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Polycyclic Aromatic Hydrocarbons in *Sardina pilchardus*: Levels and Health Risk Assessments through Dietary Exposure in Morocco

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ABSTRACT

Polycyclic Aromatic Hydrocarbons (PAHs) are a series of organic contaminants that have become ubiquitous in the environment. For the general population, exposures of humans to environmental pollutants, such as PAHs, can occur by various pathways, such as drinking water, inhalation of ambient air and ingestion of food (e.g., seafood). The exposure to high concentrations of PAHs together with other factors could contribute to the decreased health of fish living in marine ecosystem and the assessment of the impact on biota is of considerable concern. The aim of the present research was to study the bioaccumulation of four selected PAH in Sardina pilchardus collected from ten locations along the Moroccan coasts. PAHs determinations were performed using Gas chromatography-mass spectroscopy (GC/MS). The carcinogenic toxicity (TEQBaP), exposure daily intake (EDI), margin of exposure (MOE) and excess cancer rate (ECR) of four priority PAHs were evaluated. The results of this study have confirmed that 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.

Keywords: Polycyclic aromatic hydrocarbons, Chemical contamination, *Sardina pilchardus*, Moroccan coastal, Human health risk assessments.

INTRODUCTION

Fish is widely consumed in many parts of the world by humans because it constitutes an important source of proteins, minerals and vitamins. Increased seafood consumption to achieve an adequate intake of omega-3 poly-unsaturated fatty acid may simultaneously increase the contaminant intake to levels of toxicological concern. Assessment of exposure to PAHs is important due to the widespread presence of PAHs in the environment and their toxicological relevance. For a better risk evaluation, the World Health Organization and other international organizations have suggested to apply the tolerable intake and health risk factors for various toxic compounds [1]. There is a multitude of classes for potentially toxic contaminants that affect the quality of fish products of which include (PAHs). Such compounds contain two or more fused aromatic rings in linear, angular or clustered arrangements and are widespread in aquatic environments [2]. PAHs are highly persistent and toxic and it is confirmed that the long-term exposure to these chemicals will induce health problems due to their mutagenic and potentially carcinogenic properties [3,4]. PAHs originate from both natural processes and anthropogenic activities [5].

Several studies have highlighted the levels of PAHs in food and relate subsequent consumption as an important exposure pathway [6]. Human exposure to PAHs mainly occurs through food which can be contaminated by environmental PAHs that are present in air, soil, water or industrial food processing methods [7]. PAHs can be present in seafood as a result of bioaccumulation in fatty tissues and pyrolysis of oils dripping into flame. The residual levels of PAHs in aquatic organisms depend on the contamination of their habitat and the ability of these organisms to metabolise the contaminants. Fish metabolize PAHs into epoxides, phenols, quinones and subsequently in water soluble compounds [8,9].

Generally, adverse effects of PAHs have been shown in animal samples, but the risk also exists for humans [10,11]. Some compounds are classified as persistent organic pollutants, sixteen of which are regarded as priority pollutants by the U. S. Environmental Protection Agency [12]. According to the Commission Regulation No. 1881/2006, benzo[a]pyrene (B[a]P) could be used as a marker for the occurrence of these carcinogenic PAHs in food. However due to the multiplicity of PAHs and their complex existence, the European Food Safety Authority (EFSA) have identified some PAH compounds as representatives of the lot based on factors such as availability of information about them compared to others, degree of toxicity and degree of concentration [13]. Commission Regulation No 835/2011 [14] amended Regulation No 1881/2006 in regards to maximum levels for polycyclic aromatic hydrocarbons in food compounds and established the four PAHs (PAH4) to be the suitable indicators of PAHs in food [15]. In the present paper, B[a]P,(Chr), B[a]F, B[a]A concentrations are analysed in sardines of the largest Moroccan cities: Tetouan, Tangier, Kenitra, Casablanca, Safi, Essaouira, Agadir, TanTan, Laayoun, and Dakhla. The sampling was carried out during 2014 and 2015. The aim of the present study is the identification of contaminated sites and the measurement of contamination levels in marine species with regard to providing a continuing assurance of the coasts quality in accordance to public health. Therefore it was designed to conduct the

health risk assessment of PAHs exposure using the carcinogenic quotients.

MATERIELS AND METHODS

Sampling

Samples were collected on ten sites: Dakhla, Laayoun, Tantan, Agadir, Essaouira, Safi, Casablanca, Kenitra, Tangier, and Tetouan, with the locations plotted on the geographical map in Figure 1. The fish were collected over a period extending from January to December 2015.

Experimental

Sample preparation is an important stage in the analytical process, especially for analysis at low concentrations in complex matrices. Most of the analytical procedures described in the literature consist of many steps, extraction, purification, concentration, identification and quantification. The samples were extracted by solvent extraction followed by purification; the identification and quantification were performed by using GC/MS. The extraction and cleanup procedure of matrix samples can be found elsewhere in detail [16].

Statistical tests

A multivariate analysis of variance (MANOVA) was applied to test the spatial distribution of species and the influence on the measured parameters. Statistical analysis was thus based on the homogeneity of variance with Levene's test. Correlations were sought out by a principal component analysis (PCA) between polycyclic aromatic hydrocarbons [17].

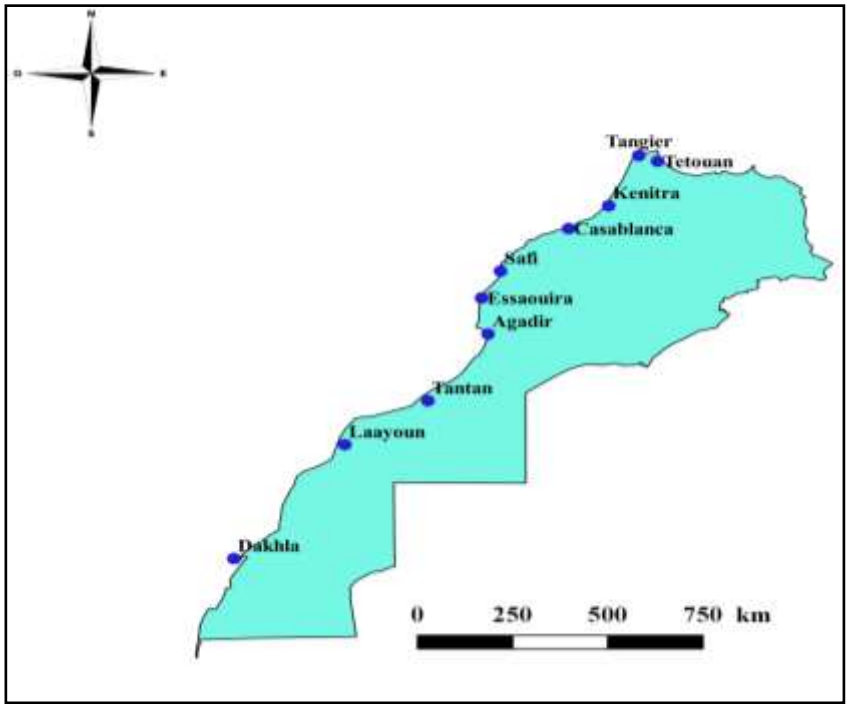

Figure 1: Geographical location of sampling stations along the Moroccan coasts

Human health risk estimations

Concentration of PAHs in the fish species was determined for individual PAH, namely Benzo(a)anthracene, Chrysene, Benzo(a)pyrene, Benzo(b)fluoranthene. To assess human health risks from exposure to PAHs through consumption of fish, human intake models were applied.

Exposure daily intake (EDI)

The Exposure daily intake (EDI) of PAHs in the sardine was assessed for general population using equation (1) [18].

$$EDI = Ci \times IR / BW \quad (1)$$

Where Ci is the PAH concentration (µg/Kg) in the edible portion of the fish, the average daily ingestion rate for fish in Morocco was obtained from data of the Food Agriculture Organization [19]. IR is the fish consumption rate (g/day). BW is the average body weight (60 Kg) for the general population.

Benzo(a)pyrene-equivalent carcinogenicity TEQB(a)P

In order to assess the possible human exposure risks associated with carcinogenic PAHs in fish samples, the toxic equivalence factors (TEFs) was calculated relative to a Reference standard benzo[a]pyrene. The TEF methodology was developed by the U. S. Environmental Protection Agency (EPA) to evaluate the relative toxicity and assess the risks of a mixture of PAH. The TEFs developed by [20] was applied (Table 1). TEQBaP was calculated as shown in the equation (2) below:

$$\Sigma B(a)Peq = \Sigma Ci \times TEFi \quad (2)$$

Where TEQBaP is the total TEQ level converted as benzo (a) pyrene by using the toxic equivalency factor (TEF) of PAHs in fish and Ci is the

concentration of each individual PAH in the fish. The TEF for these PAHs is an estimate of the relative toxicity of each PAH compared to benzo(a)pyrene, which is assigned a reference value of 1 [21].

Risk assessment for carcinogenic exposure (ECR)

The cancer risk (CR) resulting from dietary exposure to PAHs via seafood consumption was determined using equation (3).

$$ECR = \sum B(a)Peq \times IR \times EF \times ED \times CSF / BW \times AT \quad (3)$$

Where EFr is exposure frequency (365 days year-1), ED (years) is the exposure duration, CSF (mg kg-1 day-1) represents the cancer slope factor. The value of CSF is 7.3 mg/kg/d, BW (kg) is the body weight and ATn is the averaging time of exposure (365 days/ year x number of exposure years) [22,23].

Margin of exposure (MOE)

The Scientific Committee has recommended using a different approach for providing advice to risk managers, known as the margin of exposure (MOE) approach. The MOE is defined as the benchmark dose lower confidence limit (BMDL10) to the estimated human intake of the compound. The BMDL10 represents a 10% tumour response in the animal study [14]. The calculated BMDL10 value was 0.34 mg/Kg of body weight (bw) per day. The MOE from each sample was estimated using the following equation (4) [24].

$$\text{Margin of exposure (MOE)} = \text{BMDL10}/(\text{Daily Intake}) \quad (4)$$

Table 1: Name, abbreviation, number of rings, and TEFs of the 16 PAHs

PAHs compounds	Abbreviation	Number of rings	TEF*
Naphthalene	NA	2	0.001
Acénaphtylène	Ap	3	0.001
Acénaphthene	Ac	3	0.001
Fluorine	F	3	0.001
Anthracene	Ant	3	0.01
Phanthrene	Phe	3	0.001
Fluoranthene	Fl	4	0.001
Pyrene	Pyr	4	0.001
benzo(a)anthracene	BaP	4	0.1
chrysène	Chr	4	0.01
Benzo(k)fluoranthène	BkF	5	0.1
Benzo(b)fluoranthène	BbF	5	0.1
Benzo(a)pyrène	BaP	5	1
indéno(1,2,3-c,d)pyrène	Ip	6	0.1
Dibenzo(a,h)anthracène	DBahA	5	5
Benzo(g,h,i)pérylene	BghiP	6	0.01

* TEF toxic equivalence factors for cancer potency relative to B(a)P

RESULTS AND DISCUSSION

Levels of PAHs

In marine species, the relationships between species distribution and environmental factors are essential to understand the many aspects of their ecology towards effective conservation, management and assessment of possible impacts from anthropogenic activities. One of these approaches is the maximum entropy model (Maxent) [25]. The spatial and temporal dynamics of the sardine stock of the Moroccan Atlantic coast has been the subject of several studies. During the fall and winter season, the sardines tend to migrate south along the prevailing currents from north to south and during the warm seasons, they migrate in the opposite direction towards the north [26]. One of the fundamental factors that characterize the marine environment of small pelagics is surface temperature (SST).

The accumulation of PAHs in marine organisms can negatively affect health of human populations who consume this seafood. In this study, from the Moroccan coast of the Mediterranean sea and from the Atlantic ocean, the study was conducted based on the impact of the discharge of PAHs analysed in the fishing products in the following different cities: Tetouan, Tangier, Kenitra, Casablanca, Safi, Essaouira, Agadir, TanTan, Laayoun and Dakhla. Levels of PAHs in the sardines are shown in Table 2. The results reported in this work were based on the analysis of four PAHs in fishing products.

In the different regions studied, the results showed that the most contaminated sites are Tangier, Tetouan, Safi and Agadir for these areas are seat of many ports, and urban, industrial and touristic activities. Our results are in accordance with [27]. They have been conducted to underline the considered cities of the northern Moroccan coast as major sources of urban and industrial discharges (hot spots). Benzo(a)pyrene levels were higher in the sardines from Tangier, Tetouan, and Safi. In sardines from the Moroccan coast of the Atlantic ocean, the mean Benzo(a)pyrene values ranged from 0.15 µg/kg in Dakhla to 0.78 µg/kg in Safi. From the Mediterranean Sea, the mean benzo(a)pyrene levels were 1.15 µg/kg in Tangier and 0.80 µg/kg in Tetouan. Benzo(a)anthracene in the sardine samples ranged from 0.17 µg/kg in Dakhla to 0.84 µg/kg in Tangier. Chrysene ranged from 0.12 in Dakhla to 0.66 in Tangier and Benzo(b)fluranthene ranged from 0.008 µg/kg in Essaouira to 0.58 µg/kg in Tetouan. Significant differences observed in the sites ($p<0.05$) are shown on Table 3. The MANOVA test indicated that there is a statistically significant impact of sampling areas on PAH concentrations, which proves the spatial variation influence on the concentrations of these molecules. The composition profile of PAHs by ring number is depicted in Figure 2, showing the 4-ring PAH, which accounts for 3-15% of the total PAHs and was the highest in Tangier. Percentage PAHs contribution of the 5-ring PAH ranged from 4% to 16%, which are more toxic. In particular, the lowest percentage of 5 ring PAHs (4%) was detected in TanTan. The observed pattern may be explained in terms of physicochemical properties of PAHs and proximity to the source [28].

Table 2: Descriptive statistics of PAHs concentration in Sardine originating from the Moroccan coasts

	Benzo(a)pyrene	Benzo(a)anthracene	Chrysene	Benzo(b)fluoranthene	∑ HAP
Tetouan	0.80 ± 0.05	0.76 ± 0.05	0.64 ± 0.04	0.58 ± 0.04	2.78 ± 0.18
Tangier	1.15 ± 0.88	0.84 ± 0.05	0.66 ± 0.04	0.42 ± 0.03	3.07 ± 1
Kenitra	0.62 ± 0.20	0.70 ± 0.34	0.47 ± 0.19	0.10 ± 0.01	1.89 ± 0.74
Casablanca	0.76 ± 0.62	0.40 ± 0.35	0.60 ± 0.04	0.84 ± 0.40	2.6 ± 1.41
Safi	0.78 ± 0.01	0.80 ± 0.05	0.52 ± 0.22	0.60 ± 0.11	2.7 ± 0.39
Essaouira	0.54 ± 0.23	0.78 ± 0.01	0.38 ± 0.04	0.008 ± 0.05	1.70 ± 0.33
Agadir	0.57 ± 0.17	0.86 ± 0.52	0.33 ± 0.03	0.45 ± 0.36	2.21 ± 1.08
TanTan	0.17 ± 0.01	0.2 ± 0.04	0.29 ± 0.01	0.16 ± 0.05	0.82 ± 0.11
Laayoun	0.20 ± 0.04	0.31 ± 0.03	0.27 ± 0.13	0.42 ± 0.02	1.2 ± 0.22
Dakhla	0.15 ± 0.07	0.17 ± 0.01	0.12 ± 0.04	0.31 ± 0.03	0.75 ± 0.15

Table 3: Analysis of variance of benzo(a)pyrene by Sampling stations

	Sites	
HAP	**F**	**Sig.**
Benz[a]anthracene	6.93	.000
Benzo[b]fluoranthene	4.38	.000
Benzo[a]pyrene	3.52	.000
Chrysene	2.03	.000

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

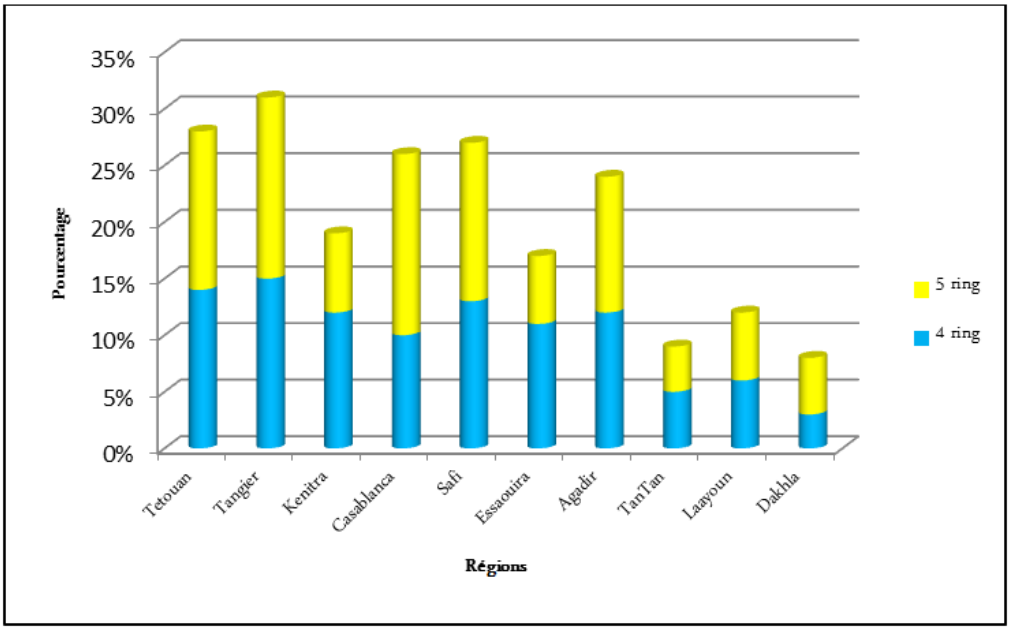

Figure 2: Ring distribution of PAHs in different regions of the Moroccan coast

The results of our study showed that concentrations of PAH in Sardines were generally lower than those reported in other studies. Summed PAH concentrations ranged from 1.9 to 37 µg/kg, wet weight, were reported by [29] in fish muscle tissue taken from the coastal waters of Madagascar. [30] Reported that the total concentrations of PAHs ranged from 2.29 to 14.18 µg/kg ww for sardines. The PAH levels in various food compounds consumed in Spain have indicated mean values of 1.32, 17.72, 5.3 and 5.0 µg/kg ww for sardines. In another study, summed PAH concentrations in chub mackerel, wet weight, were 1.8 to 20 µg/kg [29]. Since vertebrate fish have the ability to rapidly metabolize PAHs [31].

Sardines from areas such as Tangier, Tetouan and Safi specifically had the highest PAH level, which can be can only be explained by related manufactories, industrial and urban activities. These results show that the Tangier coast is the most contaminated area in the northern Mediterranean coast of Morocco, although they did not exceed the limit allowed by the European Commission. Sardines live in the open seas or oceans and have a lower exposure to higher molecular weight PAHs, which remain mainly in the sediments. Loads in the studied marine organisms reflect PAHs bioavailability, the influence of each species, and the possible relation to fat content since PAHs are lipophilic compounds. The knowledge of the total lipid content of fish and fishery products is important to evaluate their nutritional value. According to [32] the lipid content of tissues can be a determinant factor in the bioaccumulation of PAHs. Principal Component Analysis was performed on PAH components and sites. The multifactorial analysis allowed the classification and processed the information relating to the PAHs studied and the correlations between all the variables. This PCA is carried out on a matrix of data (PAHs and regions).

The values of the two components F1 and F2 and their contribution to the total inertia are shown in the Figure 3. All factors with Eigen values over 1 were extracted and were rotated using the Varimax method with Kaiser Normalization. The two factors justified 86% of data variance. The factorial map of the PCA (Figure 3) shows a positive correlation of the four molecules, with a strong positive correlation of benzo(a)pyrene, benzo(a)anthracene, Benzo(b)fluoranthene and chrysene to the first factor.

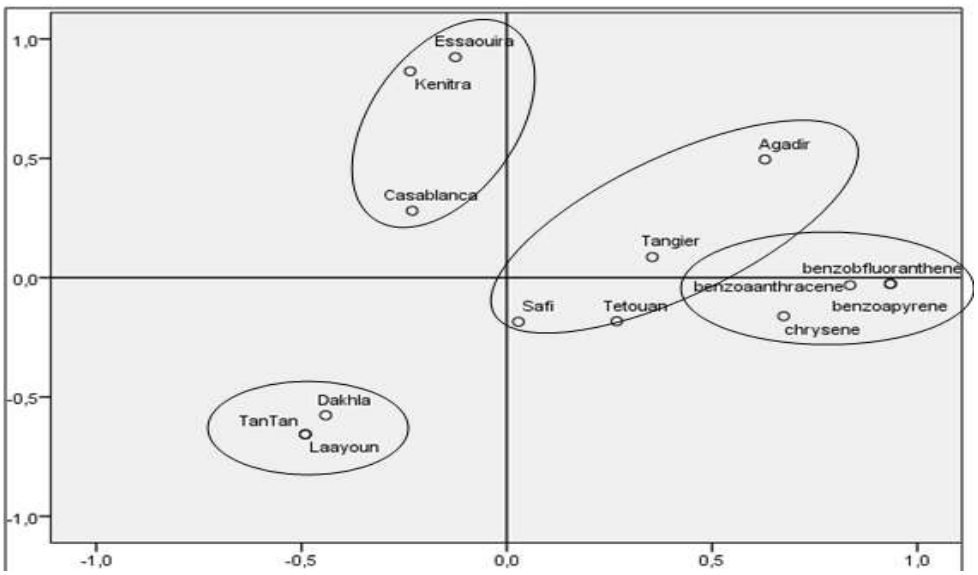

Figure 3: Analysis principal components of Σ4 PAHs

Human health risk assessment

In Morocco, there is a high rate of consumption of fishes. Several organizations such as the European Union (EU) have established maximum concentration limits of PAHs in seafood above which the health of humans who consume them is possibly threatened. The mean concentration of each contaminant in each species was compared to the EU's guidelines values for human consumption. The PAH4 index was assessed in this study based on the of review by the European Food Safety Authority (CONTAM) Panel in 2011 relating to occurrence and toxicity of PAHs in food, which concluded that PAH4 is a more suitable indicators of PAHs in Food. The TEQBaP approach was implemented to assess the carcinogenicity of PAH contamination of the fish species of a given PAH mixture. The total TEQ of PAHs in the different species of the Moroccan coastline ranged between 0.20 µg/Kg in samples obtained from TanTan and Dakhla to 1.28 µg/Kg in the samples from Tangier. The observed TEQBaP values were generally below the reference dose for all the fish species assessed (Table 4). The TEQ values for the survey fish values were also higher than values reported by [33]. Using the concept of EDI to assess the health risk of toxicants is imperative. Table 4 summarizes the Exposure daily intake (EDI) of PAH's in the analysed fish samples for the general population. EDI values estimated from individual PAH concentrations in *Sardina pilchardus* from Morocco ranged from 0.22 to 0.92. EDI values for ∑ PAHs in the fish species were higher in reported studies from Spain with EDI values of 626-712 ng/d and India with EDI values of 1.77-10.7 ng/kg/bw/day [23]. The total intake of 18 PAHs was at 294.47 [ng/kg bwt/day] in Malaysia [34]. After converting PAH concentrations to their B(a)P equivalents, risks were estimated using the BaP cancer slope factor. The CR resulting from lifetime exposure to the four more carcinogenic PAHs via seafood consumption was calculated and compared to the acceptable guideline value set by the USEPA. According to this organism, one out of a million (1×10^{-6}) chance of developing cancer over a lifetime is the level of risk considered to be acceptable or inconsequential, whereas a lifetime cancer risk of one in ten thousand (1×10^{-4}) or greater is considered serious. The CR of organic pollutants via consumption of fish and seafood products ranged from 5.9×10^{-7} to 2.8×10^{-6}, the risk of developing cancer is within the safety limits [22]. Similar studies in India and Ghana have reported estimated excess cancer risk from consumption of fish above the USEPA guideline [23]. The MOE approach is considered to be the most scientifically credible and practical approach, because it takes into account both the dietary exposure and the available data on the dose-response relationship [3]. The EFSA Scientific Committee considered that an MOE of 10,000 or more, based on animal cancer bioassay data, would be of low concern from a public health. In this study, the results show that the values of the MOE for PAH4 ranged for 369565 to 1545 454. This indicated that the consumption of Sardine from the Moroccan coast will be of relatively low public health concern.

Table 4: Estimated daily intake of PAHs and risk factors by sampling area

Sites	B(a)Peq	EDI	ECR	MOE
Tetouan	0.94	0.83	2.05×10^{-6}	409638
Tangier	1.28	0.92	2.8×10^{-6}	369565
Kenitra	0.70	0.56	1.5×10^{-6}	607142
Casablanca	0.89	0.78	1.9×10^{-6}	435897
Safi	0.92	0.81	2.1×10^{-6}	419753
Essaouira	0.62	0.51	1.3×10^{-6}	666666
Agadir	0.70	0.66	1.4×10^{-6}	515151
TanTan	0.20	0.24	4.3×10^{-7}	1416666
Laayoun	0.27	0.36	5.9×10^{-7}	944444
Dakhla	0.20	0.22	4.8×10^{-7}	1545454

CONCLUSION

Food Safety is of growing concern and PAHs residues if present in fish above recommended levels could pose serious public health concerns of the human populations who consume this seafood. This study provides the database of PAHs concentrations in relevant marine seafood species. Current levels of PAH contamination in fish from the Atlantic and Mediterranean coasts are low. Results concerning PAHs contamination of highly consumed and commercially valuable species could allow consumers to make decisions about which fish they might eat in order to reduce risks from contaminants exposure and increase health benefits. The outcome of this study has provided useful information about the level of PAHs in most of the commercially sardine. For this, to occur it is important to develop a focused and scientifically robust funded monitoring and assessment programs to allow such impacts are to be adequately investigated.

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