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## Polycyclic Aromatic Hydrocarbons Concentration Levels in the Cephalopod Species along the Moroccan Atlantic Coast

Fatima Zahra Ndadani<sup>a\*</sup>, Fouzia Zkhiri<sup>b</sup>, Abderrazzak Rachidi<sup>c</sup>

<sup>a,b</sup>Laboratory of Virology, Microbiology, Quality, Biotechnology, Eco-toxicology and Biodiversity, Department of Biology, Faculty of Sciences and Techniques Mohammedia, University Hassan II of Casablanca, B.P.146, Mohammedia, Morocco.

<sup>c</sup>National Office for Health Safety of Food Products, Regional Laboratory of Analysis and Research, Casablanca, Morocco.

### Abstract

The Moroccan coastal is constituted of two contrastive coasts, the Atlantic Ocean and Mediterranean Sea. The purpose of this study is to assess the concentrations of four polycyclic aromatic hydrocarbons (PAHs) in three cephalopod species landing on the main ports of the Moroccan Kingdom along the Atlantic coast. PAHs are ubiquitous environmental contaminants with multiple sources that present a toxic hazard due to their carcinogenic and genotoxic effects. Dietary intake is one of the most common exposure pathways of PAHs. The analysis of these pollutants is carried out by gas chromatography linked to mass spectrometry. The results obtained in this study show a variation of the levels of PAHs by the sampling sites (significant positive relationships  $p \leq 0.05$ ). Within these results, the PAH content in the cephalopod species did not exceed the limit allowed by the European Commission. The health risks by consumption of these species were assessed and revealed no threat to the consumer.

**Keywords:** Polycyclic aromatic hydrocarbons; GC/MS; Cephalopods; Atlantic coast; Morocco.

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\* Corresponding author.

## **1. Introduction**

The Atlantic Ocean is rich in high value fish, cephalopods, and crustaceans due to the inclusion of the Northeast, Eastern, and Central Atlantic in the highest principal marine fishing areas in the world [1]. Among seafood species, cephalopods represent one of the most interesting ecological and commercial classes. World food fish aquaculture production in 2014 consisted of (22%) of mollusks [2]. Fish products are often contaminated by a variety of chemical pollutants that are found among a wide range of toxic substances within the aquatic environment, with a major concern having been focused on polycyclic aromatic hydrocarbons due to their toxicological relevance [3, 4]. PAHs are a class of organic contaminants that attract increasing attention for their widespread occurrence in the environment. They are a ubiquitous group of several hundred chemically related compounds that are environmentally persistent and have various structures and varied toxicity. The aromatic series includes unsaturated hydrocarbons, which collect all compounds containing one or more aromatic rings [5, 6]. The main sources of contamination within coastal environments are anthropogenic sources resulting from incomplete combustion of grass, soda and aluminum production, catalytic cracking towers, and activities related with petroleum refineries. Some PAHs in the environment may originate from natural sources, such as land based runoff produced by burning, natural losses, seepage of petroleum or coal deposits, and volcanic activities [7, 8, 9]. Due to their lipophilic and hydrophobic nature, PAHs are concentrated in biota and can allow bio-magnification up the food chain [10, 11]. Their mechanism of toxicity is considered to be interference between the functions of cellular membranes and the enzyme systems associated with them. PAHs have been included in the priority pollutant lists due to their mutagenic and carcinogenic properties. According to The European Food Safety Authority (EFSA), the following four PAHs, benzo[a]anthracene (B[a]A), chrysene (Chr), benzo[b]fluoranthene (B[b]F), and benzo[a] pyrene (B[a]P), are the only possible indicators of PAHs in food. The EFSA indicated that B[a]P cannot be maintained as the only PAH marker in seafood as was done previously, therefore identifying this group of four PAHs as the most appropriate indicators based on data relating to occurrence and toxicity [12]. In light of the above-mentioned issues, the aim of this study is to analyze the four PAHs enacted in Regulation in order to assess the quality of Cephalopods and health risk of these contaminants. Statistical analysis is done based on analysis of variance and correlations to reveal the levels of these pollutants by geographic origin.

## **2. Materials and methods**

### ***2.1. Extraction and gas chromatography coupled to mass spectrometry analysis***

#### ***2.1.1. Sampling***

The samples were collected on eight sites: Dakhla, Laayoun, Tantan, Sidi Ifni, Agadir, Safi, Casablanca, and Kenitra, with the locations plotted on the geographical map in Figure 1. Sampling was conducted quarterly from January to December 2015, and covers a variety of marine species.

#### ***2.1.2. Reagents and solutions***

All solvents and reagents used in this study were of GC grade, n-Hexane (96%, C<sub>6</sub>H<sub>14</sub>), dichloromethane

(CH<sub>2</sub>Cl<sub>2</sub>) and iso-octane were procured from Scharlau (Spain). Anhydrous magnesium sulfate (MgSO<sub>4</sub>) and florisil were purchased from trade market (aromatic GB), with standard PAHs, Benzo (a) anthracene obtained from Sigma (US), Benzo (b) fluoranthene purchased from Supelco (US). Benzo (a) pyrene and Chrysene were obtained from Isotec (US).

### 2.1.3 Extraction and analytical methods

Each of the sampling stages of storage and transport can directly impact the quality of analytical results. Considerable care has been applied to minimize risks of contamination and degradation of the samples. The samples were kept frozen at -20 ° C until analysis. Fish samples (25 g) were collected, immersed in n-hexane / dichloromethane, the extraction was carried out by sonication. The extract was then eluted with dichloromethane and then purified on a column of florisil and anhydrous magnesium sulphate (MgSO<sub>4</sub>). This extract was evaporated and the residue was collected by isooctane. A GC-MS system consisted of (QP2010 plus, Shimadzu, Japan) gas chromatography with a GC column (DB-1MS 30m long, 0.25mm internal diameter, 0.25 μm ) was used for PAH determination. Selected ion monitoring (SIM) mode was employed for identification and quantification of four individual PAH compounds benzo (a) pyrene, benzo (a) anthracene, benzo (b) fluoranthene and chrysene. The analysis of these molecules by mass spectrometry using the electron impact ionization eV=70 [13, 14, 15, 16].

### 2.1.4. Statistical analysis

Statistical analysis was used to evaluate the analysis data and was performed using SPSS v. 21. Significant differences among sites were determined by One-way analysis of variance (ANOVA). ANOVA was performed to test the spatial distribution on the measured parameters. Correlations were sought by a principal component analysis (PCA) between polycyclic aromatic hydrocarbons.

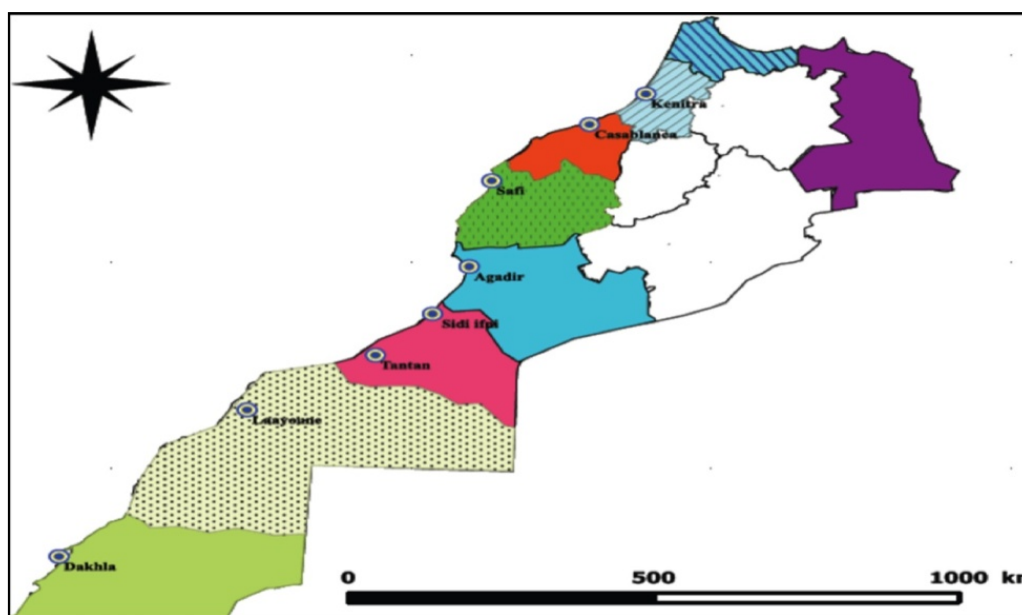


Figure 1: Geographical location of the different sampling sites.

### 3. Results and Discussion

#### 3.1. Spatial distribution of PAHs

Despite the health benefits of a seafood diet, there is a concern related to frequent seafood consumption due to the potential risk arising from exposure to aquatic pollutants [17, 18, 19]. In this study, the four PAHs enacted in Regulation (835/2011) were measured in cephalopods in the Atlantic coast, with their mean concentrations and ranges summarized in Table 1. The total mean content of PAHs was found in octopus (2,68 $\mu$ g/kg), cuttlefish (0,41 $\mu$ g/kg), and squid (0,96  $\mu$ g/kg). The other studies showing the lowest concentrations of  $\Sigma$ 7PAH were, squid (0,24  $\mu$ g/kg), cuttlefish (0,23  $\mu$ g/kg) [18]. Data obtained in this study for squid are lower in comparison with the results reported by [20] (9,16- 45,4  $\mu$ g/kg) in Indic ocean, (26,9-58,6  $\mu$ g/kg) in Atlantic ocean, (15,1  $\mu$ g/kg) in Pacific ocean. Other surveys of PAHs in marine organisms from the Adriatic Sea, the Gulf of Naples, and the Tyrrhenian Sea show determined sums in the collected specimens, of 14.7 and 16.3-71.2  $\mu$ g/kg (from the Adriatic Sea and Tyrrhenian Sea respectively). Squid is known for distinct horizontal and vertical migrations essential to feeding, they remain close to the bottom during the day, and then disperse into the water column at night [20].

**Table 1:** Descriptive statistics of PAHs concentration in cephalopod species originating from the Atlantic coast of Morocco.

	Octopus	Cuttelfish	Squid
Benzo(a)pyrene	0,49 $\pm$ 0,58 (ND-1,90)	0,06 $\pm$ 0,07 (0,06-0,17)	0,14 $\pm$ 0,24 (0-0,43)
Benzo(a)anthracene	1,30 $\pm$ 1,17 (0,23-4,30)	0,26 $\pm$ 0,05 (0,05-0,34)	0,46 $\pm$ 0,40 (0-0,58)
Beno(b)fluoranthene	0,57 $\pm$ 0,88 (ND-3,35)	ND	0,10 $\pm$ 0,16 (0-0,29)
Chrysene	0,31 $\pm$ 0,39 (0-0,82)	0,08 $\pm$ 0,09 (0-0,16)	0,26 $\pm$ 0,44 (0-0,77)
$\Sigma$ PAH	2,68 $\pm$ 3,04 (0,22-10,38)	0,41 $\pm$ 0,22 (0,11-0,69)	0,96-0,86 (0,46-1,95)

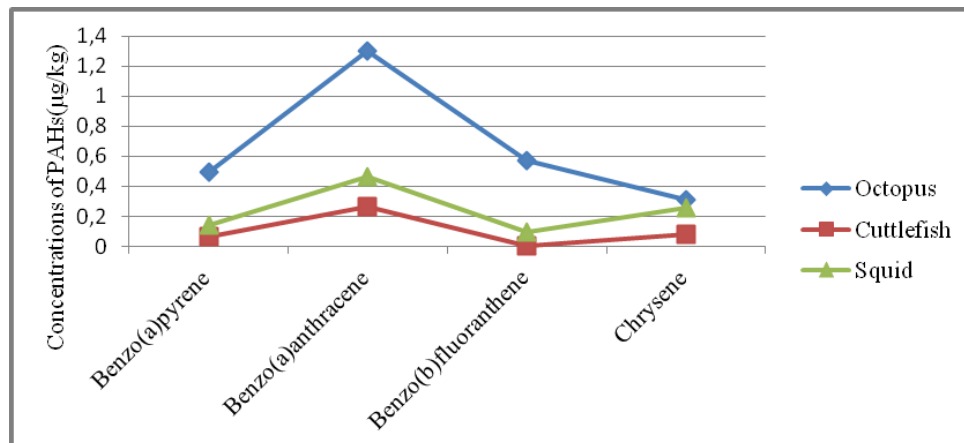
[ND: Not detected; Mean values  $\pm$  standard deviation; Values in parentheses indicate the minimum and maximum levels].

Statistical comparisons revealed that total PAH concentrations are significantly different ( $p < 0.05$ ) between sites. The analysis of ANOVA test indicated that there's a statistically significant impact of sampling areas on PAH concentrations, which proves the spatial variation influence on the concentrations of these molecules (Table 2). Overall, the detected levels are in agreement with those attained for others species in other studies [21, 22]. Figure 2 shows the distribution of PAHs in the selected cephalopod species in all locations, with the lowest PAH levels obtained in squid, and the highest concentrations in octopus, even though the average levels were under the recommended maximum permissible limit. Studies show that in aquatic food chains, vertebral fish have the ability to quickly metabolize PAHs, by contrast mollusks have the poorest metabolic capacity, the bioavailability, uptake, and fate of PAHs in water, sediments, and food were also affected by a variety of parameters [23].

**Table 2:** Analysis of variance of PAHs by sites.

PAH	F	Sig.
Benz[a]anthracene	1,464	,000
Benzo[b]fluoranthene	4,736	,000
Benzo[a]pyrene	1,365	,000
Chrysene	3,268	,000

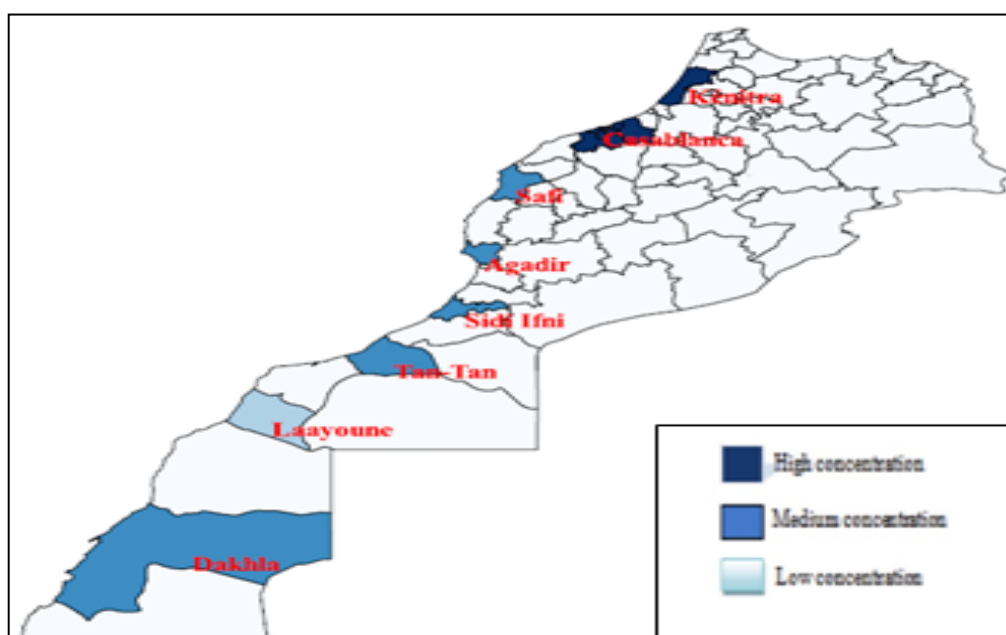
[F: F-statistic; Sig: Significance means p value; p value  $< 0.05$ : Significant difference; p value  $> 0.05$ : No Significant difference].



**Figure 2:** Variation of PAHs by fishery products in different sampling stations

Figure 3 reveals the impact of spatial variation approved on chemical contamination. The spatial distributions of pollution sources were related to geographic location, with the results showing that the most contaminated sites are Kenitra and Casablanca, due to the fact that these areas are the seats of many ports with urban and industrial activities and the pollutants found in the specimens might have come from an oil refinery close to the cities. Mollusks from areas such as Dakhla and Agadir had the medium PAH levels, which can only be explained by a

natural input of PAHs due to upwelling. As for Safi, it is located near main chemical industries [22, 24, 25]. The results obtained by [21] show that Moroccan Mediterranean coast is more polluted than the Atlantic one. This difference is probably due to the characteristics of the Mediterranean Sea (limited exchanges with others seas and oceans, and limited dynamic tidal effects). Previous studies have indicated that the PAH contamination within vertebrate marine organisms differs significantly from invertebrates, and explained that cephalopods can be an important vector for lipophilic organic compounds. The results show that anthropogenic chemicals reach deep into the ocean where they are accumulated by prey species and are available to higher trophic levels, this is confirmed by the high levels of contaminants determined according to the depth and remoteness of the sampling environment [26,27].

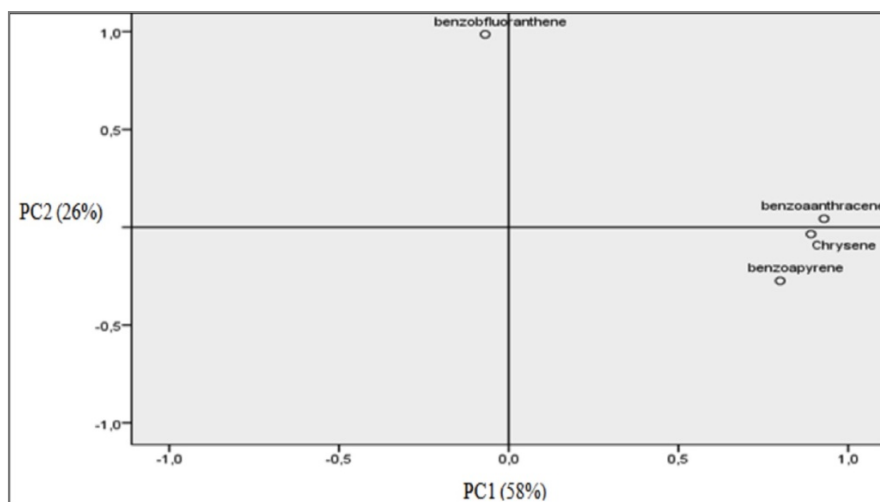


**Figure 3:** Spatial variation of PAHs contamination along the Atlantic coast

### 3.2. Principal component analysis

Principal Component Analysis (PCA) was performed on individual PAH components to reduce and extract a small number of latent factors for studying the relationship among the different molecules. The projection of the spatial variability explained that two particular components with a higher value than 1 were extracted and rotated using the varimax method. The two factors justified 84% of data variance with factor 1 explaining 58% of the total variance, and factor 2 accounting for 26% of total PAH.

The factorial map of the PCA (Figure 4) shows a positive correlation of the four molecules, with a strong positive correlation of benzo (a) pyrene, benzo (a) anthracene, and chrysene to the first factor. Benzo (b) fluoranthene has a positive association to the second factor. It has been reported that B(a)P, B(a)A, B(b)F and Chr are indicators of incomplete combustion as B(a)A, B(b)F and Chr are indicatives of coal combustion. B(b)F is a component of fossil fuels, with its major sources being anthropogenic [11, 21].



**Figure 4:** Analysis principal components of  $\Sigma$ 4 PAHs

#### 4. Conclusion

This study is part of the chemical residue monitoring surveys implemented by the veterinary services of National Office for Health Safety of Food Products, with its target being the preservation of consumer health. The results of this research provide information on  $\Sigma$ 4PAHs concentrations in the cephalopod species from the Moroccan coast along the Atlantic Ocean. The samples analyzed are compared with consumption thresholds mentioned by Regulation (EC) No 835/2011 of the European Commission. The concentrations of PAHs in all species of Moroccan Atlantic coast were considerably lower than the maximum levels set by Commission Regulation, therefore concluding that the products are not hazardous to the consumer. Marine pollution is a major concern in the population and amongst scientists due to its effects on the environment and the human health. The Mediterranean and Atlantic coasts of Morocco play an important socio-economical role in fishing and tourism and represent a dynamic environment; however, this national industry is facing changes of fishery resources and therefore requires continuous monitoring in the future.

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