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Levels of Polycyclic Aromatic Hydrocarbons (PAHs) in muscles of commercial fishes from Iraqi waters

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Abstract - The concentrations of polycyclic aromatic hydrocarbons (PAHs) were measured in the muscles of eight kind of fishes from National Iraqi Waters, Five marine species *viz*., *Euryglossa orientalis*, *Otolithes ruber*, *Lethvinus nebulosus*, *Epinephelus coioides* and *Johnieops sina* which were collected from Iraqi marine waters, and three species *viz*, *Cyprinus carpio, Carasobarbus luteus* and *Plantiliza abu* which were collected from Shatt Al-Arab river fresh water. All fishes were collected during spring and autumn seasons within the year 2015. Recorded total polycyclic aromatic hydrocarbons in fish species from Iraqi national waters were varied between 0.054 and 155.44 ng/g, while for individual fishes: Anthracene recorded the highest values varied between 17.629 and 155.44 ng/g dry wet. Lower total PAHs value recorded was 197.54 ng/g dry wet. in *O. ruber* and higher total PAHs value recorded was 381.415 ng/g dry wet. in *E. coioides*, followed by *P. abu* which recorded higher total PAHs value of 275.938 ng/g dry wet. Large differences in the values of PAHs recorded during autumn 2015 were achieved which were lower than those recorded during spring. According to the ratios between low and high molecular weight PAHs, there are two sources for PAHs in the investigated fishes, the first was pyrogenic which was appeared in the fishes *L. nebulosus* and *E. orientalis* only for 0.355 and 0.274 ng/g respectively during spring 2015 and second anthrapogenic which appeared in all fishes except *L. nebulosus* and *E. orientalis*. Therefore during spring season the dominated source was the anthropogenic one. For the results of Autumn season, the ratios indicate predominant the values which were lower than 1 for mostly all studied fishes except that for *E. orientalis* which was equal to 1.040 indicating that most of the PAHs were from anthropogenic source. Although, concentration of petroleum hydrocarbons (PAHs) in fishes from national Iraqi waters along North West Arabian Gulf were recorded in most studied fishes, but they markedly higher than that in the background and previously reported studies in the same and nearby areas. From a public health point, petroleum hydrocarbon residue levels, especially the carcinogenic ones, in all fish samples analyzed in this study are considerably lower than the hazardous levels and doesn't exert any health effect upon human being upon consumption.

Key Words: PAHs, Marine and Fresh water fishes, HPLC, NW Arabian Gulf and Shatt Al-Arab River.

Introduction

PAHs consist of hydrogen and carbon arranged in the form of two or more fused benzene rings. There are thousands of PAH compounds, each differing in the number and position of aromatic rings, and in the position of substituents on the basic ring system. Environmental concern has focused on PAHs that range in molecular weight from 128.16 (naphthalene, 2-ring structure) to 300.36 (coronene, 7-ring structure).

Unsubstituted lower molecular weight PAHs compounds, containing 2 or 3 rings, exhibit significant acute toxicity and other adverse effects to some organisms, but are non carcinogenic; the higher molecular weight PAHs, containing 4 to 7 rings are significantly less toxic, but many of the 4 to 7 ring compounds are demonstrably carcinogenic, mutagenic, or teratgenic to a wide variety of organisms, including fish tendency to biomagnified in food chains, despite their high lipid solubility, probably because most PAHs are rapidly metabolized (Abdel-Shafy and Mansour, 2016).

PAHs has two types of anthropogenic sources which are petrogenic and Pyrogenic. The Petrogenic sources such as crude oil, asphalt and gasoline are introduced to the environment through accidental oil spills, discharge from routine tanker operation, as well as municipal and urban run-off. On the other hand, pyrogenic sources are incomplete combustion of organic matter, biomass burning and mobile sources important released to environmental in the form of exhaust and solid residue (Zakaria *et al.*, 2002). Sixteen PAHs are included on lists of priority chemical contaminants by the World Health Organization and the U.S. Environmental Protection Agency (EPA) due to their mutagenic and carcinogenic properties (Dong *et al.*, 2012). These toxicants may enter local environments in a variety of ways, including natural seeps, discharges from tanks and vessels, loss associated with escalating oil and gas development, and catastrophic spills, as well as anthropogenic sources such as industrial production, transportation and waste incineration generate significant amount (Yang *et al.*, 1988). Fishery can be exposed to oil from natural sources such as the natural seeps, or by activities associated with industry, camps, docks-minor or major oil spills. Since fish are able to metabolize and eliminate PAHs from their bodies, PAHs are more likely to be present in fish tissues from higher polluted environments or of fairly recent exposure (Al-Saad, 1995; Al-Khion, 2012).

The aliphatic and polycyclic aromatic hydrocarbon fractions of dissolved petroleum are readily absorbed by most aquatic organisms because of their high lipid solubility and are bio-concentrated in fish and shellfish (Gobas *et. al.*, 1999). Oil may enter fish through the skin or gills (Enuneku *et al.*, 2015). The Arabian Gulf is liable to pollution by petroleum hydrocarbons due to heavy oil tankers (Ostadam, 1980). This subject was reflected upon increasing levels of Petroleum hydrocarbons including poly cyclic hydrocarbons in the fishes of the Gulf (Khan *et al.*, 1995). The North Western part of Arabian Gulf and Shatt Al-Arab River has been subject to inputs of oil pollution from a variety of sources (Ashraf and Mian, 2010). Petroleum hydrocarbons (PAHs) are the chemical building blocks of petroleum oil and coal. On the other hand, Polycyclic Aromatic Hydrocarbons (PAHs) are the most toxic portions of oil. Inner water ways of Iraq faced pollution by PAHs originally from sewage. Husein *et al.* (2014 a) studied the levels of PAHs in surface water of Shatt Al-Hilla in the middle sector of Iraq and reported a maximum cumulative value of 1.69 µg/l for fluorene and minimum cumulative value of 0.300 µg/l for acenaphthene.

The aim of the present study was to evaluate the distribution of Polycyclic Aromatic Hydrocarbons (PAHs) in five commercial marine fish species from national Iraqi waters and three fish species from fresh waters of Shatt Al-Arab river, Northern Arabian Gulf, as well as investigate whether these fishes are suitable for consumption and exert no poisoning effect upon human being.

Shatt Al-Arab River and National Iraqi water ways are located on the top part of the Arabian gulf within the locations 30°28'04" N 47°55'35" E and 35°36' 31" N,

50°48'23" E respectively, as shown in Figure (1). Both are affected directly by oil spills from land in most of the Arabian Gulf countries as well as Iran, in addition to the spillage from oil tankers mooning in the area. During this study, fish samples were collected from fish markets which represent two sites, fresh water species from Shatt Al-Arab River and marine species from National Iraqi waters.

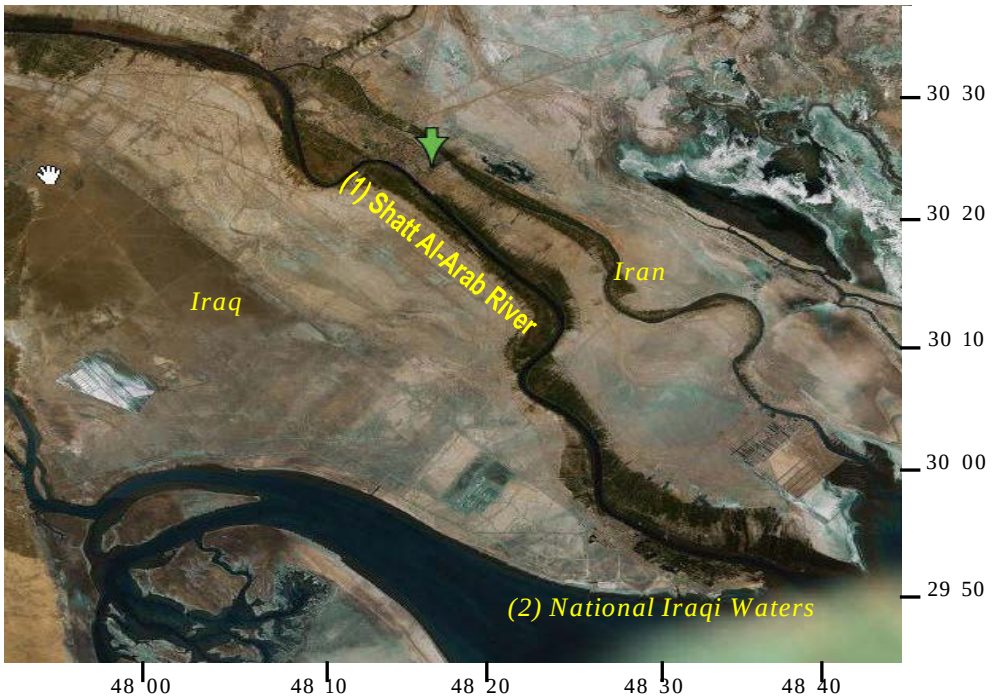

Figure 1. Location map for Northern Arabian Gulf Showing the fishing sites: 1) Shatt Al-Arab River, and 2) National Iraqi waters.

Materials and Methods

Samples of fishes (*Euryglossa orientalis*, *Otolithes ruber*, *Lethvinus nebulosus*, *Epinephelus coioides* and *Johnieops sina*) as Iraqi marines waters, and (*Cyprinus carpio, Caraso barbusluteus* and *Planiliza abu*) as from Shatt Al-Arab river were bought from fish markets during Spring and Autumn seasons within the year 2015. During a certain time of the year, Marine fishes samples were also taken from fishermen fishing off Fao Coast, and fresh water fish samples were also taken from fishermen fishing off Shatt Al-Arab River. After collection, the fish samples were wrapped in aluminum foil, stored in cool boxes, and frozen upon return to the laboratory of Marine Science Centre, and classified according to Fischer and Bianchi (1984) and Coad (2010). Then measured the total length and weight of the selected fish for this study was measured, and values are listed in Table (1).

The extraction of petroleum hydrocarbons from fishes sample were done according to the method of Grimalt and Oliver (1993). In a brief description, exactly 10 gm of dried fishes muscles were placed in a pre-extracted cellulose thimbles and Soxhlet extracted with 150 ml methanol/ benzene (1:1 ratio) for 24 hours. At the end

of this period the extracted samples were transferred to storage flasks, and each sample was further extracted with fresh solvent. The combined extractions for each sample were reduced in volume to about 10 ml by means of a rotary vacuum evaporator, and were then saponified for 2 hours with a solution of 4NKOH in 1:1 methanol:benzene. After extraction of the unsaponified matter with hexane, the extract was dried over anhydrous Na_2SO_4. The concentrated extract was cleaned up by column chromatography. The column filled with 8 gm of 5% deactivated alumina (100-200 mesh) on the top, and silica (100-200 mesh) in the bottom. The extract was then applied to the head of the column, and eluted with 50 ml n-hexane to isolate the aliphatic fraction, and 50 ml benzene to isolate the aromatic fraction. Polycyclic aromatic hydrocarbons were estimated by HPLC device Model Shimadzu UFLT equipped with a gradient pump and manual injector. The separation column had internal diameter of 4.6 mm and total length of 25 mm, the bonded phase was Carbon-18 with pore size of 5-100 μm. The separation mode was reverse phase. The detector type was UV-Visible spectrometer, PAHs compounds were detected at 254 nm. The ambient temperature was 25 ^oC. Shimadzu LC solution computer software was used for control and analysis with a computerized data recorder and standard solutions for estimated PAHs.

Table 1. Range of total length (cm) and weight (g) for studied fishes collected from National Iraqi marine and fresh waters during spring and autumn 2015.

Location of Fish Samples	Species		Spring 2015		Autumn 2015	
	Scientific Name	Common Name	Total Length	Weight	Total Length	Weight
Iraqi Marine Waters	*Euryglossa orientalis*	Mazlak	15.8-23.5	71.09-164.93	18.0-26.4	89.42-314.83
	Otolithes ruber	Nuwaiby	19.8-22.0	78.56-112.16	20.9-25.3	76.87-135.00
	Lethvinus nebulosus	Shari	20.6-25.2	105.86-232.92	29.0-32.0	333.30-407.46
	Epinephelus coioides	Hamoor	24.2-35.6	154.61-400.30	33.2-34.4	590.01-638.20
	Johnieops sina	Taataoo	14.2-20.7	35.88-142.39	14.7-18.8	40.52-122.66
Shatt Al-Arab Fresh Waters	*Cyprinus carpio*	Common Carp	29.3-31.2	377.12-543.92	22.6-36.2	218.30-668.10
	Carasobarbus luteus	Himry	17.5-20.1	78.89-107.61	14.4-18.1	58.21-86.28
	Planiliza abu	Khishni	10.5-16.2	14.32-34.81	11.5-15.1	15.32-33.89

Results

The HPLC chrmatograms for PAHs detected in an investigated fishes are shown in Figure (2) below for most of fishes collected during April and October 2015. The recorded PAHs concentration in fish species from Iraqi national waters were varied between 0.0541 and 155.44 ng/g. For individual fishes, Anthracene recorded the highest values varied between 17.629 and 155.44 ng/g dry wet.

During spring season at 2015, mostly all detected PAHs were recorded in the studied fishes (Table 2). Anthracene and Chrysen were recorded in both marine and fresh fishes Benzo(a)Anthracene did not recorded in *O. ruber* and *P. abu*, Pyrene did not recorded in *Lethyinus nebulosus* and *Planiliza abu*. On the other hand, Benzo(g,h,i)perylene was recorded in *P. abu* only, Benzo(a)pyrene was recorded in

O. ruber and *P. abu* only, while Indole + 2 methylnaphthalene, 1-methylnaphthalene, dibenzofurane, Carbazole, dibenzo(a,h)anthracene and indene(1,2,3,c,d)pyrene did not recorded any value in all studied fishes. Lower total PAHs value recorded was 197.54 ng/g dry wet. in *O. ruber* and higher total PAHs value recorded was 381.455 ng/g dry wet. in *E. coioides*, followed by *P. abu* which recorded higher total PAHs value of 275.938 ng/g dry wet. Large differences in the values of PAHs recorded during autumn 2015 which were achieved as shown in Table (3). No values recorded for all investigated PAHs in *J. sina* and *C. lutes* fishes. Low molecular weight PAHs did not recorded any value in each of the fishes *O. ruber, L. nebulosus* and *E. coioides.* For total PAHs recorded, lower value was 23.046 ng/g dry wet in *E. coioides* and higher value was 407.835 ng/g dry wet, in *P. abu*.

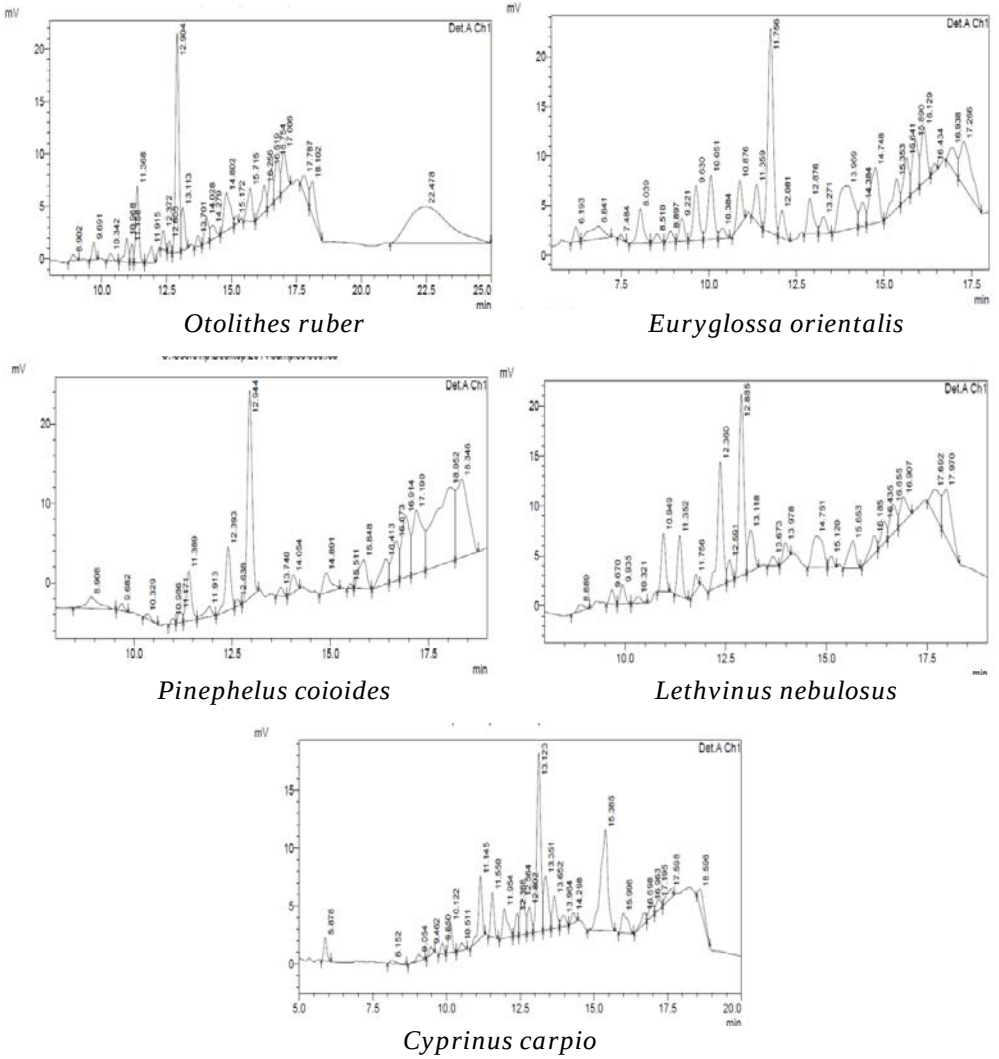

Figure 2. HPLC chromatograms of PAHs in selected fishes within this study.

Table 2. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in fish muscles (ng/gm dry weight) during Spring 2015.

PAHs Compound	Iraqi marine waters					Shatt Al-Arab fresh water		
	Euryglossa orientalis	*Otolithes ruber*	*Lethvinus nebulosus*	*Epinephelus coioides*	*Johnieops sina*	*Cyprinus carpio*	*Carasobarbus luteus*	*Planiliza abu*
Naphthalene		2.511			5.496	14.996	6.443	
Indole+2methyl naphthalene								
1-methyl naphthalene								
Biphenyl								
Acenaphthylene	0.541			50.477	38.088			
Acenaphthene	14.750	7.848	8.512					
Dibenzofuran								
Fluorene	0.054			85.782	13.026	86.805	83.201	
Phenanthrene	10.095	5.571	11.416		9.258	5.880	9.823	22.830
Anthracene	17.629	97.306	48.958	58.276	96.228	60.933	50.542	155.440
Carbazole								
Benzo (a) anthracene	54.205		33.080	90.932	62.603	46.723	47.775	
Fluoranthene			13.694	9.816		13.475		20.676
Pyrene	29.937	16.184		12.978	11.984	6.464	7.208	
Chrysene	25.211	12.639	31.386	34.489	31.197	27.415	30.440	17.453
B(k)fluoranthene		39.706	75.517					27.542
B(a) pyrene		2.525						5.573
Di benzo(a,h) anthracene								
B(b) fluoranthene	47.928	13.253	46.446	38.705				2.802
Indeno(1,2,3,cd) pyrene								
Benzo(g h i)perylene								23.622
Low molecular weight	43.069	113.236	68.886	194.532	162.096	168.614	150.009	178.270
High molecular weight	157.281	84.307	194.123	186.920	105.784	94.077	85.423	97.668
Total	200.350	197.54	263.009	381.455	267.880	262.691	235.432	275.938
H /L	0.274	1.343	0.355	1.041	1.532	1.792	1.756	1.825

Table 3. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in fish muscles (ng/gm dry weight) during Autumn 2015.

PAHs Compounds	Iraqi marine waters					Shatt Al-Arab fresh water		
	Euryglossa orientalis	*Otolithes ruber*	*Lethvinus nebulosus*	*Epinephelus coioides*	*Johnieops sina*	*Cyprinus carpio*	*Carasobarbus luteus*	*Planiliza abu*
Naphthalene								
Indole+2methyl naphthalene								
1-methyl naphthalene								
Biphenyl								
Acenaphthylene	58.598							62.790
Acenaphthene	7.438							
Dibenzofuran								
Fluorene								45.067
Phenanthrene						1.809		3.816
Anthracene	70.575							91.874
Carbazole								
Benzo (a) anthracene	45.071	4.346	9.875	3.389		13.307		138.468
Fluoranthene	2.628	1.416	1.781	7.206		1.582		12.805
Pyrene	7.514					1.724		18.898
Chrysene	7.554	3.764	7.357	0.785				22.162
B(k)fluoranthene	5.331		11.166			14.320		
B(a) pyrene	25.162	16.442	15.668	8.857				7.945
Di benzo(a,h) anthracene		94.252	144.636					
B(b) fluoranthene	35.508	2.817	1.807	2.809		4.938		4.010
Indeno(1,2,3,cd) pyrene								
Benzo(g h i)perylene								
Low molecular weight	136.611	0	0	0		1.809		203.547
High molecular weight	128.768	123.040	192.290	23.046		35.871		204.288
Total	265.379	123.040	192.290	23.046		37.680		407.835
H /L	1.061	0.00	0.00	0.00		0.050		0.996

The results listed in Tables (2 and 3) indicate that, according to the ratios between low and high molecular weight PAHs, there are two sources for PAHs in the investigated fishes, the first is pyrogenic where LMW/HMW ratio was <1 which was appeared in the fishes *L. nebulosus* and *E. orientalis* only for 0.355 and 0.274 respectively during Spring 2015, and second anthrapogenic where LMW/HMW ratio was >1 which appeared in all fishes except *L. nebulosus* and *E. orientalis*. Therefore during spring season, the dominated source was the anthropogenic one (Nasir *et al.*, 2016). For the results of Autumn season the ratios indicate predominant the values which were lower than 1 for mostly all studied fishes except that for *E. orientalis* which was equal to 1.040 indicating that most of the PAHs were from anthropogenic source (Al-Hejuje *et al.*, 2015 a, b; Nasir *et al.*, 2016).

Discussion

The detection of polluting hydrocarbons in marine organism was a complicated task since marine organisms can also produce hydrocarbons (Al-Saad *et al.*, 2008). Therefore, the total amount of petroleum hydrocarbons detected in marine organisms cannot be taken as an index for pollution by petroleum products only (Al-Saad, 1995). PAHs never occur as individual compounds in the environment (Mottier *et al.*, 2000), they occur as a mixture of many other polynuclear aromatic hydrocarbons.

PAHs represented by Oil in the Arabian Gulf may enter fish through the skin or gills. In addition, pollutants such as tar balls may ingress through the intestine by water gulped (Ashraf and Mian, 2010). Benzo(a)pyrene as a carcinogenic PAH, (Hussein *et al.*, 2014-b), recorded lower values in the range 2.525 and 5.573 ng/g dry wet. in *O. ruber* and *P. abu* respectively during Spring season 2015, and its values were increased to reach 16.442 and 7.945 ng/g dry wet. in *O. ruber* and *P. abu* respectively during autumn season 2015. Moreover, during autumn season the high molecular weight PAHs are the most abundant in most of the investigated fishes (Hussein *et al.*, 2015 b). There is a tendency of accumulating high molecular weight PAHs during autumn season as compared to spring season (Ashraf and Mian, 2010).

Comparison between the total PAHs reported within this study and other studies for fishes and other organisms, as shown in Table (4), it was found that values recorded in this study were higher than those recorded elsewhere, but for the carcinogenic ones are lower than the hazardous levels.

Table 4. Comparison of values of total (PAHs) in fishes samples with other comparable values obtained in the nearby countries.

Location	Conc. (ng/g)	Ref.
NW Arabian Gulf	6.78-23,83	Al-Saad *et al.*, 2006
Hor Al-Howaiza	0.1-92.7	Al-Khatib, 2008
Iraqi coast regions	12.19-86.48	Al-Khion, 2012
Al-Kahlaa River/Iraq	1.095 -16.661	Jazza1 *et al.*, 2015
Mumbai Harbour, India	17.43-70.44	Dhananjayan & Muralidharan, 2012
Niger Delta/African	3.67-171	Nwaichi and Ntorgbo, 2016
Shatt Al-Arab River,	37.68-407.835	This study
National Iraqi Waters	23.046-381.455	This study

Conclusion

In conclusion, concentration of petroleum hydrocarbons (PAHs) in fishes from national Iraqi waters along North West Arabian Gulf were recorded in most studied fishes, but they markedly higher than that in the background and previously reported studies in the same and nearby areas. From a public health point, petroleum hydrocarbon residue levels, especially the carcinogenic ones, in all fish samples analyzed in this study are considerably lower than the hazardous levels and does not exert any health effect upon human being upon consumption. Among the eight fish species within this study, *E. coioides* and *P. abu* accumulated the higher PAHs compounds, and Anthracene was mostly reported in muscles of all of those fishes in Spring season, this will suggests that *E. coioides* and *P. abu* as a living organisms and Anthracene as a PAH candidate could be used as a good biological and hazardous indicators for petroleum hydrocarbon pollution in the study area.

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مستويات المركبات الهيدروكاربونية الأروماتية متعددة الحلقات في عضلات أسماك تجارية من المياه العراقية.

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المستخلص - تم قياس تركيزالهيدروكربونات الأروماتية متعددة الحلقات في عضلات ثمانية أنواع من الأسماك البحرية وأسماك المياه العذبة. صنفت الأنواع البحرية وهي المزلك *Euryglossa orientalis*، النويبي *Otolithes ruber*، الشعري *Lethvinus nebulosus*، الهامور *Epinephelus coioides* والطعطعو *Johnieops sina* التي تم جمعها من المياه البحرية العراقية، وأنواع المياه العذبة وهي الكارب الاعتيادي *Cyprinus carpio*، الحمري *Carasobarbus luteus* والخشني *Plantiliza abu* التي تم جمعها من نهر شط العرب، إذ جمعت هذه الأسماك خلال فصلي الربيع والخريف 2015. سجلت الهيدروكربونات الأروماتية متعددة الحلقات في أنواع الأسماك تراكيز تراوحت بين 0.054 و 155.44 نانوغرام/غرام، وسجل مركب ألانثراسين Anthracene أعلى القيم إذ تراوح ما بين 17.629 و155.44 نانوغرام/غرام من الوزن الجاف. سجل أقل قيمة لمجموع المركبات 197.54 نانوغرام/غرام من الوزن الجاف في النويبي *O. ruber* وأعلى قيمة سجلت كانت 381.415 نانوغرام/غرام من الوزن الجاف في الهامور *E. coioides*، يليها الخشني *P. abu* التي سجلت أعلى قيمة 275.938 نانوغرام/غرام في حين أن القيم المسجلة خلال الخريف 2015 كانت أقل من تلك التي سجلت خلال الربيع. وجد نوعان من مصدر المركبات الاروماتية، الأول طبيعي Pyrogenic والتي ظهرت في أسماك الشعري والمزلك بتراكيز 0.355 و 0.274 نانوغرام/غرام على التوالي خلال الربيع 2015 والنوع الثاني بشرية المنشأ anthropogenic والتي ظهرت في كل الأسماك عدا الشعري والمزلك، لذلك كان فصل الربيع مصدر anthropogenic هو السائد. أما في فصل الخريف تشير النتائج ان القيم كانت أقل من 1 في الأسماك المدروسة عدا المزلك حيث كانت 1.040 مشيرة إلى أن معظم الهيدروكاربونات الاروماتية متعددة الحلقات ذات اصل anthropogenic. كما ان تراكيز الهيدروكاربونات الأروماتية متعددة الحلقات قد سجلت في معظم أسماك المياه العذبة والبحرية العراقية المدروسة ولكنها أعلى بقليل من الخلفية القياسية وبعض الدراسات السابقة لنفس المنطقة والمناطق القريبة منها. ومن وجهة النظر الصحية، إن مستوى متبقيات الهيدروكاربونات النفطية، خصوصا السامة منها، في الأسماك المدروسة يعتبر أقل من مستوى الخطورة ولا يمثل أي تأثير على الأنسان عند الأستهلاك.