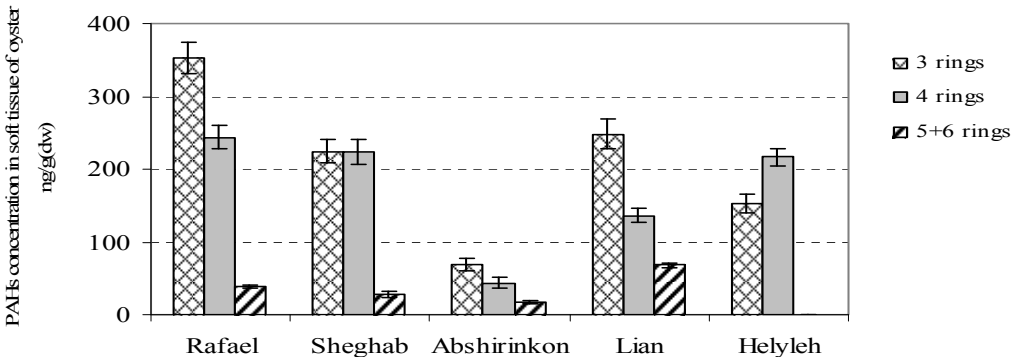

Fig. 2: Concentration of 3, 4 and 5+6 rings PAHs in soft tissue of *B.helblingii* in studied station.

3 rings PAHs are sum of acenaphtylene, acenaphthene, fluorene, phenanthrene and anthracene, 4 rings PAHs include: fluoranthene, pyrene, benzo[a]anthracene and chrysene, 5+6 rings PAHs consist of: benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[ghi] perylene and indeno[1,2,3-cd]pyrene.

4. Discussion

European Commission Union, considered Benzo(α)pyrene as marker for the occurrence and the effect of carcinogenic PAHs in food [8]. According to this guidance the maximum concentration level of PAHs in bivalves is 10ng g^{-1}(fresh weight). In order to compare the results with this guideline, the concentration of Benzo(α)pyrene measured in dry weight was converted to wet weight basis using 0.24 conversion coefficient [13]. The calculated concentration of Benzo(α)pyrene was 3.58ng g^{-1}(wet weight). This value was lower than maximum concentration level determined for human food. According to the results the mean concentration of tPAHs in *B.helblingii* was 421.86 ng g^{-1}(dw). This was compared to tPAHs concentrations in some other

marine organisms from several locations of the world (Table 3). Generally, PAHs concentration in some previously studied fishes was lower than oysters. This could be related to metabolisms of PAHs by fish liver [6, 20]. Comparison of *B.helblingii* with oysters from other locations showed that tPAHs concentration in *B.helblingii* was within the range of other studies. Benzo(α)pyrene in *B.helblingii* was higher than other marine organisms compared in table 3.

Significant difference was observed between tPAHs concentration in various stations. Rafael and Sheghab are located in the Bushehr city and are exposed to harbor and urban wastes. Therefore compared to other stations, higher concentrations of PAHs in these locations is an expected issue. The Lowest tPAHs concentration was observed in Abshirinkon, which could be due to its long distance from harbor and urban area.

Based on the number of rings in the molecule, the order of PAHs compounds in oysters from Bushehr intertidal coasts was as: 3rings PAHs>4rings PAHs>5+6 rings PAHs. One reason for abundance of 3rings PAHs in *B.helblingii* could be related to their uptake pathway. The fewer rings number in PAHs molecule causes their more solubility in the seawater. More soluble compounds have more persistency in seawater. They are more bioavailable and could easily be taken up through gill membrane or food ingestion. Bummard et al similarly found that low weight compounds (3 and 4 rings PAHs) are more abundant in marine organisms than 5 and 6rings PAHs [3].

Table 3. Concentration of PAHs in various marine organisms from different location of the world.

Studied species	tPAHs	Benzo(α) pyren	Location	References
Fish				
Epinephelus coioides	65.66	-	Qatar	[17]
Epinephelus coioides	23.9	-	Bahrain	[17]
Lethrinus nebulosus	43	-	Qatar	[17]
Lethrinus nebulosus	25	-	UAE	[17]
Mullus barbatus	24.43	0.35	Mediterranean	[3]
Serranus Scriba	58.11	0.53	Mediterranean	[3]
Crustacea				
Mysid euphausiids	364.5	11.86	Mediterranean	[3]
Bivalve				
Crassostrea.sp	524	-	Bay of Biscay	[5]
Ostera edulis	125*	2.27	Lebanon	[10]
Crassostrea Virginia	312	-	Mobile Bay	[15]
Mytilus galloprovincialis	98.80	1.5	Mediterranean	[4]
Barbatia helblingii	421.86	14.94	Bushehr coast	This study

UAE: United Arab Emirates. -Not reported.

The order of PAHs compounds in *B.helblingii* is compared to some previously studied aquatic organisms in Table 2. The PAHs composition pattern in *B.helblingii* was same as two fish species *Scomber scomberus* and *Micromesistius poutassou* and the Lobster *Nephrops norvegicus* in the Adriatic sea [16]. 4 rings PAHs were dominant in the *Merluccius merluccicus* lives in 70-370 m depth from sea surface and *Mullus barbatus* which lives in the sediment of the Adriatic sea (Table 4).

Table 4. compostion pattern of PAHs in various selected marine organisms based on number of rings

species	**pattern**	**Location**	**References**
fish			
Scomber scomberus	3>4>5,6 *	Adriatic sea, Italy	[16]
Micromesistius poutassou	3>4>5,6 *	Adriatic sea, Italy	[16]
Merluccius merluccicus	4>3>5,6*	Adriatic sea, Italy	[16]
Mullus barbatus	4>3>5,6*	Adriatic sea, Italy	[16]
Crustacea			
Nephrops norvegicus	3>4>5,6 *	Adriatic sea, Italy	[16]
Bivalve			
Mytilus galloprovincialis	4>3>5,6*	Mediterranean sea	[3]

Mytilus Chilensis	3>4>5,6	*	Corral Bay, Chile	[9]
Crassostrea Virginica	3>4>5,6	*	Terminos Lagoon, Mexico	[2]
Barbatia helblingii	3>4>5,6	*	Bushehr coast	This study

*Number of benzene rings

The oysters *Crassostrea Virginica* (in Mexico) and *Mytilus Chilensis*(in Chile) have shown the same PAHs pattern as *B.helblingii*, whereas in *Mytilus galloprovincialis* from the Mediterranean sea, 4 rings PAHs have been more concentrated than 3 rings. Different order of PAHs in this case seems to be related to different in PAHs sources. Unlike other samples the clams from the station Helyleh were found to contain high concentrations of 4rings PAHs. Dickhut et al suggested that even PAHs with the same molecular weight have different dynamic transport in different environmental condition [7]. It means that rather than PAHs concentration, many other factors are involved in PAHs bioavailability for clams.

5. Conclusions

Although Bushehr coastal zone is the main route for oil carrying tankers, and many activities related to oil export is performed there, this study showed that generally PAHs concentration in the Ark clams in this region is not higher than available standards, but the PAHs level in the clams located near the ports, were higher than other locations. Compared to fish and oyster species studied from other parts of the world, ark clams from Bushehr province in Iran were moderately to highly contaminated. According to the results PAHs contamination and bioavailability in Bushehr coastal waters is to be noticed and regularly monitored.

6. Reference

[1] P. H. Albers, Petroleum and individual polycyclic aromatic hydrocarbons In: D.J, Hoffman, et al (eds.). *Handbook of Ecotoxicology*, 2003, 342-360.

[2] E.N. Barroso, G.G. Bouchot, O.Z. Perez, J.L. Sericano. Polynuclear aromatic hydrocarbons in American oysters Crassostrea virginica from the Terminos Lagoon, Campeche, Mexico. *Mar. Poll. Bull*. 1999, 38: 637-645.

[3] P. Baumard, H. Budzinski, P. Garrigues, J.C. Sorbe, T. Burgeot, J. Belloca,. Concentration of PAH in various marine organisms in relation to those in sediments to throphic level. *Mar. Poll. Bull*. 1998. 36: 951-960.

[4] P. Baumard, H. Buzinski, Q. Michon, P. Garrigues, J. Burgeot Tand BellocqOrigin and bioavailability of PAHs in the Mediterranean Sea from mussel and sediment records, *Estuar.Coast.Shelf Sci.* 1998, 47: 77-90.

[5] E. Cortazar, L. Bartolomé, S. Arrasate, A. Usobiaga, J.C. Raposo, O. Zuloaga, N. Etxebarria. Distribution and bioaccumulation of PAHs in the UNESCO protected natural reserve of Urdaibai, Bay of Biscay. *Chemosphere*. 2008, 72: 1467–1474.

[6] R. D'Adamo, S. Pelosi, P. Trotta, G. Sansone. Bioaccumulation and biomagnification of polycyclic aromatic hydrocarbons in aquatic organisms. *Mar. Chem*. 1997, 56: 45-49.

[7] R.M. Dickhut, E.A. Canuel, K.E. Gustafson, K. Liu, K.M. Arzayus, S.E. Walker, G. Edgecombe, M.O. Gaylor, , E.H. MacDonald. Automotive sources of carcinogenic polycyclic aromatic hydrocarbons associated with particulate matter in the Chesapeake Bay Region. *Environ. Sci. Technol*. 2000, 34: 4635-4640.

[8] EC- European Comission regulation. Setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur.Union*. 2006, 364: 5-24.

[9] H.P. Fleming, J. Adalberto, P. Asencio, E. Gutierrez. Polycyclic aromatic hydrocarbons in sediments and mussels of Corral Bay, south central Chile. *Environ.Monit*. 2004, 6: 229 – 223.

[10] C. Kelly, D. Santillo, P. Johnston, G. Fayad, K. L. Baker, R. J. Law. Polycyclic aromatic hydrocarbons in oysters from coastal waters of the Lebanon 10 months after the Jiyeh oil spill in 2006. *Mar. Poll. Bull.* 2008, 56: 1215–1233.

[11] E. Manoli, C. samara, I. konstantinou, T. Albanis. Pollution survey of polycyclic aromatic hydrocarbons in the bulk precipitation and surface waters of northern Greece. *Chemosphere*. 2000, 41, 1845- 1855.

[12] MOOPAM, Standard methods for chemical analysis of petroleum hydrocarbons, regional organization for the protection of marine environment. 1999, Third addition. Kuwait.

[13] I.C.T. Nisbet and P.K. LaGoy. Toxic equivalency factor (TEFs) for polycyclic aromatic hydrocarbons (PAHs). *Region.Toxico.Pharmaco*. 1992, 16: 290-300.

[14] Orbea, A., Cajaraville, M.P. Peroxisome proliferation, and antioxidant enzymes in transplanted mussels of four Basque estuaries with different levels of polycyclic aromatic hydrocarbon and polychlorinated biphenyl pollution. *Environ. Toxico.Chem.* 2006, 25: 1616–1626.

[15] R.B.G..Peachey. Tributyltin and polycyclic aromatic hydrocarbon levels in Mobile Bay, Alabama: A review. *Mar. Poll. Bull*. 2003, 46: 1365–1371.

[16] M. Perugini, P. Visciano, A. Giammarino, M. Manera, W.Di Nardo. Polycyclic aromatic hydrocarbons in marine organisms from the Adriatic Sea, Italy.*chemospher*. 2007, 66: 1904-1910.

[17] I. Tolosa, S.I. Mora, S.w. Fowler, J.P. Villeneuve, J. Bartocci, C. Cattini. Aliphatic and aromatic hydrocarbons in marine biota and coastal sediments from the Gulf and the Gulf of Oman. *Mar. Poll. Bull*. 2005, 50: 1619-1633.

[18] US Environmental Protection Agency. Method 8310, Polynuclear Aromatic Hydrocarbons. 1986, 13pp.

[19] US Environmental Protection Agency. Method 3540C, Soxhlet Extraction. 1996, 8pp.

[20] U. Varanasi, W.L. Reichert, J.E. Stein, et al. Bioavailability, and biotransformation of aromatic hydrocarbons in benthic organisms exposed to sediment from an urban estuary. *Environ. Sci. Technol.* 1985, 19: 836-841.