Quantitative Analysis and Risk Assessment of Polycyclic Aromatic Hydrocarbons (Pahs) in Marine Organisms from Moroccan Coastal

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ABSTRACT

Fishery products play an increasingly vital role in meeting nutritional needs. They represent valuable sources of proteins and minerals beneficial to health. Polycyclic Aromatic Hydrocarbons (PAHs) are among the toxic chemical contaminants that accumulate in marine organisms. In this context that this study aims to assess specifically the pollutants that can impair the quality and safety of fish products in Morocco. Four elements of PAHs were studied in order to investigate the direct impact of sampling areas and species on PAH levels in the marine environment. The PAHs were assayed by Gas Chromatography Coupled to a Mass Spectrometer (GC/MS). The method of calculation of the reports and the statistical analysis made it possible to determine the origin of the contamination of the fishes and the factors that give rise to their spatial evolution. The study showed that the cities of Tetouan, Eljadida, Dakhla, Safi, Essaouira and Kenitra are relatively the most contaminated by PAHs and are the most threatened because of the developed industry in these regions. Nevertheless, the grade of PAH studies and the level of contamination remain below the limits recommended by European Regulation.

Keywords: Fish, GC/MS, Moroccan coastal, Polycyclic aromatic hydrocarbons, Chemical contamination

INTRODUCTION

Morocco is endowed with a coastline extensive and rich in various fishing resources that are concealed within its coastal waters. This coastline is an important attraction for socio-economic activities of national interest. However, it is threatened by overexploitation and various sources of pollution [1,2]. There is a multitude of classes of potentially toxic contaminants that affect the quality of water and fish products of which include (PAHs) [3,4].

They pose major environmental problems because of their toxicity, mainly due to their carcinogenic and mutagenic properties. They are listed in the lists of the World Health Organisation, the International Agency for Research on Cancer, and the European Commission [5,6]. We have been particularly interested in these chemical elements because of their accumulation and persistence in aquatic biota. They are likely to present dangers to the sustainable exploitation of fishery resources.

The persistence of these substances is related to their resistance to natural degradation or metabolism and their involvement in the phenomenon of biomagnification and bioaccumulation in the trophic chain [7,8]. In principle, PAHs are a group of organic compounds that can come from both natural and anthropogenic sources. In vertebrates, these compounds are metabolized quickly, therefore, they are usually not detected other than in sediments and in invertebrates [8-11]. Biota on the Mediterranean coast and the Atlantic coast may be more sensitive to chronic or accidental releases of PAHs from oil production [12,13].

Although the Moroccan coastline includes a wide range of organic and inorganic pollutants, analysis of these pollutants proved to be essential in order to study the specific way their harmful effects impact the quality and safety of fishery products. Therefore, the implementation of plans to monitor contaminant residue in fishing products was crucial in order to ensure that consumer products are free of any health risk [2,14].

A comprehensive study of the coastal was undertaken, and as part of this study, the most feared pollutants for the marine environment were cited. The samples selected for this study focuses on seven species of frozen fish e.g., Lobster (*Palinurus elephas*), Whiting (*Merlangius merlangus*), Anchovy (*Engraulis encrasicolus*), Conger (*Conger conger*), Mackerel (*Scomber scombrus*), Horse mackerel (*Trachurus trachurus*) and Hake (*Merluccius merluccius*), furthermore four PAHs were selected as target analytes: Benzo[a]Pyrene (B[a]P), Benzo[a]Anthracene (B(a)A), Benzo[b]Fluoranthene (B[b]F) and chrysene, as Enacted by Regulation (EC) No 835/2011.

The main objective of this study was the chemical evaluation of fishing products. We are considering the quantitative analysis of PAHs in fish that are commonly caught in order to estimate the health risk assessment.

MATERIALS AND METHODS

Choice of biological material and sampling

This environmental study covers the two fronts of the Moroccan coastline. We carried out a study of 200 samples of different species of frozen fish, these samples were stored at -20°C e.g. Lobster (*P. elephas*), Whiting (*M. merlangus*), Anchovy (*E. encrasicolus*), Conger (*C. conger*), Mackerel (*S. scombrus*), Horse mackerel (*T. trachurus*) and Hake (*M. merluccius*) which represent a well-defined spatial distribution. Monthly biological sampling campaigns were carried out from January 2014 to December 2015 and 13 coastal sites (Nador, Tetouan, Larache, Kenitra, Casablanca, Eljadida, Safi, Essaouira, Agadir, Sidi Ifni, TanTan, Laayoune, Dakhla) were selected in relation to their close proximity to major effluent discharges and in more or less remote areas (Figure 1).

Chemicals

Solvents and reagents were of GC grade, n-Hexane (96%, C_6H_{14}), Dichloromethane (CH_2Cl_2) and isooctane were obtained from Scharlau (Spain). Anhydrous Magnesium Sulfate ($MgSO_4$) and Florisil® from trade market (aromatic GB). For the individual standard Polycyclic Aromatic Hydrocarbons (PAHs), Benzo[a]anthracene obtained from Sigma (US), Benzo[b]fluoranthene purchased from Supelco (US), Benzo[a]pyrene and chrysene obtained from Isotec (US).

Extraction and GC/MS analysis of polycyclic aromatic hydrocarbons

Approximately 25 g of specimen were homogenized and ground into a mixture of 100 ml hexane: Dichloromethane (v/v) and the extraction were carried out by sonication for 2 h (Branson 3200). The purification was passed through a glass column containing 5 g of Florisil and $MgSO_4$, then the column was eluted with 45 ml of dichloromethane, thus evaporating the organic extract in the evaporator rotary (Buchi Rotavapor R-200; Heating BathB-490). The recovery of the residue was carried out by 5 ml of iso-octane, and finally the filtrate was transferred to the GC vials. The extract was analyzed by Gas Chromatography Coupled to a Mass Spectrometer (GC/MS). The detection was carried out in Scan mode, which makes it possible to have the retention time and the mass spectrum of each compound. Then, Selected Ion Monitoring (SIM) mode was used to reduce interference and increase the detection limit. The GC/MS was equipped with (QP2010 plus, Shimadzu, Japan) column length (30 m, 0.25 mm, film 0.25 μm).

The sample solutions were injected using the "splitless" injector system on the GC/MS instrument. Helium was employed as a carrier gas with a flow rate of 1 ml/min. The column temperature program was as follows: 90°C (1 min), 10°C/min to 180°C and 5°C/min to 310°C (10 min). Acquisition was carried out in SIM mode. The injection volume was 1 μl and Electron Ionization (EI) at 70 eV was used in the analysis. Ion source temperature was 200°C [15-18].

Statistical analysis

The statistical processing of the data was carried out using the SPSS 21.0 software. The PAH concentrations in the samples were expressed as (mean ± SD). The difference between concentrations was assessed using the two-way Analysis of Variance (ANOVA) followed by post hoc test that were applied to provide the impact of sites and species. The similarity of the concentrations of PAHs between the sampling sites was studied by a Hierarchical Ascending Classification (HAC) which shows the distribution of the contaminants according to the sampling sites. The concentrations were expressed in (μg/Kg) of the fresh product.
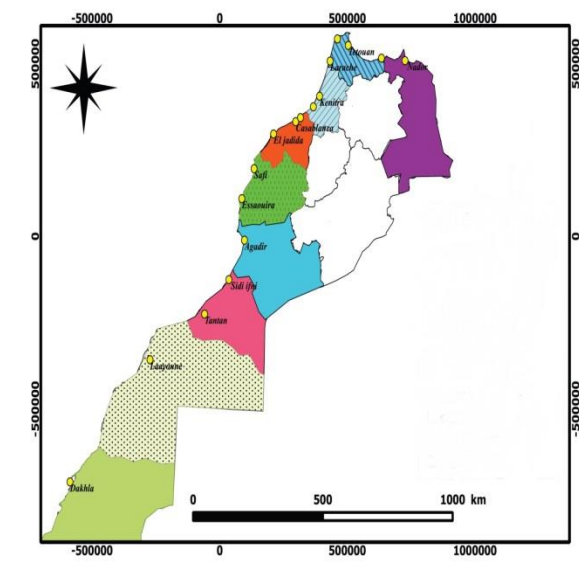

Figure 1: Geographical location of sampling stations along the Moroccan Coastal

RESULTS AND DISCUSSION

The results reported in this work were based on the analysis of four PAHs in fishing products (Figure 2). These results made it possible to compile statistics by calculating the mean values and the standard deviations, which correspond to the concentrations calculated according to the sampling sites (Table 1).

PAH concentrations reached a maximum level of 6.06 µg/kg in Tetouan, while the minimum level of 0.78 µg/kg was recorded in TanTan. Hydrocarbons are detected at all sites with the exception of B[a]A and B[b]F, respectively, in Sidi Ifni and Eljadida. Mean B[a]P concentrations show that the highest levels were reported in Tetouan (3.67 ± 1.28) and the lowest concentrations were found to be (0.11 ± 0.20) in TanTan. For chrysene the grades range from (0.10 ± 0.22) in TanTan to (0.94 ± 1.24) in Essaouira. B[b]F shows concentrations ranging from (0.03 ± 0.09) in Laayoune to (1.99 ± 1.67) in Dakhla. The spatial variation of B[a]A content ranges from (0.16 ± 0.14) in Larache to (1.55 ± 0.52) in Tetouan.
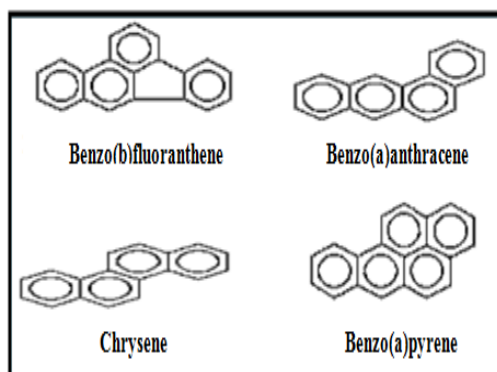

Figure 2: Chemical structure of four PAHs

The study of the geographical distribution of the organic contamination of the samples by the PAHs shows that the sites of the order of Tetouan, Dakhla, Eljadida, Essaouira, Safi and Kenitra present the highest contamination. Indeed, it is due to the degree of pollution to which fish from these sites are exposed. PAHs are poorly biodegradable, making them persistent in the environment. Their presence is highly dependent on geographical origin, and fish from industrialized areas may have higher levels of contamination [19].

To analyze the spatial distribution of pollutant concentrations, an Ascending Hierarchical Classification (AHC) was performed by aligning the different sites on the vertical axis. Figure 3 shows that variation in PAH levels makes it possible to differentiate the sampling areas and classify them into two distinct categories. The dendogram regroups the 13 sites studied according to the concentrations of contaminants. This distribution makes it possible to group the similar sites, close according to the contents of the PAHs. The AHC has produced a tree structure that has highlighted the hierarchical links between the different sites, by choosing the Euclidean distance and the Ward criterion that define the measure of resemblance. The dendogram shows two classes, with the first class collecting the cities of Nador, Larache, Agadir Casablanca, TanTan, SidiIfni and Laayoune, revealing average levels of PAH. The second class includes the cities of Tetouan, Kenitra, Eljadida, Safi, Essaouira and Dakhla. The highest levels of organic contamination were recorded in these regions. This class is directly subjected to the discharge into the sea by the chemical industries and is considered to be the main source of pollution in these areas.

Our results agree with a similar study by Sabhi et al. [20] which revealed that the Mediterranean coast of Morocco are relatively more contaminated by chemical pollutants. Tangier and Tetouan are the most threatened because of the industry developed in this area. Thus, they highlighted the inconsistent effects of effluent from the coastal cities "Hot Spots" on the immediate receiver (marine environment), which shows that cities on the Mediterranean coast are considered the main sources of urban and industrial discharges. And they also showed that the Mediterranean coast of Morocco is more polluted than the Atlantic coast. This difference may be due to the characteristics of the Mediterranean Sea and human activities (industrial activities). Thus the results reported by Ezziyyani et al. [21] are in agreement with our study and indicate that the Safi region represents an important industrial complex of phosphate transformation and receives a high organic and mineral load generated by domestic and industrial discharges. The shores of the city of Safi are potentially polluted by chemical rejections of phosphate treatments to which are added those of urban and industrial origins.

The city of Eljadida has a coastline showing various coastal formations, with on the other hand a threatening economic and population growth. Several effluents emerge and discharge into this coastline without any prior treatment [1]. The levels of PAH in Dakhla fish can be explained only by a natural intake of PAHs, probably due to upwelling [22]. In the case of Casablanca and Kenitra, the pollutants found in the samples come from the oil refinery near these cities. The Atlantic coast is under the influence of several actions, resulting in a wide modification of the biotic and abiotic parameters and consequently a variability of the marine biodiversity. Interpretation of the PAH contents from these results is difficult due to biogeochemical processes acting on their persistence and displacement. However, increases in concentrations in fish products may reflect local contamination [12].

Another study focused on levels of contamination in fish and seafood in Catalonia, Spain, showed that the levels of the four PAHs (0.07-0.20 ppb) are lower than those recorded along the Mediterranean coast from Morocco [23]. In a study carried out in Senegal, the maximum values of PAHs in mussels harvested at the coasts of the Dakar region (5308.67 and 17973.37 µg/kg dry weight in 2007 and 2008 respectively) are higher than those recorded in our study [24]. According to the study of Ramalhosa et al. [25], from the Atlantic Ocean, the PAH levels appear higher than the total concentrations determined in the Pelagic fish along the Atlantic coast of Morocco and vary between 2.29 and 14.18 µg/kg in *Sardina pilchardus*, 2.73 and 10.0 µg/kg in *T. trachurus* and 1.80-19.90 µg/kg in *Scomber japonicus* [26], showed that mackerel, European hake and blue whiting had high concentrations ranging from 44.1-63.3 ng/g [10] reported that PAH concentrations in different fish samples from the Dongjiang River and the Pearl River in China were high, ranging from 11.3 to 935 ng/g in marine fish. For fish of fresh water, the contents varying from 12.0-238 ng/g. The Bioconcentration Factor (BCF) is slightly higher for marine fish than for freshwater fish. The BCF may vary due to the diversity of fish species, dietary habits and PAH metabolism in fish. Fluctuations in PAH concentrations in various species of fish, bivalves, cephalopods and crustaceans have been demonstrated by several authors [10,12] and confirmed Spatial variation validated by the ANOVA test.

Table 1: Descriptive statistics of PAH concentrations in fish products

	PAH			
Sites	**B[a]P**	**Chrysene**	**B[b]F**	**∑PAH**
Dakhla	1.6 ± 1.88 (0.53-3.84)	0.55 ± 0.03 (0.53-0.57)	1.99 ± 1.67 (0.57-3.84)	5.24 ± 4.25
Laayoune	1.02 ± 1.12 (0-4.04)	0.22 ± 0.33 (0-0.95)	0.03 ± 0.09 (0-0.27)	2.48 ± 2.34
TanTan	0.11 ± 0.20 (0-058)	0.10 ± 0.22 (0-0.62)	0.11 ± 0.17 (0-0.58)	0.78±0.92
Sidi Ifni	0.49 ± 0.02 (0.48-0.51)	0.65 ± 0.10 (0.58-0.73)	0.43 ± 0.01 (0.42-0.44)	1.57±0.13
Agadir	0.58 ± 0.42 (0-1.74)	0.28 ± 0.26 (0-0.73)	0.48 ± 0.44 (0-1.95)	2.32 ± 1.8
Essaouira	1.78 ± 1.14 (0-4.34)	0.94±1.24 (0-3.57)	0.10 ± 0.13 (0-0.37)	4.36 ± 3.04
Safi	2.54 ± 2.58 (0-5.29)	0.60 ± 0.46 (0-1.07)	0.06 ± 0.08 (0-0.22)	4.27 ± 3.92
Eljadiada	3.57±0.45 (0-3.56)	0.30±0.02 (0-0.2)	(ND)	5.01±0.55
Casablanca	1.14±1.57 (0-4.09)	0.61 ± 0.59 (0.11-2.38)	0.14±0.01 (0-0.57)	2.84±3.21
Kenitra	2.23 ± 2.36 (0-5.11)	0.50 ± 0.37 (0-1.09)	0.04 ± 0.13 (0-0.48)	3.59 ± 3.63
Larache	0.27 ± 0.49 (0-1.18)	0.39 ± 0.37 (0-0.78)	0.06 ± 0.14 (0-0.42)	0.88 ± 1.14
Tetouan	3.67±1.28 (0.47-4.52)	0.86 ± 0.30 (0.35-1.49)	0.08±0.21 (0-0.61)	6.06 ± 2.31
Nador	0.43 ± ND (0-0.43)	0.25 ± ND (0-0.25)	0.80 ± 0.05 (0.71-0.85)	2.08 ± 0.11

ND: Not Detected; Mean values ± standard deviation; Values in parentheses indicate the minimum and maximum levels
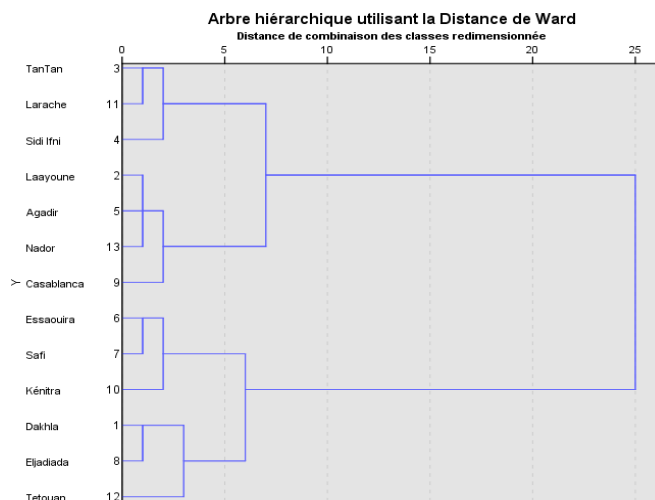

Figure 3: Ascendant hierarchical classification of organic contamination in function of geographic origin

Figure 4 shows the evolution of the PAHs as a function of the sites. This finding is also confirmed by the significant results at the threshold of 0.05 of the ANOVA test (Table 2), which allows the observation of significant differences according to the stations, respectively (F=2.91, P=0.02).

The station effect was observed taking into account the set of measurements at all stations. The study of PAH concentrations shows a significant difference between the sites, depending on the environmental conditions, such as pollution, nature and release rate also geographical distribution. The levels of PAHs present in fishing products indicate that the concentrations obtained are lower than the limit values laid down in the Regulation No. 835/2011). The analysis of variance in our study asserts that there is also a significant variation between the species (P<0.05). Moreover, this result agrees with the results of Ramalhosa et al.; Perugini et al. [25,27].

In this study, the origin of PAHs (petrogenic or pyrolytic) was determined using the following ratio: B[a]A/Chr. It is mainly a pyrolytic origin that is demonstrated for these PAHs for most sites, with the exception of Sidi Ifni where the origin of the PAH is petrogenic (Table 3). The origins of the existing PAH contents in the study sites are determined by the calculation of an index to distinguish between pyrolytic sources and petroleum sources. The ratio of the isomers of the quantified PAHs was studied to detect the source of these molecules. The B[a]A/Chrysene ratio of less than 0.20 is likely to indicate a petrogenic source, the B[a]A/Chrysene ratio ranging from 0.20-0.35 indicates a mixed origin. The B[a]A/Chrysene ratio greater than 0.35 indicates a pyrolytic origin [28]. In our study, the B[a]A/Chrysene ratio ranges from 0.41-5.5 indicating that PAHs contaminating fish are of pyrolytic origin. A ratio of 0 means that one of the compounds studied has a concentration below the limit of quantification per GC/MS [28] indicated that the ratio B[a]A/Chrysene do not appear to have sufficiently distinct energies of formation, to use them as discriminating indices.
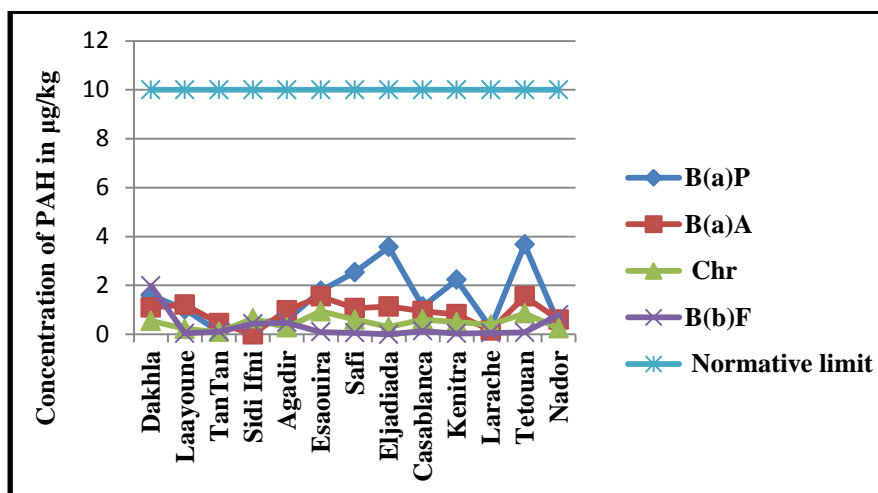
Figure 4: Variation of PAHs in fish according to regions

Table 2: Test of variance homogeneity, results of ANOVA test for the studied PAHs

PAH	Species		sites	
	D	**Sig**	**D**	**Sig**
	4.514	0.000	2.918	0.002

F: F-statistic; Sig: Significance means P value; P<0.05: Significant difference; P>0.05: No Significant difference

PAHs come from a variety of sources and are released into the environment in a systematic way. The hydrophobicity of PAHs increases with the number of aromatic rings, while their solubility and volatility decrease. They are classified into groups based on the number of aromatic rings and their molar masses. High molecular weight PAHs (in the order of 228-278 g/mol, e.g. 4-6 cycles) are B[a]A, chrysene, B[a]P and B[b]f. This means that high levels of four to five cycles' hydrocarbons are characteristic of PAHs formed during the burning of fossil fuels. According to the results obtained, the PAHs found in fish are mainly derived from pyrolytic sources. The ratios show a clear and significant pyrolytic effect on the origin of PAHs in the sampling areas on the Mediterranean Sea and the Atlantic Ocean.

PAHs of pyrolytic origin constitute the majority of PAHs introduced into the environment and are ubiquitous on a very wide geographical scale. They are emitted by chemical processes and by anthropogenic activities based on the incomplete combustion of organic matter. On the other hand, the introduction of petrogenic PAHs takes place on a more limited geographical scale, through natural seepage or accidentally during the transport of petroleum products. Concerns over chemical contamination have therefore arisen in the more populated areas, particularly due to natural processes, human, industrial and mining activities.

Table 3: **Variation of PAH contents and the origin of contamination according to sampling station**

Station	B[a]A/Chrysene	Source of contamination
Dakhla	2	Pyrolytic
Laayoune	5.5	Pyrolytic
TanTan	0.41	Pyrolytic
Sidi Ifni	0	Petrogenic
Agadir	3.5	Pyrolytic
Essaouira	1.63	Pyrolytic
Safi	1.78	Pyrolytic
Eljadiada	3.8	Pyrolytic
Casablanca	1.55	Pyrolytic
Kenitra	1.64	Pyrolytic
Tetouan	1.80	Pyrolytic
Larache	4.6	Pyrolytic
Nador	2.4	Pyrolytic

Figure 5 shows that the percentage contribution of four-rings PAHs ranges from 1 to 15% and the percentage of five-rings PAHs ranges from 3 to 14%. The highest percentage of four and five-rings PAHs was recorded in Tetouan, with the lowest percentage being 1% (Laayoune).

The five-rings PAHs were found more in Tetouan, Safi, Eljadida, Kenitra, Casablanca and Dakhla. The four-ring PAHs were concentrated in Essaouira, Agadir, TanTan, Laayoune and Larache. Four and five-rings PAHs were identified in both Nador and Sidi Ifni. The distribution of these substances according to the number of cycles was explained in terms of the physicochemical properties and the biological activity of the receiving medium [19]. Figure 6 shows the contents of the four PAHs and B[a]P, they are observed more frequently and at higher concentrations in the regions already mentioned at the level of the Atlantic coast and the Mediterranean coast. By comparing the results obtained from several sites in fish, the sum of the four PAHs ranges from 0.78 ppb-6.06 ppb and the B[a]P content ranges from 0.11 ppb-3.67 ppb.

Factors that can increase the adverse effects of chemical contamination include the increase of human settlements and their activity, extraction of natural resources, chemical spills and climate change, allowing an integrated picture of chemical contamination and its effects on fish and its preservation in Morocco.
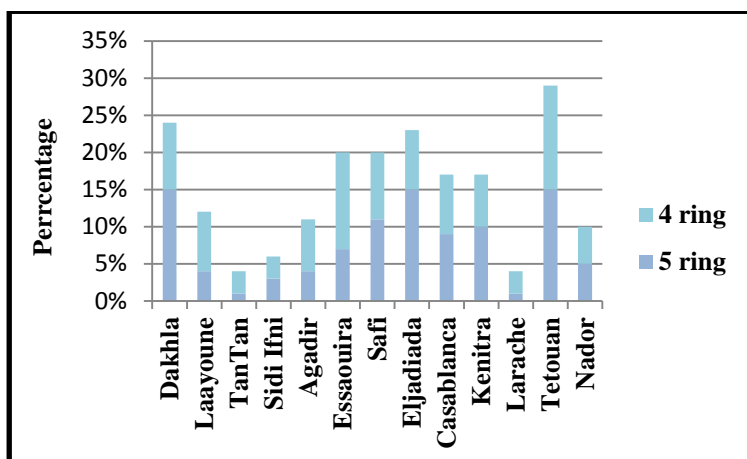
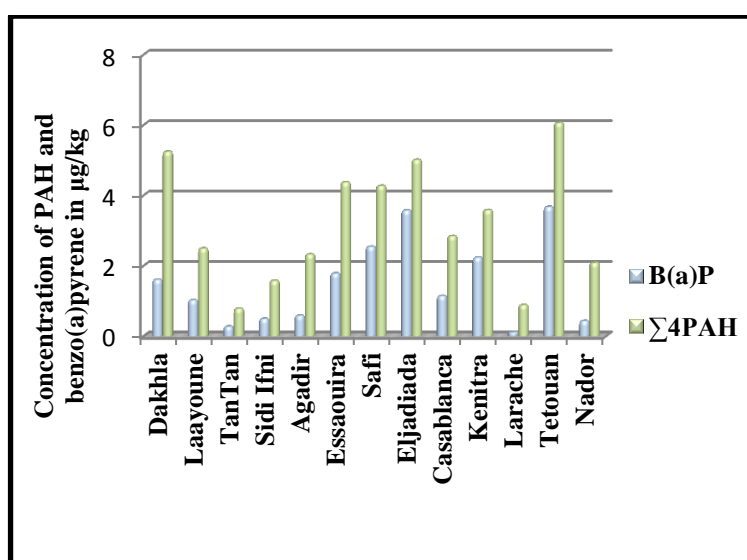**Figure 5: Variation of PAH percentages (4 rings, 5 rings) in fishery products according to regions**

Figure 6: Variation of the concentrations of ∑4PAH and of benzo(a)pyrene in fishing products according to sampling sites

CONCLUSION

In this work, we studied the distribution of PAHs in fish samples harvested from the Moroccan coastal. Our study analyzed the importance of the determination of contaminants in aquatic ecosystems in relation to other factors that could impact the protection of fish and the sustainable use of fishing resources. The results obtained show that the bioaccumulation is variable according to the station and the species. Some areas have maximum levels because of the activities developed there. The northern sector comprises the largest urban agglomerations and contains various industrial units (petrochemicals, agro-food and textiles). The central sector is characterized by urban agglomerations and important industrial infrastructures located in the coastal zones of El jadida and Safi. The southern sector is experiencing less urban and industrial development.

The fish are contaminated by hydrocarbons but the concentrations do not exceed the maximum levels laid down by the regulation of the European Union, which does not entail a public health problem. Monitoring plans are the main means of limiting the impacts of pollution on the environment and biodiversity. In this sense, the National Office for food safety has several networks along the Moroccan coast, and carries out campaigns to assess the health status of certain coastal resources. The growth of the industry will raise concerns about the impact of the large increase in contaminants. In conclusion, it can be said that any potential risk associated with fish consumption can be minimized by compliance of official recommendations. However, it is necessary to adopt more stringent regulations in order to avoid negative impacts on marine flora and fauna. It is imperative to monitor these environments against the harmful effects of human and industrial activities in order to preserve human health and environmental quality.

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