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Identification and Detection the Aromatic and Aliphatic Hydrocarbons in *Epinephelus Marginatus* Fish Samples Collected from Benghazi Coast

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Abstract

The contents of aliphatic and aromatic compounds were determined in *Epinephelus marginatus* fish samples collected from Benghazi coasts (Libya). The GC-Mass analysis method was used to estimate the hydrocarbon

compounds. The results recorded presence different types of aliphatic and aromatic. Different contents were recorded in the studied tissues of gills, Muscles and Livers.

Keywords: *Epinephelus Marginatus*, Hydrocarbons, Libya

Introduction

Hydrocarbon resources are resources that contain hydrocarbon molecules which means it consists both hydrogen and carbon that can take the form of a solid, liquid, or gas. Hydrocarbons are the main components of fossil fuels, also known as nonrenewable energy resources, including coal, oil, and natural gas. The natural sources of hydrocarbons include coal, petroleum, and natural gas. Hydrocarbon resources are the largest source of primary energy, contributing to over 85% of the world's primary energy (Ravindra *et al.*, 2008) [32]. Aliphatic compounds can be saturated, joined by single bonds (alkanes), or unsaturated, with double bonds (alkenes) or triple bonds (alkynes). Besides hydrogen, other elements can be bound to the carbon chain, the most common being oxygen, nitrogen, sulfur, and chlorine. The least complex aliphatic compound is methane (CH₄) (Smith & March, 2007) [39]. Aliphatic compounds can be cyclic or acyclic meaning they can contain close chains or rings of carbon atoms in their molecule. As the hydrocarbons are non-polar they tend to be insoluble in water and other polar solvents. They prefer to dissolve in non-polar solvents such as benzene and diethyl ether. Thus, hydrocarbons can be described as hydrophobic or lipophilic. The hydrocarbons are less dense than water meaning that they float on the surface of water (Morrison and Boyd, 1992) [26]. The most important source of alkanes is natural gas and crude oil. Alkanes are separated in an oil refinery by fractional distillation and processed into many different products (Sephton and Hazen, 2013) [36].

Aromatic hydrocarbons, or arenes, are aromatic organic compounds containing solely carbon and hydrogen atoms. The configuration of six carbon atoms in aromatic compounds is called a "benzene ring", after the simple aromatic compound benzene, or a phenyl group when part of a larger compound (Fetzer, 2000) [14]. Are those chemical compounds that contain one or more rings with pi electrons delocalized all the way around them.

Polycyclic Aromatic Hydrocarbons (PAHs), containing two or more fused benzene rings, are one of the important classes of anthropogenic organic contaminants. Some of them have been identified as carcinogenic and mutagenic, PAHs are derived from petroleum based products (petrogenic PAHs), and also incomplete combustion of fossil fuels or biomass (pyrogenic PAHs). The members of POME group have performed analyses of PAHs in sediments of rivers, lakes, estuaries and coastal environments. For example, the distribution of PAHs and the degree of contribution of petrogenic and pyrogenic sources in each location in many coasts were revealed using the indices of the ratio of PAHs to their alkyl homology, e.g., methylphenanthrene to phenanthrene (MP/P) ratio (Burns and Salot, 1986, Chiou *et al.*, 1993) [3, 5] in addition, his group also identified the source of the oil included in tar balls found at coasts from the ratio of hopanes, a group of terpenes (El-Khaier, 1993) [9]. The PAHs assimilated by marine organisms can be passed upwards through food chain towards man, this will lead to

serious concern of human health (Chiou *et al.*, 1993) [5] Industrial activities in the coastal region of Libya is confined to large cities, like Tripoli, Misurata and Benghazi, with some centers of specialized industrial complexes (petrochemicals, oil and gas refineries, steel industry). The petrochemical complex in Abukammash (near Farwa Lagoon in the west) is a continuous risk to the marine environment, even if no pollution crises have been reported to date, (Saleh, 2012) [35].

Some of studies and their results carried on the determination of hydrocarbons along the world, (Masato., 2020) [24] studied the Toxicities of Polycyclic Aromatic Hydrocarbons for Aquatic Animals. Studies have revealed the toxicity of PAHs, including endocrine disruption and tissue-specific toxicity, although researchers have mainly focused on the carcinogenic toxicity of PAHs. We summarize the toxicity of PAHs regarding these aspects. Additionally, the bioaccumulation properties of PAHs for organisms, including invertebrates, are important factors when considering PAH toxicity. In this review, we describe the bioaccumulation properties of PAHs in aquatic animals. (Joana *et al.*, 2011) studied the challenges in assessing the toxic effects of polycyclic aromatic hydrocarbons to marine organisms: A case study on the acute toxicity of pyrene to the European seabass (*Dicentrarchus labrax* L.) the toxicity of readily degradable substances to marine fish, and stress the importance of taking into consideration the toxicity of environmental degradation products in addition to toxic effects of the parental substance and its metabolites for marine ecological risk assessment.

(Dhafar *et al.*, 2016) [8] determined Polycyclic Aromatic Hydrocarbons in some fishes from the Iraqi Marine Waters. It is showed that the PAHs origin in fishes were Pyrogenic and Petrogenic. Some of the studied samples showed high ability to accumulate PAHs compounds like Fluorene, Anthracene, Acenaphthene and Phenanthrene in muscles compared with other fishes, there was different ability of fish to accumulate the PAHs from surrounded environments. (Nyarko *et al.*, 2015) evaluated the Polycyclic Aromatic Hydrocarbons (PAHs) levels in two commercially important fish species from the coastal waters of Ghana and their carcinogenic Health Risks. The estimated carcinogenic potency equivalent concentrations exceeded the screening value for both species from all the study areas, indicating significant carcinogenic health risks associated with the consumption of these fishes. A further study of PAHs and other contaminants in seafood, landed on the coast of Ghana, is recommended in the light of recent discovery and drilling of oil in commercial quantities in Ghanaian coastal waters.

(Hager *et al.*, 2006) [17] Assessment of Polycyclic Aromatic Hydrocarbons Contamination in Water, Sediment and Fish of Tamsah Lake, Suez Canal, Egypt. Data show that these values are considered to be alarmingly high and the studied areas have values high enough to cause lethal toxicity effect to the surrounding environment and consequently to the human health. All samples are characterized by low concentration of low molecular weight PAHs compared to high molecular weight PAHs. Sediment and fish samples are characterized by relatively high concentration of six member rings PAHs. The origin of PAHs in collected samples is either petrogenic, biogenic or mixed petrogenic and biogenic. This study aims to estimate the contents of

hydrocarbon compounds in some fish type collected from Benghazi coast.

Materials and Methods

Description of the Studied Area:

Benghazi region is one of the most important center for fishery in Libya is a major seaport and the second-most populous city in the country, as well as the largest city in Cyrenaica. Benghazi has wonderful weather; Summers are hot and rainless, the studied area is extended from Benghazi city to Dyriana town.

Sampling and Preparation of Samples:

The (*Epinephelus marginatus*) fish was collected from Benghazi city coast and Daryanah town and kept in the frozen bags then transferred to the laboratory until extraction. Different tissues (muscle, gills and liver) were separated from the sample to determine the bioaccumulation the hydrocarbons in each part. *Epinephelus marginatus* has a relative large body, oval bodied and large headed fish with a wide mouth which has a protruding lower jaw. There are eleven spines and 13-16 soft rays in the dorsal fin. This species can grow up to 150 cm in standard length but is more often 90 cm (Pollard *et al.*, 2018) [30]. *Epinephelus marginatus* has two disjunction distribution centers, the main one is in the eastern Atlantic from the west coast of Iberia south along the western coast of Africa to the Cape of Good Hope, extending east into the south-western Indian Ocean, as far as southern Mozambique, with doubtful records from Madagascar and possibly Oman. It is found throughout the Mediterranean too.

Extraction Step of Hydrocarbons for Fish Sample:

Five grams of wet weight of every tissue (muscles, gills and liver) and sediments sample were treated with 30 g of anhydrous sodium sulfate and the mixture was blended at high speed for 5 min. Then the mixture was extracted using a soxhlet with 200 ml of methanol for 8 hrs. 20 ml of 0.7 M KOH and 30 ml of distilled water were added to the flask and the reflux was continued for 2hrs to saponify the lipids. The content of the extraction flask was extracted three times in a separating funnel with 80 ml hexane. The three extracts were combined, dried with anhydrous sodium sulfate and filtered through glass wool. The hexane fraction was concentrated with a rotary evaporator down to about 15 ml at 300C and concentrated down to a volume of 1 ml with nitrogen gas stream and then subjected to cleaning up and fractionation (El Nemr *et al.*, 2007) [11].

Aliphatic and Polycyclic Aromatic Hydrocarbons Analysis:

Aliphatic and PAHs analysis (The Polycyclic Aromatic Hydrocarbons), was carried out according to the techniques which given by (UNEP, 1992). The compounds were analysed by GC/MS, Identification of each compound in fish extract was made by comparing the retention times and its spectrum with that taken from HP memory and also with the EPA standard at the central lab of the marine science institute at (Alexandria, Egypt). The sample were analyzed for PAHs following different steps including; extraction, cleaning up and fractionation, instrumental analysis and analytical quality control. The obtained data was tabulated and statistically analyzed using statistical package (SPSS).

Results

The obtained results which recorded in this study are described as following:

Aliphatic Hydrocarbons:

For the *Epinephelus marginatus* Fish the concentrations of Aliphatic hydrocarbons in were illustrated in the Table 1 and Figures of (1-4).

Table 1: The concentration (µg/g) of aliphatic hydrocarbons in the *Epinephelus marginatus* fish measured by GC/MS

| Sample | <i>Epinephelus marginatus</i> fish | | | Average |
|--------|------------------------------------|---------|---------|----------|
| | Liver | Gills | Muscles | |
| C-9 | 651.09 | 406.69 | 918.08 | 658.62 |
| C-10 | 37.49 | 28.18 | 40.75 | 35.47333 |
| C-11 | 15.33 | 10.78 | 15.6 | 13.90333 |
| C-12 | 78.37 | 86.85 | 77.98 | 81.06667 |
| C-13 | 24.22 | 13.08 | 31.46 | 22.92 |
| C-14 | 89.18 | 107.36 | 97.44 | 97.99333 |
| C-15 | 138.99 | 128.89 | 410.6 | 226.16 |
| C-16 | 66.81 | 84.88 | 90.67 | 80.78667 |
| C-17 | 88.23 | 102.91 | 347.24 | 179.46 |
| C-18 | 38.15 | 41.11 | 41.89 | 40.38333 |
| C-19 | 10.22 | 10.87 | 20.64 | 13.91 |
| C-20 | 8.2 | 20.68 | 19.97 | 16.28333 |
| Total | 1246.28 | 1042.28 | 2112.32 | 1466.96 |

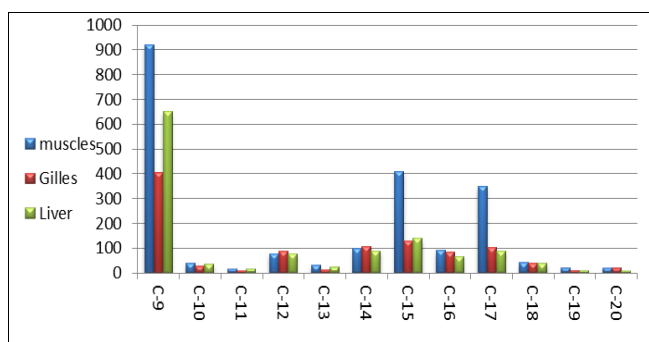


Fig 1: Distribution the concentrations (µg/g) of aliphatic hydrocarbons of *Epinephelus marginatus* fish

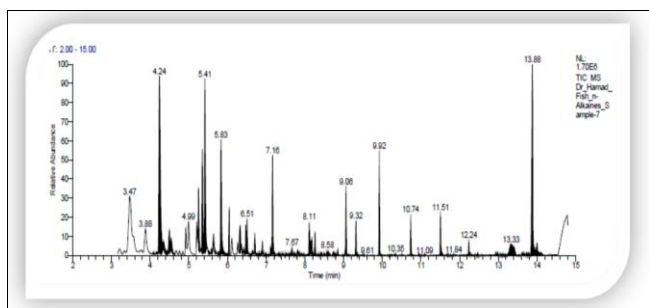


Fig 2: The GC mass analysis for liver sample of *Epinephelus marginatus* fish

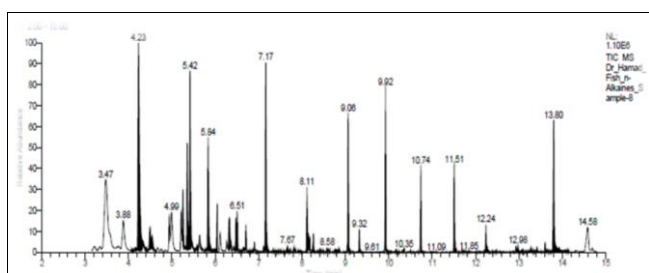


Fig 3: The GC mass analysis for Gills sample of *Epinephelus marginatus* fish

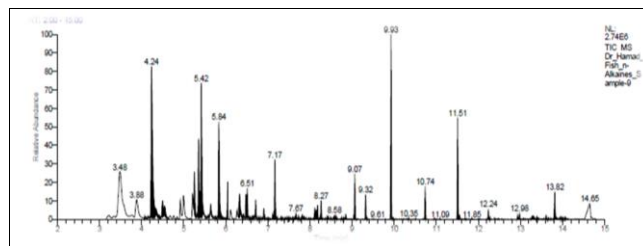


Fig 4: The GC mass analysis for muscle sample of *Epinephelus marginatus* fish

Table 2: Correlation coefficients between tissues in the *Epinephelus marginatus* fish

| | Muscles | Liver | Gilles |
|--------|---------|--------|--------|
| Muscle | 1 | | |
| Liver | 0.5643 | 1 | |
| Gilles | 0.5742 | 0.8954 | 1 |

Aromatic Hydrocarbons:

Table 3: The concentration (µg/g) of poly aromatic hydrocarbons in the *Epinephelus marginatus* fish measured by GC/MS

| Sample | <i>Epinephelus marginatus</i> fish | | | Average |
|------------------------|------------------------------------|---------|---------|----------|
| | Liver | Gills | Muscles | |
| Benzo(g,h,i)perylene | N/A | N/A | N/A | |
| Acenaphthylene | 0.32628 | 0.32632 | 0.32583 | 0.326143 |
| Fluorene | 0.30798 | 0.30865 | 0.30454 | 0.307057 |
| Phenanthrene | 0.52629 | 0.52992 | 0.52707 | 0.52776 |
| Anthracene | 0.42188 | 0.42279 | 0.42126 | 0.421977 |
| Pyrene | 0.11164 | 0.11318 | 0.11285 | 0.112557 |
| Benzo(a)anthracene | 0.71605 | 0.71604 | 0.71604 | 0.716043 |
| Chrysene | 0.49672 | 0.49751 | 0.50422 | 0.499483 |
| Benzo(b)fluoranthene | 0.36351 | 0.35732 | 0.35733 | 0.359387 |
| Benzo(k)fluoranthene | 0.35731 | 0.3635 | 0.36703 | 0.362613 |
| Benzo(a)Pyrene | 0.69937 | 0.70031 | 0.70215 | 0.70061 |
| Indeno(1,2,3-cd)pyrene | 3.66368 | 3.66373 | 3.66367 | 3.663693 |
| Dibenzo(a,b)anthracene | 0.20538 | 0.20541 | 0.20533 | 0.205373 |
| Total | 8.19609 | 8.20468 | 8.20732 | 8.202697 |

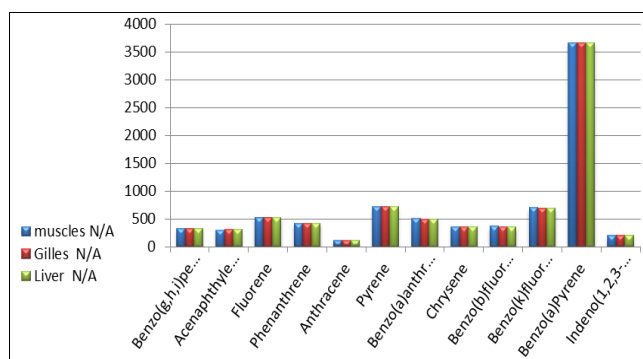


Fig 5: Distribution the concentrations (µg/g) of poly aromatic hydrocarbons of *Epinephelus marginatus* fish

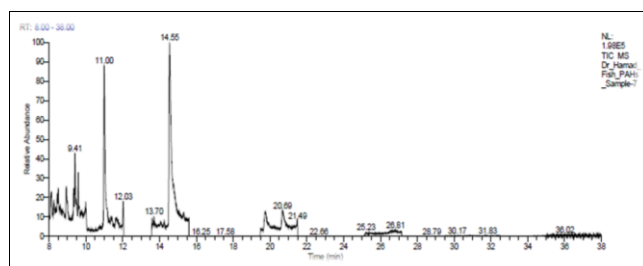


Fig 6: The GC mass analysis for liver sample of *Epinephelus marginatus* fish

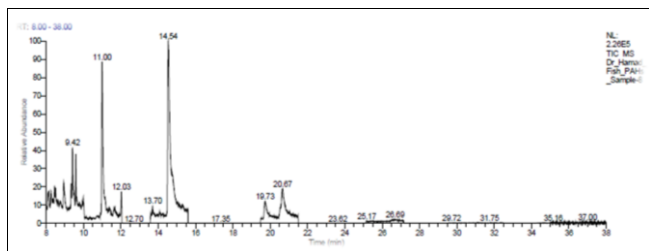


Fig 7: The GC mass analysis for Gills sample of *Epinephelus marginatus* fish

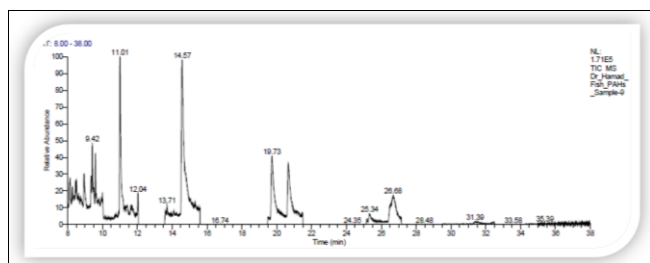


Fig 8: The GC mass analysis for muscle sample of *Epinephelus marginatus* fish

Table 4: Correlation coefficients between tissues in the *Epinephelus marginatus* fish

| | Muscles | Liver | Gilles |
|--------|---------|--------|--------|
| Muscle | 1 | | |
| Liver | 0.9609 | 1 | |
| Gilles | 0.9582 | 0.9726 | 1 |

Table 5: Total poly aromatic compounds in *Sphyaena sphyaena* fish

| | Liver | Gills | Muscles |
|--------------------------|---------|---------|---------|
| <i>Sphyaena sphyaena</i> | 0.81933 | 0.82058 | 0.82029 |

Table 6: The calculated sources ratios for fish samples

| Spices | Ratios | | | | | |
|--------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| | R ₁ | R ₂ | R ₃ | R ₄ | R ₅ | R ₆ |
| <i>Sphyaena sphyaena</i> | 1 | 0.7328 | 0.5847 | 0.5549 | 0.4985 | 0.4450 |

Table 7: HMW/LMW ratio of fish samples

| Spices | Ratios | |
|--------------------------|---------|--|
| | HMW/LMW | |
| <i>Sphyaena sphyaena</i> | 4.172 | |

Table 8: Carcinogenic compounds in the studied samples

| Species | Carcinogenic compounds |
|--------------------------|------------------------|
| <i>Sphyaena sphyaena</i> | 5.52234 |

Table 9: Sum of some ratio carcinogenic compounds in the studied species

| Species | Carcinogenic compounds | | | |
|--------------------------|------------------------|-------------|----------|----------|
| <i>Sphyaena sphyaena</i> | 5.52234 | | | |
| Species | Σ COMB | Σ COMB/PAHs | Fluo/pyr | Phe/Anth |
| <i>Sphyaena sphyaena</i> | 20.1526 | 0.8191 | 2.7438 | 1.2466 |

Discussion

Aliphatic Hydrocarbons:

The concentrations of aliphatic hydrocarbons in the fish sample are shown in the Tables of (1) and represented in Figures of (1 to 4). The results showed that the types and the n-alkanes compounds containing the aliphatic hydrocarbons

of C₉ to C₂₀. The concentrations were fluctuated between (8.2-918.08 μg/g) in the fish sample (*Epinephelus marginat*). This value is lower than those data which recorded in some Alexandria organism (451-1148 μg/g), also lower than the values of (El-Sikaily *et al.*; 2002) [12] (180 μg/g) at some Mediterranean coasts and in harmony with results of (Neussrey, 2013) study (100 μg/g), and lower than data of at Eastern Harbor of Alexandria (1782 μg/g) (Emara and shridah, 2008) [13]. The high concentrations of the detected aliphatic hydrocarbons was (1975.86 μg/g) is related to C₉ compound in the fish and. This was less than the recorded levels for clean urban sites in Scotland, UK; with an average value of 3003 μg/g (wet weight). (Mackie *et al.*, 1980) [23]. Correlation coefficients between different types of tissues revealed that gills present positively high relation with muscle and liver in fish except *Epinephelus marginatus* fish with present low correlation is form the gills, it is also the fish are suffered about the same effect of aliphatic hydrocarbons. Generally, the high levels of aliphatic are higher aromatic compounds in fish.

Poly Aromatic Hydrocarbons (PAHs):

The concentrations of speciation's and Σ PAHs in the studied fish sample is shown in Tables of (4) and represented in Figures of (5 to 8), the results showed variations between the contents of the PAHs in the studied fish organism:

The poly aromatic hydrocarbons which obtained and detected by GC-mass instrument in this study including: (Benzo(g, h, i) perylene Acenaphthylene, Fluorene, Phenanthrene, Anthracene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)Pyrene, Indeno (1,2,3-cd) pyrene, Dibenzo (a, b) anthracene.

The concentrations of PAHs in the fish organisms were fluctuated between: (0.112-3.66367 μg/g) in liver, gills, muscles, the high concentrations were recorded in the gills tissue in fish sample (*Epinephelus marginatus*) was (3.6637 μg/g), the maximum value of ΣPAHs 10.9911 μg/g was recorded for compound *Indeno (1, 2, 3-cd) pyrene* in the sample. In general, the ΣPAHs contents which recorded in this study were lower than that recorded for the Alexandria coast (Egypt), 61.87 μg/g and those recorded for *Derna* coasts, (Neussery, 2013) [28], Also lower than that results recorded in the marin organism and Algae samples at Abu-Qir harbor (6140 μg/g), (Emara and Shridah, 2008) [13], also lower than (7780 μg/g) value at some spain coasts.

The joint FAO/WHO Expert Committee on food additives (Simko *et al.*, 2004) [38], has developed a notification that concentration of Bap should be not exceed the limit of 10 μg/g in fish tissue for human consumer. This value is higher than those values which obtained in present study i.e., ranged between (0.6993-0.7004 μg/g) for fish sample. The concentrations of carcinogenic Benz (a) pyrene was also low inl studied fish sample. This value are much below the average values of 5.59 and 0.86 μg/g obtained from the Gulf of Suez and Gulf of Aqaba (Said and Hamed, 2006) [34].

If the ratio of (flu/pyr) < 1 was attributed petrogenic sources and if the ratio of (flu/pyr) > 1 is related to pyrolytic origin (Sicre *et al.*, 1997) [37], Combustion of coal and wood gave (Fluo/pyr) ratios of 0.013 and 0.042, respectively. While crude oil and fuel oil had values of 0.6-0.9 (Gschwend and Bites 1981) [16], In the present study, all sites had (Fluo/Pyr), ratio < 1 reflecting petrogenic origin. The ratio of major

combustion specific compounds $\sum\text{COMB}$ = (Flu, Pyr, BaA, Chr, BbF, BkF, BaP, InP and BghiP) to the sum of total PAHs ($\sum\text{COMB}/\sum\text{PAHs}$) were (0.8191 $\mu\text{g/g}$) of the fishes (*Epinephelus marginatus*), in study and the $\sum\text{COMB}$ concentrations displayed values (20.1643 $\mu\text{g/g}$), for (*Epinephelus marginatu*). Benzo (a) pyrene (BaP), the most potent carcinogenic PAHs, and the sum of six carcinogenic PAHs; ($\sum\text{PAHs}_{\text{CARC}}$, ($\sum\text{PAHs}_{\text{CARC}}$ = Flu, Pyr, BbF, BkF, BaP and BghiP) for the studied species, was given in (Table 9). It was stated that the physical and chemical properties of some PAHs, like chemical reactivity (photo-oxidation and oxidation) can contribute to modify the original distribution pattern of emission sources (Butler and crossley, 1981)^[4]. In marine ecosystems, PAHs can undergo degradation by photo-oxidation in superficial water layer. The concentrations of carcinogenic Benz(a)pyrene was also high in studied fish with an average value of 0.62 $\mu\text{g/g}$, this value are much the Gulf of Suez and Gulf of Aqaba (said and hammed 2006). If the ratio of (flu/pyr) < 1 was attributed to petrogenic sources and if the ratio of (flu/pyr) > 1 is related to pyrolytic origin (Sicre *et al.*, 1997)^[37], Combustion of coal and wood gave (Fluo/pyr) ratios of 2.2791 and 2.7438, respectively. While crude oil and fuel oil had values of 0.6-0.9 (Gschwend and Bites 1981)^[16], In the present study, all sites had (Fluo/Pyr), ratio < 1 reflecting petrogenic origin. The ratio of major combustion specific compounds $\sum\text{COMB}$ = (Flu, Pyr, BaA, Chr, BbF, BkF, BaP, InP and BghiP) to the sum of total PAHs ($\sum\text{COMB}/\sum\text{PAHs}$) was (0.8191) of the Algae (*Epinephelus marginat*), in study and the $\sum\text{COMB}$ concentrations displayed values (20.1643 $\mu\text{g/g}$), for (*Epinephelus marginatu*).

The estimated diagnostic isomeric ratios of PAHs should be cautiously used due to the interaction of physical and biogeochemical processes which may alter PAH profiles during processes of transport and flux. The fingerprints of PAHs from pyrolytic or petrogenic origin may be used to differentiate these origins using molecular indices based on ratios of selected isomers of PAH concentrations (Colombo *et al.*, 1989)^[6]. The present study suggesting that the values of PAHs were 24.6015 to 24.6118 $\mu\text{g/g}$ is to some extent safe and will have weak, but not harmful, effect on sediment marine. this is in accordance with reported conclusion (Mazmanidi *et al.*, 1976)^[25] who stated that the total hydrocarbon concentration in seawater which can produce a harmful effect on the aquatic organisms is about 50 $\mu / 1$, the present investigation briefly concludes that the sources of PAHs in the studied area are mainly from incomplete combustion at high temperatures of recent and fossil organic matter Pyrolytic origin with little evidence of petrogenic origins. Atmospheric deposition, industrial discharges and land runoff water are the main factors responsible for pyrolytic PAHs.

Various PAHs concentration diagnostic ratios have been used to identify and quantify the contribution of each source of pollution to the specified environmental regions (Krom *et al.*, 1989)^[21], Fluoranthene/pyrene (Fluo/Pyr) ratio indicated the origin of PAHs found that the high partitioning of PAHs to sedimentary organic matter was mainly due to the significant aromatic fraction of the organic matter, they considered the sedimentary organic matter as a natural "heterogeneous polymer" where PAHs interact more favorably with the aromatic regions (Hwang *et al.*, 2003)^[18]. If the ratio of (flu/pyr) < 1 was attributed petrogenic sources and if the ratio of (flu/pyr) > 1 is related to pyrolytic origin

(Sicre *et al.*, 1997)^[37], Combustion of coal and wood gave (Fluo/pyr) ratios of 2.2791 to 2.7438 $\mu\text{g/g}$, respectively. While crude oil and fuel oil had values of 0.6-0.9 (Gschwend and Bites 1981)^[16], In the present study, all sites had (Fluo/Pyr), ratio < 1 reflecting petrogenic origin.

The ratio of major combustion specific compounds $\sum\text{COMB}$ = (Flu, Pyr, BaA, Chr, BbF, BkF, BaP, InP and BghiP) to the sum of total PAHs ($\sum\text{COMB}/\sum\text{PAHs}$) were (0.8195) of the fish sample (*Epinephelus margin*), in the study Area and the $\sum\text{COMB}$ concentrations displayed values 20.164 for (*Epinephelus marginatu*). The sum six carcinogenic PAHs; $\sum\text{PAHs}_{\text{CARC}}$ (IARC, 1983)^[19] ($\sum\text{PAHs}_{\text{CARC}}$ = Flu, BaA, BbF, BkF, BaP, and BghiP) were varied fish sample with concentration 5.5266 for studied sample, Table (9).

The Low Molecular Weight/High Molecular Weight Ratio:

Table (6) showing the ratio of low molecular weight / high molecular weight compound (Lipiatou *et al.*, 1997)^[22], suggesting that the low molecular weight represent by polyaromatic hydrocarbons compound that have (2-3) rings and high molecular weight represent by polyaromatic hydrocarbons compound that have (4-5) rings. The ratio of (LMW/HMW) (Naphthalene + Acenaphthylene + Acenaphthene + Fluorene + Phenanthrene + Anthracene / Fluoranthene+ Pyrene + Benzo(a)anthracene + Chrysene + Benzo(b)fluoranthene + Benzo(k)fluoranthene + Benzo(a)pyrene + Dibenzo(a,h)anthracene + Benzo(ghi)Perylene + ndeno(1,2,3-cd)Pyrene), This ratio in all station for fish sample was > 1 that indicate to the source of TPAHs related to ptrogenic origin (Garrigueset *et al.*, 1993) Correlation coefficients between individual PAHs concentrations in fish (*Epinephelus marginatu*) was found to positively high relation ($r > 0.50$) for most of the origin components.

Correlation coefficients between different types of tissues revealed that gills present positively high relation with muscles and liver in of fish as given in Table (3.23), indicating that the initial source of contamination came from the gills which naturally receive of contamination passed through it and then accumulated intensively in liver. also noted that *Epinephelus marginatus* fish are low contaminated.

The difficulty in the identifying of PAHs origins come from the possible coexistence of many contamination sources, and the information processes that PAHs can undergo in the air, water or wastes before deposition in the analyzed sediments. Some compounds could exhibit comparable evolution kinetics that could be used to identify the origin of organic matter in the environment (Colombo *et al.*, 1989)^[6]. The distributions of aromatic compound differ according to the sources of production (Port and Al Baiges, 1993), and, chemical composition and temperature combustion of the organic matter (Neff, 1979)^[27]. Pyrene. Phenanthrene and benzo (b) fluoranthene are components of fossil fuels and a portion of them is associated with their combustion. Benzo (a) pyrene is usually emitted from catalyst and non-catalyst and automobiles. Benzo (a) anthracene and chrysene are often resulted from combustion of both diesel and natural gas (Rogge *et al.*, 1993)^[33].

The sources of PAHs can also be identified by the ratios of individual PAH compounds-based oil peculiarities in PAH composition and distribution pattern as a function of the emission source (Gschwend and Bites. 1981; Colombo *et*

al., 1989)^[16, 6], If the source is from fuel-combustion it named pyrolytic and if it is from crude oil it named petrogenic contamination. Ratio values such as phenanthrene/anthracene (Phe/Ant) and fluoranthrene/pyrene (Flu/Pyr) had been used by previous workers (Soclo *et al.*, 2000^[40]; El Sikaily *et al.*, 2002^[12]; El Nemr *et al.*, 2003^[10], El Nemr *et al.*, 2007^[11] and 2008), Petroleum often contains more phenanthrene relative to anthracene as phenanthrene that is more a thermodynamically stable tricyclic aromatic isomer than anthracene. So, a Phe/Ant ratio is observed to be very high in PAH petrogenic pollution, but low ratio in pyrolytic contamination cases (Gschwend and Bites, 1981; Soclo *et al.*, 2000; Yang 2000)^[16, 40, 42].

Crude oil had a Phe/Ant ratio of around 50, and motor vehicle exhaust had a ratio of around four (Yang, 2008), Low Phe/Ant ratio values (less than 10) indicated the major PAH input was from combustion of fossil fuel (Gschwend and Bites, 1981; Colombo *et al.*, 1989)^[16, 6], (Budzinski *et al.*, 1997)^[2] suggested that sediments with Phe/Ant > 10 were mainly contaminated by petrogenic inputs and Phe/Ant < 10 was typical of pyrolytic sources. In the present study, the ratio of individual PAHs compounds varied between fish sample indicating that the sources of PAHs contamination might be different. (Table 6) However the different Phe/Ant ratio values might be related to weathering such as photo-degradation, chemical degradation or biodegradation and also the composition for fish, The (Flu/pyr), can indicated the origin of PAH since (Sicre *et al.*, 1987), In the present study, the samples had (Flu/Pyr), ratio values more than 1 However, the results obtained from the Phe/Ant and Flu/Pyr ratios, indicated a mixed pattern of petrogenic and pyrolytic contamination at most tissue samples. Other six diagnostic ratios between individual PAHs concentrations were calculated and used to make the identification of the PAHs origin more precisely:

R-1 = $\text{InP} / (\text{InP} + \text{BghiP})$; R-2 = $\text{Flu} / (\text{Flu} + \text{Pyr})$; R-3 = $\text{BaP} / (\text{BaP} + \text{Chr})$; R-4 = $\text{Phe} / (\text{Phe} + \text{Ant})$, R-5 = BbF / BkF and R-6 = $\text{Ant} / (\text{Ant} + \text{Phe})$ (Mandalakis *et al.*, 2002; Guo *et al.*, 2002; Hwang *et al.*, 2003^[18] and El Nemr *et al.*; 2008).

R-1 ratio is less than 0.4 for petroleum and > 0.5 for petroleum/combustion mixture. Literature values of R-1 ratio are above 0.5 for grass combustion, wood soot and creosote (Yunker *et al.*, 2002)^[43], R-3 ratio values are less than 0.2 for combustion and between 0.6 and 0.9 for petroleum while ratios between 0.1 and 0.6 are due to petroleum/combustion mixture. R-6 ratio < 0.1 usually is taken as an indication of petroleum while a ratio > 0.1 indicates a dominance of combustion (Budzinski *et al.*, 1997)^[2], R-6 ratios > 0.1 represent the combustion of diesel oil, shale oil, coal and some crude oil samples. R-2 ratio of 0.4 is usually defined as the petroleum. while ratio or 0.5 is for petroleum/combustion transition point (Budzinski *et al.*, 1997)^[2], however, this boundary appears to be less definitive than 0.1 for R-6.

Generally, R-2 ratio is below 0.4 for most petroleum samples and above 0.5 in kerosene, grass, most coal and wood combustion samples and creosote, but ratios between 0.4 and 0.5 is characteristic for liquid fossil fuel such as gasoline, diesel, fuel oil and crude oil Combustion find emissions from cars and diesel trucks (Tsapaksi *et al* 2003). When applying the results obtained from the above ratios, (Tables 6) gave three groups indicating three sources; the first represents the samples of petrogenic sources, the

second group represents the stations of pyrolytic sources (most ratios in the studied samples) and the third group represents the stations of a mixture of petrogenic and pyrolytic source (Yang, 2000)^[42].

References

1. Baumard P, Budzinski H, Garrigues P. Polycyclic aromatic hydrocarbons in sediments and mussels of the western Mediterranean Sea. *Environmental Toxicology and Chemistry*. 1998; 17:765-776.
2. Budzinski I, Jones J, Bellocq J, Pierard C, Garrigues P. Evaluation of sediment contamination by polycyclic aromatic hydrocarbon in the Gironde estuary, Mar. Chem. 1997; 58:85-97.
3. Burns KA, Saliot A. Petroleum hydrocarbons in Mediterranean Sea: a mass balance. *Marine Chemistry*. 1986; 20:41-157.
4. Butler JD, Crossley F. Reactivity of polycyclic aromatic hydrocarbons adsorbed on soot particles. *Atmosphere Environment*. 1981; 15:91-94.
5. Chiou CT, McGroddy SE, Kile DE. Partition characteristics of polycyclic aromatic hydrocarbons on soils and sediments', *Environ. Sci. Technical*. 1993; 32:264-269.
6. Colombo JC, Pelletier F, Bronchu C, Khalil M, Cataggio JA. Determination of hydrocarbon sources using n-alkanes and polyaromatic distribution indices. Case study: Rio de la Plata estuary, Argentina. *Environmental Technology*. 1989; 23:888-894.
7. De Morais L, Smith-Vaniz WF, Kara. *Sphyræna sphyræna*. The IUCN Red List of Threatened Species, 2015.
8. Dhafar DH, AL-Khion, Balqees Al-Ali S, Ahmad MZ. Polycyclic Aromatic Hydrocarbons in Some Fishes from the Iraqi Marine Waters *Journal of Natural Sciences Research* www.iiste.org, 2016.
9. El-Khaier EM. Chemical studies on the Mediterranean coastal waters in the front of the Rosetta mouth of the Nile, M.Sc., thesis, Faculty of science. Alexandria university, 1993.
10. El Nemr A, Abd-Allah AMA. Contamination of polycyclic aromatic hydrocarbons (PAHs) in micro layer and subsurface waters along Alexandria coast, Egypt, *Chemosphere*. 2003; 52:1711-1716.
11. El Nemr A, Abd-Allah AMA. Contamination of polycyclic aromatic hydrocarbons (PAHs) in microlayer and subsurface waters along Alexandria coast, Egypt, *Chemosphere*. 2007; 52:1711-1716.
12. El Sikaily A, Khaled, El Nemr A, Said TO, Abd-Allah AMA. Determination of hydrocarbons in Bivalves from the Egyptian Mediterranean coast. *Med. Mar. Sci*. 2002; 312:121-131.
13. Emara HI, Shridah MA. Some heavy metals distribution in the Eastern Harbour and El-mex waters of Alexandria. *Symposium of marin chemistry in Arab Region*. Egypt, 2008.
14. Fetzer JC. *The Chemistry and Analysis of the Large Polycyclic Aromatic Hydrocarbons*. Polycyclic Aromatic Compounds. New York: Wiley. 2000; 27(2):p143.
15. Froese, Rainer, Pauly, Daniel. *Epinephelus marginatus* in FishBase. December 2019 version, 2019.
16. Gschwend PM, Bites RA. Fluxes of polycyclic aromatic hydrocarbons to marine and lacustrine sediments in the

- northeastern United States. *Geochimica et Cosmochimica Acta*. 1981; 45:2359-2367.
17. Hager R, Ali N, EL-Gendy SH, Roushdy MN. Assessment of Polycyclic Aromatic Hydrocarbons Contamination in Water, Sediment and Fish of Temsah Lake, Suez Canal, Egypt, 2006.
 18. Hwang HM, Wade TL, Sericano JL. Concentrations and source characterization of polycyclic aromatic hydrocarbons in pine needles from Korea, Mexico, and United States', *Atmosph. Environ.* 2003; 37:2259-2267.
 19. IARC. (International Agency of research on cancer), IARC monographs on the evaluation of the carcinogenic risk of chemicals to human. Poly nuclear aromatic hydrocarbons. Part I, chemical. Environmental. And experimental data. Agency for research on cancer, Lyons. 1983; 32:1-477.
 20. Jakov, Dulčić, Alen S. On the occurrence of the Yellowmouth Barracuda *Sphraena viridensis* Cuvier 1829 (Pisces: Sphraenidae) in the Adriatic Sea (PDF). *Annales, Series Historia Naturalis*. 2004; 14(2):225-227.
 21. Krom MD, Erez J, Parter CB, Eliner S. Phytoplankton nutrient uptake dynamics in earthen marine fish ponds under winter and summer conditions. *Aquaculture*. 1989; 76:237-253.
 22. Lipiatou E, Tolosa I, Simo R, Bouloubassi I, Dachs J, Marti S, *et al.* Mass budget and dynamics of polycyclic aromatic hydrocarbon in the Mediterranean Sea. *Deep-Sea Research Part II*. 1997; 44:881-905.
 23. Mackie PR, Hardy RKJ, Bruce C, McGil AS. Tissue hydrocarbon burden of muscles from various sites around the Scottish Coast, in A. Bjorseth and AJ Dunnis (eds). *Polycyclic aromatic hydrocarbons chemistry and biological effects*. Columbus, Ohio, Battelle Press, 1980, 379-393.
 24. Masato H, Nobuo S. Toxicities of Polycyclic Aromatic Hydrocarbons for Aquatic Animals, *International Journal of Environmental Research and Public Health*, 2020.
 25. Mazmanidi ND, Kovaleka GI, Kotov AM, Bazhashvili TR, Diasamadizi NM, Zambakhidzi NP. The effect of petroleum products on The Black Sea hydrobiology. *Marine Pollution Bulletin*. 1976; 6:201-208.
 26. Morrison RT, Boyd RN. *Organic Chemistry* (6th ed.) New Jersey: Prentice Hall, 1992. ISBN 978-0-13-643669-0.
 27. Neff JM. *Polycyclic aromatic hydrocarbons sources fates and biological effects*. London: Applied Science, 1979.
 28. Neussery MM. Study the concentrations of aliphatic and aromatic hydrocarbons in some fishes tissues collected from Derna coast city (Libya), M.Sc. Thesis Libyan academy of post graduate studies, Libya, 2013.
 29. Nyarko BO, Botwe, Klubi E. Department of Oceanography & Fisheries, University of Ghana, P. O. Box LG 99, Legon, Accra, Ghana *West African Journal of Applied Ecology*. 2011; 19.
 30. Pollard DA, Afonso P, Bertoincini AA, Fennessy S, Francour P, Barreiros J. *Epinephelus marginatus*. IUCN Red List of Threatened Species, 2018. e.T7859A100467602.
 31. Porte C, Albaigés J. Bioaccumulation patterns of hydrocarbons and polychlorinated biphenyls in bivalves, crustacean and fishes. *Archives of Environment Contamination and Toxicology*. 1993; 26:273-281.
 32. Ravindra K, Sokhi R, Van Grieken R. Atmospheric polycyclic aromatic hydrocarbons: Source attribution, emission factors and regulation. *Atmospheric Environment*. 2008; 42(13):2895-2921.
 33. Rogge WE, Hildemann L, Mazurek MA, Cass GR, Simoneit BRT. Sources of fine organic aerosol: Noncatalyst and catalyst-equipped automobiles and heavy-duty diesel trucks. *Environmental science and Technology*. 1993; 27:636-651.
 34. Saed, Hamed MA. Mobility of polycyclic aromatic hydrocarbons in water of the Egyptian Red Sea coasts. *Bulletin Environmental Contamination Toxicology*. 2006; 77:126-136.
 35. Saleh FS. Heavy metals distribution in fishes samples at some Derna coast regions. Higher academy of post graduate studies. Benghazi. Libya, 2012.
 36. Sephton MA, Hazen RM. On the Origins of Deep Hydrocarbons. *Reviews in Mineralogy and Geochemistry*. 2013; 75(1):449-465. Bibcode: 2013RvMG.75.449S.
 37. Sicre MA, Bayona JM, Grimault JO, Saliot A, Albaiges J. Mass balance and dynamics of polycyclic aromatic hydrocarbons in the Mediterranean Sea. *Deep-Sea Res.* 1997; 44:881-905.
 38. Simko P, Anyakora C, Ogheche A, Palmer P, Coke H. Determination of poly nuclear aromatic hydrocarbons in marine samples of Siokola fishing settlement *J Chrom.* 2004; 2:21-28.
 39. Smith Mi B, March, Jerry. *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure* (6th ed.), New York: Wiley-Inter science, 2007, p23. ISBN 978-0-471-72091-1.
 40. Soclo HH, Garrigues PH, Ewald M. Origin of marine polycyclic aromatic hydrocarbons (PAHs) in coastal sediments: Case studies in Cotonou (Benin) and Aquitaine France) areas. *Marine Pollution Bulletin*. 2000; 40:387-396.
 41. Tsapakis M, Stephanou E, Karakassis I. Evaluation of atmospheric transport as a non point source of polycyclic aromatic hydrocarbons in marine sediments of the eastern Mediterranean, *Mar. Chem.* 2003; 80:283-298.
 42. Yang GP. Polycyclic aromatic hydrocarbons in sediment of South China Sea, *Environ Pollut.* 2000; 108:163-171.
 43. Yunker MB, McDonald RW, Vingarzan R, Mitchell R, H, Goyette D, Sylvestre S. PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. *Organic Geochemistry*. 2002; 33:489-515.