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The Occurrence and Levels of Polycyclic Aromatic Hydrocarbons (PAHs) in Frozen Fishes from the Local Market

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A B S T R A C T

Study of the levels of thirteen polycyclic aromatic hydrocarbons (PAHs) in the muscles, liver and gills of 5 frozen fish species from the Libyan market. Gas chromatographic analysis were employed for PAHs determination. Observed mean PAHs levels in the samples ranged from below detection limit (BD) of analytical instrument to 35.7 ng.g-^{1} in *seriala dumerili*, from BD to 30.5 ng. g^{-1} in *pagellus acarne* , from BD to 24.7 ng. g-^{1} in *Mullus barbatus*. From BD to 27.9 ng. g^{-1} in pagellus bogaraveo and from BD to 28.0 ng.g^{-1} in Gilthead seabream. chrysene, Benzo(a)anthracene, Dibenzo(a,h)anthracene and Benzo(g,h,i)perylene in a detectable concentration ranging from 0.35 ngg^{-1} in the muscles of *seriala dumerili* to 4.81 ngg-1 in the gills of *pagellus acarne.*

1 Introduction

Human health is significantly influenced by diet. Enough nutrients should be provided by proper nutrition, which should also have low concentrations of hazardous microorganisms and toxic substances. Fish is high in essential fatty acids, minerals, vitamins, and proteins and unsaturated essential fatty acids (PUFAs), especially omega-3. Epidemiological studies have revealed that there are long-term benefits. Consuming fish has been demonstrated to lower the risk of coronary heart disease. (Chow, C. K et al., 2008). In contrast to the possible health benefits of dietary fish consumption, a problem associated with regular fish consumption is the danger of chemical pollution exposure. PAHs are a wide group of chemical molecules found in the environment. Due to their mutagenic and carcinogenic qualities, they are on the priority pollution list of the European Union and the United States Environmental Protection Agency (US EPA) (USEPA, 2000). Most people are exposed to PAHs through their diet, with the exception of smokers and those who are exposed at work. Bordajandi, (Lene & Faranak 2008) through the marine environment's food chain, PAHs are available to marine life. Pollutants in contaminated soils have the ability to bioaccumulation in aquatic organisms because they are waterborne and lipophilic substances that easily cross lipid membranes. While fish and shellfish comprise very little of the average person's diet, for some, they account for a sizable portion of their daily intake of PAHs. (Domingo, 2007). The European Union recently defined a maximum level of 2 ng/g wet weight for benzo(a)pyrene (the marker used for the carcinogenic risk of PAHs) in fish muscle meat with the goal of minimizing adverse effects on human health(European Union, 2005). Dibenzo(a,l)pyrene has recently been given a carcinogenic potency of around 100 times that (Okona-Mensah et al., 2005). of benzo(a)pyrene However, there have been few studies that have

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included the determination of dibenzo(a,l)pyrene in food (Lene & Faranak , 2008) . Isolation of PAHs from biological matrices is frequently problematic from an analytical standpoint. Extraction and clean-up operations to produce extracts that are ready for precise analytical analysis (Bartle, 1991). PAHs extraction from fish products has been studied extensively utilizing Soxhlet-based techniques (Huggett et al., 2003), pressured liquid extraction, and supercritical fluid extraction (Ali & Cole, 2002). . Microwave-assisted extraction, for example, has primarily been employed for environmental samples (Castro D et al., 2009). Although most current procedures utilize less organic solvent than traditional extraction, they nonetheless require extensive cleaning of glassware and extraction vessels prior to the next use. The major goal of this work was to extract 13 PAHs from frozen fish samples (table 1), which are considered priority pollutants by the US EPA. The extracts would be GC/MS analyzed using the Xcalibur. (Thermo Fisher Scientific, 2009). Another objective was to determine the levels of PAHs in the muscles, liver, and gills of Mullus barbatus, Pagellus acarne, Pagellus bogaraveo, seriala dumerili, and Gilthead seabream.

2 Materials and Methods

During the winter of 2016, the aforementioned fish species were taken from frozen fish markets in Benghazi, Libya. Among the fish samples are: (*Mullus barbatus, pagellus acarne, pagellus bogaraveo, seriala dumerili and Gilthead seabream*). The fish samples were immediately packaged in polyethylene bags, placed in an isolated polystyrene ice box, and then transported to the lab. Prior to analysis, the tissues of each species' fish muscle, liver, and gills were removed using stainless steel tools on spotless glass surfaces. Using an electronic balance, 10 g of each tissue were precisely weighed.

The method that was employed 10g of freeze-dried fish species tissues are placed in 75mL of 6.7 % (KOH/CH_3OH) and refluxed for one hour to determine the aromatic hydrocarbons infecting the fish species. The extraction was done in a 1000 ml separating funnel using n-pentane. After complete drying, repeated washings with water and reverse extraction of the washings with n-pentane were performed. Extraction is carried out and the results are collected in a graduated container. After cleaning with silica gel, the vial is covered and the extract is used for the analysis.

The Hewlett Packard 5890 series II GC gas chromatograph with a flame ionization detector (FID) was used to analyze all the samples. The instrument was set to splitless mode with a 3µl splitless injection. The injection port temperature was maintained at 290oC, and the detector was kept at 300oC. The samples were analyzed using an HP-1 fused silica capillary column with 100% dimethyl polysiloxane. The column had dimensions of 30m length, 0.32mm inner diameter, and a 0.17µm film thickness. The oven temperature was programmed to increase from 60 to 290oC at a rate of 3 oC min−1 and then maintained at 290oC for 25 minutes. Nitrogen was used as the carrier gas, flowing at a rate of 1.2 ml min−1.

To quantify the PAHs, a stock solution containing various PAHs, including naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, benzo(a)anthracene, hrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, pyrene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(ghi)perylene, and indeno(1,2,3-cd)pyrene, was used. This stock solution was diluted to create a series of calibration standards with concentrations of 0.1, 0.25, 0.5, 0.75, 1.0, 2.0, 5.0, and 10µgml−1. The detection limit for each PAH was approximately 0.01µgml−1. To ensure analytical reliability and recovery efficiency, six analyses were performed on PAH reference materials, HS-5 and 2974, provided by EIMP-IAEA. The laboratory results demonstrated a recovery efficiency ranging from 92 to 111% for all studied pollutants (13 PAH fractions), with a coefficient of variation (CV) of 8–14%.

3 Results and Discussion

Some of the previously mentioned poly aromatic hydrocarbons were detected in our samples although some are in a trace but they were within the instrumentation detection limits the best way to discuss the results is by categorise them into analysed part of sample.

The analysis of fish muscles

Shown in table and figure 2 that Benzo(a)pyrene, Benzo(b)fluoranthene, Benzo(k)fluoranthene and Indeno(1,2,3-cd)pyrene are absent in all samples while Fluorene was the highest in all samples followed by Acenaphthylene, Phenanthrene and Anthracene while others are in a trace.

Muscles of all samples were contaminated, *G. seabream* was the highest in contamination and p. *acarne* was the lowest

The analysis of fish liver

Shown in table 2 that Benzo(a)pyrene, Benzo(b)fluoranthene, Benzo(k)fluoranthene and

Indeno(1,2,3-cd)pyrene are absent in all samples while Fluorene was agine the highest in all samples followed by Acenaphthylene, Phenanthrene and Anthracene while others are in a trace.

Liver of all samples were contaminated, ***M. barbatus* was the highest in contamination and G.** *seabream* was the lowest.

The analysis of fish gills

Shown in table 3 the gills of *p. acarne* showed a trace of Benzo(k)fluoranthene which was not detected in all other samples while Benzo(a)pyrene, Benzo(b)fluoranthene, and Indeno(1,2,3-cd) pyrene are absent in all samples Gilles of all samples were contaminated, ***p. acarne* was the highest in contamination and *M. barbatus*** was the lowest.

Contamination by Fluorene

Fluorene, a polyaromatic hydrocarbon (PAH) with three rings, is categorized as a Priority Pollutant. It is commonly found in various sources, including incomplete combustion products and fossil fuels. Fluorene is considered a toxic pollutant under section 307(a) of the Clean Water Act and is regulated with effluent limitations.

Research studies have indicated that the toxicity of oil is influenced by its di-aromatic and tri-aromatic hydrocarbons, which encompass three-ring hydrocarbons like fluorene. These specific hydrocarbons, including fluorene, contribute to the overall toxicity of the oil. (EL-Saeid et al., 2008).

In table 4 it is clear that fluorene is present in high concentration in all samples

Contamination by Phenanthrene

Phenanthrene is found in fossil fuels and is present in substances resulting from incomplete combustion. It is widely distributed in aquatic environments and has been detected in various sources such as surface water, tap water, wastewater, and dried lake sediments. Phenanthrene has also been identified in seafood obtained from polluted waters, as well as in smoked and charcoal-broiled food items. Additionally, phenanthrene occurs naturally in fossil fuels and has been detected in spruce needles, tree leaves, grass, and other plants. Human exposure to phenanthrene primarily occurs through inhalation of polluted air and ingestion of food or water contaminated by combustion byproducts. Table 3

Contamination by Pyrene

Pyrene is produced as a byproduct of incomplete combustion, such as the burning of gasoline or wood. It has a tendency to attach itself to very fine particles that become airborne. Over time, these particles, along with the pyrene, settle back onto the ground or into bodies of water like ponds, lakes, or rivers. Once introduced into the environment, pyrene can persist in soil, water, or air.

Limited studies have indicated that pyrene exhibits toxicity to aquatic organisms at concentrations lower than those typically found in surface water. Pyrene has the potential to damage cell DNA and disrupt endocrine activity. Of particular concern is its ability to accumulate in aquatic sediments, which can pose a risk to organisms residing in or near the bottom of lakes and rivers. Table 4

APAH compound	Chemical Formula	M. Weight (g mol−1)	Boiling Point (◦C)
Benzo(a)pyrene	$C_{20}H_{12}$	252.3	495
Benzo(b)fluoranthene	$C_{20}H_{12}$	252.3	481
Benzo(k)fluoranthene	$C_{20}H_{12}$	252.3	480
Indeno(1,2,3-cd)pyrene	$C_{22}H_{12}$	276.3	530
Acenaphthylene	$C_{12}H_{10}$	154.2	279
Fluorene	$C_{13}H_{10}$	166.2	295
Phenanthrene	$C_{14}H_{10}$	178.2	340
Anthracene	$C_{14}H_{10}$	178.2	340–342
Pyrene	$C_{16}H_{10}$	202.3	393–404
Chrysene	$C_{18}H_{12}$	228.3	448
Benzo(a)anthracene	$C_{18}H_{12}$	228.3	438
Dibenzo(a,h)anthracene	$C_{22}H_{14}$	278.3	535
Benzo(g,h,i)perylene	$C_{22}H_{12}$	276.3	550

Contamination by other PAH in trace concentration

Even if some of the well-known PAH are below the detection limits or absent in samples under study, the present of large size PAH as chrysene, Benzo(a)anthracene, Dibenzo(a,h)anthracene and Benzo(g,h,i)perylene in a detectable concentration ranging from 0.35 ngg^{-1} in the muscles of *seriala dumerili* to 4.81 ngg^{-1} in the gills of *pagellus acarne*. The toxicity of these PAH are higher and it had been mentioned in many articles that it could have Genotoxicity to humans (Pan et al., 2013) and Carcinogenic activity.

4 Conclusions

The study showed without any doubt that the contamination of frozen fish in the Libyan market without addressing the origin of these fish, and we advise officials to take strict measures to import and distribute these goods.

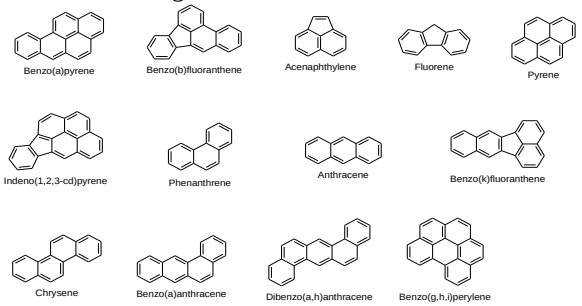

Figure 1 structure of PAH under study

PAH compound	*M. barbatus*	*p. acarne*	*p. bogaraveo*	*s. dumerili*	*G. seabream*
	Muscles				
Benzo(a)pyrene	BD	BD	BD	BD	BD
Benzo(b)fluoranthene	BD	BD	BD	BD	BD
Benzo(k)fluoranthene	BD	BD	BD	BD	BD
Indeno(1,2,3-cd)pyrene	BD	BD	BD	BD	BD
Acenaphthylene	9.53	3.31	4.99	3.30	10.9
Fluorene	15.9	20.3	27.1	18.8	28.0
Phenanthrene	8.93	5.58	6.88	3.57	6.23
Anthracene	6.23	2.82	3.65	2.65	5.00
Pyrene	5.44	5.16	5.74	6.37	5.44
Chrysene	0.35	0.36	0.36	0.35	1.25
Benzo(a)anthracene	0.77	0.77	1.27	0.77	1.29
Dibenzo(a,h)anthracene	1.14	1.13	1.13	1.13	1.13
Benzo(g,h,i)perylene	0.89	0.86	0.75	0.74	0.75

Table (2) levels of PAH in Muscles of samples

PAH compound	*M. barbatus*	*p. acarne*	*p. bogaraveo*	*s. dumerili*	*G. seabream*
	liver				
Benzo(a)pyrene	BD	BD	BD	BD	BD
Benzo(b)fluoranthene	BD	BD	BD	BD	BD
Benzo(k)fluoranthene	BD	BD	BD	BD	BD
Indeno(1,2,3-cd)pyrene	BD	BD	BD	BD	BD
Acenaphthylene	3.28	3.31	3.32	3.31	3.34
Fluorene	24.7	22.6	20.8	21.9	19.5
Phenanthrene	6.36	5.26	5.37	4.76	3.03
Anthracene	5.38	3.55	3.38	3.19	2.24
Pyrene	4.93	6.05	5.82	5.23	5.17
Chrysene	0.35	0.36	0.75	0.84	1.42
Benzo(a)anthracene	0.77	0.77	0.77	0.77	1.69
Dibenzo(a,h)anthracene	1.19	1.14	1.14	1.13	1.14
Benzo(g,h,i)perylene	0.93	0.74	0.74	0.74	0.74

Table (3) levels of PAH in liver of samples

PAH compound	*M. barbatus*	*p. acarne*	*p. bogaraveo*	*s. dumerili*	*G. seabream*
	Gilles				
Benzo(a)pyrene	BD	BD	BD	BD	BD
Benzo(b)fluoranthene	BD	BD	BD	BD	BD
Benzo(k)fluoranthene	BD	0.63	BD	BD	BD
Indeno(1,2,3-cd)pyrene	BD	BD	BD	BD	BD
Acenaphthylene	4.74	6.40	3.29	11.1	4.94
Fluorene	22.5	30.5	27.9	35.7	23.4
Phenanthrene	4.33	14.1	8.99	9.79	5.22
Anthracene	2.58	9.56	7.15	7.20	3.59
Pyrene	5.41	9.29	9.14	6.15	8.25
Chrysene	0.36	0.35	1.68	1.09	2.20
Benzo(a)anthracene	0.77	4.81	2.16	1.52	BD
Dibenzo(a,h)anthracene	1.13	1.14	1.13	1.13	1.13
Benzo(g,h,i)perylene	0.87	0.75	0.74	0.74	0.74

Table (4) levels of PAH in gills of samples

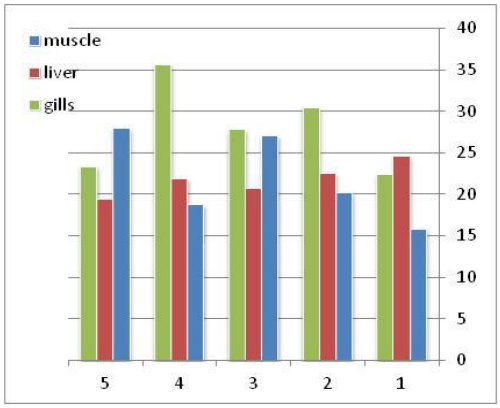

Figure (2) Concentration of **Fluorene** in fish samples

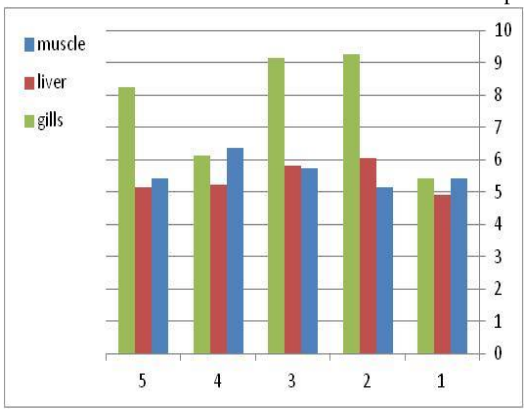

Figure (3) Concentration of **pyrene** in fish samples

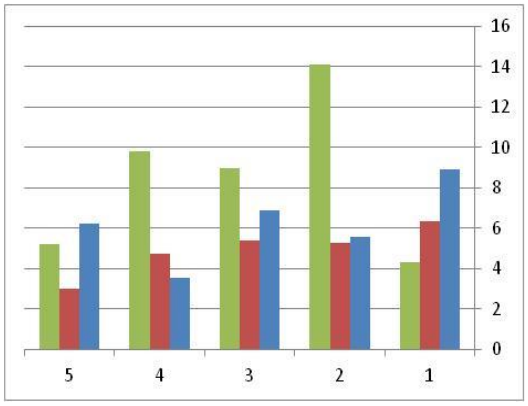

Figure (4) Concentration of **phenanthrene** in fish sampl

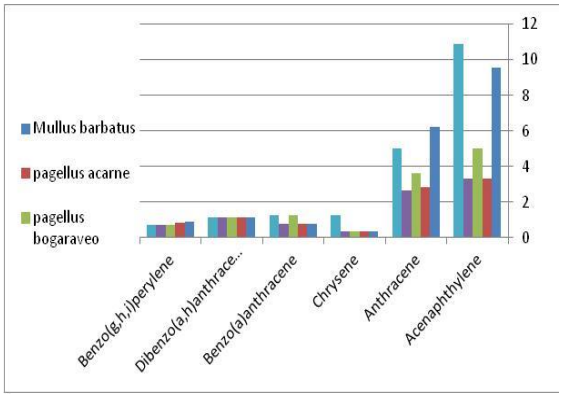

Figure (5) Concentration of trace PAH in the muscles of fisl samples

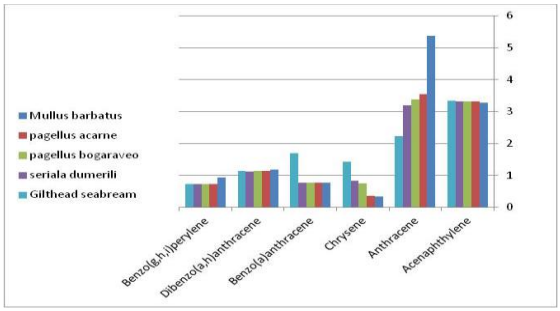

Figure (6) Concentration of trace PAH in the liver of fisl samples

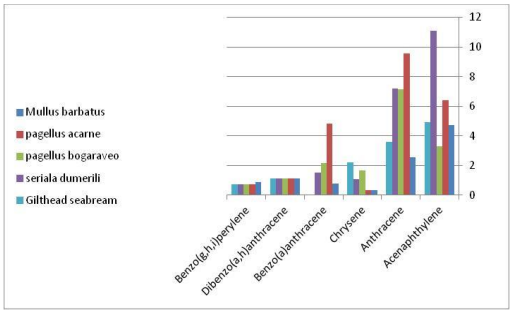

Figure (7) Concentration of trace PAH in the gills of fish samples

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