



Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in some Imported Fishes

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Abstract: Samples of frozen fish available in Kirkuk markets were Shad *Tenualosa ilisha* (Hamilton), Mackerel *Trachurus trachurus* (Linnaeus), Rainbow trout *Oncorhynchus mykiss* (Walbaum) and striped catfish (*Pangasianodon hypophthalmus* (Sauvage)). They collected for the period 1st May 2015 to 30th January 2016 at weights average between 650-1250 g. The results showed many of carcinogenic and non-carcinogenic hydrocarbons compounds; Rainbow trout contained seven and nine of hydrocarbons compounds in the extracts of hexane and ethanol respectively, among them, there were five carcinogenic hydrocarbons in the ethanol extract and three in the hexane, their values ranged between 0.234-19.707 and 0.419-2.972 ng.g⁻¹ dry weight respectively. Numbers of the hydrocarbons ranged between 4-6 in other fish species. This may due to the differences of nature of feeding among the fish species and the differences in their fat content. In Shad fish, the results have recorded one carcinogenic compound in the ethanol and five in the hexane, the concentration of Benzo (a) pyrene reached 24.23 ng.g⁻¹ dry weight which is the most dangerous component among carcinogenic compound. Mackerel contains five compounds of hydrocarbons in the hexane; Four of them belong to the non-carcinogenic group and six in ethanol, five of them are non- carcinogenic. This study showed that differences in the numbers and concentrations of aromatic hydrocarbons in the studied fish. This is due to several reasons such as occurrences of fish near to the sources of hydrocarbons pollution. the nature of fish feeding and living.

Key words: Aromatic Hydrocarbon, Shad, Mackerel, Striped catfish, Rainbow trout.

Introduction

Fish is the biggest group among the animal kingdom that is consumed. about 1000 species from 30000 are used as commercial food that is consumed dried, canned, salted or smoked (Sándor *et al.*, 2011). Fish has a nutritional value because of it contains sahigh quality of

proteins and is a good source of calcium, iron and phosphorus as well as a vitamin B (Rahimi *et al.*, 2010).

Consuming fish protein is increasing more than other animal protein, this is because fish is characterized by low cholesterol levels (Verbeke *et al.*, 2007). The United States are

the world's third largest state in consuming of food in the world, and about 20% of sea food including of their food according to FAO data (Boadi *et al.*, 2011). However, fish food is healthy, it may be more subject to chemical pollutants from its environment, (Hajeb *et al.*, 2009).

Iraq imports large amounts of frozen fish, fillets, fish portion, headless fish products or whole fish from different countries such as India, Bangladesh, Vietnam, Turkey, UAE and Morocco, these fish may be exposed to hydrocarbon pollutants, and by eating fish products that were contaminated with aromatic hydrocarbons and by leads that were poisoning (Bordajandi *et al.*, 2004).

Distribution of petroleum hydrocarbons in commercial fish species in Iraqi coastal area North-West Arabian Gulf and in Shatt Al-Arab River were studied (Al-Saad *et al.*, 1989; Al-Saad, 1995; Al-Saad *et al.*, 1997; Hantoush *et al.*, 2001; Al-Ali *et al.*, 2016), while Al-Abdul-Nebi *et al.* (2013), studied

the aromatic hydrocarbons in muscles of some imported fish (frozen and canned) from local market of Basrah province, southern of Iraq.

The present study aims to identify the levels of hydrocarbons in four species of fish, including, shad, striped cat fish, rainbow trout and mackerel imported through the northern border of Erbil, Duhok and Kirkuk. The Poly Aromatic hydrocarbons (PAHs) were determined as a carcinogenic hydrocarbon.

Materials and Methods

Samples collection

Frozen fish from the local markets of the of Kirkuk city were collected from the period, 1st May 2015 until 30th January 2016. Fish samples included four brands representing different origin (Iraq, India, Thailand and Turkey), these included different samples from the most popular fish in the local markets as it is explained in table (1).

Table (1): Types and concentration of Poly Aromatic Hydrocarbons (PAHs) in the studied fish.

Sample No.	Species	Company	Origin	Remarks
1	Shad (<i>Tenualosa ilisha</i>)	Tahani	India	Packed in Iraq within the validity period
2	Catfish (<i>Pangasianodon hypophthalmus</i>)	Al- Baraa	Iraq	within the validity period
3	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Kaskin Oglu	Turkey	Within the validity period
4	Mackerel (<i>Trachurus trachurus</i>)	Americana	Thailand	Packed in UAE, within the validity period)

The average weight of fishes were (650-1250) grams, the fish were transferred in ice boxes then to the central laboratory of the Directorate of Agriculture and Irrigation, Kirkuk Governorate to prepare the samples. The head, skin and viscera of the fish were removed manually using sharp knives. These parts of body were placed in plastic bags, And freeze.

Fat extraction

Solvent extraction method was conducted to extract fat from freeze dryer fish tissues according to the method of Al-Saad (1995), two solvents (98% ethanol) as a polar solvent and 100% hexane as a non-polar solvent were used. The extracted lipid samples were transferred to the laboratories of the Department of Environmental Chemistry of Marine Science Centre, University of Basrah

for the purpose of hydrocarbons measurement. Hydrocarbon separation was performed used gas chromatograph (HPLC, Agilent 6890N -USA), compared to the standard curve as shown in Fig (1).

Determination of Polycyclic Aromatic Hydrocarbons (PAHs)

A method of Grimalt & Olive (1993) was used to extract hydrocarbons. A mixture of standard compounds of polycyclic aromatic compounds was used to determine the concentrations of hydrocarbons compounds in different fish samples.

The blank sample was extracted as mentioned in previous. Concentrations were estimated using the equation described in APHA (2005).

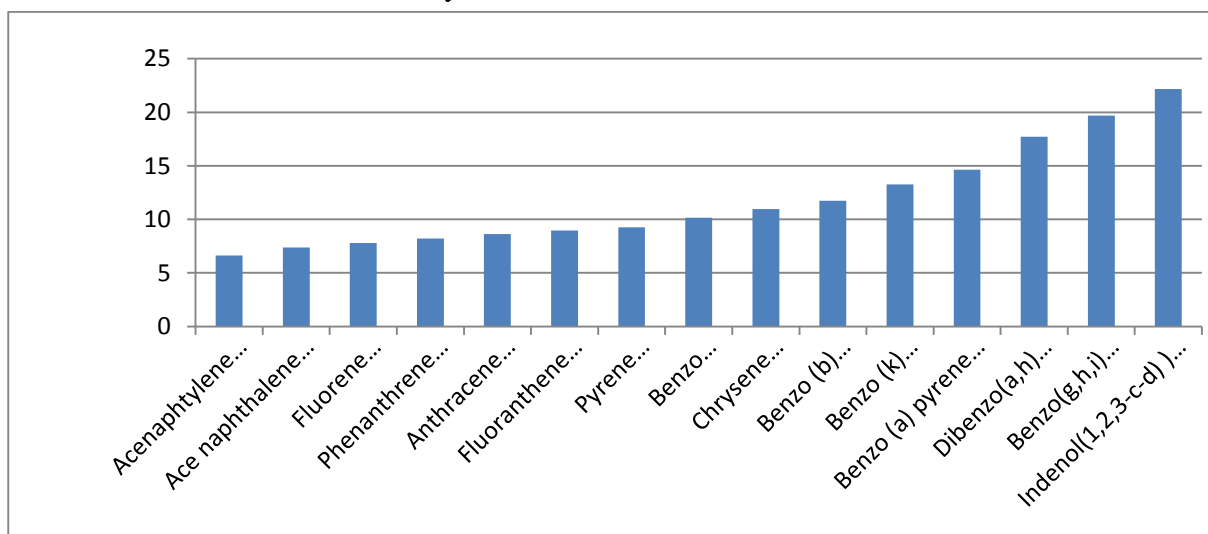


Fig. (1): The standard curve of Aromatic hydrocarbons.

Results & Discussion

Polycyclic Aromatic Hydrocarbons (PAHs)

The results exhibited in table (2). That indicated the present many Polycyclic Aromatic Hydrocarbons (PAHs) compounds, Rainbow trout fish contained 7 and 9 hydrocarbon compounds in the hexane and ethanol extracts, respectively, including five

carcinogenic but within the allowable limits, ranged between 0.234 - 19.707 ng.g⁻¹ for chrysene and Benzo {k} fluoranthene, respectively, while other fish species contains 4 to 8 aromatic hydrocarbon compounds in their tissue. This may be due to the Differences of food in the nature, and the difference in the level of fat contents. Shad

and Rainbow trout classified as fatty fish that contain more than 9% fat. Mackerel fish was classified as medium fatty fish more than 3% fat, while striped catfish was classified as lean fish, which content less than 3% fat (Hindi *et al.*, 2001).

Haritash & Kaushik (2009) indicated that hydrocarbons are low water solubility compounds, their solubility increases in water by increasing their molecular weight (Blahova *et al.*, 2010).

The highest compound was Anthracene hydrocarbon compound, with average of 84.196 ng.g⁻¹ in the ethanol extract of Mackerel and acenaphthylene compound in

the hexane extract at average 185.1 ng.g⁻¹ in Rainbow trout fish.

The hexane extracts indicated that there were five carcinogenic hydrocarbons out of eight compounds recorded the highest values, all of them were found in Shad fish: benzo {a} anthracene (0.915) ng.g⁻¹ , Benzo {b} fluoranthene (4.201) ng.g⁻¹, Benzo {k} fluoranthene (3.348), Dibenzo{a,h}anthracene (9.3 ng.g⁻¹) and Benzo{a}pyrene (24.230 ng.g⁻¹), which is the most dangerous carcinogenic compounds .They exceed the limits that allowed by the European Commission, That recommending not to increase more than 5 ng.g⁻¹.

Table (2): Types and concentration of Polycyclic Aromatic Hydrocarbons (PAHs) in the studied fish.

Aromatic Hydrocarbon	Shad	Shad	Striped cat fish	Striped cat fish	Rainbow trout	Rainbow trout	Mackerel	Mackerel
	Hexane	Ethanol	Hexane	Ethanol	Hexane	Ethanol	Hexane	Ethanol
Naphthalene	0	0	0.427	0	0	0	0	0
Acenaphthylene	0	0	37.246	0	185.1	0	12.509	0
Acenaphthalene	4.452	1.519	3.453	76.559	156.213	6.45	0	0.391
Fluorene	0	1.883	11.176	60.009	0	0	32.332	11.720
Phenanthrene	2.667	0.496	0.814	0	0	0.648	2.188	2.294
Anthracene	0	14.262	34.097	0	57.593	0	28.11	84.196
Fluoranthene	0	0	0.498	0	0	0.599	0	5.711
Pyrene	6.280	0	0	0	5.478	0.205	0	0
Benzo{a}anthracene	0.915	0	0	0	0	0.664	0	0
Chrysene	0	0	0	0	1.592	0.234	0	0.778
Benzo{b}fluoranthene	4.201	0	0	0	0.419	0.543	2.782	0
Benzo{k}fluoranthene	3.348	0	0	0.070	0	19.707	0	0
Benzo{a}pyrene	24.230	0	0	3.836	2.972	0	0	0
Dibenzo{a,h}anthracene	9.300	0	0	0	0	0	0	0
Benzo{g,h,i}pyrene	0	0	4.063	0.562	0	0.579	0	0
Indenol {1,2,3-c, d} pyrene	0	7.610	0	0	0	0	0	0
16 PAHs		25.770	91.774	141.036	418.367	29.629	77.921	105.090

Polycyclic Aromatic Hydrocarbons (PAHs) in the Shad fish:

Table (3) revealed the Polycyclic Aromatic Hydrocarbons compounds (PAHs) in the muscles of Shad fish, eight hydrocarbons compounds totally 55.393, in the hexane extract, and five Aromatic hydrocarbons compounds in the ethanol extract, as total 25.77. These results were less than those obtained by Alomirah *et al.* (2009), which was 68.7 ng.g⁻¹. The high contents of total hydrocarbons In current study, may due to the nature of the feeding habit of Shad fish and its biology as they are migratory fish inhabiting

the seas then migrates to the estuaries of the rivers in summer, in this migration fish has been exposed to various sources of hydrocarbon contaminants in seas, oceans and rivers.

It is also noted from the table (3) that the average concentration of low carcinogenic aromatic hydrocarbons reached 4.466 ng.g⁻¹ in the hexane extract. This result is consisted with Lobet *et al.* (2006). The average concentration of low carcinogenic aromatic hydrocarbons in the ethanol extract reached 4.54 ng.g⁻¹. This finding was consistent with Lobet *et al.* (2006).

Table (3) Types and concentration of Polycyclic Aromatic Hydrocarbons (PAHs) in Shad fish.

Low carcinogenic aromatic hydrocarbons		
PAH Compounds	Shad (Ethanol)	Shad (Hexane)
Acenaphthalene	1.519	4.452
Fluorene	1.883	0
Phenanthrene	0.496	2.667
Anthracene	14.262	0
Fluoranthene	0	0
Pyrene	0	6.280
Total	18.160	13.399
Mean	4.540	4.466
Carcinogenic Aromatic hydrocarbons		
PAH Compounds	Shad (Ethanol)	Shad (Hexane)
Benzo{a}anthracene	0	0.915
Chrysene	0	0
Benzo{b}fluoranthene	0	4.201
Benzo{k}fluoranthene	0	3.348
Benzo{a}pyrene	0	24.23
Dibenzo{a,h} pyrene	0	9.300
Benzo{g,h,i}pyrene	0	0
Indenol {1,2,3-c, d}	7.610	0
Total	7.610	41.994
Mean	7.610	8.300
16 PAHs	25.770	55.393

The average concentration of these compounds in sardine was 5.3 ng.g⁻¹. For carcinogenic hydrocarbons, Benzo {a} pyrene was higher than the other in the hexane extract.

Polycyclic Aromatic Hydrocarbons (PAHs)in the striped cat fish

Table (4) exhibited the concentration of total hydrocarbons in striped cat fish 141.038 ng.g⁻¹

¹, totally in ethanol extract, and 91.774 ng.g⁻¹ in the hexane extract, the total non-carcinogenic compounds were higher than carcinogenic in both extracted solvents. The values of Benzo {a} pyrene was 3.836 ng.g⁻¹ and is close to the permissible limits of the European Commission 2 ng.g⁻¹. The average concentration of total aromatic hydrocarbons

Table (4): Types and concentration of Polycyclic Aromatic Hydrocarbons (PAHs)in the striped cat fish.

Low carcinogenic aromatic hydrocarbons		
PAH Compounds	striped cat (Ethanol)	striped cat (Hexane)
Naphthalene	0	0.427
Acenaphthylene	0	37.246
Acenaphthalene	76.559	3.453
Fluorene	60.009	11.176
Phenanthrene	0	0.814
Anthracene	0	34.097
Fluoranthene	0	0.498
Pyrene	0	0
Total	136.568	87.711
Mean	68.284	12.530
Carcinogenic Aromatic hydrocarbons		
PAH Compounds	striped cat (Ethanol)	striped cat (Hexane)
Benzo{a}anthracene	0	0
Chrysene	0	0
Benzo{b}fluoranthene	0	0
Benzo{k}fluoranthene	0.070	0
Benzo{a}pyrene	3.836	0
Dibenzo{a,h} pyrene	0	0
Benzo{g,h,i}pyrene	0.562	4.063
Indenol {1,2,3-c, d}	0	0
Total	4.468	4.063
Mean	1.489	4.063
16 PAHs	141.036	91.774

was 12.53 ng.g⁻¹ in the hexane extract and approximated this value has converged with results this result with the study of Al-Saleh & Al-Doush, (2002), The concentration of total aromatic hydrocarbons in Emperor *Lethrinum miniatus* was 13.880 ng.g⁻¹, while this study recorded different average concentration of total aromatic hydrocarbons in the ethanol extract. It reached 28.20. ng.g⁻¹.

In Table (4), the acenaphthene extract with ethanol was higher than for the other carcinogenic compounds. It was also found that Benzo (g, h, i} perylene, extracted by hexane, was higher than for the other carcinogenic compounds, while the benzo-a} pyrene extract with ethanol was higher than Benzo {g, h, i} pyrene.

Polycyclic Aromatic Hydrocarbons (PAHs)in Rainbow trout:

Table (5) explained that the total number of polycyclic aromatic hydrocarbons compound in the ethanol extract was nine, five were carcinogenic hydrocarbons and four belonged to the non-carcinogenic hydrocarbons group, Seven types of hydrocarbons appeared in the hexane extract, three carcinogenic hydrocarbons group and four to non-carcinogenic hydrocarbons, The Benzo {a} pyrene was approximately 2.972 with the limits allowed by the European Commission, which recommends that the permissible limits of 2 ng.g⁻¹, This may be due to the fact that Rainbow trout fish are migratory fish between the oceans and estuaries that may be exposed during their migration to various oil pollution factors. It is also noted from the table that the average concentration of aromatic hydrocarbons in the hexane extract has reached 59.76 ng.g⁻¹. This result not consisted with that obtained by Webster *et al.* (2012) in

their study of hydrocarbon estimation in fish. The average concentration of aromatic hydrocarbons in the ethanol extract was 3.292 ng.g⁻¹ with Lobet *et al.* (2006). The total concentration of these compounds in sardines was 5.3 ng.g⁻¹.

Table (5) revealed the Acenaphthylene compound in the hexane extract greater than for the other benzo-k} fluoranthene compounds in the ethanol extract, which is higher than for the other carcinogenic compounds.

Polycyclic Aromatic Hydrocarbons (PAHs)in mackerel fish

Table (6) showed that the total number of polycyclic aromatic hydrocarbons compound in the hexane extract was five, one of them were carcinogenic, which is Benzo {b} fluoranthene, reaching 2.782 ng.g⁻¹ and four belonged to the non-carcinogenic hydrocarbons group. Six types of aromatic hydrocarbons were recorded in the ethanol extract, one of which belonged to the carcinogenic hydrocarbons group and five to non-carcinogenic hydrocarbon. Chrysene which is the only carcinogenic aromatic hydrocarbon in ethanol extract reaching 0.778 ng.g⁻¹. It is also noted from the table that the average concentration of aromatic hydrocarbons in the hexane extract has reached 77.921 ng.g⁻¹ and 105.09 ng.g⁻¹ in the ethanol extract. This result is different compared with that obtained by Webster *et al.* (2012) in their study of hydrocarbon estimated in marine fish of Scotland which was 5.6 ng.g⁻¹. The table also showed that the Anthracene which belong to non-carcinogenic group in the ethanol extract was higher than the other non-carcinogenic compounds.

Table (5): Types and concentration of Polycyclic Aromatic Hydrocarbons (PAHs) in Rainbow trout fish.

Low carcinogenic aromatic hydrocarbons		
PAH Compounds	Rainbow trout (Ethanol)	Rainbow trout (Hexane)
Naphthalene	0	0
Acenaphthylene	0	185.100
Acenaphthalene	6.450	156.213
Fluorene	0	0
Phenanthrene	0.648	0
Anthracene	0	57.593
Fluoranthene	0.599	0
Pyrene	0.205	5.478
Total	7.902	404.384
Mean	1.975	101.096
Carcinogenic Aromatic hydrocarbons		
PAH Compounds	Rainbow trout (Ethanol)	Rainbow trout (Hexane)
Benzo{a}anthracene	0.664	0
Chrysene	0.234	1.592
Benzo{b}fluoranthene	0.543	0.419
Benzo{k}fluoranthene	19.707 a	0
Benzo{a}pyrene	0	2.972
Dibenzo{a,h} pyrene	0	0
Benzo{g,h,i} pyrene	0.579	0
Indenol {1,2,3-c, d}	0	0
Total	21.727	9.966
Mean	4.345	3.322
16 PAHs	29.629	418.367

Table (6): Types and concentration of Polycyclic Aromatic Hydrocarbons (PAHs) in the Mackerel fish.

Low carcinogenic aromatic hydrocarbons		
PAH Compounds	Mackerel (Ethanol)	Mackerel (Hexane)
Naphthalene	0	0
Acenaphthylene	0	12.509
Acenaphthalene	0.391	0
Fluorene	11.720	32.332
Phenanthrene	2.294	2.188
Anthracene	84.196 a	28.110
Fluoranthene	5.711	0
Pyrene	0	0
Total	104.312	75.139
Mean	20.862	18.784
Carcinogenic Aromatic hydrocarbons		
PAH Compounds	Mackerel (Ethanol)	Mackerel (Hexane)
Benzo{a}anthracene	0	0
Chrysene	0.778 a	0
Benzo{b}fluoranthene	0	2.782
Benzo{k}fluoranthene	0	0
Benzo{a}pyrene	0	0
Dibenzo{a,h} pyrene	0	0
Benzo{g,h,i}pyrene	0	0
Indenol{1,2,3-c,d}pyrene	0	0
Total	0.778	2.782
Mean	0.778	2.782
16 PAHs	105.09	77.921

Conclusions

It conducted that, contamination of fishes by hydrocarbons was clearly obvious, (PAHs, containing 5 compounds in the shad fish and striped cat fish (in the ether extract). Nine compounds in Rainbow trout fish (in the hexane extract), while the number of PAHs in the hexane extract was 5 in mackerel and 8 in

shad and striped cat fish. The number of carcinogens compounds in the ether extract ranged from 1 in shad and mackerel and 5 in trout. In the hexane extract, the number of carcinogens compound was 1 in striped cat fish and mackerel and 5 in shad. The highest value of the PAHs content was in Rainbow trout 418.367 ng.g⁻¹ in the ether extract and

141.038 ng.g⁻¹ in the striped cat fish (hexane extract).

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