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The Levels and Sources of Aliphatic and Polycyclic Aromatic Hydrocarbons in *Blue Runner* Fish from Benghazi Coast, Libya

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ABSTRACT

As a part of an ecological health study, polycyclic aromatic hydrocarbons (PAHs) and n-alkanes (n-C_9 to n-C_{20}) profiles were assessed in the muscles, livers and gills tissues of *Blue runner* fish collected from Benghazi coast extending east to Daryanah coast, Libya. Target compounds were analytically determined with gas chromatography-mass spectrometry (GC-MS). The presented results showed that total n-alkanes varied from 829.59 to 1693.51 µg/g, with an average value of 1178.987 µg/g, and the livers exhibited a higher n-alkanes accumulation capacity compared to gills and muscles tissues. The n-alkanes were biogenic in origin, based on the CPI index. The findings also indicated that total concentrations of PAHs ranged from 8.19 to 8.21µg/g, with an average of 8.20 µg/g. Benzo(a)pyrene, a first class PAHs carcinogen, showed a level above the approved safe limits. Some ratios were calculated for PAHs pollution to assess their sources. Thus, the data imply that the fish were contaminated of pyrogenic origin with a potential cancer risk.

Keywords: ***Blue runner*** fish, n-alkanes, polyaromatic aromatic hydrocarbons, Libya.

INTRODUCTION

Human health depends greatly on diet. A balanced diet should contain a sufficient concentration of nutrients and should not be contaminated by chemicals. Environmental chemical contamination is a major global food safety problem (Thompson & Darwish, 2019). Fish in general are a healthy food source, but on the other hand, contaminated fish can negatively affect human health. The risk comes from frequent consumption of the contaminated fish (Forstner & Wittman, 1981; Jorgensen & Pedersen, 1994).

Hydrocarbons like aliphatic hydrocarbons (AHs) and PAHs are among the most organic compounds found in terrestrial and aquatic environment. Naturally, they are exist in low concentration, and the larger amounts enter the environment from polluted sources**.** The most important sources of AHs and PAHs is crude oil (Kachel, 2008; Ravindra, et al., 2008; Muthukumar, et al., 2013). The lipophilicity of AHs and PAHs make them easier to accumulate in the adipose tissues of marine organisms (Gobas, et al., 1999; Bouloubassi, et al., 2001). Hence, dietary intake of contaminated fish with petroleum hydrocarbons is a serious and public health concern. Wherefore, the content, origin, and health risks of AHs and PAHs in marine organisms were examined by several authors (Ali, et al., 2006; Nyarko, et al., 2011; Al-Khion, et al., 2021).

The US Environmental Protection Agency (US EPA) has classified PAHs as hazardous environmental chemicals (Bouloubassi, et al., 2001; Ramalhosa, et al., 2009) due to their toxicity and carcinogenicity (Olayinka, et al., 2019; Honda & Suzuki, 2020; Soliman, et al., 2023). In general, aliphatic hydrocarbons are less harmful and have a high biodegradable rate in aquatic plants and animals compared to PAHs. The biodegradation of PAHs depends on the number of aromatic rings, and the rate of degradation decreases as the number of aromatic rings increases (Patnaik, 1992; Meador, et al., 1995).

Libya is one of the oil producing countries in the world. Recently, contamination of the marine environment with oil pollutants (represented by ship movement, oil transport and refining) on the Libyan coast has become an important issue. Besides, industrial discharges of coastal cities flow into the sea without treatment, leading to high levels of pollutants and endangering marine life. Libyan studies were conducted on the presence of petroleum hydrocarbons (AHs and PAHs) in sediment from Zwitina coast (Zeyadah, et al., 2023), shells from Toubrouk (Hasan, et al., 2022) and Derna coasts (Mohammed & Alsharef, 2023). In addition, few researches were studied the bioaccumulation of AHs and PAHs in some fish from Libya marine waters (Hamad & Nuesry, 2014).

As a case study, we chose an area of high environmental and industrial interest, Benghazi sea. The Benghazi coast is an important area for fisheries in Libya, but on the other hand, it receives amounts of petroleum products. Therefore, the presence of aliphatic and polyaromatic hydrocarbons contamination in marine life is to be expected in this zone. Previous researches were estimated the content of these organic compounds in seawater, sediments and marine organisms in the region (Shaltami, et al., 2021; El-Fergani, et al., 2023a; El-Fergani, et al., 2023b).

The objective of this manuscript is to determine the level of contamination with n-alkanes (which are subset of AHs) and polyaromatic hydrocarbons in soft tissues (muscles and livers) and in hard tissues (gills) of *Blue runner* fish collected from the coastal area extending from Benghazi to Daryanah town, as well as identifying the sources of these compounds.

2. MATERIALS AND METHODS

2.1. Study Area. Benghazi is the main city in Cyrenaica on the Mediterranean coast, in eastern Libya. It represents the second largest city in the country, with a population of about 1,207,250 inhabitants distributed over about 314 km^2. It is an industrial city with fishing activities and an important port. This study was conducted in the coastal area extending from Benghazi to Daryanah town, 32 km east of Benghazi (Figure 1).

Figure1. The area of study (Benghazi – Daryanah coast).

2.2. **Sample Collection**. In summer 2021, ***Blue runnear*** fish were collected from Benghazi coast extending east to Daryanah coast. ***Blue runners** (Caranx crysos*) fish are coastal species, reaching a maximum length of 70 cm and a weight of half a kilogram. They are widespread throughout the Mediterranean sea, and have been recorded from almost all countries located on its shores, as well as in the Atlantic (Fischer, et al., 1981). The fish samples were immediately transported to the lab on ice packs. Three tissues, livers, gills and muscles of the fish were carefully removed to analysis the bioaccumulation of the hydrocarbons.

2.3. Analytical Procedures. The analysis of AHs and PAHs was performed according to the technique proposed by (UNEP/IOC/IAEA, 1992). In order to extract organic compounds, about 5 grams of wet weight of each sample were ground with anhydrous sodium sulphate (30 g). The mixture was then extracted using Soxhlet apparatus and 200 mL methanol as a solvent extraction for 8 h. To remove fatty acids, a saponification step was achieved by adding 20 mL of KOH (0.7 M) and 30 mL of distilled water in the Soxhlet extractor over 2 h. After 2 h, the content of the flask was extracted three times in a separating funnel using 80 mL of hexane and the extract was then dried with anhydrous $NaSO_4$, filtered, and concentrated to a final volume of 1 mL with a rotary evaporator and a gentle flow of N_2 gas. The hexane extract (1 mL) was fractionated into n-alkanes and aromatic fractions by passing through a silica gel column. Subsequently, the n-alkanes fraction (F1) was eluted with 25 ml of hexane, and the PAHs fraction (F2) was eluted with 60 ml of hexane/dichloromethane mixture (80:20 v/v). Finally, the fractions (F1 and F2) were concentrated using a flow of N_2 gas for instrumental analysis. The samples were analyzed for n-alkanes and polyaromatic hydrocarbons by a gas chromatograph (Hewlett Packard 5890 series II) along with a mass spectrometer. The compounds were identified by comparing measured mass spectral data and retention times with the US Environmental Protection Agency (EPA) standard at the Desert Research Center, Cairo, Egypt.

2.4. Statistics. The obtained data were tabulated and statistically analyzed using IBM SPSS Statistics (IBM-Statistical Package for Social Sciences).

3. RESULTS AND DISCUSSIONS

Qualitative and quantitative determination of n-alkanes and polycyclic aromatic hydrocarbons in fish was performed for use as chemical markers to determine the health of the sea in the study area.

3.1. Levels of Aliphatic Hydrocarbons (AHs)

The concentrations of short chain n-alkanes (n-C9 to n-C20) in three tissues of ***Blue runner*** fish are summarized in Table 1 and Figure 2. n-Alkanes (n-C9 to n-C20) were found to be in the range of 9.83 - 582.33µg/g (wet weight). The total concentrations of twenty identified aliphatic hydrocarbons were between 829.59-1693.51µg/g with an average 1178.987µg/g. These values were similar to those levels measured in different fish species from the same region (El-Fergani, et al., 2023b), while they were higher than values reported from the coast of Derna in eastern Libya (Hasan and Nuesry, 2014). This could be due to the fact that Benghazi is an industrial area compared to Derna which does not have any industrial importance and its port is small. Additionally, the levels of detected n-alkanes were high compared to other marine organisms collected from Mediterranean coasts adjacent to Libya coast. Egyptian Mediterranean coast recorded total aliphatics average 180 ng /g (El-Sikaily, et al., 2002) and the range of total aliphatic hydrocarbons in Tunisian Gulf fish were fluctuated between 0.1 to1.6 µg/ g. (Mzoughi, et al., (2010).

The results showed that nonane (n-C9) was present in large amounts in all tissues. Moreover, the livers had the highest total n-alkanes among the three tissues. The low content of total n-alkanes in the muscles and gills than livers is probably due to the lower fat content of these tissues. To estimation of the origin of n-alkanes, the carbon preference index (CPI) was calculated. The calculation showed a predominance of odd numbered carbon chains with CPI >1 and this indicated that n-alkanes were derived from natural biogenic sources (Bray and Evan, 1961; Fagbote & Olanipekun, 2013; Rushdi, et al., 2019).

Correlation coefficients between n-alkanes content in different types of tissues were calculated by a statistical analysis. n-Alkanes for gills showed a highly positive correlation with n-alkanes in both livers (0.98) and muscles (0.85) as well as, content of n-alkanes in the liver correlated significantly with n-alkanes in muscles (0.74). The result emphasized that gills were the main routes of AHs contaminants in fish due to constant contact with the external environmental pollution. Subsequently, these AHs contaminants moved and accumulated in the livers and muscles.

Table 1. The concentration (µg/ g) of n-alkanes in ***Blue runner*** fish from the study region.

Sample	***Blue runner* fish**			**Average**
	Livers	**Gills**	**muscles**	
C-9	447.58	582.33	421.13	483.68
C-10	35.24	35.88	31.94	34.35
C-11	15.56	10.84	9.91	12.10
C-12	148.28	77.78	75.25	100.43
C-13	266.68	22.95	14.54	101.39
C-14	343.19	87.74	87.74	172.89
C-15	123.39	27.63	22.30	57.77
C-16	138.80	66.04	64.49	89.77
C-17	40.58	26.22	19.11	28.63
C-18	85.14	45.70	47.45	59.43
C-19	15.22	9.83	10.60	11.88
C-20	33.85	20.92	25.13	26.63
$\Sigma(C9–C19)_{odd}$	909.01	679.8	497.59	695.46
$\Sigma(C10–C20)_{even}$	784.5	334.06	332	483.52
CPI	1.15	2.03	1.49	1.5
Total	**1693.51**	**1013.86**	**829.59**	**1178.98**

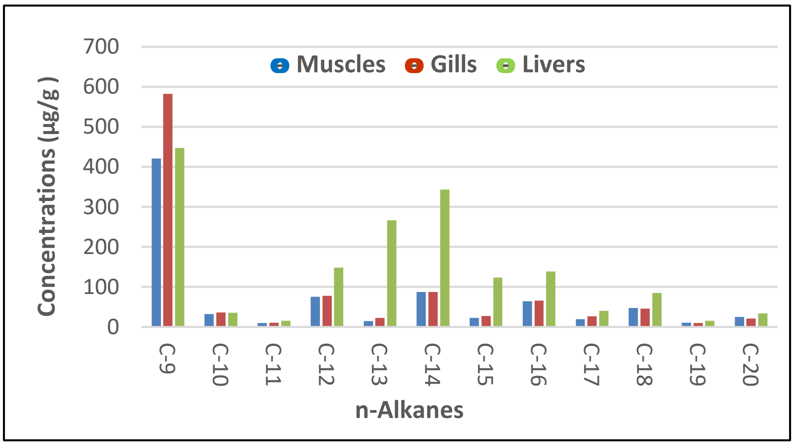


Figure 2. Distribution of n-alkanes concentrations (µg/g) of ***Blue runner*** fish

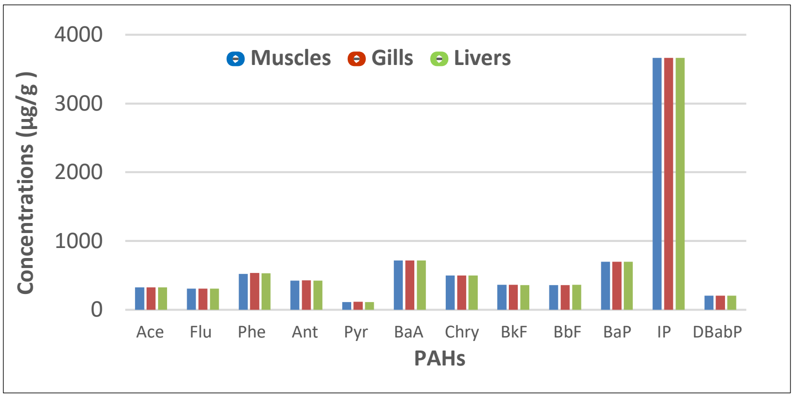
3.2. Levels of Poly Aromatic Hydrocarbons (PAHs)

Table 2 and figure 3 show the concentrations of 13 PAHs, namely benzo(g,h,i)perylene, fluorene, acenaphthylene, phenanthrene, anthracene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, Indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene. The concentrations of PAHs in the fish tissues (livers, gills and muscles) showed the same pattern in the range of 0.11 - 3.66 µg/g, with a total about 8.20 µg/g. For the individual PAH compounds, Indeno(1,2,3-cd)pyrene had the highest concentration (3.66 µg/g) while benzo(g,h,i)perylene was not detected in the three tissues. Similar results were reported for PAHs in *Epinephelus marginatus* fish collected from the same area (El-Fergani, et al., 2023b). In addition, the PAHs concentrations obtained in the present investigation were lower than that recorded for the coast of Derna (Hasan and Nuesry, 2014), and our values were also lower than the results recorded in seafood from Tobrouk coast (Hasan, et al., 2022).

Among the analyzed 13 PAHs, benzo(a)anthracene, chrysene, benzo(k)fluoranthene, benzo(b)fluoranthene, Benzo(a)pyrene, indeno(1,2,3-cd)pyrene and dibenzo(a,h)anthracene were considered clearly carcinogenic compounds (USEPA, 1984). Benzo[a]pyrene which is the best indicator of the occurrence of PAHs in food, and is identified as the most powerful and widely studied carcinogen (Alexander, et al., 2008; Menzie, et al., 1992; Emoyan, et al., 2020) had a value of 0.699 µg/g. This value was exceeded the safe limit recommended by the European Union for human consumption of fish (2 µg/kg) (Varanasi, et al., 1989), suggesting that water organisms around the coast of Benghazi may pose risks to human health.

In order to assess possible sources of PAHs, several studies were used a number of concentrations of individual PAHs to differentiate between the two sources. In the present work, four diagnostic ratios Phe/Ant, BaA/(BaA + Chry), Ant/(Ant+Phe) and Inp/(Inp+BghiP) were deduced to determine the sources of PAHs whether pyrolytic (from incomplete fuel combustion) or petrogenic (from crude oil) sources. The Phe/Ant (1.24) value was below than 10 suggested a pyrogenic origin. Meanwhile, BaA/(BaA + Chry) (0.58) and Ant/(Ant+Phe) (0.44) ratios were respectively greater than 0.35 and 0.1, supported the pyrogenic origin of PA hydrocarbons. The observed Inp/(Inp+Bghip) (1) ratio reported a value more than 0.5, further indicated the pyrogenic origin (Gilbert, et al., 2006; Yu, et al., 2014). The ratio value of low molecular weight (LMW) (2-3 rings) to high molecular weight (HMW) (>4rings) was also used to identify the sources of PAHs. The calculated value of the (LMW/HMW) ratio (0.23) was < 1 and this result was typical of pyrogenic sources (Rocher, et al., 2004). Based on the result, the PAHs were derived from pyrolytic sources. This finding confirms the finding of Shaltami, et al., (2021), who assessed the origin of PAHs in Benghazi port, concluded that pyrogenic sources were the main source of PAH contamination.

Table 2. The concentration (μg/ g) of PAHs in ***Blue runner*** fish from the study region.



Sample	Abbreviation	***Blue runner*** fish			Average
		Livers	Gills	Muscles	
Benzo(g,h,i)perylene	BghiP	N/A	N/A	N/A	N/A
Acenaphthylene	Ace	0.3265	0.32584	0.32588	0.326073
Fluorene	Flu	0.30594	0.30688	0.30678	0.306533
Phenanthrene	Phe	0.5297	0.53425	0.52299	0.52898
Anthracene	Ant	0.42569	0.4265	0.42307	0.425087
Pyrene	Pyr	0.11257	0.11826	0.1122	0.114343
Benzo(a)anthracene	BaA	0.71604	0.71604	0.71604	0.71604
Chrysene	Chry	0.49907	0.49715	0.49672	0.497647
Benzo(k)fluoranthene	BkF	0.35733	0.3635	0.3635	0.361443
Benzo(b)fluoranthene	BbF	0.36351	0.35733	0.35732	0.359387
Benzo(a)Pyrene	BaP	0.69935	0.69936	0.6994	0.69937
Indeno(1,2,3-cd)pyrene	IP	3.66357	3.66374	3.66368	3.663663
Dibenzo(a,b)anthracene	DBabP	0.20533	0.20544	0.20537	0.20538
Total		**8.2046**	**8.21429**	**8.19295**	**8.203947**

Figure 3. Distribution of the poly aromatic hydrocarbons concentrations (μg/g) of ***Blue runner*** fish.

CONCLUSION

In This study, the muscles, livers and gills tissues of ***Blue runner*** fish collected from the coast of Benghazi city to Daryanh town were analyzed for n-alkanes and polyaromatic hydrocarbons by using GC-MS. Thus, the fish samples were contaminated by short straight-chain alkanes (n-nonane to n-icosane) of biogenic origins and polyaromatic hydrocarbons (LMW and HMW) of pyrogenic origins with a potential cancer risk.

RECOMMENDATION

Since organic contaminants can transfer to seafood and thus affect health, we recommend that environmental studies and risk assessments should be carried out regularly.

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