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High levels of Polycyclic Aromatic Hydrocarbons in the Date Mussel (*Lithophaga lithophaga*) from Bizerte coast (northern Tunisia): Sources and human health risk implications

Ferdaous Jaafar Kefi[1], Yassine Elmegdiche[2], Jihène Maatoug Béjaoui[1], Youssef Lahbib[1,3,*], Imed Chraief[4], Mohamed El Hammami[4], Najoua Trigui El Menif[1]

Abstract
The date mussel *Lithophaga lithophaga* is protected by law in Tunisia. Still, illegal consumption of this luxury seafood has increased over time due to high demand and high prices, which have made the species regularly available in the seafood market of Bizerte, putting wild stocks at risk of decline. To raise public awareness of the risks to human health associated with the consumption of this bivalve, 16 priority polycyclic aromatic hydrocarbons (PAHs) were quantified in the soft tissue of the species, which was collected from two sites of high fishery pressure in the bay and lagoon of Bizerte. Total PAHs concentrations differ significantly between the studied sites ranging from 0.45 to 546.05 μg g^{-1} d.w., and were associated with port activity and the petroleum industry. The benzo(a)pyrene toxic equivalent factor and excess cancer risk showed both high values exceeding permissible limits in the polluted site. These findings provide valuable information regarding the distribution of PAHs in mussels from wild ecosystems that could be useful to prevent consumer intoxication and improve awareness against illegal harvesting of this species.

Keywords
Lithophaga lithophaga; PAHs; Human risk; Public awareness; Tunisia

[1] *Laboratory of Environmental monitoring (LR01ES14), Faculty of Sciences of Bizerte, University of Carthage, 7021 Zarzouna, Bizerte, Tunisia*
[2] *Laboratory of Hetero-Organic Compounds and Nanostructured Materials (LR18ES11), Faculty of Sciences of Bizerte, University of Carthage, 7021 Zarzouna, Bizerte, Tunisia*
[3] *Higher Institute of Heritage Crafts, University of Tunis, Impasse Bachrouche, Monfleury, Tunis, Tunisia*
[4] *Biochemistry Laboratory "Nutrition-Functional Foods and Vascular Health" (LR12ES05), Faculty of Medicine, University of Monastir, Monastir, Tunisia*
***Correspondence**: lahbibyoussef@yahoo.fr (Y. Lahbib)

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are semi-volatile organic compounds that accumulate in marine organisms due to their hydrophobic and lipophilic characters (León et al., 2013; Bandowe et al., 2014).

PAHs are ubiquitous group of several hundred related compounds that have been identified worldwide in water, soil, and dust particles. Most of them are introduced into the marine environment by anthropogenic activity and pyrolytic processes and are also released through wastewater, petroleum industries, vehicle traffic, and oil spills (Meador et al., 1995, Serpe et al., 2010). They are also transported into the marine environment via atmospheric pathways from industrial emissions, or through exhaust fumes of internal combustion engines and domestic heating systems (Balcıoğlu, 2016).

Most PAHs are persistent organic pollutants (POPs) with low solubility being rapidly adsorbed by suspended materials, sediments, and living organisms. They have been known to affect a variety of biological processes and can be potent cell mutagens and carcinogens making them priority pollutants (Nisbet and Lagoy, 1992; EFSA, 2008; Smith and Lynam, 2010; Jung et al., 2010). For this reason, PAHs are considered a useful indicator for assessing the eco-health of aquatic environments (Meador et al., 1995; Perugini et al., 2007).

Bivalves are common sentinels used in biomonitoring programs to measure toxicity and contaminants' bioavail-

ability levels. Their worldwide distribution, sedentary nature, filter-feeding behavior, and limited ability to metabolize organic pollutants make them ideal species for monitoring investigations.

During the last decades, *Lithophaga lithophaga* has been intensively exploited in some European countries such as the former Yugoslavia and Spain, and in parts of the Moroccan Mediterranean coast (Poutiers, 1987; Shafee, 1999). Due to the species organoleptic quality and its high price, it is often collected and sold even where this is prohibited by law (Devescovi and Iveša, 2008). Ecotoxicological investigations focusing on PAHs and persistent organic pollutants (POPs), suggested that the species can be used as indicator in biomonitoring programs (Dujmov and Sučević 1990; Deudero, 2007).

In Tunisia, studies on the date mussel are scarce and limited to shell disturbances, biological status and organotins contamination (Jaafar Kefi et al., 2012, 2014). The present paper focuses on 16 PAHs included in US-EPA recommendation to get chemical data regarding the date mussel *L. lithophaga*. Seasonal levels, sources and human health risk assessment were investigated.

2. Materials and methods

2.1 Sampling area

Because the date mussel is a protected species with a restricted geographical distribution in Tunisia, only two sites in Bizerte shores were sampled (Figure 1). The first station is the marina of Bizerte, which was selected as an experimental group due to its high date mussel productivity and high Hydrocarbons (HC) contamination (polluted site, PS) generated by boating traffic, mainly oil tankers operated by the Tunisian lubricants company. The second site, chosen as control site (CS), is called Echaara and located at the northern part of Bizerte lagoon known as less contaminated area. Sampling was conducted seasonally from September 2015 to August 2016 at three meters depth. Samples (5 males and 5 females per site and season, total number = 80) with common shell length (40–60 mm) and fresh weight range (2.9–8.7g) were washed with seawater and transferred to laboratory in polyethylene bags prior to analysis.

2.2 Chemical analysis

The mussel tissues (pooled samples) were lyophilized in a freeze dryer (VaCo2, Germany) then crushed into fine particles. The extraction method described by Serpe et al. (2010) for HPLC-FL was adopted with few modifications for the GC-MS. This concerns the use of hexane as extraction solvent, direct purification with a mini column of silica-alumina, and the use of hexane-dichloromethane (9:1) as eluting solvent. Before extraction, an aliquot of 2 g was taken and mixed with a perdeuterated surrogate standards (Benzo[a]fluoranthene-d12, and pyrene-d10)

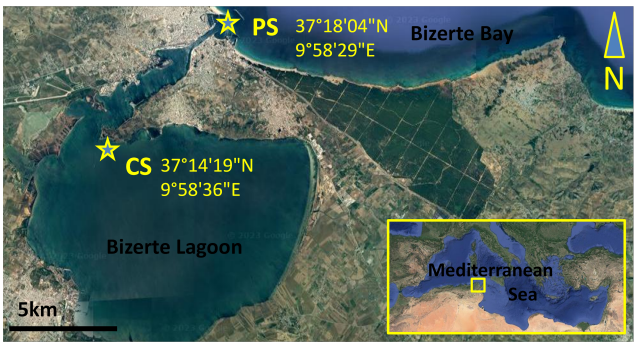

Figure 1. Sampling sites of *Lithophaga lithophaga* in the Bizerte coast. PS, polluted site (Bizerte Bay), and CS, control site (Echaara).

in order to determine recoveries during the analytical procedure. Mixture was saponified with 10 ml of a solution of potassium hydroxide (2 N in ethanol) at 80°C in a water bath for 4 h. The extraction was carried out three times by adding 20 ml of hexane.

The extracts were concentrated close to 3 ml in a rotary evaporator at 40°C. A silica–alumina (2:1 ratio) gel column was implemented to clean up the extracts. PAHs were eluted with 50 ml of hexane and dichloromethane (9:1 ratio) and then concentrated up to 1 ml by rotary evaporator.

PAHs were determined by GC_MS (Hewlett Packard model HP 5890 II series) being operated in Selected Ion Monitoring (SIM) mode. The analytical column was HP- 5 MS (60 m × 0.25 mm × 0.25 µm) used under the following chromatographic condition: injector temperature 300°C, column program of temperatures: 80°C (1 min); 80-100°C (1 min); 100-130°C (3.75 min); 130-320°C (18 min); and then the temperature was held constant at 320°C for 10 min. Nitrogen was used as the carrier gas with a flow rate of 1 ml min^{-1}.

In prepared extracts seventeen PAHs were analyzed: Naphtalene (Naph), 2-Methylnaphtalene (2-Methnaph), Acenaphtalene (Act), Acenaphtene (Ace), Fluorene (Fl), Anthracene (An), Phenanthrene (Phe), Fluoranthene (Ft), Pyrene (Py), Benzo(a)anthracene (B(a)A), Chrysene (Chy), Benzo(b)fluoranthene (B(b)Ft), Benzo(k)fluoranthene (B(k)Ft), Benzo(a)pyrene (B(a)P), Indenol(1,2,3-cd)pyrene (Ind[1,2,3-cd]Py), Dibenzo(a,h)anthracene (D(a,h)An) and Benzo (g,h,i)perylene (B g,h,i) Pe). All of the results were expressed on a dry-weight basis. Individual PAH compound was confirmed by the retention time and the abundance of quantification/confirmation ions. Quantification of individual compounds was based on the internal standard method relative to perdeuterated PAHs added to the samples prior to the extraction. The response factors of the compounds were measured by injecting SRM 2260 solution spiked with the same solution containing the perdeuterated PAHs as the one used for spiking the mussels. Recoveries ranged from 72.5 to 99.76%. The detection limit

(LOD) of the method was 1 ng g^{-1} dw. The PAH standard obtained as single compounds from Promochem (Wesel, Germany). All solvents used for sample processing and analyses (Dichloromethane, Hexane, Acetonitrile) were of analytical grade and of highest purity (from Scharlau, Spain). Analyses were made in three replicates.

2.3 PAHs ratios

PAH ratios refer to the relative concentrations of specific PAH compounds compared to others in a sample (see Table 2 on page 5). These ratios are often used as diagnostic tool to help identify the sources of PAHs in environmental samples. Indeed, the PAH emission profile for a given source depends on the processes producing the PAHs. Petrogenic PAHs that are dominated by low molecular weight compounds (LMW) are usually formed during low temperatures, while pyrolytic PAHs that are enriched with higher molecular weight compounds (HMW) are produced at higher temperatures. The following PAH ratios were calculated to identify PAH sources: Phe/An, Ft/Py, An/An + Phe, Ft/Ft + Pyr, B(a)An/B(a)An + Chy, and LMW/HMW (Budzinski et al., 1997; Baumard, 1998; Soclo et al., 2000; Magi et al., 2002; Yunker et al., 2002).

2.4 Human health risk assessment

2.4.1 Calculation of total toxic BaP equivalent (TEQ)

The human health risk associated with the consumption of date mussels was estimated by calculating the sum of benzo[a]pyrene equivalent concentrations. Toxic equivalence factors (TEFs) were applied to quantify the carcinogenicity of other PAHs relative to benzo(a)pyrene and to estimate benzo(a)pyrene-equivalent doses (BaPeq dose). The carcinogenicity of the TEF is quantified based on several contamination experiments in rats and mice considering dose levels and tumour incidence rates. A mathematical model of the dose-response relationship was applied to the data for each compound and compared the results to those obtained for B[a]P (Nisbet and Lagoy, 1992).

The benzo[a]pyrene equivalent concentration (BaPeq) (potency equivalent concentrations) was defined as the TEQ of all PAHs and was calculated as follow:

$$\mathrm{TEQ(B(a)Peq)} = \sum \mathrm{TEFi} \times \mathrm{Ci} \tag{1}$$

where Ci (µg g^{-1}) is the concentration of a single PAH (i) compound in date mussel tissues and TEF is the toxicity equivalence factor of this compound.

TEF values were taken from Nisbet and Lagoy (1992) and modified by US-EPA (1993).

In addition, the excess cancer risk (ECR) model was used to calculate the level of lifetime cancer risk of dietary exposure according to Ke et al. (2017):

$$\mathrm{ECR} = \frac{\mathrm{CSF} \times \mathrm{TEQ} \times \mathrm{CR} \times \mathrm{ED}}{\mathrm{BW} \times \mathrm{AT}} \times 10^{-6} \tag{2}$$

where:

CSF is the oral cancer slope factor of BaP of 7.3 mg kg^{-1} day^{-1}

TEQ is the potency equivalent concentrations

CR is the consumption rate (intake rate) of seafood. Average daily seafood consumption in Tunisia is 25 g d^{-1} (FAO, 2012).

ED is the exposure duration (adults = 30 years) and AT is average exposure time (assuming 75.47 years) (Bandowe et al., 2014).

BW body weight (kg). Average body weight of an adult African is assumed to be 60.7 kg (Walpole et al., 2012).

2.5 Statistical analysis

Analysis of variance (ANOVA) was performed on PAHs levels (mean ± SD, n = 8) using the software Statistica 8.0. After testing ANOVA assumptions, statistical significance was evaluated through one way ANOVA. Whenever ANOVA detected significant differences, post-hoc comparisons were made using the Tukey's HSD test. In all statistical analyses, significance level was considered at $P < 0.05$.

3. Results

3.1 PAHs levels in the date mussel

The obtained results showed that date mussel accumulates all the 16 PAHs listed as priority by the US-EPA (Table 1). Pyrene, benzo(a)anthracene and benzo(a)pyrene are the most abundant congeners in the date mussel. PAHs were mainly reported in the Bizerte Bay (polluted site, PS) as compared to Echaara (control site, CS) where most values were under the LOD. In the PS, the lowest concentration of total PAHs was 0.45 µg g^{-1} d.w., while the highest concentration was 546.05 µg g^{-1} d.w. PAHs concentration in CS ranged from 0.17 to 37.03 µg g^{-1} d.w.

PAHs levels were similar among all the seasons in males (Tukey's HSD, $p > 0.05$) while in females, the summer was marked by higher values (Tukey's HSD, $p < 0.001$) (Figure 2). Comparison between males and females showed significant variations in PAHs concentrations only in summer being higher in females than males for both sites (Tukey's HSD, $p < 0.00$).

Considering the PS, PAHs of high molecular weight (HMWPAHs) (4–6-rings) were predominant with an average of 63.234%. The highest concentrations were registered in summer with an average of 341.717 µg g^{-1} for HMW-PAHs and 204.333 µg g^{-1} for PAHs of low molecular weight (LMW-PAHs). Maximum levels were recorded in females for both PAHs classes. HMW-PAHs and LMW-PAHs levels were lower in winter (0.304 µg g^{-1}) and spring (0.067 µg g^{-1}) respectively.

The content of Pyrene, Benzo(a)Pyrene, and Fluorenthene was the highest of all PAHs analyzed (Table 1).

Table 1. PAHs mean concentrations (μg g^{-1} d.w.) in *Lithophaga lithophaga* in polluted (PS) and control (CS) sites (PS/CS).

PAHs (PS/CS)	Autumn		Winter		Spring		Summer	
	Males	Females	Males	Females	Males	Females	Males	Females
Naph	ND/ND	ND/ND	0.01/ND	ND/ND	ND/ND	0.02/ND	ND/ND	55.90/1.87
2-Methnaph	ND/ND	ND/ND	0.10/ND	ND/ND	ND/ND	0.02/ND	ND/ND	48.68/1.65
Act	ND/ND	ND/ND	0.09/ND	ND/ND	ND/ND	ND/ND	ND/ND	ND/ND
Ace	ND/ND	ND/ND	ND/ND	0.01/ND	ND/ND	ND/ND	ND/ND	ND/ND
Fl	ND/ND	ND/ND	0.09/ND	0.01/ND	ND/ND	ND/ND	ND/ND	46.93/3.41
An	ND/ND	ND/ND	0.10/ND	0.01/ND	ND/ND	0.02/ND	ND/ND	52.82/2.02
Phe	0.19/ND	0.17/ND	0.09/ND	0.12/ND	0.17/ND	0.02/ND	0.14/ND	ND/ND
Ft	0.24/ND	0.18/ND	0.49/ND	0.16/ND	0.35/ND	0.20/ND	0.19/ND	ND/ND
Py	1.02/0.23	1.05/0.17	0.09/ND	0.02/ND	1.21/0.20	1.06/0.13	1.02/0.11	49.38/8.42
B(a)An	0.05/ND	0.16/ND	0.14/ND	0.01/ND	0.30/ND	0.02/ND	0.140/ND	51.99/2.57
Chy	0.18/ND	ND/ND	0.11/ND	0.02/ND	ND/ND	0.09/ND	ND/ND	ND/ND
B(b)Ft	0.17/ND	ND/ND	0.12/ND	0.01/ND	ND/ND	0.02/ND	ND/ND	ND/ND
B(k)Ft	0.04/ND	ND/ND	0.11/ND	0.02/ND	ND/ND	0.02/ND	ND/ND	46.70/1.62
B(a)P	0.07/ND	0.63/0.03	0.10/ND	0.04/ND	0.80/0.04	0.59/0.11	0.59/0.06	77.85/9.35
Ind[1.2.3-cd]py	ND/ND	ND/ND	0.09/ND	0.01/ND	ND/ND	0.02/ND	ND/ND	63.86/3.70
D(a.h)An	0.05/ND	ND/ND	0.09/ND	0.01/ND	ND/ND	0.02/ND	ND/ND	ND/ND
B(g.h.i)Pe	ND/ND	ND/ND	0.09/ND	0.01/ND	ND/ND	0.02/ND	ND/ND	51.948/2.421
∑PAHs	2.01 ± 0.16^{a}/ 0.23 ± 0.01^{a}	2.18 ± 0.38^{a}/ $0.210.02^{a}$	1.99 ± 0.03^{a}/ ND	0.45 ± 0.04^{a}/ ND	2.83 ± 0.24^{a}/ 0.24 ± 0.02^{a}	2.09 ± 0.30^{a}/ 0.24 ± 0.03^{a}	2.08 ± 0.27^{a}/ 0.17 ± 0.02^{a}	546.05 ± 39.85^{b}/ 37.03 ± 11.26^{b}
∑PAHs (mixed sexes)	$2.09 \pm 0.11^{a}/0.22 \pm 0.02^{a}$		1.22 ± 1.08^{a}/ND		$2.45 \pm 0.53^{a}/0.24 \pm 0.00^{a}$		$274.07 \pm 384.64^{b}/18.60 \pm 26.06^{b}$	

Standard deviations SD are only reported for ∑PAHs. ND: not detected. Superscript letters depict statistical comparison among concentration values for each site:
[a] no significant difference, $p > 0.05$,
[b] significant difference, $p < 0.001$.

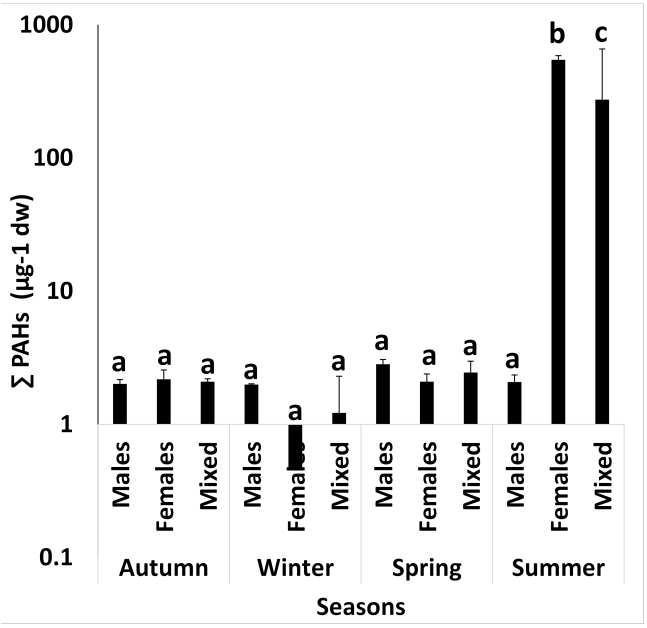

Figure 2. Seasonal variation of PAHs concentrations in *Lithophaga lithophaga* from the polluted site (PS) (logarithmic scale). Superscript letters depict statistical comparisons (ANOVA, Tukey's HSD test).

3.2 PAHs sources in the Bizerte Bay

PAHs ratios are traditionally used to distinguish PAHs sources and to estimate the importance of petrogenic and pyrogenic-derived PAHs (Santos et al., 2017). PAHs may be generated by combustion at a very high temperature of organic matter, petroleum leak or degradation of the organic matter. In the PS, comparison of LMW/HMW ratio to 1, indicated that PAHs sources are both pyrolytic (LMW/HMW values < 1) and petrogenic (LMW/HMW values > 1) (Table 2). In addition, ratios of phenanthrene to anthracene (Phe/An) and fluoranthene to pyrene (Ft/Py) were used (Table 2). PAHs of pyrolytic origin are generally characterized by Phe/An values < 10, whereas PAHs of petrogenic sources often result in high Phe/An ratios (>10). The ratio of Phe/An ranged from ND to 10.778. This indicates a predominance of pyrolytic sources (Table 2). Moreover, Ft/Py ratio indicated mixed pattern of contamination (petrogenic and pyrogenic) (Table 2). Ft/Pyr values < 1 were attributed to petrogenic sources and values greater than 1 were obviously related to a pyrolytic origin. The Ft/Pyr values ranged from 0.163 to 10.681 which is an indication of contamination from petroleum sources.

Another three PAHs ratios were used in the present study (Table 2): an An/An + Phe ratio <0.100 usually is taken as an indication of petroleum while a ratio >0.100 indicates a dominance of combustion. An Ft/Ft + Pyr ratio <0.400 is defined as petrogenic. Most mussel samples are characterized by Ft/Ft + Pyr values <0.400 which is characteristic of strong petroleum input.

Furthermore, a B(a)An/ B(a)An + Chy ratio below 0.200 indicates that contaminants are from petroleum, whereas a B(a)An/ B(a)An + Chy between 0.200 and 0.350 shows that the origin is petroleum combustion. In contrast, a B(a)An/ B(a)An + Chy ratio greater than 0.350 is typically observed for combustion materials including grass, coal and wood (Table 2). Results affirm that wood or coal combustion is the major source of PAHs in date mussel samples.

Our results suggested that PAHs pollution in the Bizerte Bay (PS) is originated from a "mixed" origin source. PAHs in date mussels, are both pyrolytic and petrogenic with a dominance of petroleum sources.

3.3 Ecological risk determination

Total TEQ-BaP calculated for samples from PS varied from 0.053 to 95.3 µg TEQ-BaP g^{-1} (Table 3). These values are recorded in females during winter and summer respectively. PAH_4 (B(a)An, B(a)P, B(b)Ft, and Chy) and PAH_7 (PAH_4 + B(k)Ft, D(a.h)An, Ind[1.2.3-cd]py) are both specific groups used as indicators of toxicity and carcinogenic risk in food. The results obtained for these two groups were similar. This could be explained by the fact that TEFs for carcinogenic PAHs are high. The greatest TEQ-BaP levels were registered in summer for the 2 PAHs groups (83.045 and 94.101 µg TEQ-BaP g^{-1} for PAH_4 and PAH_7 respectively). Lowest values are recorded in winter for both PAHs and are of 0.04 µg TEQ-BaP g^{-1} for PAH_4 and of 0.053 µg TEQ-BaP g^{-1} for PAH_7 (Table 3). The ECR values resulting from dietary exposure to 16 PAHs through consumption of this seafood varied from 1.06×10^{-6} recorded in autumn for males to 6.38×10^{-4} in summer for females being in agreement with the TEQ-BaP values and clearly

Table 2. Assessment of PAHs sources based on diagnostic ratios.

PAHs ratio	PAH sources	Autumn		Winter		Spring		Summer	
		Male	Female	Male	Female	Male	Female	Male	Female
Phe/An	< 10 Pyrogenic	ND	ND	0.900	10.778	ND	0.700	ND	ND
Ft/Py	< 1 Petrogenic	0.237	0.169	5.332	10.681	0.290	0.163	0.180	ND
An/An+Phe	< 0.1 Petrogenic	ND	ND	0.526	0.085	ND	0.588	ND	1
Ft/Ft+Py	< 0.4 Petrogenic	0.192	0.144	0.842	0.914	0.225	0.140	0.153	ND
B(a)An/B(a)An+Chy	< 0.2 Petrogenic 0.2–0.35 combustion of fuels > 0.35 combustion of carbon, wood	0.204	1	0.548	0.374	1	0.187	1	1
LMW/HMW	< 1 Pyrogenic	2.623	1.775	0.891	2.355	1.565	1.601	1.842	0.350

Table 3. Assessment of the Toxic benzo(a)pyrene equivalent and the excess cancer risk (ECR) for total PAHs (TEQ PAHs) in polluted site.

PS	Autumn		Winter		Spring		Summer	
	Male	Female	Male	Female	Male	Female	Male	Female
∑PAHs	2.017	2.178	1.989	0.454	2.834	2.089	2.082	546.050
TEQ PAHs	0.149	0.643	0.241	0.053	0.834	0.619	0.608	95.300
ECR	1.1E-06	4.6E-06	1.7E-06	3.8E-07	6.0E-06	4.4E-06	4.3E-06	6.83E-04
$\sum PAH_7$	0.557	0.785	0.761	0.117	1.105	0.775	0.732	240.389
TEQ PAH_7	0.147	0.641	0.238	0.053	0.833	0.617	0.606	94.101
$\sum PAH_4$	0.464	0.785	0.477	0.078	1.105	0.724	0.732	129.831
ECR_7	1.1E-06	4.6E-06	1.7E-06	3.8E-07	6.0E-06	4.4E-06	4.3E-06	6.7E-04
$TEQPAH_4$	0.094	0.641	0.131	0.040	0.833	0.597	0.606	83.045
ECR_4	6.7E-07	4.6E-06	9.4E-07	2.9E-07	6.0E-06	4.3E-06	4.3E-06	6.0E-04

showed high to serious levels.

4. Discussion

4.1 PAHs levels in the date mussel

PAHs levels showed high variability between the two studied sites. As expected, the high maritime traffic and petroleum transport around the Mariana of Bizerte (PS) were the main causes of high concentration levels recorded. In the control site of Echaara (CS), low concentration levels were recorded and are attributed to local traffic of few fishing boats as well as to currents coming from the Bizerte channel that may bind contaminants from PS.

There are 16 polycyclic aromatic hydrocarbons that have been listed as priority pollutants in the measured results and were all recorded in the date mussel. These compounds are Naphthalene, Acenaphthalene, Acenaphthene, Fluorene, Anthracene, Phenanthrene, Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(b) fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Indenol(1, 2,3-cd) pyrene, Dibenzo(a,h)anthracene and Benzo(g,h,i) perylene. When compared with other studies, the concentration values recorded presently for the polluted site (1.22–274.07 μg g^{-1}, mixed sexes) were higher than those reported by Dujmov and Sučević (1990), in date mussels collected from the East coast of the Adriatic Sea and other bivalves collected from different localities (Table 4). PAHs levels detected in Adriatic *L. lithophaga* ranged from 1 to 20 μg g^{-1} (d.w.). Moreover, concentrations obtained for Tunisian date mussel exceed the recommended limit (0.5 μg g^{-1} d.w.) fixed for the 16 priority PAHs in bivalves (AFSSA, 2003), for all the sampling seasons, especially during summer. This can be related to the date mussel lifestyle and the longevity of this species, which can live for more than 54 years (Galinou-Mitsoudi and Sinis, 2003; Katsanevakis et al., 2008). In addition, *L. lithophaga* is endolothic and filter-feeding species; its presence inside rock in contact with water-sediment interface for a long time makes it a good accumulator.

Levels of PAHs demonstrated a marked seasonal pattern. Seasonal body concentrations decreased in the following order summer > spring > autumn > winter. According to Sheehan and Power (1999), seasonal variations of pollutant content can be controlled by many factors such as temperature changes, physiological status and cycles in industrial activities. Weinstein (1995) noted that water temperature variation influenced seasonal PAHs distribution in *Crassostrea virginica* by altering their adsorption to lipids. Higher temperatures reduced PAHs adsorption and lead to less bioaccumulation, which did not corroborate observations reported in the date mussel.

In *M. edulis*, pollutants levels may be expected to follow a variable seasonal pattern depending on nutrient availability and sexual cycle (Sheehan and Power, 1999). Thus, xenobiotic levels detected in tissue samples may be found to vary in a seasonal manner. In the present study, high levels of PAHs were recorded during summer in both sites. This can be related to the gonad development of *L. lithophaga*, which is coinciding with vitellogenesis and species spawning period. Indeed, high levels of PAHs are observed in females during full maturity and spawning periods, while low levels are registered in winter during the sexual rest. A decrease in PAHs values can be related to the release of gametes, which are enriched with lipids (Porte and Albaigés, 1994). Therefore, this discharge causes the elimination of a large amount of polluting lipophilic organic substances. Moreover, Dyrynda et al. (2000) noticed an increase in PAHs levels in *M. edulis* tissues (Milford Haven, Wales, UK) during autumn and winter. This PAHs increase coincides with the lipid storage period. However, a reduction in PAHs was observed in spring and summer in the same species being explained by the depuration of these compounds during spawning.

Presently, in the polluted site, males accumulate higher PAHs levels in winter. This result can be attributed to females' ability to achieve rapid depuration during spawning, unlike males, which explains the lower PAH concentrations observed in females during winter. Furthermore, eggs are more enriched in PAHs compounds while sperm is not. Additionally, bivalves exhibit minimal enzymatic capacity for metabolizing PAHs (Thorsen et al., 2004). According to these authors, oysters may lose up to 50% of their total body burden of some PAHs and pesticides in a single

Table 4. PAHs concentrations detected in different bivalve species [ng g^{-1}]; d.w. – dry weight, w.w. – wet weight.

Localities	Species	PAHs	Levels [ng g^{-1}]	Source
Baltic Sea	*M. edulis*	–	90–3900 (d.w.)	Baumard et al. (1998)
Germany, France, Spain	*M. edulis*	–	92.5–361 (d.w.)	Baumard et al. (1998)
French coasts	*M. edulis*, *C. gigas*	16	142–254 (d.w.)	RNO (2000)
Croatia	*M. galloprovincialis*	10	42.2–134 (w.w.)	Bihari et al. (2007)
Greece (Saronic Gulf)	*M. galloprovincialis*	17	219–1482 (d.w.)	Valavanidis et al. (2008)
Egypt (Abu Qir Bay)	*Tapes decussata*, *Mactra corallina*	16	11.4–3880 (d.w.) 242–2830 (d.w.)	Khaiyri et al. (2009)
western basin of the Mediterranean Sea	*M. galloprovincialis*	16	22–106 (d.w.)	Andral (2011)
Italy	*M. galloprovincialis*	11	0.2–16 (w.w)	Serpe et al. (2010)
Spain	*M. galloprovincialis*	13	23.8–390 (d.w.)	León et al. (2013)
Tunisia (Nothern coasts)	*M. galloprovincialis*	24	45.6–241.6 (d.w.)	Barhoumi et al. (2014)
Tunisia (Lagoon of Bizerte)	*M. galloprovincialis*	19	47.5–390 (d.w.)	Mzoughi and Chouba (2012)
Adriatic Sea	*L. lithophaga*	–	1000–20,000 (d.w.)	Dujmov and Sučević (1990)
Tunisia (Bay of Bizerte)	*L. lithophaga*	17	454–546,050 (d.w.)	Present study

spawn. In general, PAHs bioavailability, type, sources, potential trophic transfer in food chains, and animal lifestyle may affect PAHs concentrations (Mazeas, 2004; Thorsen et al., 2004; Frouin et al., 2007). Organic compounds with low solubility are easily accumulated by filter-feeding organisms and tend to be concentrated in lipids-enriched tissues due to their ability to be dissolved in the lipid fraction. Besides, organisms living in littoral zones seem to be more exposed to PAHs than those living in deep waters. Migratory behavior, food and species localization in water column may also influence their exposure to contaminants (Mazeas, 2004).

PAHs variations may be also influenced by maritime traffic and industrial activities. The Bizerte Bay that showed high contamination is located just at the mouth of the Bizerte channel (that joins Bizerte lagoon to Mediterranean Sea) and its presence near the marina of Bizerte (Sport Nautique) and the commercial harbor of the Tunisian Company for Refining Industries (STIR), submit the area to various kinds of organic pollutants. Field observations clearly showed the presence of oil films on the water surface as well as petroleum black traces products at the outfall of STIR and the bottom of the eastern wave breaks. Indeed, industrial activities are responsible for 100% of oil spills in the lagoon complex and most hydrocarbons (98%) are originated from STIR/SOTULUB (Tunisian Company of Lubricants) discharges and the fishing port of Bizerte (DGEQV, 2003). Among industrial sources known for their significant contribution to the lagoon complex pollution by hydrocarbons are El Fouledh (Tunisian steel company), STIP (Tunisian Company of Pneumatic Industries) and SOCOMENA (Tunisian Company of Naval Construction and Mechanical Repair DGEQV, 2003). In the control site of Echaara, although the PAHs contamination is low compared to the polluted site, PAHs presence seems to be the result of boating traffic and hydrodynamism.

Date mussel tissues were dominated by the HMWPAHs (4–6-rings). Several studies have shown that bivalves accumulate 4, 5 and 6 ringed PAHs rather than 2 and 3 ringed compounds (Porte and Albaigés, 1994; Baumard et al., 1998). This can be explained by the fact that HMWPAHs are characterized by low water solubility and are hydrophobic compounds, their availability depends on water turbidity and thus, they can be concentrated at higher proportion in filter-feeding bivalves (Pichaud, 2005). Among HMWPAHs detected in mussel tissues, Benzo(a)Anthracene, Benzo(a) Pyrene, Benzo(k)Fluorentene, Chrysene and Indeno[1,2,3-cd]Pyrene are considered to be mutagenic and genotoxic compounds (EFSA, 2008).

The content of Pyrene, Benzo(a)Pyrene and Fluorenthene was the highest of all PAHs analyzed. Levels obtained for date mussel are different to those obtained for *M. galloprovincialis* collected in the Bizerte lagoon where no B(a)P was detected (Mzoughi and Chouba, 2012; Barhoumi et al., 2014). This suggested a species pattern in bioaccumulation and metabolism between the two mytilids.

4.2 PAHs sources

PAHs in date mussels are both pyrolytic and petrogenic with a dominance of petroleum sources. PAH ratio results are like those found by Barhoumi et al. (2014), in the Bizerte channel. Indeed, this area is exposed to anthropogenic pressure and influenced by intense boating traffic and urban and industrial effluents from the city of Bizerte.

4.3 Ecological risk assessment

In order to evaluate the toxicity of a PAHs mixture, the concept of equivalent toxicity was developed. This concept was used to estimate the toxic potential of a mixture of chemical compounds belonging to the same class of contaminants (Polycyclic Aromatic Hydrocarbons). Benzo[*a*] pyrene was chosen to be used as a marker for the occurrence and effects of carcinogenic PAHs in food. The applica-

tion of (BaP)-toxic equivalent factor to PAH concentrations can provide a more accurate risk assessment from environmental exposure to PAHs (Jung et al., 2010).

In carcinogenic mechanisms, exposure to PAH mixture can lead to the formation of reactive oxygen species (ROS) which affect the immune system and induce differential effects in covalent DNA binding due to the PAH metabolites (Knafla et al., 2006). This allowed the Scientific Committee on Food to list a number of PAHs as genotoxic carcinogens (US-EPA, 2008).

Benzo(a)Pyrene is the most potent compound of the PAHs based on carcinogenicity being listed as carcinogenic group 1 by the IARC (Frouin et al., 2007). Since February 2005, the European Regulation 208/2005/EC fixed at 10 ng g^{-1} wet weight the maximum admissible concentrations for B(a)P in shellfish. In the present study, levels of BaP ranged between 7.16 and 14.97 10^3 ng g^{-1}. Maximal level registered in date mussel body exceeds value setting by the European Commission which can constitute a risk to consumer.

Moreover, the European Food Safety Authority panel (EFSA, 2008), concluded that BaP and the PAH_4 (benzo[a]pyrene, chrysene, benz[a]anthracene and benzo[b]fluoranthene) are better indicators of the occurrence of carcinogenic PAHs in food.

Following the EC recommendation (Regulation 835/2011), the maximum admissible concentrations were fixed in bivalves at 2 ng g^{-1} and 12 ng g^{-1} for BaP and the PAH_4 respectively. Our results showed high PAH_4 values that ranged between 15 and 24.967 10^3 ng g^{-1}.

In conclusion, the results of the present study indicate that the date mussel *L. lithophaga* from the Bay of Bizerte accumulates the 16 PAHs listed as priority by the US-EPA and that the PAHs sources are both petrogenic and pyrogenic. High levels of PAHs, TEQ-BaP and ECR were recorded in females during summer likely linked to the gonadal cycle of *L. lithophaga* (vitellogenesis and spawning period). Although, the determination of fatty acid composition in the gonad-digestive gland of this bivalve revealed that date mussel tissues are rich in polyunsaturated fatty acid especially (ω_3) (personal data, unpublished), *L. lithophaga* seems to be not suitable for human consumption.

The finding of this study provided valuable data on the occurrence, distribution, and source apportionment of PAHs in *L. lithophaga* from the Bay of Bizerte. These results could be instrumental in identifying and managing the ecological risks posed by PAHs in *L. lithophaga* tissues. Precautionary measures are recommended to protect consumers from potential intoxication, especially given the limited number of health risk assessments on PAHs via seafood consumption in a country like Tunisia, situated in the central Mediterranean basin.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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