PAHs Concentration in Ark clam (*Barbatia helblingii*) From South Persian Gulf, Bushehr, Iran

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***Abstract*— In order to study PAHs concentration in Ark clam Barbatia helblingii, in Bushehr coastal zone, samples of clams was collected from five stations. Following digestion of clam's soft tissues in methanol, their PAHs content was extracted with hexane solvent and then measured by HPLC. Result showed that the total PAHs concentration in samples taken from Rafael, Sheghab, Abshirinkon, Lian and Helyleh 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). Maximum and minimum concentration of PAHs was measured in clams collected from Rafael and Abshirinkon respectively. Among different PAHs compounds, 3 rings PAHs were the most abundant. The mean concentration of tPAHs in clams collected from Bushehr coast was 421.86 ng g-1. PAHs contamination in Bushehr originates from both pyrolitic and petrogenic sources. Compared to other studies from other parts of the world, the level of contamination was moderately high.**

***Index Terms* — Barbatia helblingii, bushehr, PAHs, persian gulf**

I. 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 (PAHs) constitute about 0.2 to 7% of the total hydrocarbons in the 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 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 alternation in gametogenesis, gender determination, and growth [14], together with the increased risk of cancer and mutation are their major adverse effects 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 ecosystem of the Bushehr province a 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 Bushehr coasts providing a good candidate for monitoring of PAHs bioavailability in the area. This study was performed in order to investigate PAHs levels in *B.hellblingii* from Bushehr shore line.

II. Material and Methods

Clams samples were collected from five different stations along the Bushehr coast 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 I. About 30 Ark clams with the same size (35±5mm) were collected from each station. They were enveloped in aluminum foils, 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. To run PAHs digestion and extraction procedures, 5 g of each dried sample was digested with 1 ml decachlorobiphenyl (16 µ L-1) as surrogate standard [18] in 200 ml methanol using Soxhlet apparatus. PAHs content of this mixture were extracted by 30 ml double distilled water and 60 ml Hexane using separator funnel. Extraction was repeated twice with 2 volumes of hexane (50 ml). The extract was concentrated by rotary evaporator and then passed through column clean up including 10mg of activated alumina, silica and anhydrous sodium sulfate [12], [19]. After evaporation of the whole volume of solvent, 1ml acetonitrile was added to extract [16] and the mixture was injected to HPLC. In order to analyze PAHs, the HPLC system prepared with UV detector and C_{18} reverse phase. A linear gradient started with acetonitrile (40%) and water (60%) which converted to 100% acetonitrile in 31 minutes by 2.0 ml/min flow rate. PAHs calibration mix with 4790-U catalog number (Supelco) and surrogate standard decachlorobiphenyl with catalog number 48318 (Supelco) were used for system calibration. PAHs calibration mix contained 16 different aromatic compounds including: naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, acenaphtylene, benzo[a]pyrene, pyrene dibenzo[a,h]anthracene, benzo[ghi]-perylene and indeno[1,2,3-cd]pyrene. The chromatogram of standard calibration curve is shown in Fig. 2.

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The obtained data were subjected to Shapiro-wilk normality test. Since the data were normally distributed, the difference between tPAHs concentrations in different stations was compared by one way analysis of variance (ANOVA).

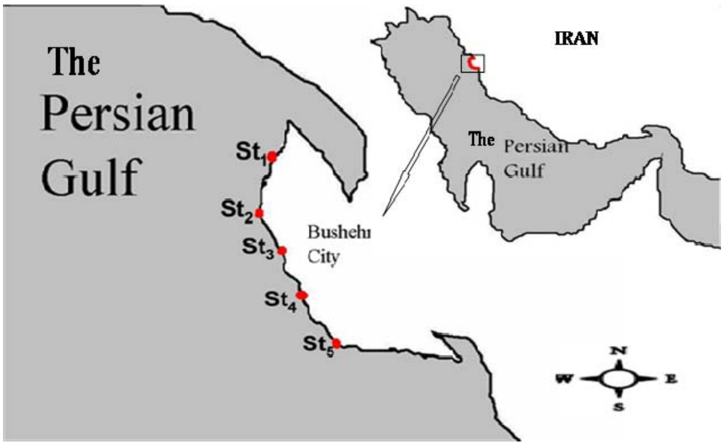

Fig. 1.The position of sampling stations.

TABLE I: DESCRIPTION AND GEOGRAPHICAL LOCATION OF SAMPLING STATIONS IN THE BUSHEHR COAST.

Stations	Latitude	Longitude	Description
St1 (Rafael)	28º 57 ' 49.5 "	50º 48 ' 43.2 "	landing port for fishing vessels
St2 (Sheghab)	28º 55 ' 38.7 "	50º 48 ' 26.7 "	Building construction, residential area
St3 (Abshrinkon)	28º 54 ' 12.7 "	50º 49 ' 9.0 "	urban dump
St4 (Lian)	28º 52 ' 20.0 "	50º 50 ' 33.3 "	landing port for fishing vessels, small industries
St5 (Helyleh)	28º 50 ' 3.3 "	50º 52 ' 31.9 "	landing port for fishing vessels, urban dump

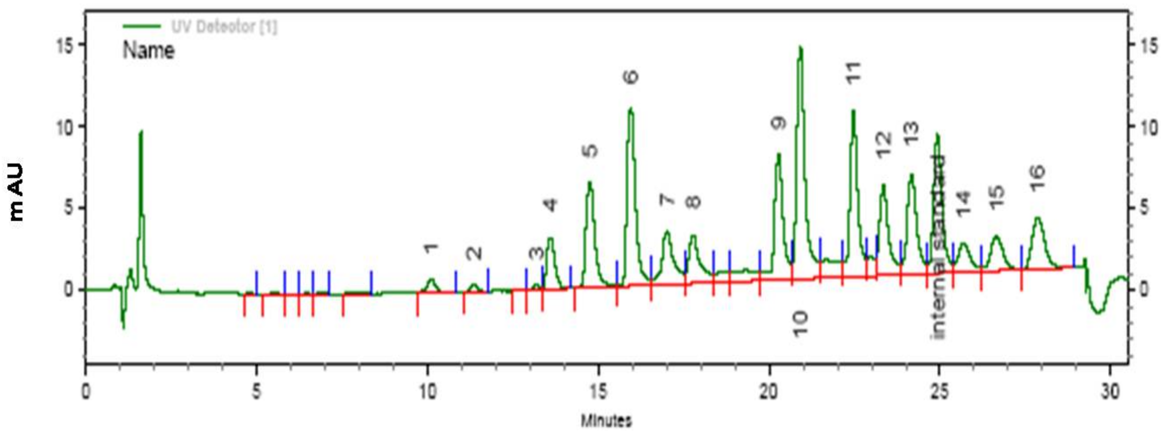

Fig. 2. Standard calibration curve.

III. RESULTS AND DISCUSSION

Concentrations of PAHs in clam's soft tissue are shown in Table II. Some PAHs compounds such as naphthalene, dibenzo[a,h]anthracene, benzo[ghi]perylene and indeno-[1,2,3-cd] pyren were absent in the studied samples while some others such as pyrene and phenanthrene were found in high concentrations. Phenanthrene and pyrene had considerable concentrations in Rafael, Sheghab and Lian.

Relatively the elevated level of Acenaphtylene in was observed in Abshirinkon, while Phenanthrene and fluoranthene were higher in Helyleh which, might be related to different sources of PAHs in the stations. In recent years researchers used isomers ratio indices of some single PAHs compounds in order to Investigations and identify the source of PAHs in the marine ecosystems [21-23]. The Ant/Ant+Phe ratio (Anthracene /Anthracene+Phenanthrene) is one of the common indices which have been applied in this propose. Where this ratio is more than 0.1 indicates that PAHs originate from pyrolitic sources while, if the ratio is less than 0.1 it means that PAHs have came from petrogenic sources. Another isomer index for determining PAHs sources is Flu/Flu+Pyr ratio (Fluoranthene/Fluoranthene+Pyrene). Once this ratio is more than 0.5 indicates PAHs originate from pyrolitic sources, but petrogenic sources exhibite Flu/Flu+Pyr ratio less than 0.5 [5], [22]. Calculating of the above mentioned indices for the data presented in table II revealed that in four stations (Rafael, Sheghab, Abshirinkon and Lian PAHs originated from both pyrolitic and petrogenic sources. The mentioned stations are located near harbours or ports and received sea-based PAHs (commonly from petrogenic sources). Since these stations are placed in Bushehr city, land based and urban discharges may also countribute in PAHs inputs (mainly pyrolitic PAHs). Unlike other stations, Helyleh is less urbanized and located far from the city, harborsand ports. This station receives PAHs mainly from petrogenic sources which are carried there by water current. The calculated values of Ant/Ant+Phe and Flu/Flu+Pyr ratios are shown in Fig. 3 and 4 respectively.

According to the results tPAHs concentration in the soft tissue of ark 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 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. Rafael and Sheghab coasts 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 the city and less also the absence of contamination sources.

TABLE II: PAHS CONCENTRATION IN ARK CLAM'S 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.

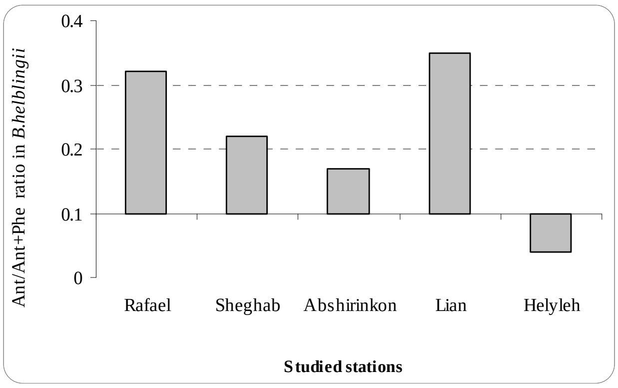

Fig.3. Ant/Ant+Phe ratio index in clams from studied stations in Bushehr coasts.

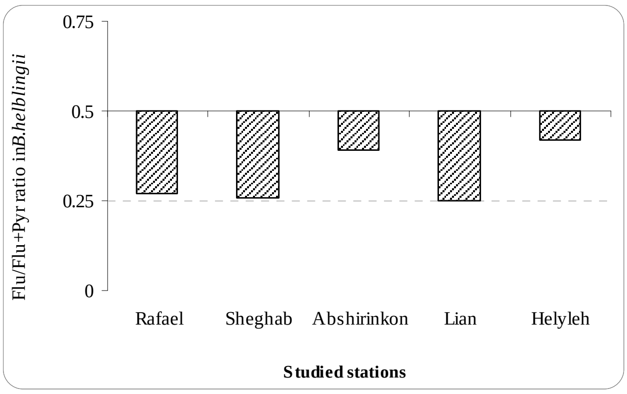

Fig. 4. Flu/Flu+Pyr ratio index in clams from studied stations in Bushehr coasts.

The European Commission Union, considered Benzo(a)pyrene as a marker for the occurrence and 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(a) pyrene measured in dry weight was converted to fresh weight using 0.24 conversion coefficient[13]. The average

Benzo(a)pyrene concentration in Arc clams was 3.58ng g-1 (fresh weight), which was lower than its maximum concentration level in food.

Based on the results the mean concentration of tPAHs in Arc clams was 421.86 ng g-1. This amount is compared to tPAHs concentration in marine organisms from several locations in the world in Table III. According to the table, generally PAHs concentration in fishes is lower than oysters. These could be related to metabolisms of PAHs by fish liver [6], [20] as well as difference in uptake root and the level of contaminant in their environment.

Comparison between *B.helblingii* and oysters from different locations of the world showed that tPAHs concentration in *B.helblingii* was higher than *Crassostrea Virginia* (Mobile Bay), *Mytilus galloprovincialis* (Mediterranean Sea), *Saccostrea cucullata*(Oman) and *Circentia callipyga* (Qatar) in Persian Gulf. On the other hand it was lower than *Ostera edulis* (coastal waters of the Lebanon) and *Crassostrea.sp*. (Bay of Biscay). Thus tPAHs concentration in *B.helblingii* was within the range of tPAHs concentrations in other oysters in other studies. Comparison of Benzo(a)pyrene concentration in *B.helblingii* with other organisms in Table III showed that concentration of this compound was higher than other marine organisms. Mean concentration of 3rings, 4rings and 5+6rings PAHs in clams were 209.3, 172.9 and 30.5 ng g-1 respectively (Fig. 5). The study of composition pattern of PAHs in *B.helblingii* showed that based on the number of rings in the molecule, the order of PAHs compounds in clams from Bushehr intertidal coasts is 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 the more solubility of the compound in seawater. Dissolved compound in seawater could easily be taken up through gill membrane. Bummard et al believe that 3 and 4 rings PAHs are more concentrated rather than 5 and 6rings PAHs in organisms [3].

The order of PAHs compounds in *B.helblingii* is compared to some previously studied aquatic organisms in Table IV. 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.

TABLE III. CONCENTRATION OF PAHS IN VARIOUS MARINE ORGANISMS FROM DIFFERENT LOCATION OF THE WORLD.

Studied species	tPAHs	Benzo(a) 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]
Saccostrea cucullata	66	-	Oman	[17]
Circentia callipyga	105	-	Qatar	[17]
Barbatia helblingii	421.86	14.94	Bushehr coast	This study

UAE: United Arab Emirates. -Not reported.

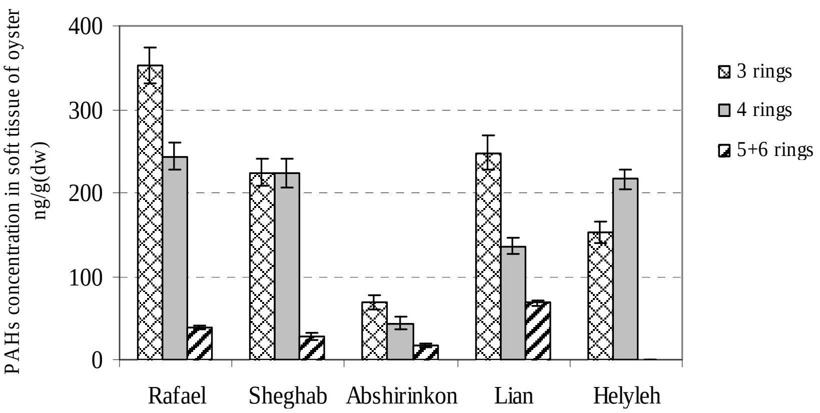

Fig. 5. Concentration of 3, 4 and 5+6 rings PAHs in soft tissue of B.helblingii in studied station. 3 rings PAHs are sum of acenaphtylene, acenaphthene, fluorene, phenanthrene and anthracene, 4 rings PAHs include: fluoranthene, pyrene, benzo[a]anthracene and chrysene, 5+6 rings PAHs consist of: benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[ghi] perylene and indeno[1,2,3-cd]pyren

TABLE. IV: 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

IV. CONCLUSIONS

Although Bushehr coastal water 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 from Bushehr is not higher than available standards, while the PAHs concentrations in the clams located near the ports or city were higher than other locations. Compared to fish and oyster species studied from other parts of the world, PAHs contamination in ark clam from Bushehr, Iran was moderately high. The PAHs contamination in station Helyleh mainly originates from petrogenic sources, while the major part of PAHs in the other stations originated from both; pyrolitic and petrogenic sources. According to the results of the present study PAHs contamination and bioavailability in Bushehr coastal waters is to be noticed and regular monitoring is recommended.

REFERENCES

[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.

[21] D. Fabbri, I. Vassuraa, C.g. Sunb, C.E. Snapeb, C. McRaec, A.E. Fallick. "Source apportionment of polycyclic aromatic hydrocarbons in a coastal lagoon by molecular and isotopic characterization". Mar. Chem. 2003. 84: 123– 135.

[22] G. Mille, L. Asia, M. Guiliano, L. Malleret, P. Doumenq. "Hydrocarbons in coastal sediments from the Mediterranean Sea (Gulf of Fos area, France)". Mar. Poll. Bull. 2007. 54: 566–575.

[23] j.w. Readman, G. Fillmann, I.Tolosa, J. Bartocci, J.P. Villeneuve, C. Catinni, L.D. Mee. "Petroleum and PAH contamination of the Black Sea". Mar. Poll. Bull. 2002 44: 48–62.