

First assessment of Polycyclic Aromatic Hydrocarbons contamination and associated human health risk in Mullet (*Liza aurata*) from Tunisia: case of Bizerte and Ghar El Melh Lagoons

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Abstract

Data about the presence of polycyclic aromatic hydrocarbons in biota from Tunisian coastal ecosystems is scarce. To our knowledge, this is the first work to report these chemicals in the biota of Ghar El Melh Lagoon. In *Liza aurata* muscle from Bizerte and Ghar El Melh Lagoons (Tunisia), levels of 15 priority polycyclic aromatic hydrocarbons were measured.

Total PAH levels in mullet from Bizerte and Ghar El Melh Lagoons were comparable to or lower than those found in other fish specimens from around the globe. The nature of the detected chemicals reveals petrogenic and pyrolytic anthropogenic contamination in both studied lagoons.

Threat to human health caused by mullet consumption was assessed and it was observed that PAH intakes don't threaten to local population's health.

Full Text

Coastal ecosystems, which are a source of remarkable goods and services, face advanced demographic pressure (Ben Othman et al. 2013) resulting in increased contamination by metallic, organic and inorganic pollutants. One of the main environmental risk factor for human health is pollution of aquatic ecosystems (Jafarabadi et al. 2019). Among the organic pollutants having impacts on marine ecosystems are Polycyclic Aromatic hydrocarbons (PAHs) (Ben Othman et al. 2013). Polycyclic Aromatic Hydrocarbons are a family of semi-volatile chemicals that include at least two bonded benzene rings in a variety of dispositions and are mostly derived from petrogenic, biogenic, and pyrogenic origins (Jafarabadi et al. 2019). In the environment, more than 100 PAH congeners (which include parent PAHs and alkylated derivatives) have been reported. Among them, 16 PAHs have been listed as priority hazardous pollutants (Jafarabadi et al. 2019), according to the International Agency for Research on Cancer, chrysene, benzo[a]anthracene, dibenzo[a,h]anthracene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[g,h,i]perylene, benzo[b]fluoranthene, and indeno[1,2,3-cd]pyrene are all possibly carcinogenic to humans (Jafarabadi et al. 2019). Petrogenic sources, such as polycyclic aromatic hydrocarbons related to oil spillage and pyrogenic sources, which include PAHs originating from fossil fuel burning, biomass burning, waste incineration, and asphalt processing, are the two most common origins of PAHs in the environment. Due to their stability, long distance transportation and bioaccumulation characteristics, these pollutants are widely dispersed in all parts of the environment, such as the air, water, sediments, as well as biological tissues (Habibullah-Al-Mamun et al. 2019). PAHs can be introduced into the environment through a variety of anthropogenic causes, such as incomplete wood, coal, or petroleum benzine burning, ship loading/unloading, wastewater sludge, municipal effluent release, runoff, and atmospheric rainfall (Jafarabadi et al. 2019).

PAHs are widespread in marine environments like estuaries, coastal zones, and the deep sea because of human activities and their relative long half-life (Habibullah-Al-Mamun et al. 2019). PAHs can reach the aquatic ecosystems in a variety of manners, such as navigation and fisheries, shipbuilding and shipbreaking, manufactory and urban effluent pollution, drainage from the land and rivers, atmospheric deposition, petrochemical spillage etc. PAH pollution in the environment has become a major focus of interest around the world because of their carcinogenic and mutagenic effects (Habibullah-Al-Mamun et al. 2019).

Fish consumption has risen around the world since it provides dietary protein low in minerals, cholesterol, polyunsaturated fatty acids (PUFAs), and vitamins, especially omega-3 PUFAs (Ramalhosa et al. 2012; Iwegbue et al. 2015; Effiong et al. 2016). It has been shown that Omega-3 PUFAs have a primordial role in risk minimization of neurological problems and heart disease (Iwegbue et al. 2015; Effiong et al. 2016). Musaiger and D'Souza (2008) reported that they can diminish the possibility of renal cell carcinoma for women and prostate cancer for men, and also Alzheimer's disease and dementia. Pregnancy results can be improved with omega-3 PUFAs, like less preterm and decreased preterm and low-birth-weight births (Iwegbue et al. 2015). Although the possible positive effects of dietary fish consumption, there is a problem associated with regular fish consumption which is the possibility of hazardous exposure to substances, like metals, PAHs, pesticides, PCBs, PBDEs, and other chemicals, which fish may accumulate in their tissues (Iwegbue et al. 2015). Consequently, the degree of PAH pollution in seafood mainly consumed by humans, especially, must be investigated on a priority basis.

The Lagoon of Bizerte from the northern region of Tunisia is an environmentally important area (Ben Ameer et al. 2021). However, this ecosystem is contaminated directly and indirectly through municipal and industrial wastes release and runoff by numerous harmful compounds in abiotic and biotic matrices like Organochlorine pesticides (Ameer et al. 2013, 2012), organohalogen chemicals as polychlorinated biphenyls (Ben Ameer et al. 2012, 2013; Barhoumi et al. 2014a,b; Ben Ameer et al. 2021), PAHs (Ben Ameer et al. 2012; Barhoumi et al. 2016; Trabelsi and Driss, 2005), Polybrominated diphenyl ethers and their methoxylated analogs (Barhoumi et al. 2014a,b; Barhoumi et al. 2016; El Megdiche et al. 2017; Ben Ameer et al. 2021), emerging halogenated flame retardants and halogenated norbornenes (Mekni et al. 2019) and heavy metals (Ben Garali et al. 2010).

The Lagoon of Ghar El Melh is an aquatic ecosystem in northern Tunisia that has ecologic and economic importance. It is a Ramsar wetland with various dwellings for migrating fish and birds (Dhib et al. 2017). Indeed, this ecosystem is subjected to stresses, in fact the anthropogenic activities (releases of municipal and industrial wastewater, releases of the drainage system and the fishing operations) (Moussa et al. 2005), led to the decline of its biodiversity and particularly, the decrease of fishery resources, contributing to a decrease in fishing income (Moussa & Ben Khemis, 2005; Béjaoui et al. 2018). In fact, previous works have reported that this water body is influenced by contamination with heavy metals and organic pollutants (Chouba et al. 2007; Ben Ameer et al. 2011; Louiz et al. 2018; Oueslati et al. 2018; Zakhama-Sraieb et al. 2019).

In spite of the presence of all these possible pollutants, recent informations on the existence of PAHs in biota from Tunisian coastal regions are very limited, only three surveys were conducted in the assessment of PAH concentrations in Tunisian aquatic fish. Indeed, Bizerte Lagoon has only been the topic of three researchs assessing PAH levels in two fish species and mussel (Barhoumi et al. 2014a; Barhoumi et al. 2016). In addition, there is only one work which had interested to the study of PAHs bioaccumulation in Ghar El Melh Lagoon's biota (Ben Ameer et al. 2021).

To obtain additional information on the state of their contamination and to evaluate possible hazards for consumers of fish, this work examined residue concentrations of PAHs in a benthopelagic fish species (*Liza aurata*) among their comestible marine organisms. In addition, this work is the first to assess PAH concentrations in this commercially valuable fish species from Tunisian Lagoons.

Materials And Methods

Study areas

The lagoon of Bizerte, a coastal lagoon is located in the north of Tunisia (latitude 37°80'-37°14' N; longitude 9°46'-9°56' E) (Fig. 1). It extends over an area of 150 km² with an average depth of 7 m and with a width of 11 km and a maximum length of 13 km. In addition to urban planning, (162,900 inhabitants living in agglomerations around the lagoon) (Afli and Zaabi, 2008), Bizerte lagoon is influenced by a large number of industrial units covering several sectors such as port activities, oil refineries, steel industry, shipbuilding, petrochemicals and several other industries (textiles, food processing, etc.). The lagoon is the seat of a strong fishery and shellfish production, mainly mussels (90%) but also oysters and clams (Afli and Zaabi, 2008).

Ghar El Melh Lagoon is situated in the Southern Mediterranean basin, north of Tunisia (37°06'-37°10'N and 10°08'-10°15'E) (Fig. 1). It's a lagunar complex classified as Ramsar site (Ramsar, 2007) sheltering a significant number of migratory birds (Ayache et al. 2009). It includes three wetlands of about 35 km² (the main lagoon, Sabkhet El Ouafi and Sabkhet Sidi Ali El Mekki) and with depth varying between 0.2 and 3.8 m (Dhib et al. 2013, 2017). This wetland communicates with the opened sea by a single channel, to the east, called "El Boughaz" with an average depth of 0.8 m (Dhib et al. 2013). Currently, due to domestic and industrial wastewater discharges, discharges from the drainage system and fishing activity, the lagoon is experiencing a decline in its specific richness and in particular a reduction in fishery resources, leading to the fall of the revenues of fishing of the lagoon (Moussa and Ben Khemis, 2005).

Sample collection and preparation

Sampling at both lagoon ecosystems was conducted during July 2018, with 30 fish samples taken from each surveyed site. The samples were collected from the landings, put under ice and transported in a cooler to the laboratory where they are stored in the freezer (-20°C). Once arrived at the laboratory, the samples were cleaned to remove debris from broken shells, parasites, were weighed and their size was measured. After evisceration and cleaning of the fish, the specimens were then ground and kept at -20 ° C pending the extraction step.

Analytical Methodology

Chemicals

15 individual USEPA priority PAHs standards including naphthalene (Nap), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Ch), benzo[b]fluoranthene (BbF), benzo [k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DahA), benzo[g,h,i]perylene (BghiP) and indeno[1,2,3-cd] pyrene (I(1,2,3cd)P) having purities varying from 95 to 99.9%, were provided by Supelco (Bellefonte, PA, USA). Stock solutions from each individual PAH were prepared in methanol and were after diluted with acetonitrile (ACN) to obtain solutions for calibration in the interval of 15–400 ng mL⁻¹. All used solvents (acetone, ACN, methanol, hexane and dichloromethane) were pesticide grade and were provided by Fisher (UK). Sulphuric acid (95–97%) was provided from Biotechnica. High grade silica gel (60–230 mesh) was also purchased from Merck Company (Germany), activated at 250°C and kept in a desiccator till its utilization. Anhydrous sodium sulphate (Na₂SO₄) was provided by Fluka and was of analytical quality. It was kept at 130°C after being heated to 300°C. The Ultra-pure water utilised throughout this work was obtained from a MilliQ system (Milford, MA, USA).

Sample extraction

The extraction of target compounds from the fish muscle samples was conducted using the method reported by Li et al. (2013) with some modifications. 15 g of muscle tissue were extracted 3 times in an ultrasonic bath with 40 mL of a hexane/acetone mixture in the proportions (1:1, v/v). Each extraction lasted 30 minutes. The obtained extract was concentrated by evaporation in a rotary evaporator to a volume of approximately 20 mL. One milliliter of this solution was taken for fat assessment. The extracted fat was weighted after solvent evaporated at 105°C in an oven. The obtained extract was purified in 2 steps: precipitation of fat by acid attack and purification by adsorption chromatography. The fat was precipitated by concentrated sulfuric acid attack (10 mL x 3). After precipitation of the fat, the organic fraction was washed with 20 mL of an aqueous solution of 5% sodium carbonate (Na₂ CO₃) in order to neutralize traces of the acid. To eliminate the remaining co-extracted substances, which may disrupt chromatographic analyzes, the hexanic extract was subjected, after evaporation to a volume of 1 mL, to an adsorption chromatography on a mini column filled with silica. The glass purification column has an internal diameter of 0.5 cm and contains 2 g of silica and 2 g of sodium sulfate. Initially, the adsorbent was washed by 10 mL of hexane and then the extract was transferred to the head of the column and eluted with 40 mL of hexane and dichloromethane in the proportions 3:1(v/v). Finally, the eluate was concentrated in a rotary evaporator to a volume of 10 mL, then it was evaporated to dryness in a micro Kuderna Danish, redissolved to 0.5 mL of hexane and filtered through a 0.45 µm nylon filter before being analyzed by liquid chromatography.

Instrumental analysis

The analysis of PAHs in the sample extract was finished using an analytical HPLC device fitted with a fluorescence detector and a SUPELCOSIL LC-PAH C18 column (4.6×250 mm, 5µm particle size) particular to the PAH assessment. The used instrument operating conditions are those described by Barhoumi et al. (2016).

Quality assurance and quality control

The specimens were checked for quality assurance as described by (Barhoumi et al. 2016). The absence of detection of target analytes was revealed by procedural blanks. For the chemicals examined, the applied methodology results in recoveries ranging from 32 to 77 percent. Recovery correction was applied to all specimens. Repeatability expressed as relative standard deviation (RSD), was lower than 10% for all compounds, this proves that the method's reproducibility is acceptable. In order to quantify the substances under investigation, a multi-level calibration curves in the linear response interval of the detector (in the range 15–400 ng mL⁻¹) were done and good linearity was obtained ($R^2 > 0.995$). Limits of quantification (LOQs) calculated as ten times the signal to noise ratio (Ben Ameer et al. 2011), ranged from 0.002 to 0.80 ng/g wet weight.

Risk assessment for PAHs in fish

To assess the health hazards with ingesting PAHs from Bizerte Lagoon and Ghar El Melh lagoon fish, the Estimated Daily Intake (EDI), Hazard Quotient (HQ), Hazard Index (HI), Cancer Risk (CR), Potency equivalent concentration (PEC), Screening Value (SV) and Excess Cancer Risk (ECR) of PAHs were calculated.

Estimated Daily Intake

To determine the extent of exposure to PAHs by fish ingestion, we determined the mean estimated daily intake values (ng / kg body weight / day) of all studied substances using the formula described by Tongo et al. (2017).

The daily value of fish consumption (25 g) was provided by the Institut National de Nutrition et de Technologie Alimentaire (<http://www.institutdenutrition.rns.tn>).

Hazard Quotient

Hazard related with nutritional exposure to non-carcinogenic PAHs was assessed using a hazard quotient method. The hazard quotient was calculated using the formula outlined by Barhoumi et al. (2016).

Hazard Index

The hazard index was determined using the the formula described by Barhoumi et al. (2016).

Cancer Risk

The cancer risk index was calculated using the formula reported by Barhoumi et al. (2016).

Potency equivalent concentration

The PEC values were determined on the basis of the equation outlined by Barhoumi et al. (2016).

Screening Value

The SV was calculated using the formula described by Tongo et al. (2017).

Excess Cancer Risk

The ECR value was calculated by using the Equation described by Tongo et al. (2017).

Statistical analysis

For statistical analysis, the collected findings were processed using Statistical tool (SPSS 10.0 for Windows, SPSS Inc.). The examination of the significance of the relationship across parameters was performed by the use of the spearman rank correlation. The threshold for statistical significance was set at $p < 0.05$.

Results And Discussion

The mugelid species *Liza aurata* was chosen because of its nutritional value, and its consideration as a highly edible food for many populations. The mullets are very widespread teleost fish in Tunisia. They are caught all along the coast, from the north to the extreme south of the country, and they are a considerable part of the local fish market. In addition, mullets constitute one of the most important resources of Tunisian lakes, whose exploitation, particularly by means of fixed fisheries, allows abundant catches. These fish are sought after by consumers for more than one way. Their flesh is appreciated and even more the gonads of sexually mature females. It is therefore essential to know the state and the degree of the contamination of this bioindicator species by PAHs.

Morphometric (weight and height determination) and constituent (lipid content) data were collected from all examined individuals and reported in Table 1. The average weight of mullet in the lagoon of Bizerte and Ghar El Melh (LGM) is respectively 204.95 and 141.72 g. The average length is 24.54 cm and 23.88 cm respectively for

the mullet from Bizerte Lagoon and Ghar El Melh Lagoon. The average value of the percentage in lipids is 0.26 and 0.23% respectively in the samples from Bizerte and Ghar El Melh Lagoons. No significant difference was found between the two studied sites in term of the length and lipid percentage values in the mullet samples ($p > 0.05$). Only a statistically significant difference in weight of the samples was detected between the two studied sites ($p < 0.05$).

Table 1
Biometry and lipid content of *Liza aurata* from Bizerte Lagoon (BL)
and Ghar El Melh Lagoon (GML)

Sites	Number of samples	Weight g (\pm SD)	Length cm (\pm SD)	Lipid % (\pm SD)
BL	20	204.95 (9.10)	24.54 (1.43)	0.26 (0.17)
GML	20	141.72 (10.29)	23.88 (0.69)	0.23 (0.09)
SD, standard deviation				

The PAH levels registered in mullet samples collected from the Bizerte Lagoon and the Ghar El Melh Lagoon are given in Table 2. The compounds Ace, Fl, Phe, An, Ft, Py, B (a) an, Chry, B (b) ft, B (k) ft, B (a) P, D (a, h) an, and B (g, h, i) P were detected in the muscle of fish collected from the two studied sites while Naph and In were detected only in Bizerte Lagoon. The other congeners were detected at a frequency ranging from 37.5–100% in Bizerte Lagoon and from 28.6–100% in Ghar El Melh Lagoon.

Table 2
PAH levels (ng g⁻¹ ww) in *Liza aurata* muscle from Bizerte and Ghar El Melh Lagoons

	BL				GML			
	Mean	SD	Range	DF (%)	Mean	SD	Range	DF (%)
Nap	0.94	(0.63)	nd-2.03	75	0.00			
Ace	0.84	(0.44)	0.13–1.75	100	0.75	(0.31)	0.273–1.178	100
Fl	2.65	(0.76)	2.18–4.44	100	3.85	(0.83)	2.353–4.886	100
Phe	0.50	(0.56)	0.03–1.24	100	0.40	(0.49)	nd-1.178	85.7
Ant	0.03	(0.02)	nd-0.06	87.5	0.06	(0.13)	nd-0.356	85.7
Flu	6.79	(0.96)	6.02–8.60	100	0.07	(0.07)	0.004–0.167	100
Pyr	0.15	(0.13)	0.07–0.15	100	0.03	(0.01)	0.009–0.052	100
BaA	0.11	(0.09)	0.003–0.26	100	0.06	(0.03)	0.027–0.112	100
Ch	0.14	(0.13)	0.01–0.40	100	0.14	(0.08)	nd-0.257	85.7
BbF	0.06	(0.09)	0.004–0.28	100	0.03	(0.01)	0.009–0.038	100
BkF	0.03	(0.04)	0.004–0.11	100	0.01	(0.01)	nd-0.021	85.7
BaP	0.02	(0.04)	nd-0.11	75	0.02	(0.01)	nd-0.045	85.7
DahA	0.048	(0.07)	nd-0.19	75	0.23	0.60	nd-0.016	28.6
BghiP	0.027	(0.04)	nd-0.082	62.5	0.30	0.61	nd-0.016	42.9
I(1.2.3cd)P	0.027	(0.05)	nd-0.12	37.5	0.00			
∑PAHs	12.51	1.93	9.41–14.42		5.95	(1.17)	4.83–8.28	
<i>nd</i> , not detected; <i>SD</i> , standard deviation; <i>DF</i> , detection frequency								

Total PAH concentrations in mullet samples from Bizerte Lagoon vary in a concentration range of 9.41 to 14.42 ng.g⁻¹ ww and a concentration average of 12.27 ng.g⁻¹ ww, while those in fish from the Ghar El Melh Lagoon vary between 4.83 ng.g⁻¹ and 6.38 ng.g⁻¹ ww and a concentration average of 5.49 ng.g⁻¹ ww.

In Bizerte Lagoon, the observed individual PAH levels range from 0.02 ng.g⁻¹ ww for B(a)P to 6.79 ng.g⁻¹ ww for Ft, on the other hand, at Ghar El Melh Lagoon they vary between 0 ng.g⁻¹ ww for In and 3.85 ng.g⁻¹ ww for Fl, therefore, these results show a general contamination of two investigated ecosystems by PAHs.

The total PAH concentrations were found at high levels when compared to those found in Ghar El Melh Lagoon (Table 2). A statistically significant difference in concentrations was registered between the studied sites ($p < 0.05$).

The examination of the concentrations of each individual PAH detected in the two studied sites (Fig. 2), revealed that fluoranthene (Ft) exhibits the highest concentration with a percentage compared to the sum of the concentrations of total PAHs of 55.33% in the sample fish from Bizerte Lagoon, while in the samples from Ghar El

Melh Lagoon it is fluorene which exhibits the highest level with a percentage compared to the sum of the concentrations of total PAHs of 70.14. The compound with the lowest level is B (a) P in Bizerte Lagoon samples with a percentage of 0.17 relative to the sum of the total PAH concentrations, while in Ghar El Melh Lagoon mullet muscle the D (a, h) an has the lowest content with a percentage of 0.05 compared to the sum of the concentrations of total PAHs.

Depending on the aromatic ring number that they contain, PAHs are classified into three groups namely : PAHs with 2 to 3 rings (Ant, Fl, Nap, Phe, Ace) and showing low molecular weight (LMW-PAHs), PAHs with 4 rings (Flu, BaA, Ch, Pyr) and showing medium molecular weight (MMW-PAHs) and PAHs with 5 to 6 rings (BaP, BbF, DahA, BkF, BghiP, I(1,2,3cd) P) and showing high molecular weight (HMW-PAHs). The PAH profiles in the BL and GML mullet samples are shown in Fig. 2. Very similar profiles have been obtained in other published investigations (Barhoumi et al. 2016; Frapiccini et al. 2018).

From Figs. 2A, it can be seen that in the BL mullet muscle, the MMW-PAHs were the most predominant with a percentage of 58%. LMW-PAHs were the second predominant group of PAHs, accounting for 40%. Finally, the least dominant class of PAHs were those with high molecular weight detected in the BL and GML samples with a value of 3%. According to Fig. 2B, it can be seen that PAHs with low molecular weight were the most predominant in GML mullet muscle. Their contribution to the sum of the PAHs is 85%. After, medium molecular weighted PAHs were the second predominant group of PAHs, accounting for 10%.

Although most of the studies focusing on the determination of PAHs in biota have shown that naphthalene constitutes the most dominant PAH in the group of LMW-PAHs (Darilmaz and Kucuksezgin, 2012; Mashroofeh and Pourkazemi, 2015; Moraleda-Cibrián and Rosell-Melé, 2015; Barhoumi et al. 2016), this chemical was not registered in all of GML mullet studied samples. Not detecting this compound could be due to its volatilization during the evaporation and concentration steps of the adopted analytical protocol.

The relatively PAH higher concentrations such as Ace, Fl and Phe with low coefficients of octanol-water partition ($\text{Log Kow} < 5$) in the studied biota could be associated to their high bioavailability and solubility compared to congeners with higher molecular weight and coefficients of octanol-water partition ($\text{Log Kow} > 5$) (Liang et al. 2007). The assimilation of low molecular weight PAHs might be associated to their solubility in water (Perugini et al. 2007).

The PAHs of low molecular weight and medium molecular weight were the most represented congeners in this study. This could be due to the fact that high molecular weight PAHs with low solubility bind and stored in sediment particles and absorbed preferentially by the marine organisms' digestive system (Barhoumi et al. 2016). The predominance of low molecular weight PAHs was registered in similar studies on contaminated marine species, such as pelagic and benthic fish from the Atlantic Ocean (Ramalhosa et al. 2012), freshwater fish from China (Xu et al. 2011) and fish species from the Mediterranean Sea (Baumard et al. 1998).

The prevalence of PAHs with low and medium molecular weights in muscles of fish species is probably caused by the transformations of the heavy PAHs metabolism by the cytochrome P450 system in the fish liver (Meador et al. 1995; D'adamo et al. 1997; Livingstone 1998). Added to that, it has been reported that in PAHs with a $\text{log Kow} < 5$, no biological transformation was recorded in fish species like for anthracene, naphthalene and phenanthrene (Thomann and Komlos, 1999). Therefore, increased amounts of PAHs with medium molecular weight may result from direct contact with water through the skin and the gills (Perugini et al. 2007; Ramalhosa et

al. 2012). It was shown that 99% of the ingested PAHs by fish can be rapidly converted to metabolic compounds during twenty four hours of absorption and changing the PAH concentrations and profile in fish tissues (Barhoumi et al. 2016). Moreover, Barhoumi et al. (2016) stated that the PAH half lives in fish species is extremely brief, ranging from 6 to 9 days for Flu, Ant, Phe and Fl, leading to the hypothesis that the existence of these chemicals in fish tissues is an indication of current contamination.

The comparison of the studied chemicals levels obtained in this work with those reported in literature is difficult because PAH concentration unit and the number of PAH compounds analyzed, vary from one study to another. Table 3 illustrates the concentrations of PAH observed across the world in certain fish specimens.

Table 3
PAH concentrations (ng g⁻¹ ww) in fish specimens world-wide

Location	Species	∑PAHs	Reference
Zanzibar coastal waters	Goby (<i>Periophthalmus sobrinus</i>)	0.032	Rashid et al. (2018)
Niger Delta	Goby (<i>Periophthalmus koeleuteria</i>)	(nd-171)	Nwaichi and Ntorgbo (2016)
Thane creek (India)	Lizard fish	156.8	Tiwari et al. (2017)
Poyang Lake (China)	Carp Bighead carp (<i>Aristichthys nobilis</i>) Silver carp (<i>Hypophthalmichthys molitrix</i>)	105.4	Zhao et al. (2014)
Zhanjiang Harbor (China)	<i>Thryssa hamiltonii</i>	256	Sun et al. (2018)
Lake Small Bai-Yang-Dian (China)	Crucian carp, snakehead fish, grass carp and silver carp	28.11	Xu et al. (2011)
Ovia River. (Southern Nigeria)	<i>Clarias gariepinus</i>	378.56	Tongo et al. (2017)
Catania Gulf (Italy)	Sole (<i>Solea solea</i>)	27.52	Ferrante et al. (2018)
Catalonia (Spain)	Sole	2.52	Martí-Cid et al. (2008)
Mediterranean Sea (Adriatic Sea)	<i>Raja miraletus</i> (brown ray) <i>Lepidorhombus whiffiagonis</i> (megrim) <i>Lophius piscatorius</i> (angler)	(125.4–231.5) (108.8–213.7) (103.5–228)	Storelli et al. (2013)
Faroe-Shetland Channel (West of Scotland)	<i>Micromesistius poutassou</i> , <i>Argentina silus</i> , <i>Pollachius virens</i> , <i>Molva dypterygia</i> , <i>Reinhardtius hippoglossoides</i> , <i>Lepidorhombus whiffiagonis</i> , <i>Trachurus trachurus</i> , <i>Aphanopus carbo</i> , <i>Coryphaenoides rupestris</i>	(< LOD-4.44)	Webster et al. (2018)
Ismailia city (Egypt)	Mullet, bolti	(19.7–154.3)	Loutfy et al. (2007)
Hong Kong	Freshwater and marine fish	(15.5–118)	Cheung et al. (2007)

The range of concentrations is indicated by the values in brackets

Location	Species	Σ PAHs	Reference
Pearl river delta (China)		(30.94–410.06)	Dong et al. (2006)
Rosemary Bank Seamount (West of Scotland)	Bluewhiting, greater argentine, saithe, blue Ling, Greenland halibut, megrim, horse mackerel, black scabbard, roundnose grenadier	(< LOD–0.76)	Webster et al. (2018)
Ariake Sea (Japan)	Herbivore mudskippers, omnivore mud-skippers, flatfish, horse mackerel, mackerel, sardine	(< 0.04–2.3)	Nakata et al. (2003)
Daya Bay (South China)	Sole	170	Sun et al. (2016)
Victoria harbour (Hong Kong)	Rabbitfish (<i>Siganus Oramin</i>)	(1100–4300)	Fang et al. (2009)
Warri River at Ubeji, Niger Delta (Nigeria)	Tilapia	1 098.5	Asagbra et al. (2015)
Niger Delta		6800	Anyakora et al. (2005)
Coastal area of Bangladesh	Sole (<i>Cynoglossus lingua</i>)	2123.2	Habibullah-Al-Mamun et al. (2018)
Atlantic Ocean	Sardine Horse mackerel Spanish mackerel	(2.29–14.18) (2.73-10.01) (1.80–19.90)	Ramalhosa et al. (2012)
Port Phillip Bay (Australia)	Crocodile fish (<i>Platycephalus bassensis</i>)	< 0.1	Gagnon et al. (2016)
Sweden		5.4–23 16	Brorström-Lundén et al. (2010)
Portugal	Horse mackerel Spanish mackerel Sardine	(0.73–4.8) (3.34–5.03) (3.64–6.60)	João Ramalhosa et al. (2009)

The range of concentrations is indicated by the values in brackets

Location	Species	Σ PAHs	Reference
North Sea	Flatfish, haddock	(< LDD–3.8)	Webster et al. (2011a,b and 2012)
Ghar El Melh Lagoon (Tunisia)	Mullet (<i>Liza aurata</i>)	5.95 (4.83–8.28)	Present study
Bizerte Lagoon (Tunisia)	Mullet (<i>Liza aurata</i>)	12.51 (9.41–14.42)	Present study
The range of concentrations is indicated by the values in brackets			

The concentrations of PAHs in BL and GML fish is lower than those registered by other authors in fish species from other aquatic ecosystems such as the Niger Delta (Nwaichi and Ntorgbo, 2016), Lake Poyang in China (Zhao et al. 2014), the Ovia river in southern Nigeria (Tongo et al. 2017), the Catania Gulf in Italy (Ferrante et al. 2018), the Thane stream in India (Tiwari et al. 2017), the Port of Zhanjiang in China (Sun et al. 2018), the Mediterranean Sea (Adriatic Sea) (Storelli et al. 2013), Lake Baiyangdia (North China) (Xu et al. 2011), the City of Ismailia (Egypt) (Loutfy et al. 2007), Hong Kong (Cheung et al. 2007), the Pearl River Delta (China) (Dong et al. 2006), Daya Bay (South China) (Sun et al. 2016), Victoria Harbor in Hong Kong (Fang et al. 2009), the Niger Delta (Anyakora et al. 2005), the Warri River in Nigeria (Asagbra et al. 2015) and the coastal areas of Bangladesh (Habibullah-Al-Mamun et al. 2019).

The level of PAH contamination of assessed fish during this work is higher than those found in fish from Port Phillip Bay (Australia) (Gagnon and Holdway, 2002), Faroe-Shetland Canal in West Scotland (Webster et al. 2018), North Sea (Webster et al. 2011, 2012), Rosemary Seamount in West Scotland (Webster et al. 2018), Ariake Sea in Japan (Nakata et al. 2003) and Portugal (João Ramalhosa et al. 2009). Similar PAH concentrations were found in different fish species in the Atlantic Ocean (Ramalhosa et al. 2012) and Sweden (Brorström-Lundén et al. 2010).

The identification of PAH sources of contamination is approximate because it depends on several factors such as individual metabolism and the fate of PAHs, the location of the species in the trophic chain, turbidity, etc. The PAH compounds could contaminate the environment by different routes, namely the pyrolytic route occurring through the incomplete combustion at high temperatures of recent organic matter and fossils, the petrogenic route occurring during petroleum formation by slow maturation of organic matter and the brief diagenetic degradation of biogenic precursors such as, steroids, pigments and di- and tri-terpenes (Wakeham, Schaffner et al. 1980).

Diagnostic ratios were used in this study to distinguish between petrogenic and pyrolytic PAHs (Table 4). These ratios used in the study to determine the PAH sources in mullet muscle of BL and GML are: Phe/Ant, Ft/Py, Ft/(Ft + Py), Ant/(Ant + Phe), Chry/B (a) an, B (a) an/(B (a) an + Chry), LMW PAHs/HMW PAHs and total PAHs/PAHs.

Table 4

Molecular markers for PAHs with pyrolytic and petrogenic origins (Zeng and Vista 1997; Akyüz and Çabuk 2008, Barhoumi et al., 2016)

	Petrogenic origin		Pyrolytic origin	Mixed origin	This study	
		Petroleum ^c . ^b	Biomass ^c . ^c		BL	GML
Phe/Ant	> 15	< 10	< 10	10–15	16.80	6.78
Flu/Py	< 1	> 1	> 1		45.05	2.68
Flu/Flu + Py	< 0.40	0.40–0.50	> 0.50		1.15	1.03
Ant/Ant + Phe	< 0.10	> 0.10	> 0.10		1.50	1.40
Chry/B(a)an	< 1	> 1			1.29	2.15
B(a)an/ (B(a)an + Chry)	< 0.2	> 0.35			1.14	1.14
LMW/HMW	> 1	< 1	< 1		23.6	39.4
CombPAHs/ΣPAHsa	< 1	> 1	> 1		7.33	0.42
aThe proportions of PAHs originating from combustion (Flu, Pyr, BaA, Ch, BbF, BkF, BaP, BghiP, I(1,2,3cd)P) to the total 15 PAHs						
^b Petroleum burning						
^c Biomass burning						

Phe/Ant ratios > 10 indicate pollution of petrogenic origin, while those with a Phe/Ant ratio < 10 are specific of pyrolytic contamination (Budzinski et al. 1997; Yang, 2000). In the present study, the Phe/Ant ratio obtained in the mullet muscle of the BL is greater than 10, it equals to 16.80 (Table 4), which indicates that the PAHs contaminating the samples are of petrogenic origin. Whereas in GML fish muscle, this ratio is less than 10 (6.78), therefore PAHs contaminating the samples are of pyrolytic origin.

A ratio of Fluoranthene/Pyrene ≥ 1 is linked to pyrolytic origins while values of this ratio less than 1 are attributed to a petrogenic source (Doong and Lin, 2004). In our study, the Ft/Py ratio is greater than 1 in BL and GML mullet muscle, indicating a source of pyrolytic contamination at both sites.

A Ft/(Ft + Py) ratio < 0.5 indicates a petrogenic source. According to the obtained results (Table 4), the Ft/(Ft + Py) ratio is greater than 0.5 in the BL and GML samples with respective values of 1.15 and 1.03, which indicates that the PAHs contaminating the samples are of pyrolytic origin.

A An/(An + Phe) ratio < 0.1, indicates a petrogenic source, while a value of this ratio greater than 0.1 reveals that the contamination is mainly controlled by pyrolytic PAHs. In our study, the An/(An + Phe) ratios obtained in mullet muscle of BL and GML are greater than 0.1 with respective values of 1.50 and 1.40, which indicates that the contaminating PAHs in the samples are of pyrolytic origin.

A Chry/B(a)an ratio > 1 would be indicative of contamination of petrogenic origin, while a value of this ratio less than 1 would indicate a pyrolytic origin of PAHs (Zeng and Vista, 1997). According to the obtained results

(Table 4), the Chry/B (a) an ratios are greater than 1 in BL and GML samples with respective values of 1.29 and 2.15, which indicates that the contamination derived from pyrolytic sources.

A $B(a)an/(B(a)an + Chry)$ ratio < 0.2 indicates a petrogenic source of PAHs, while a value greater than 0.35 indicates that the contamination is mainly from combustion (pyrolytic PAHs) (Yunker et al. 2002). In this work, the results show $B(a)an/(B(a)an + Chry)$ ratios greater than 0.35 indicating that the PAHs contaminating the samples are of pyrolytic origin.

According to Akyüz and Çabuk (2008), a $B(a)an/(B(a)an + Chry)$ ratio value of 0.2 to 0.35 shows that PAHs contaminating the studied samples are from coal combustion, whereas a value of this ratio more than 0.35 indicates that the PAHs derive essentially from road traffic. As a result, PAHs contaminating mullet samples of BL and GML are primarily derived from road traffic.

The predominance of the LMW PAHs (2 to 3 cycles) characterizes petroleum sources. A majority of low molecular weight PAHs are observed for fuels (diesel and super), lubricating oils and tire debris. Conversely, PAHs from pyrolytic sources are mainly composed of 4 or more cycles (HMW).

LMW PAH HMW PAH ratio values less than 1 indicate a pollution of pyrolytic source, but ratio with values greater than 1 show that the contamination could be essentially related to petrogenic PAHs (Soclo et al. 2000). According to the obtained results (Table 4), PAHs found in the mullet muscles of BL and GML are essentially derived from petrogenic origin.

A $CombPAHs/\Sigma PAHs$ ratio less than 1 is related to a petrogenic source, and a value greater than 1 is related to a pyrolytic origin of PAHs (Barhoumi et al. 2016). Consequently, the sources of contamination by the PAHs are pyrolytic in the mullet samples of BL and GML.

From the used diagnostic ratios, it is deduced that PAHs present in BL and GML fish samples would derive from both petrogenic and pyrolytic origins.

The pyrolytic sources that might contaminate BL and GML by PAHs include: domestic heating (wood, coal, fuel oil or gas), automobile traffic (combustion of automobile fuel), discharges of wastewater from treatment plants of the wastewater (industrial and domestic), the spreading of residual sludge from wastewater treatment plants, industries (company cements of Bizerte, sugar refining plant in Bizerte, the Tunisian Society of Refining Industries in Bizerte (STIR), Tunisian Company of Lubricants in Bizerte (SOTULUB), metallurgical industries and household waste incineration.

The petrogenic sources that could contribute to the pollution of the two studied lagoons by PAHs are: port activities, gasoline and oil discharges (effluents from STIR, SOTULUB and the Tunisian Society of Mechanical and Naval Constructions and Repairs (SOCOMENA)) and fishing activities. Indeed, the port of Bizerte-Menzel Bourguiba and the old fishing port, located at the bottom of the GML and the new one of greater capacity are at the origin of the adsorption of PAHs on the sediments by oil emptying and water cleaning of boats.

The potential damage to human health from harmful, bioaccumulative and persistent PAHs could be assessed through the comparison of the dietary consumption with thresholds toxicity (CSF and RfD) (Barhoumi et al. 2016).

Several PAHs and, in particular, their metabolic derivatives have been stated to be of major concern because of their known carcinogenicity, yet reports on dietary health hazard evaluation for PAHs are quite restricted (Oliveira et al. 2018; Zhao et al. 2014).

BaP is the only PAH with a well-defined toxicological profile, and it is the most commonly encountered carcinogen among all PAHs (Sun et al. 2016; Zhao et al. 2014). The European Union suggested 2 ng/g ww for BaP in fish as the maximum admissible concentration (Sun et al. 2016). BaP was identified in 85% and 86% in the studied species, from BL and GML respectively, with average levels of 0.02 ng/g ww in mullet from both investigated sites (Table 5). The concentrations of BaP in mullet from the both studied areas were under the maximum allowable level established by the European Union.

Table 5
EDI, PEC, CR and ECR of PAHs in *Liza aurata* from BL and GML

	BL					GML				
	EDI (ng/Kg)	B(A)Pteq (mg/Kg)	CR	ECR	EFSA- EDI	EDI (ng/Kg)	B(A)Pteq	CR	ECR	EFSA- EDI
Nap*	0.337	3.37 E ⁻¹⁰	NA	6,32 E ⁻¹²	NA	0.000	0	NA	0	NA
Ace*	0.299	2.99 E ⁻¹⁰	NA	2,56 E ⁻⁰⁹	NA	0.269	2.69 E ⁻¹⁰	NA	5,05 E ⁻¹²	NA
Fl*	0.946	9.46 E ⁻¹⁰	NA	8,11 E ⁻⁰⁹	NA	1.376	1.38 E ⁻⁰⁹	NA	2,59 E ⁻¹¹	NA
Phe*	0.178	1.78 E ⁻¹⁰	NA	1,52 E ⁻⁰⁹	NA	0.144	1.44 E ⁻¹⁰	NA	2,70 E ⁻¹²	NA
Ant*	0.011	1.06 E ⁻¹⁰	NA	9,08 E ⁻¹⁰	NA	0.021	2.12 E ⁻¹⁰	NA	3,98 E ⁻¹²	NA
Flu *	2.425	2.42 E ⁻⁰⁹	NA	2,08 E ⁻⁰⁸	NA	0.025	2.47 E ⁻¹¹	NA	4,65 E ⁻¹³	NA
Pyr*	0.054	5.382 E ⁻¹¹	NA	4,61 E ⁻¹⁰	NA	0.009	9.22 E ⁻¹²	NA	1,73 E ⁻¹³	NA
BaA***	0.040	3.97 E ⁻⁰⁹	2.90 E ⁻⁰⁸	3,41 E ⁻⁰⁸	NA	0.023	2.30 E ⁻⁰⁹	1.68 E ⁻⁰⁸	4,32 E ⁻¹¹	NA
Ch***	0.051	5.13 E ⁻¹⁰	3.75 E ⁻¹⁰	4,40 E ⁻⁰⁹	NA	0.050	4.95 E ⁻¹⁰	3.61 E ⁻¹⁰	9,30 E ⁻¹²	NA
BbF***	0.020	1.99 E ⁻⁰⁹	1.45 E ⁻⁰⁸	1,71 E ⁻⁰⁸	NA	0.009	9.28 E ⁻¹⁰	6.77 E ⁻⁰⁹	1,74 E ⁻¹¹	NA
BkF**	0.010	9.57 E ⁻¹⁰	6.98 E ⁻¹⁰	8,20 E ⁻⁰⁹	NA	0.005	4.55 E ⁻¹⁰	3.32 E ⁻¹⁰	8,55 E ⁻¹²	NA

NA, Not available

	BL					GML				
BaP**	0.008	7.65 E ⁻⁰⁹	5.59 E ⁻⁰⁸	6,56 E ⁻⁰⁸	3.9–6.5	0.006	5.81 E ⁻⁰⁹	4.24 E ⁻⁰⁸	1,09 E ⁻¹⁰	3.9–6.5
DahA**	0.054	2.71 E ⁻⁰⁷	3.96 E ⁻⁰⁷	2,32 E ⁻⁰⁶	NA	0.081	4.06 E ⁻⁰⁷	5.92 E ⁻⁰⁷	7,62 E ⁻⁰⁹	NA
BghiP***	0.023	2.30 E ⁻¹⁰	NA	1,97 E ⁻⁰⁹	NA	0.107	1.07 E ⁻⁰⁹	NA	2,01 E ⁻¹¹	NA
I(1.2.3cd)P**	0.016	1.62 E ⁻⁰⁹	NA	1,21 E ⁻⁰⁸	NA	0.000	0	NA	0	NA
		PEC = 0.80 E ⁻⁰³					PEC = 1.20 E ⁻⁰³			
ΣPAH2	0.06				10.7–18	0.06				10.7–18
ΣPAH4	0.16				19.5–34.5	0.09				19.5–34.5
ΣPAH8	0.18				28.8–51.3	0.28				28.8–51.3
NA, Not available										

According to the European Food Safety Authority (EFSA), B(a)P alone is not an appropriate indicator for the incidence of PAHs in food and proposed the sum of i) two PAHs (B(a)P, Chry; ΣPAH2), ii) four specific compounds (B(a)P, B(a)A, B(b)F, and Chry; ΣPAH4) and iii) eight PAHs (B(a)P, B(a)A, B(b)F, Chry, B(k)F, B(g,h,i)P, DB(a,h)A, and Ind; ΣPAHs8) as the most suitable markers (Oliveira et al. 2018). With this system, it is assured that the exposition to feasible/potential carcinogenic PAH due to food intake is maintained at concentrations that do not induce public health concern even if B(a)P is not identified (Oliveira et al. 2018).

Because *Liza aurata* is consumed worldwide, this species is a suitable bioindicator for environmental monitoring studies and for the studies of human exposure to contaminants through food. The PAH EDIs in the studied fish species were calculated to understand the exposure magnitude to the studied contaminants through the consumption of the *Liza aurata* in the general population from the northern regions in Tunisia (Bizerte and Ghar El Melh) (Table 5). Moreover, the HI, HQ, PEC, SV and ECR values of PAHs were also determined to assess the potential risk to health related to the consumption of this species from the two investigated lagoons (Table 5).

EDI estimations, got from the levels of PAHs in *Liza aurata* were indicated in Table 5. The EDI estimated on the foundation of BaP levels is lower than the range determined by EFSA in the BL and GML mullet muscles. Likewise, the EDI evaluated for ΣPAH2 (B(a)P and Chry), ΣPAH4 (Chry, B(b)F, B(a)A and B(a)P) and ΣPAH8

(B(a)A,B(a)P, DB(a,h)A, B(k)F, B(b)F, Ind, Chry, and B(g,h,i)P) (Oliveira et al. 2018) is lower than the range determined by the EFSA.

According to Ferrante et al. (2018), this result indicates that local population from Bizerte and Ghar El Melh is not especially exposed to PAHs that may derive from mullet collected from the investigated locations, because they did not exceed the maximum value of the range calculated by the EFSA for the consumer diet.

Carcinogenic risk on human health from ingestion of mullet was also evaluated by the use of specific PAH carcinogenic capabilities (B(A)Pteq), PEQ, SV, ECR, and HQ. The PEC of total PAHs mean values were 2.92 E^{-07} and $4.19 \text{ E}^{-07} \text{ mg/Kg}$ respectively in BL and GML (Table 5).

The calculated values of PEC in the present work (BL = $0.80 \text{ E}^{-03} \text{ ng/g ww}$; GML = $1.20 \text{ E}^{-03} \text{ ng/g ww}$) were lower than the ones in aquatic biota (such as crustacean species and fish) in Italy from the Adriatic Sea ($0.03\text{--}2.8 \text{ ng.g}^{-1} \text{ ww}$) (Perugini et al. 2007), from markets in Hong Kong ($0.02\text{--}0.37 \text{ ng.g}^{-1} \text{ ww}$) (Cheung et al. 2007), Haimen bay in China ($499.1 \text{ ng.g}^{-1} \text{ ww}$) (Shi et al. 2016), in China from Daya Bay ($0.57\text{--}10 \text{ ng.g}^{-1} \text{ ww}$) (Sun et al. 2016), in Turkey from Marmara Sea and Istanbul Strait ($1.6\text{--}41 \text{ ng.g}^{-1} \text{ ww}$) (Karacık et al. 2009), Poyang Lake in China (Zhao et al. 2014). However, their values were comparable to those in fish muscular tissue from the Bay of Algeciras in Spain ($0.09 \text{ ng.g}^{-1} \text{ ww}$) (Rojo-Nieto et al. 2014) and from Ovia River in Nigeria ($0.006 \text{ ng.g}^{-1} \text{ ww}$) (Tongo et al. Akpeh, 2017).

Fish advisories proposed by the USEPA to be used in the screening value (SV) of the PEC of total PAHs represented the level of the pollutant in edible fish tissue that can have possible harmful hazards on human health (Habibullah-Al-Mamun et al. 2019). In this work, the evaluated values of the PEC for the studied seafood species were lower than the suggested PEC ($0.67 \text{ ng.g}^{-1} \text{ ww}$) for human uptake (Habibullah-Al-Mamun et al. 2019) and the measured SV value of $3.3 \text{ }\mu\text{g.g}^{-1}$, implying that the intake of this PAH-polluted fish species would not have harmful health effects.

The values of HQ and HI for noncarcinogenic hazard of exposure to PAHs by the nutritional exposition to mullet muscle were estimated. HQ values ranged from 2.70×10^{-5} to 5.93×10^{-3} and from 3.07×10^{-5} to 5.74×10^{-3} respectively in BL and GML. HI values were 0.018 and 0.011 respectively in BL and GML. The assessed values of the HI and HQ were inferior to 1 and this result reveals that the exposure to complex PAH mixtures or individual PAHs across seafood consumption will have no harmful impact on consumers in the towns of Bizerte and Ghar El Melh.

The calculable ECR derived from lifespan exposition to PAHs fish based regime uptake was assessed. ECR values for single PAHs varied from 6.32×10^{-12} to 2.32×10^{-6} in BL fish specimens and 1.73×10^{-13} to 7.62×10^{-8} in GML fish samples (Table 5). The comparison of the ECR values from this study with the USEPA guideline value of 10^{-6} (Barhoumi et al. 2016; Habibullah-Al-Mamun et al. 2019; Tongo et al. 2017) showed that our results were lower. USEPA states that the level of the threat where there is a cancer risk for life of 1/1000000 ($\text{ECR} = 10^{-6}$) for a lifetime of 70-year is deemed to be acceptable, whereas the risk of cancer for extra lifetime of 1/10000 or more ($\text{ECR} = 10^{-4}$) is deemed to be severe (Barhoumi et al. 2016; Habibullah-Al-Mamun et al. 2019; Tongo et al. 2017). Based on ECR values obtained in this study, the lifespan of the exposition to PAHs via fish uptake would not lead to a hazard of cancer.

The values of the CR derived from nutritional exposure to 16 US EPA PAHs through the uptake of this fish species varied from 3.75×10^{-10} to 5.59×10^{-8} for Bizerte residents and from 3.32×10^{-10} to 5.92×10^{-7} for Ghar El Melh residents.

The US EPA established the peak acceptable risk level (ARL) of 10^{-5} , whereas the priority risk level of 10^{-4} is deemed to be severe (Sun et al. 2016). The mean CR values in the current work which were 7.24×10^{-8} for Bizerte population and 1.10×10^{-7} for Ghar El Melh population, were lower than the ARL and the priority risk level, showing prospective risk of carcinogenicity to citizens in both towns.

These data showed no negative health impacts (carcinogenic or non- carcinogenic) due to the consumption of fish muscular tissues from Bizerte and Ghar El Melh Lagoons with respect to PAHs.

Indeed, it is crucial to highlight that the prospective health hazards in this work were hinge on unprocessed and crude food, and it is recognized that PAHs could be also produced during cooking, mainly during roasting, frying and grilling (Oliveira et al. 2018). It has been shown that the existence of another class of carcinogenic chemicals in food and certain residents habits, may contribute to certain hazards, particularly if synergistic impacts happen (Oliveira et al. 2018). In addition, the bioaccessibility of each dangerous chemical can have a significant decrease in the absolute quantity taken up by the gastrointestinal system and thus in nutritional exposure (Oliveira et al. 2018).

Since biological factors could affect the accumulation of persistent organic pollutants in aquatic biota, PAHs concentrations were correlated with some specific fish characteristics. The concentrations of PAHs in mullet from Ghar El Melh Lagoon were not correlated with either weight ($r_s = -0.457$, $p = 0.303$), length ($r_s = -0.154$, $p = 0.741$), or lipid percentage ($r_s = -0.029$, $p = 0.950$). For mullet samples from the Bizerte Lagoon, total PAH levels also showed no significant correlation with either weight ($r_s = 0.450$, $p = 0.263$), length ($r_s = 0.309$, $p = 0.456$), or lipid percentage ($r_s = -0.117$, $p = 0.782$). These findings could be similar to those reported by Borghesi et al. (2009), Deshpande et al. (2002), Ferrante et al. (2018), Gao et al. (2009) and Mariottini et al. (2008). These outcomes are identical to those stated by Barhoumi et al. (2016) and Frapiccini et al. (2018).

These obtained data suggest the decrease of PAH levels with a rise in the size of the body, in accordance with results on red mullet collected from the Mediterranean sea in Spain and with the findings registered by (Pellini et al. 2018). The authors reported that the affluence of microplastic pollutants, capable of adsorbing PAHs (persistent hydrophobic compounds), diminished with increasing body size of the sole in the Adriatic Sea (Frapiccini et al. 2018). In fact, a decreased initiation of the biotransformation of xenobiotics with age was reported in fish species with comparatively low resistance of PAHs to biotransformation and elevated degrees of depuration by adult organisms (Frapiccini et al. 2018).

Although that some works have reported that the lipid percentage is relevant factor for examining the apportionment of lipophilic pollutants, like PAHs (Barhoumi et al. 2016; Jafarabadi et al. 2019) also the level of hydrophobic pollutant uptakes in fish could rise with an increased percentages of the lipid in the biological membranes (Jafarabadi et al. 2019).

The negative correlation found between lipid percentage and PAH concentrations found in this work which is similar with the outcomes of other authors (Frapiccini et al. 2018; Hellou and Law, 2003), supports the hypothesis that contaminants are diluted with rising fat content (Larsson et al. 1991) and shows that lipid content could not

be the only main factor in the bioaccumulation of PAH in fish tissue (Frapiccini et al. 2018; Sun et al. 2016; Zhao et al. 2014).

Generally, numerous parameters such as environmental parameters (e.g. temperature, oxygen, salinity etc.), duration and route of exposure, rates of depuration and absorption, dietary quality, differences between species (filter-feeding, sessile and carnivorous) may impact the PAHs accumulation and depuration in fish (Barhoumi et al. 2016). In addition, endogenous and exogenous parameters like temperature, accessibility of food, storage of energy stocks and reproductive phase are accountable for seasonal variations in lipid content (Jafarabadi et al. 2019).

Conclusion

USEPA priority PAHs were detected in muscle of *Liza aurata* sampled from Bizerte and Ghar El Melh Lagoons.

This study is the first one that reports the results of an investigation into PAHs in Ghar El Melh Lagoon Biota. PAHs were detected in mullet from the both studied lagoons. In the mullet samples from BL, the most concentrated PAH congeners were those with medium molecular weight, while in GML the PAHs with low molecular weight were the highest dominant congeners.

The levels of concentration of the studied contaminants were comparable or lower than the concentrations detected in other fish species from various regions worldwide.

PAHs around BL and GML derived from the mixed contamination origins of petrogenic and pyrogenic processes.

Evaluations hinge on certain existed guidelines revealed non significant risks to human health related to nutritional intake of PAHs from the consumption of *Liza aurata*.

Over all, this study provides a regional baseline assessment of PAH incidence and concentrations in *Liza aurata* and confirms the role of this species as bioindicator organisms capable to distinguish between various levels of environmental contamination, justifying the use of this species in biomonitoring programs.

Declarations

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Author contributions

* Walid Ben Ameer conceived the presented study.

* Yassine El Megdiche and Takoua Mhadhbi contributed to sample collection and preparation.

* Walid Ben Ameer carried out the chemical compounds extraction from samples with help from Maaned Khadija and Souad Trabelsi.

* Walid Ben Ameer carried out the chromatographic analysis of the studied compounds with help from Sihem Ben Hassine and Ghanmi Safouen.

* Walid Ben Ameer, Ali Annabi and Bechir Hammami analyzed the data and wrote the manuscript with support from Soukaina Ennaceur, Mohamed Ridha Driss and Soufiane Touil.

* All authors provided critical feedback and helped shape the research, analysis and manuscript.

* All authors discussed the results and contributed to the final manuscript.

Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability

The datasets generated for this study are available on request to the first and corresponding authors.

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Figures

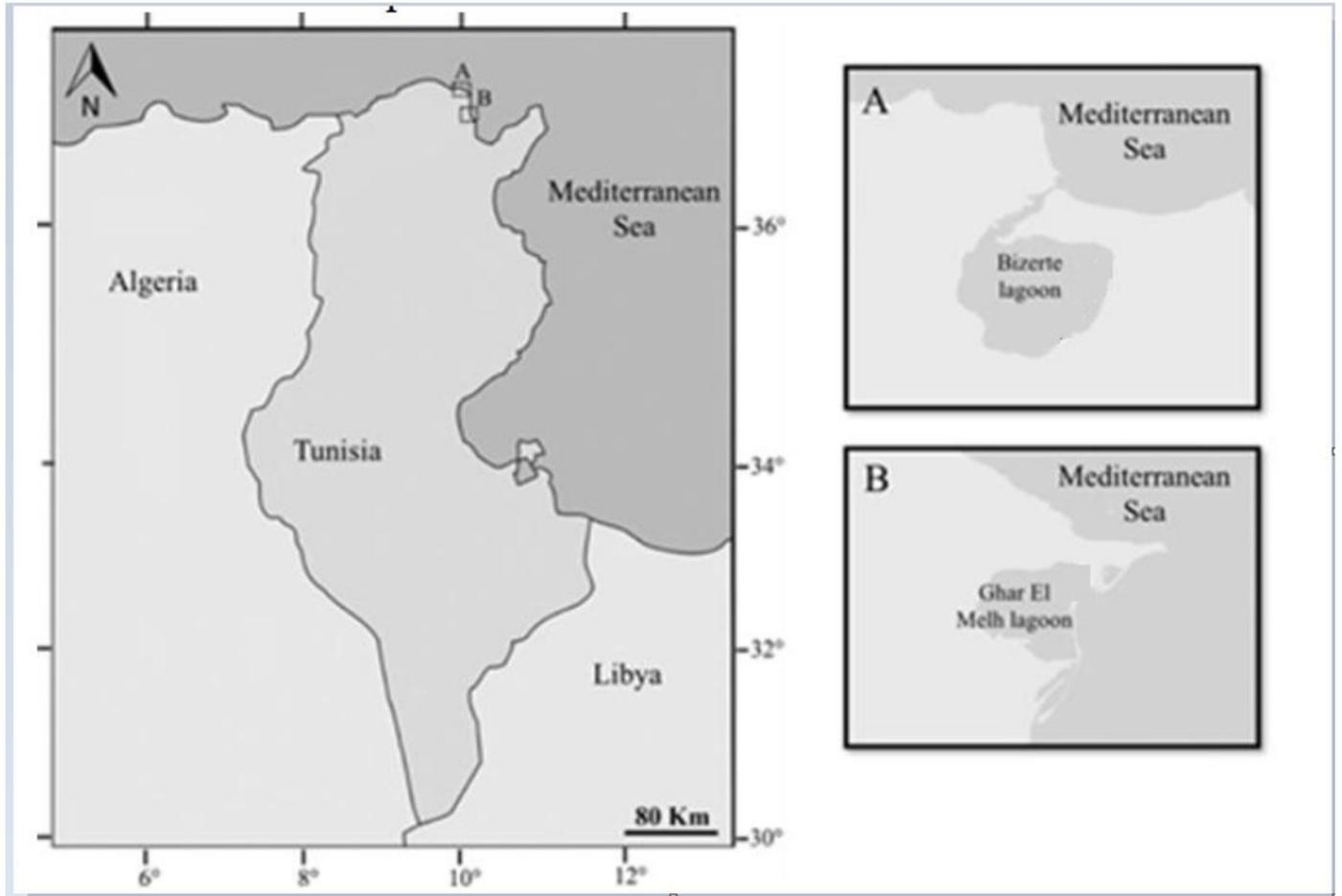


Figure 1

Map showing sampling areas. A Bizerte lagoon; B Ghar El Melh lagoon

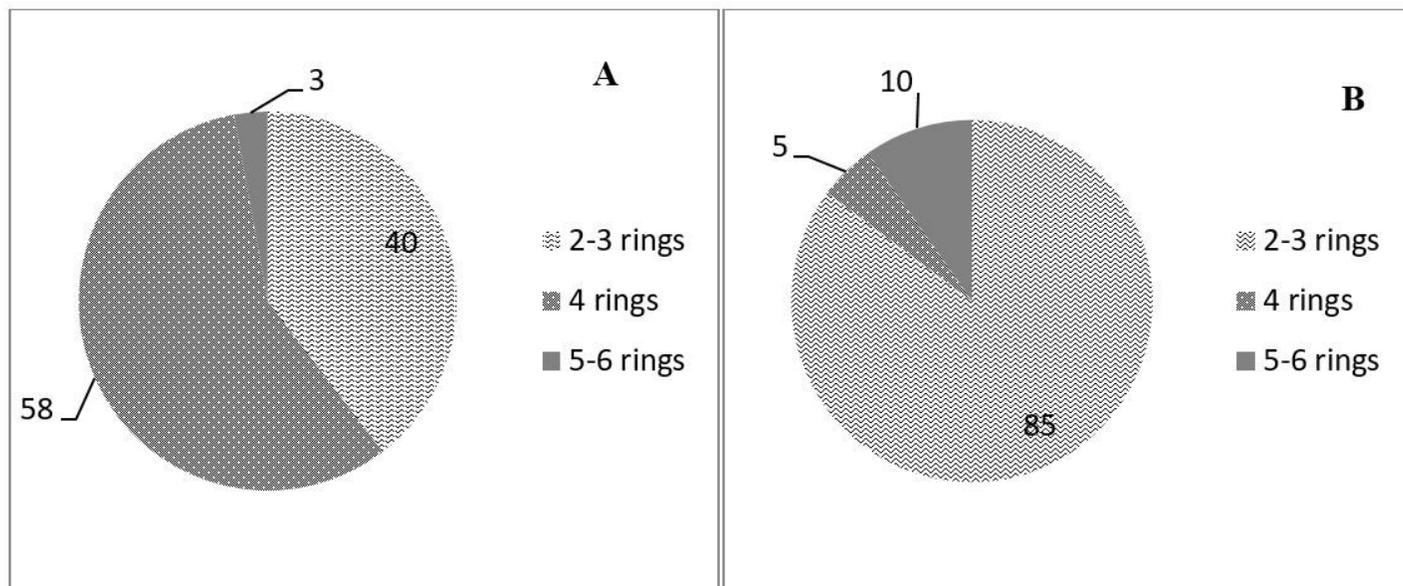


Figure 2

Content profile (%) of PAHs in fish muscle from the Bizerte Lagoon (BL) and the Ghar El Melh Lagoon (GL) according to the number of rings: 2-3, 4-5, and 6-ring PAH in Bizerte Lagoon (BL) and Ghar El Melh Lagoon (GL) fish muscle. A Bizerte lagoon; B Ghar El Melh lagoon