Polycyclic aromatic hydrocarbons (PAHs) in surface sediments from the Bizerte Lagoon, Tunisia: levels, sources, and toxicological significance

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Abstract To assess the status of polycyclic aromatic hydrocarbon (PAH) contamination in sediments from the Bizerte Lagoon (northern Tunisia), 18 surface sediment samples were collected in March 2011 and analyzed for 14 US Environmental Protection Agency priority PAHs by high-performance liquid chromatography. The total concentrations of the 14 PAHs (ΣPAHs) ranged from 16.9 to 394.1 ng g^{-1} dry weight (dw) with a mean concentration of 85.5 ng g^{-1} dw. Compared with other lagoons, coasts, and bays in the world, the concentrations of PAHs in surface sediments of the Bizerte Lagoon are low to moderate. The PAHs' composition pattern was dominated by the presence of four-ring PAHs (45.8 %) followed by five-ring (26.8 %) and three-ring PAHs (12.7 %). The PAH source analysis suggested that the main origin of PAHs in the sediments of the lagoon was mainly from pyrolytic sources. According to the numerical effect-based sediment quality guidelines of the USA, the levels of PAHs in the Bizerte Lagoon should not exert adverse biological effects. The total benzo[a]pyrene toxicity equivalent values calculated for the samples varied from 3.1 to 53.7 ng g^{-1} dw with an average of 10.6 ng g^{-1} dw.

Keywords Polycyclic aromatic hydrocarbons · Surface sediment · Bizerte Lagoon · Diagnostic ratios · Sediment quality guidelines

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) with two or more fused rings are an important class of persistent organic pollutants and are ubiquitous in the environment. PAHs are introduced into the environment mainly via anthropogenic inputs and the biological conversion of biogenic precursors (Means et al. 1980). Anthropogenic input was regarded as the main source for the elevated concentration of PAHs in environmental matrices including fossil fuel combustion, refuse incineration, coal gasification and liquefaction processes, petroleum cracking, and the production of coke, carbon black, coal tar pitch, and asphalt (Countway et al. 2003). Industrial

wastewater, sewage, road runoff/street dust, and petroleum-related activities are other important sources of PAHs. A minor portion of PAHs also originates from the discharges of non-combusted fossil fuel products (Bouloubassi and Saliot 1991; Näf et al. 1992; Manoli et al. 2000). Because of their low solubility and high hydrophobicity, PAHs entering the aquatic environment preferentially adsorb onto particulates and finally accumulate in the sediments (Karickhoff 1981; Chiou et al. 1998). They are therefore considered a potentially dangerous group of chemicals and a threat to the environment and its bioresources, especially those adjacent to major urban centers with high PAH inputs. The recognition of these facts prompted the US Environmental Protection Agency (USEPA) to include 16 PAHs within the priority pollutants list (Manoli et al. 2000). Due to their persistent, toxic, mutagenic, and carcinogenic characteristics (NRC 1983; Connel et al. 1997), the sources and distribution of PAHs have been the focus of numerous investigations in coastal/estuarine regions in industrialized countries in areas with anthropogenic impact all over the world (Kennicutt et al. 1994; Budzinski et al. 1997; Fernandes et al. 1997; Baumard et al. 1998a, b; Zakaria et al. 2002; Hartmann et al. 2004; Cachot et al. 2006; Fang et al. 2007; Yan et al. 2009).

In the last few decades, progressive economic and demographic development at the vicinity of the Mediterranean coastline and lagoons has resulted in substantial changes taking place in northern Tunisia. Industrialization has increased, which results in that the lagoon basins and streams have unfortunately became open-dumping disposal sites, as was the case of the Bizerte Lagoon in Tunisia. In this area, several studies have investigated the application of biochemical tools for pollution biomonitoring (Dellali et al. 2001, 2004; Khessiba et al. 2001, 2005; Roméo et al. 2006; Mahmoud et al. 2010). However, little information is available on the sources and distribution of PAHs in surface sediments of Bizerte Lagoon (Mzoughi et al. 2002; Trabelsi and Driss 2005). In light of this data, our present work aims to (1) investigate the spatial distribution of PAHs at a relatively large scale through the analysis of 18 surface sediment samples from the Bizerte Lagoon, (2) distinguish the possible sources of PAHs using diagnostic ratios of PAHs, and (3) assess the potential ecological risk of benthic organisms residing in this area.

Materials and methods

Study area

The Bizerte Lagoon is a Mediterranean lagoon located in the northern coast of Tunisia (latitude, 37°80′–37°14′ N; longitude, 9°46′–9°56′ E). It covers an area of 128 km^2 and has a mean depth of 7 m. It communicates, in the north, with the Mediterranean Sea by a 7-km-long canal and in the south with the Ichkeul Lake by the Tinja River. The main tributaries of this lagoon are the rivers Tinja, Mrezig, Garek, Ben Hassine, and Gueniche (Fig. 1). The lagoon is subjected to the influence of several physical factors that strongly fluctuate during the year. In winter, winds induce a vertical mixture of the water column, rains are strong, and the freshwater flow coming from the Ichkeul Lake is important. On the other hand, in summer, inputs of seawater into the lagoon are important and the water column warming can induce its stratification (Sakka Hlaili et al. 2003). The human population around the lagoon is estimated at 163,000 inhabitants (2004 census) of which approximately 70 % are concentrated in the city of Bizerte. The other main important towns bordering the lagoon are Menzel Bourguiba (which has a naval port and a metal factory), Menzel Abderrahman, and Menzel Jemil. Some other industries (an iron and steel plant, a cement factory, and a refinery) are established nearby (Essid and Aissa 2002). The majority of these surrounding towns and industries discharge their waste into the lagoon. The exchange of water between the Mediterranean and the lake determines the salinity of the lagoon, which varies between 32.5 and 38.5‰. The water temperature range between 10 °C during winter (wet season) and 29 °C in summer time (dry season). The residence time of water bodies and contaminants in the Bizerte Lagoon is governed by several factors such as tide, wind, atmospheric pressure, and temperature. A recent study, based on numerical modelling, was performed by Harzallah (2003) focusing on water and heat exchange between the lagoon and the Mediterranean Sea. This model showed that the residence time of the contaminants is about 7 months. Actually, urban and industrial discharges in the Bizerte Lagoon take about 10 days to reach the canal entrance but several months to reach the Mediterranean Sea. This relatively high output time
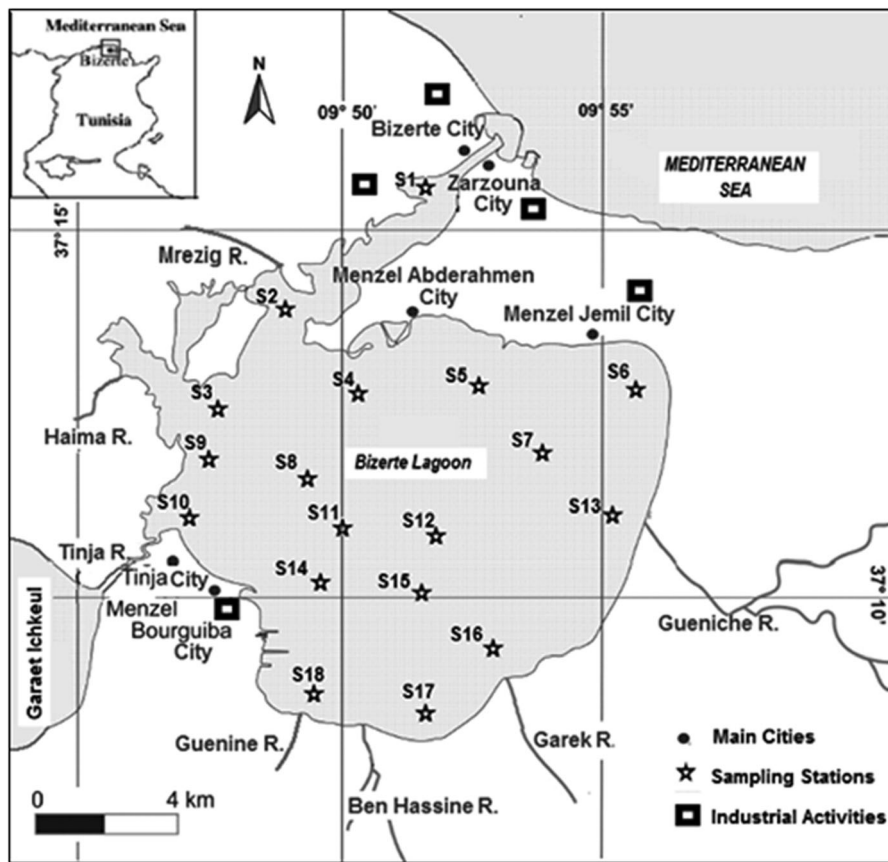
Fig. 1 Sampling locations in the Bizerte Lagoon

is due to the length of the canal which is about 7 km long.

Sample collections, storage, and preparation

Surface sediments were collected at 18 locations in March 2011 (Fig. 1). The selection of the sampling sites was based on different criteria related to the geomorphology of the lagoon, the hydrological regime, and the localization of the urban and industrial discharges. Water depth at the sampling sites ranged from 2.5 to 12.1 m, with a mean value of 6.7 m. The top 0–10 cm of sediment was collected using a stainless steel grab sampler. Individual sediment samples were mixed well and immediately frozen at −20 °C in pre-cleaned glass jars on board the boat used for sampling. In the laboratory, samples were defrosted and freeze dried, and the sediments were then passed through a stainless steel sieve (2-mm mesh) and stored at 4 °C until their analysis.

Chemicals

Standards (solids) of 14 USEPA priority PAHs [naphthalene (Nap), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DahA), and benzo[g,h,i]perylene (BgP)] and a certified PAH standard mixture containing all 14 PAHs (PAH-Mix 45 at 10 μg mL^{-1} in cyclohexane) were purchased from Supelco (Bellefonte, PA, USA) and Dr. Ehrenstorfer (Germany), respectively. The purity of individual standards was higher than 96 %. The first standard of PAHs was dissolved in acetonitrile (ACN) to prepare calibration solutions in the range 15–400 ng mL^{-1}, and the second solution was diluted with isooctane for the standard addition method. All solvents used for sample processing and analyses (dichloromethane (DCM) and

acetonitrile) were of high-performance liquid chromatography (HPLC) grade and were purchased from Fisher (UK). The water used was from a MilliQ system (Milford, MA, USA). Glass pearls (Roth) were used to fill the headspace of the pressurized fluid extraction cells (Dionex). A marine sediment certified reference material (EC-7, National Water Research Institute, Canada) was used in the evaluation of the analytical method.

Sample extraction

An ASE 200 accelerated solvent extractor (Dionex, France) with 22 mL extraction cells was used to perform the extraction of samples. Five grams of dry sediment was accurately weighed into the stainless steel extraction cell that had been prefitted with a filter paper. The headspace was filled with glass pearls. The cell was placed in the carousel and the extraction was carried out using DCM. The parameters used during the extraction procedure are listed in Table 1. When the extraction was complete, the extract was completely evaporated under a gentle nitrogen stream and dissolved in 0.5 mL of acetonitrile. The final acetonitrile extracts were then filtered through a 0.45-μm PTFE syringe filter before being analyzed.

HPLC analysis

Quantitative analysis of PAHs was carried out using an analytical HPLC unit (JASCO, Japan) equipped with a JASCO PU-2089 HPLC pump, a type 7125 Rheodyne injector (with a 20 μL loop), and a fluorescence detector (FP-2020) with excitation and emission wavelengths that could be varied throughout the analysis. The optimized parameters of the fluorescence detector for the

Table 1 Accelerated solvent extraction (ASE) parameters

Parameters	Values
Temperature	100 °C
Pressure	100 bars
Static time	8 min
Heat time	5 min
Solvent	Dichloromethane (100 %)
Cycle	2
Flush volume	60 %
Purge time	60 s

determination of each PAH compound are illustrated in Table 2. Chromatographic separation and resolution were best achieved by using a SUPELCOSIL LC-PAH (Supelco, Inc. Bellefonte, PA) reverse-phase C18 column (4.6×250 mm, 5 μm particle size) specific for PAH analysis. Data acquisition and processing were controlled by Chrom NAV (JASCO) software. The mobile phase was acetonitrile/water in gradient mode at a flow rate of 1.5 mL min^{-1} with the column thermostated at 22.0 ± 0.5 °C. The gradient elution program started with an initial mobile phase at 50:50 ACN/water (*v/v*), changing linearly to 100 % ACN in 20 min and, after 10 min, changing back to the initial phase (50:50). Total run time of an analysis was 40 min. The injection volume was 20 μL.

Quality assurance and quality control

PAHs were identified by comparison of their retention time with those of the authentic standards; quantification was accomplished by using the standard addition method from an average sample obtained from a mixture of all the samples analyzed. The method is described in detail elsewhere (Bader 1980). The principle is presented as follows: a calibration curve is obtained by the addition of a known amount of analytes to the unknown specimen. Serial concentrations of the analyte are added to the sample; the intensity measured is then plotted versus the concentration added. The intercept point of the regression line and the concentration axis equals the amount of analyte in the original sample. In this work, the standard addition graph for all PAHs in sediments extracted was linear in the concentration range 1.97–28.75 ng mL^{-1} ($r^2 > 0.99$).

To perform the quality control, procedural blanks were analyzed and any analyte of interest was detected. The external standard multipoint calibration technique (in the range 15–400 ng mL^{-1}) was used to determine the linear response interval of the detector, and in all cases, regression coefficients were higher than 0.996 for all the analytes. Detection limits (signal-to-noise ratio greater than 3) and quantification limits (estimated for a signal-to-noise ratio of 10) for each PAH ranged from 0.01 to 0.54 ng mL^{-1} and 0.04 to 4.65 ng g^{-1} dry weight (dw), respectively. The whole analytical procedure was validated by analyzing the EC-7 certified reference material (CRM) from the National Water Research Institute (Canada). For all the studied PAHs, recoveries of higher than 80 % were obtained for the CRM.

Table 2 Optimized fluorescence conditions for PAH determination

PAH component	Abbreviation	Retention time (min)[a]	Excitation wavelength (nm)	Emission wavelength (nm)	Time (min)
Naphthalene	Nap	7.52	224	330	0–11.10
Acenaphthene	Ace	9.96	224	330	0–11.10
Fluorene	Fl	10.38	224	330	0–11.10
Phenanthrene	Phe	11.60	252	370	11.10–12.40
Anthracene	Ant	12.91	252	402	12.40–13.60
Fluoranthene	Flu	14.16	288	462	13.60–14.60
Pyrene	Pyr	15.20	238	398	14.60–17
Benzo[a]anthracene	BaA	18.55	252	402	17–20.40
Chrysene	Chr	19.58	252	402	17–20.40
Benzo[b]fluoranthene	BbF	22.16	290	430	20.40–25.55
Benzo[k]fluoranthene	BkF	23.75	290	430	20.40–25.55
Benzo[a]pyrene	BaP	25.10	290	430	20.40–25.55
Dibenzo[a,h]anthracene	DahA	27.86	300	420	25.55–32
Benzo[ghi]perylene	BgP	29.47	300	420	25.55–32

[a] Retention time values may vary between 0.1 and 0.2 min

Other analyses

Total organic carbon (TOC) contents of sediments were determined by combustion in an LECO CS 125 analyzer (Etcheber et al. 2007). The dried and homogenized sediment samples were acidified in crucibles with 2 N HCl to destroy carbonates, then dried at 60 °C to remove inorganic C and most of the remaining acid and water. The analyses were performed by direct combustion in an induction furnace, and the CO_2 formed was determined quantitatively by infrared absorption. TOC contents are expressed as a percentage of the dw of the sediment, abbreviated as TOC (%).

The percentage of finer grain size fractions (%<63 μm) of each sediment sample was determined gravimetrically after wet sieving (Savinov et al. 2000).

Results and discussion

PAH concentrations

Surface sediments can reflect the current sediment contaminant status. All sediment samples ($n=18$) of the Bizerte Lagoon contained detectable amounts of PAHs. The individual PAH concentrations (on a dry weight basis) were measured at each station. Table 3 showed that Ace was only found in the sites S1, S2, and S5;

DahA was not found in the sites S17 and S18. The other priority PAHs were found at most sampling sites. Indeed, the total concentration of 14 USEPA PAHs (∑PAHs) in surface sediments varied from 16.9 to 394.1 ng g^{-1} dw with a mean concentration of 85.5 ng g^{-1} dw. The largest amounts of PAHs were found along the channel (between the narrow part and the arsenal of Menzel Bourguiba), the path most frequented by boats. Maximum PAH levels were recorded in site S1 (394.1 ng g^{-1} dw), followed by sites S2 (160.2 ng g^{-1}) and S9 (102.8 ng g^{-1}). Stations S1 and S2 are located along the channel connecting the lagoon with the Mediterranean Sea. These areas are highly populated and characterized by industrial activities including lead and cement manufacturing. In addition, S1 is located near the Bizerte Harbor where heavy traffic of tankers and commercial cargo boats is common. PAH inputs could also be attributed to the Merazig River and the incineration landfill located close to S2. In fact, the Merazig River, compared to the other rivers opening on the Bizerte Lagoon, receives the largest amounts of urban and industrial effluent discharges (Harzallah 2003). High concentrations of PAHs were also observed at station S9, probably due to the closeness of the mouth of the Tinja River which is affected by agricultural inputs. Site 4 is located near an urban area (Menzel Abderrahman City) and receives direct inputs of untreated urban sewage. S14 and S15 are located near the city

of Menzel Bourguiba with intensive industrial activity: metallurgic industry, naval construction, and tire production. In all the other stations, the PAH levels ranged from 16.9 to 73.3 ng g^{-1} dw, with a mean value of 53.6 ng g^{-1} dw. The highest concentration of 73.3 ng g^{-1} dw was recorded at S6, located near an urban and an industrial zone (Menzel Jemil City). However, the lowest concentration, 16.9 ng g^{-1} dw, was found at S17. The geochemical composition (%TOC) of this last site contributes to this observation. The distribution of PAHs in terms of the physical and chemical properties of sediments will be discussed later.

The comparison of ∑PAH concentrations with those carried out in other coastal areas is difficult due to the geological characteristics of the sampling areas, the number of samples taken, and the number of PAH compounds analyzed in the different studies, which may not be the same. However, a general idea of the pollutant levels in other coastal areas could be useful. Baumard et al. (1998b) classified four pollution levels of sediment: low, 0–100 ng g^{-1} dw; moderate, 100–1,000 ng g^{-1}; high, 1,000–5,000 ng g^{-1}; and very high, >5,000 ng g^{-1}. Compared to other coasts, bays, and lagoons in the world, the pollution levels of the PAHs in all the sediment samples

from the Bizerte Lagoon (16.9–394.1 ng g^{-1} dw) are low to moderate (Table 4).

A comparison of the temporal of PAH contamination in the Bizerte Lagoon with those in the past was also made to show a decrease of PAH concentrations (Table 5). The ∑PAH concentration in the present study was approximately three times lower than those in sediments collected in 2001 (Trabelsi and Driss 2005). The difference in pollutant levels may reflect differences in the sampling periods. Trabelsi and Driss collected sediments in December (winter period), while in the present study, sediments were collected in March (beginning of the spring period). The higher concentration of PAHs in December could be attributed to three main factors including the increasing use of domestic heating systems, lower microbial degradation, and photolytic decomposition during winter. The biodegradation of organic compounds is facilitated by the presence of environmental conditions which favor microorganism metabolic activity (Cerniglia 1993; Hinga 2003). Quan et al. (2009) demonstrated that the metabolic activity of microorganisms was inhibited at low temperatures. Furthermore, temperature could potentially affect the sorption rate of pollutants onto particle and

Table 3 Concentrations of PAHs (nanograms per gram dw), percentage of total organic carbon (TOC), and percentage of finer grain size fractions (%<63 μm) in coastal superficial sediments of the Bizerte Lagoon

Site	Nap	Ace	Fl	Phe	Ant	Flu	Pyr	BaA	Chr	BbF	BkF	BaP	DahA	BgP	∑PAHs	TOC	%<63 μm
S1	29.2	16.7	21.7	36.9	9.3	55.5	41.2	29.0	33.5	26.6	17.3	29.0	18.9	29.4	394.1	3.85	52.20
S2	11.3	1.8	2.4	12.4	2.2	22.8	21.9	14.3	20.5	16.1	8.9	13.8	3.4	8.5	160.2	0.68	11.32
S3	5.3	nd	2.3	6.0	1.3	10.7	9.8	6.4	9.6	6.8	3.1	5.2	1.0	2.1	69.7	2.16	57.04
S4	5.9	nd	4.2	6.0	1.2	10.7	9.4	6.5	9.4	9.6	3.5	5.4	1.0	4.1	76.7	1.40	89.50
S5	6.2	1.4	2.0	3.7	0.7	6.9	6.7	4.7	6.7	7.8	2.9	4.7	1.0	5.3	60.8	0.79	50.12
S6	nd	nd	nd	4.1	0.6	13.7	14.7	6.8	7.5	8.2	3.5	8.2	0.9	5.2	73.3	1.49	32.60
S7	6.5	nd	nd	3.7	0.8	5.5	4.9	2.7	4.5	12.1	1.5	2.1	1.5	4.3	50.0	1.24	12.98
S8	5.5	nd	2.2	4.0	1.0	8.6	7.3	4.7	6.5	5.6	3.1	5.0	1.4	9.5	64.4	1.58	89.62
S9	7.3	nd	2.4	6.4	1.4	13.9	12.8	6.5	9.1	12.5	6.2	9.0	1.5	13.9	102.8	1.27	65.90
S10	4.6	nd	nd	4.0	0.6	5.2	4.7	3.7	5.0	4.2	1.8	2.0	0.3	3.7	39.7	1.89	69.28
S11	6.7	nd	2.3	4.1	1.0	6.9	6.2	4.2	6.1	6.9	3.2	4.3	0.9	4.9	57.5	1.06	90.24
S12	7.1	nd	2.0	3.8	0.9	5.7	5.3	3.6	5.5	5.8	2.6	3.5	1.5	4.9	49.2	1.01	87.84
S13	nd	nd	1.6	3.6	0.9	4.3	4.1	2.5	3.6	4.0	1.8	2.4	0.5	5.5	34.9	1.16	89.40
S14	nd	nd	2.0	7.3	1.8	11.3	9.9	5.8	6.9	8.8	4.2	6.0	3.3	12.3	79.4	1.27	91.50
S15	nd	nd	1.9	6.4	1.6	12.3	11.0	6.0	7.4	10.7	5.0	7.1	1.8	12.3	83.5	1.01	95.28
S16	nd	nd	1.8	5.6	1.7	10.9	10.0	6.5	8.7	6.7	3.8	6.4	3.2	6.0	70.8	1.11	85.08
S17	nd	nd	nd	1.4	0.5	2.6	2.4	2.2	3.5	1.8	0.9	1.6	nd	nd	16.9	0.44	15.88
S18	nd	nd	nd	5.7	1.4	7.6	6.9	4.8	7.5	9.2	3.4	4.6	nd	5.0	55.8	1.03	32.10

nd not detected

Table 4 Comparison of PAH concentrations (nanograms per gram dw) in sediments of other coasts, bays, and lagoons in the world

Sites		Number of samples	Min–max	Pollution level	References
Tunisia	Bizerte Lagoon	18	16.9−394.1	Low to moderate	This study
	Bizerta Lagoon	10	83.3−447	Low to moderate	Trabelsi and Driss (2005)
	Bizerta Lagoon	6	2.5−209.1	Low to moderate	Louiz et al. (2008)
	Ghar El Melh Lagoon	12	39.59−655.28	Low to moderate	Ben Ameur et al. (2010)
	Jarzouna-Bizerte, coastal area	8	13.84−115.60	Low to moderate	Zrafi-Nouira et al. (2008)
	Sfax–Kerkennah, Coastal area	15	113.2−10720	Low to very high	Zaghden et al. (2007)
Italy	Pialassa Baiona	13	3,032−87,150	High to very high	Guerra (2012)
	Venice Lagoon	25	20±1−502±69	Low to moderate	Secco et al. (2005)
	Venice Lagoon	51	20−3,087	Low to high	Zonta et al. (2007)
	Stagnone coastal lagoon	8	72−18,381	Low to very high	Culotta et al. (2006)
France	Thau Lagoon	15	59−7,679	Low to very high	Leaute (2008)
	Berre Lagoon	2	334−853	Low to moderate	Kanzari et al. (2012)
	Arcachon Bay	7	32−4120	Low to high	Baumard et al. (1998a)
Brazil	Patos Lagoon	18	37.7−11,780	Low to very high	Medeiros et al. (2005)
	Rodrigo de la Freita Lagoon	-	405−11,734	Low to very high	Stefens et al. (2007)
Iceland	Skógalón and Skógar lagoons	13	6−309	Low to moderate	Geptner et al. (2006)
Mexico	Coastal lagoons	4	14.9−61	Low to moderate	Piazza et al. (2008)
	Todos Santos Bay	28	7.6−813	Low to moderate	Macias-Zamora et al. (2002)
China	Yuandang Lagoon	12	203.7−1,590.5	Low to high	Li et al. (2010)
	South China Sea	16	24.7−275	Low to moderate	Yang (2000)
	Daya Bay	14	42.5−158.2	Low to moderate	Yan et al. (2009)
	Kenting coast, Taiwan	5	0.20–493	Low to moderate	Cheng et al. (2011)
Benin	Cotonou Lagoon	6	25.1−1,411	Low to high	Soclo et al. (2000)
Central Vietnam	Coastal lagoons	10	112−628	Low to moderate	Giuliani et al. (2008)
USA	Chesapeake Bay	7	0.56−180	Low to moderate	Foster and Wright (1988)
	San Francisco Bay	16	2,636−27,510	High to very high	Pereira et al. (1996)
Russia	White Sea	11	13−208	Low to moderate	Savinov et al. (2000)

The contaminant levels are those assigned by Baumard et al. (1998b): low, 0–100 ng g^{-1}; moderate, 100–1,000 ng g^{-1}; high, 1,000–5,000 ng g^{-1}; and very high, >5,000 ng g^{-1}

organic matter (Wu and Gschwend 1986). When temperatures decrease, PAH solubility subsequently decreases and conversely PAH bioavailability and biodegradation decrease (Whitehouse 1984; Eriksson et al. 2003). During summer time, increasing temperatures and light intensity may favor biodegradation and photodegradation of PAHs. Interestingly, several PAH-degrading bacterial strains were recently isolated from Bizerta Lagoon sediments (Ben Said et al. 2008), which demonstrated the high PAH biodegradation capabilities of sediment microflora in this lagoon. As shown in Table 5, the ∑PAH concentration is only slightly different between 2006 (Louiz et al. 2008) and 2011 (this study), indicating that the net balance between the input

and the degradation of PAHs have not changed significantly over this time period.

Indeed, there are many other factors affecting temporal variations of PAH levels in sediments, such as the physical and chemical characteristics of the PAH, the composition and chemical characteristics of the sediment, the depositional patterns, and the partitioning processes.

Sediment geochemistry and PAH accumulation

TOC and fine fraction percentages in surface sediments from the Bizerte Lagoon ranged from 0.44 to 3.85 % and from 11.32 to 95.28 %, respectively (Table 2). The S1 and

Table 5 The annual mean concentrations (nanograms per gram dw) of individual PAHs in the sediments of the Bizerte Lagoon

Year	Nap	Ace	Fl	Phe	Ant	Flu	Pyr	BaA	Chr	BbF	BkF	BaP	DahA	BgP	∑PAHs
2001	4.5	0.9	0.8	22.7	9.8	33.0	45.7	7.2	34.8	1.8	28.2	15.1	3.2	0.6	**208.3**
2006	0.1	0.1	0.3	7.0	2.0	21.2	16.4	2.8	1.8	5.2	2.1	3.5	0.1	4.8	**67.4**
2011	5.1	1.1	2.7	6.9	1.6	11.9	10.5	6.7	9.0	9.1	4.3	6.7	2.3	7.6	**85.5**

The data presented in bold are the sum of the annual mean concentrations of individual PAHs

S3 stations showed the highest content of TOC: 3.85 and 2.16 %, respectively. The high levels of organic carbon recorded at these two stations are related to the high occurrence of urban and industrial wastewater discharges, which are rich in organic matter. For grain size analysis, with the exception of stations S2, S6, S17, and S18 that show a predominantly sand composition, all other stations were composed of particles below 63 μm in size. Station S2 is located near a naval base and a solid waste landfill. Stations S6, S17, and S18 are located near the lagoon bank in an area characterized by sandy sediments (Mouldi et al. 2008). The abundance of fine particles was assumed to come from anthropogenic inputs associated with the erosion of surrounding agricultural areas (Ben Hassine River, Garek River, Gueniche River, Tinja River, Merazig River). This result was consistent with the findings of Mouldi et al. (2008) and Boufahja (2010) who showed that the surface sediments of the Bizerte Lagoon are mostly composed of silt and clay fractions.

It is generally stated that the environmental fate and behavior of hydrophobic organic compounds are ultimately determined by the physicochemical properties of both compound and sediments, namely, sediment organic matter content, size particle distribution, PAH octanol–water partition coefficients (K_{ow}), and water salinity (Baker et al. 1991; Doong and Lin 2004). Previous studies have indicated that sediments with high organic carbon content generally contained high PAH concentrations. On the contrary, the sediments with low organic carbon content generally had low levels of PAHs. Furthermore, PAHs are mainly adsorbed onto fine particles (clay) because of their higher specific surface area (Xia and Wang 2008). In the present study, data from stations S1 and S17 are distinguished from the others and were excluded of the data set. Indeed, the highest and the lowest PAH concentration in these sites correspond to the highest and the lowest TOC content, respectively (Table 3). Regression analysis was carried out to investigate the relationship between the concentration of ∑PAHs and the percentage of TOC and fine fraction.

No significant correlation was found between ∑14PAHs and %TOC ($r=-0.330$, $P=0.212$, $n=16$). This suggests that distribution and concentrations of PAHs are more influenced by direct inputs rather than by organic matter content in the sediments. Several other studies reported similar results indicating no significant correlation between PAHs and %TOC (Mostafa et al. 2009; Arias et al. 2010). Simpson et al. (1996) suggested that PAH concentrations and organic carbon (OC) content in sediments are significantly correlated only for highly contaminated sites where total PAH concentrations exceed 2,000 ng g^{-1}. In the present study, total PAH concentrations of all sampled sediments did not exceed 2,000 ng g^{-1} (Table 3). Hence, it can be concluded that the distributions and concentrations of PAHs in sediments of the Bizerte Lagoon are not controlled by the sediment OC content. In our study, the homogeneity of the grain size composition between sediments made any correlation analysis between fine fraction and PAH concentrations difficult.

Although PAHs and TOC were correlated fairly well for the highly contaminated site (S1), it cannot be assumed that the observed positive correlation represents preferential partitioning of PAHs onto sediment according to the TOC percentages. As shown in Table 3, in most of the studied locations of the Bizerte Lagoon, high PAH concentrations are not associated with high TOC and fine particulate contents. These preliminary results reveal a complex relationship between PAH concentrations in sediments and some physicochemical parameters of sediments of the Bizerte Lagoon, thus suggesting involvement of other factors such as sources, transport, mixing, and deposition (Mai et al. 2005).

PAH composition

According to the number of aromatic rings, the 14 PAHs were divided into five groups representing two-ring, three-ring, four-ring, five-ring, and six-ring PAHs. The relative concentrations of PAHs by aromatic groups in

surface sediments from the Bizerte Lagoon are shown in Fig. 2. As illustrated, four-ring PAHs (46 % of total PAHs, on average) were the most abundant followed by five-ring PAHs which accounted for 27 % of the total PAHs. Two-ring, three-ring, and six-ring PAHs accounted for about 5, 13, and 9 % of the total PAHs, respectively. Similar contributions of four-ring PAHs (49–56 %) were found in the same region in 2001 and 2006 by Trabelsi and Driss (2005) and Louiz et al. (2008), respectively (Fig. 3).

In general, high molecular weight (HMW) four-ring to six-ring PAHs were prevalent (82 %) in the Bizerte Lagoon sediments. Usually, HMW PAHs predominated in sediment samples. The low molecular weight (LMW) two-ring and three-ring PAHs, such as Nap, Ant, and Phe, are more labile and are expected to degrade faster than larger ones (Fernandes et al. 1997; Macias-Zamora et al. 2002). The higher concentration of HMW PAHs compared to those of LMW PAHs has been commonly observed in sediments from rivers, lagoons, and marine environments (Culotta et al. 2006; Berto et al. 2009; Mostafa et al. 2009). The sources of PAHs will be further discussed with other evidence in the following section.

Sources of PAHs

Table 6 shows correlation (Spearman's) values among the 14 individual PAHs and ∑PAHs. All PAHs (except Nap, Fl, and BgP) show a good to very good correlation ($r=0.601$–0.969) with the ∑PAH values. Such result suggests that the compositions of the major PAHs are fairly constant at different sampling sites and may come from similar sources.

PAHs are introduced into the environment mainly via industrial discharge, fossil fuel combustion, petroleum spills, and automobile exhausts, as well as through nonpoint sources, such as urban runoff and atmospheric fallout (Countway et al. 2003; Liu et al. 2008).

Identifying the possible sources of PAHs is very important to understand the fate of PAHs in the environment. The PAHs' congener distribution varied with the source as well as the composition and combustion temperature of the organic material. Molecular indices based on ratios of selected PAHs may be used to differentiate PAHs from pyrogenic and petrogenic origins. Pyrogenic sources (combustion processes) are enriched in HMW PAHs, whereas petrogenic sources, such as the releasing of fuel oil or light refined petroleum products, are dominated by LMW PAHs. Thus, the LMW PAHs/HMW PAHs (LMW/HMW) ratio may be used to determine the petrogenic and pyrogenic sources of PAHs in sediments (Budzinski et al. 1997; Soclo et al. 2000; Rocher et al. 2004). LMW/HMW ratio values which are lower than 1 indicate pyrolytic origin pollution, while ratio values higher than 1 indicate that the contaminations are mainly controlled by petrogenic PAHs (Soclo et al. 2000). To estimate the origin of the PAHs in sediment samples from the Bizerte Lagoon, the LMW/HMW ratios were determined and the results were listed in Table 7. LMW/HMW ratios at all sampling sites were lower than one (<1) suggesting the predominance of pyrolytic origin. In addition, ratios of specific PAH compounds including Phe/Ant, Flu/Pyr, Chr/BaA, Ant/(Ant + Phe), BaA/(BaA + Chr), and Flu/ (Flu + Pyr) were also used to differentiate PAHs from pyrogenic, and petrogenic origins (Budzinski et al. 1997; Zeng and Vista 1997; Baumard et al. 1998a;
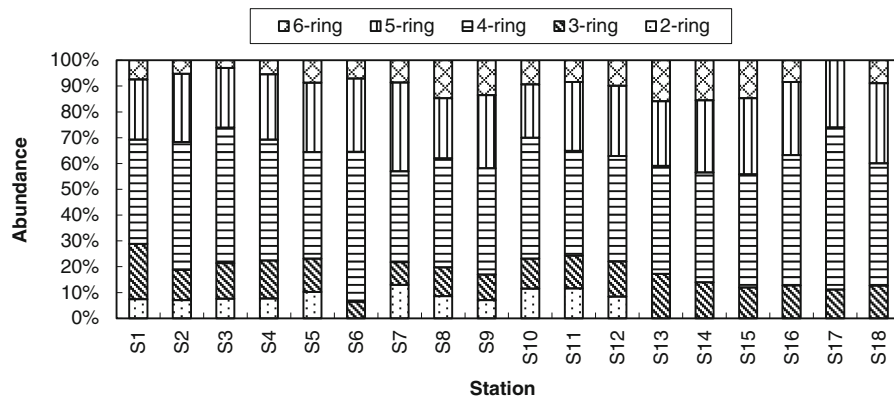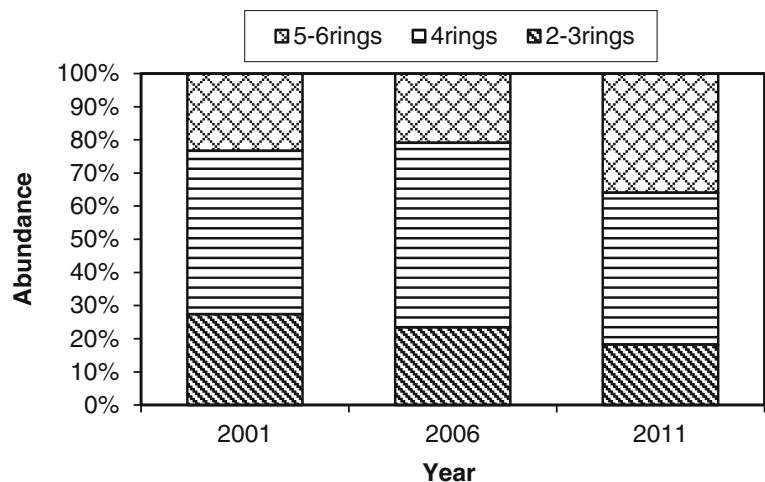

Fig. 2 Relative concentrations of PAHs by aromatic groups in sediments from the Bizerte Lagoon

Fig. 3 Relative abundance of each grouped PAHs (percent) in different time span

Yunker et al. 2002; Doong and Lin 2004; Liu et al. 2008) were also determined in this study. Table 7 showed that all of the Phe/Ant values were lower than 10 and all of the Flu/Pyr values were higher than 1,

indicating that pyrogenic inputs could be the main source of PAHs in surface sediments of the study area. The Chr/BaA ratios obtained ranged from 1.10 to 1.61, indicating a petrogenic source of contamination of the

Table 6 Spearman's rank correlation coefficients (r) for sediment individual PAHs and the sum of PAHs ($\sum$PAHs) (n=16)

	Nap	Ace	Fl	Phe	Ant	Flu	Pyr	BaA	Chr	BbF	BkF	BaP	DahA	BgP
Ace	**0.560**													
	0.024													
Fl	0.373	0.193												
	0.155	*0.473*												
Phe	0.342	**0.541**	0.347											
	0.195	*0.030*	*0.188*											
Ant	0.036	0.276	0.384	**0.877**										
	0.894	*0.300*	*0.142*	*<0.001*										
Flu	0.317	**0.497**	0.316	**0.878**	**0.718**									
	0.231	*0.050*	*0.233*	*<0.001*	*0.002*									
Pyr	0.289	**0.515**	0.243	**0.839**	**0.654**	**0.991**								
	0.278	*0.041*	*0.365*	*<0.001*	*0.006*	*<0.001*								
BaA	0.410	**0.634**	0.336	**0.914**	**0.713**	**0.960**	**0.953**							
	0.115	*0.008*	*0.202*	*<0.001*	*0.002*	*<0.001*	*<0.001*							
Chr	**0.521**	**0.682**	0.371	**0.921**	**0.704**	**0.904**	**0.890**	**0.978**						
	0.038	*0.004*	*0.157*	*<0.001*	*0.002*	*<0.001*	*<0.001*	*<0.001*						
BbF	0.456	0.481	0.142	**0.732**	**0.575**	**0.738**	**0.719**	**0.689**	**0.706**					
	0.075	*0.059*	*0.600*	*<0.001*	*0.020*	*0.001*	*0.002*	*0.003*	*0.002*					
BkF	0.373	**0.546**	0.378	**0.907**	**0.801**	**0.929**	**0.902**	**0.905**	**0.885**	**0.766**				
	0.155	*0.029*	*0.148*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.001*				
BaP	0.304	**0.545**	0.306	**0.838**	**0.698**	**0.978**	**0.978**	**0.938**	**0.884**	**0.725**	**0.954**			
	0.251	*0.029*	*0.248*	*<0.001*	*0.003*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.001*	*<0.001*			
DahA	0.102	0.335	0.321	**0.652**	**0.742**	**0.632**	**0.586**	**0.600**	**0.533**	0.461	**0.611**	**0.597**		
	0.704	*0.203*	*0.225*	*0.006*	*0.001*	*0.009*	*0.017*	*0.014*	*0.033*	*0.072*	*0.012*	*0.014*		
BgP	−0.053	0.037	0.227	0.403	**0.535**	0.451	0.397	0.272	0.194	0.421	**0.591**	0.497	**0.504**	
	0.843	*0.889*	*0.397*	*0.122*	*0.033*	*0.079*	*0.128*	*0.308*	*0.471*	*0.104*	*0.016*	*0.050*	*0.046*	
$\sum$PAHs	0.472	**0.601**	0.394	**0.918**	**0.751**	**0.967**	**0.943**	**0.952**	**0.934**	**0.813**	**0.969**	**0.960**	**0.651**	0.495
	0.065	*0.014*	*0.131*	*<0.001*	*0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.006*	*0.051*

For each parameter, the Spearman's correlation coefficient is indicated in normal characters and the P value is in italics. Data are rendered in bold when the correlation is significant (P<0.05)

**Table 7** Characteristic values of selected molecular ratios for pyrogenic and petrogenic origins of PAHs

Ratio	Pyrolytic origin	Petrogenic origin	This study	References
Flu/Pyr	≥1	<1	0.93−1.35	Doong and Lin (2004)
Phe/Ant	<10	>15	3.11−6.81	Budzinski et al. (1997)
Chr/BaA	<1	>1	1.10−1.61	Zeng and Vista (1997)
Ant/(Ant + Phe)	>0.1	<0.1	0.13−0.24	Liu et al. (2008)
BaA/(BaA + Chr)	>0.35	<0.20	0.38−0.48	Yunker et al. (2002)
Flu/(Flu + Pyr)	>0.50	<0.40	0.48−0.57	Yunker et al. (2002)
LMW/HMW	<1	>1	0.07−0.41	Soclo et al. (2000)

sediment. This index is based in part on the concentrations of Chr; however, in the literature, opinions on the sources of chrysene are divided. Rocher and Moilleron (2007) favored a pyrolytic origin, while Doong and Lin (2004) classified this PAH as the marker of petrogenic origin. The isomer pair ratios in our study, such as Ant/(Ant + Phe) and BaA/(BaA + Chr), ranged from 0.13 to 0.24 and from 0.38 to 0.48, respectively, indicating that the main origin of PAHs in sediments in this lagoon was from a pyrolytic source (Table 7). Plots of isomeric ratios BaA/(BaA + Chr) and Ant/(Ant + Phe) versus Flu/(Flu + Pyr) (Fig. 4) confirmed this predominant

source and showed that the pyrogenic PAHs originated from the incomplete combustion of coal, grass, or wood, for all the samples, except S6 that had the combustion of petroleum as a source. This station is located close to the motorway, in front of the municipal draining discharge for the city of Menzel Jemil that is a highly urbanized region. Thus, the hypothesis of a contamination by the road at this station is likely, but remains to be confirmed.

Predominance of pyrolytic PAHs in the studied area could be explained by the high number of factories using coal as a fuel. In fact, since the 1950s, about 100

Fig. 4 Plots of isomeric ratios BaA/(BaA + Chr) and Ant/(Ant + Phe) versus Flu/(Flu + Pyr)
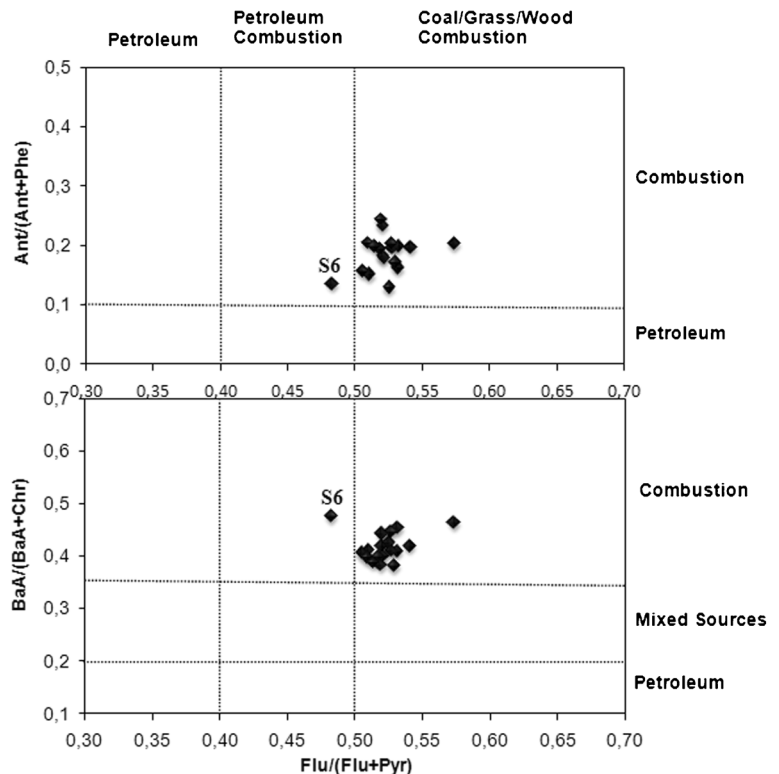
industries settled in the vicinity of the lagoon, the most important being the cement factory (1950) and the steel complex "El Fouledh" (1965). These industrial units are responsible for various pollutant discharges reaching the sediments as a result of atmospheric deposition and urban runoff (Stout et al. 2004; Van Metre and Mahler 2005). Altogether, industries generate a daily average flow of wastewater of about 31,600 m³/day in the Bizerte Lagoon. These wastewater discharges account for 3,139 kg/day of PAH inputs into the lagoon. In addition, urban sources generate a daily average flow of wastewater of about 20,000 m³/day, which corresponds to 23.5 kg/day of hydrocarbons, while harbor activities provide only 0.11 kg/day of hydrocarbons (MAERH 2003). Open burning of biomass is a common procedure for crop and forest residue disposal and land preparation. Combustion by-products are transported to the marine environment by dry and/or wet depositions from the atmosphere (Wu et al. 2001). In addition, multiple other sources of PAHs such as transport, heating systems, waste incinerators, municipal waste, etc. can explain the pyrolytic origin of the PAH contamination in the area. Dominance of pyrolytic PAHs in marine sediments has been found in most Mediterranean countries in the vicinity of industrialized or urbanized areas (Van Metre and Mahler 2005; Barra et al. 2009; Pietzsch et al. 2010; Viñas et al. 2010; Alebic-Juretic 2011; Hu et al. 2011; Hung et al. 2011; Perra et al. 2011). Despite the presence of an oil refinery in the vicinity of stations S1 and S2, petrogenic PAHs were not predominant in our work. This result agrees with the results obtained in the Galician coast (Franco et al. 2006) and in the case of the Erika accident (Tronczynsky et al. 2004).

Although distinct sources may be inferred from PAH isomer pair ratios, it is well documented that the fingerprint of PAHs may be altered by biological (e.g., bacterial activity), chemical (e.g., oxidation and reduction), and/or physical (e.g., air mass mixing and sediment resuspension) processes during transport and after deposition in marine sediments (Tolosa et al. 1996). The importance of using a battery of different ratios is highlighted here; when only considering the Chr/BaA ratio, the conclusion about the sources of PAHs would have been different. The ratios used, except this one, converge to the same conclusion: PAHs in surface sediments in the Bizerte Lagoon are mainly from pyrolytic sources.

## Assessment of sediment quality and toxicity

PAHs are ubiquitously distributed in diverse environmental matrices such as soil and sediment and deserve increasing attention due to their high stability, low solubility, and toxic, carcinogenic, and mutagenic effects. Sediment quality guidelines (SQGs) are useful tools for the assessment of contamination in marine and estuarine sediments (Long et al. 1995; Qiao et al. 2006; Quiroz et al. 2010). Long et al. (1995) developed two guideline values, an effects range low (ERL) and an effects range median (ERM), to assess the sediment quality using a ranking of low to high impact values. The measured concentrations of PAHs were compared with the ERL and ERM values (Table 8). Results obtained in this study showed that the average total PAH concentrations at all sites were below the ERL and ERM. For individual components, this result is also valid, except in the case of Ace and Fl (with a slightly higher concentration than the ERL value). Thus, concentrations of PAHs found in this study are not expected to be a threat to benthic marine organisms along the Bizerte Lagoon.

Table 8 Guideline values for PAH compounds in sediments (nanograms per gram)

PAHs	SQG[a]		This study	
Compound	ERL	ERM	Average	Maximum
Nap	160	2,100	8	29.2
Ace	16	500	7	16.7
Fl	19	540	4	21.7
Phe	240	1,500	7	36.9
Ant	853	1,100	2	9.3
Flu	600	5,100	12	55.5
Pyr	665	2,600	11	41.2
BaA	261	1,600	7	29.0
Chr	384	2,800	9	34.0
BbF	NA	NA	9	26.6
BkF	NA	NA	4	17.3
BaP	430	1,600	7	29.0
DahA	63.4	260	3	18.9
BgP	NA	NA	8	29.4
∑PAHs	4,000	44,792	85.5	394.1

NA not available

[a] Values taken from Long et al. (1995)
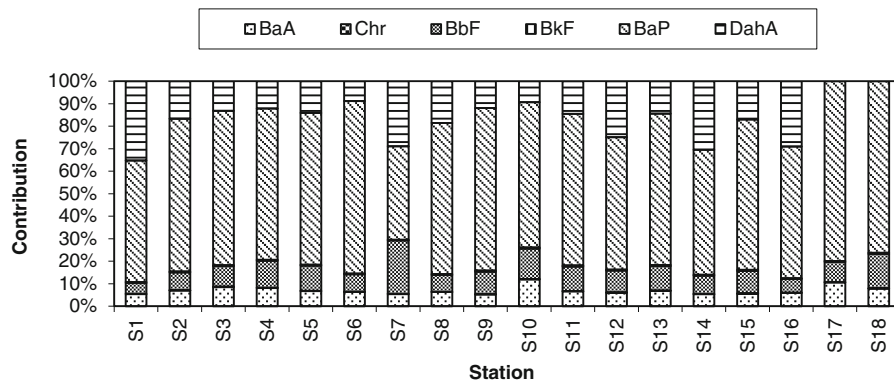
Fig. 5 Contributions of BaA, Chr, BbF, BkF, BaP, and DahA to the total TEQ value at each station

Some PAHs, and especially their metabolic products, are of great concern due to their documented carcinogenicity. These potentially carcinogenic PAHs (CPAHs) include BaA, BaP, BbF, BkF, DahA, and InP (Savinov et al. 2003). The InP was not investigated in this study. The total concentrations of CPAHs in sediments from the Bizerte Lagoon varied from 6.6 to 120.8 ng g^{-1} dw (with a mean concentration of 29.1 ng g^{-1} dw) and accounted for 30.1–39.8 % of the total PAH concentrations.

Among all the known potentially carcinogenic PAHs, BaP is the only PAH for which toxicological data is sufficient to derive a carcinogenic potency factor (Peters et al. 1999; Bowman et al. 2002). Toxic equivalence factors (TEFs) were applied to quantify the carcinogenicity of other PAHs relative to BaP and to estimate benzo(*a*)pyrene-equivalent doses (BaPeq dose). Calculated TEFs for BaA, BaP, BbF, BkF, DahA, and Chr were 0.1, 1, 0.1, 0.01, 0.1, 1, and 0.001, respectively, according to the USEPA. In this study, we converted the abovementioned six PAH concentrations into one toxic concentration for each site using the corresponding TEFs. According to IARC (1987), the total toxic benzo(*a*)pyrene equivalent (TEQ) of all these PAHs is:

$$BaP_{eq}dose_i = TEF_i \cdot dose_i$$
$$Total\ TEQ = \sum BaP_{eq}dose_i$$

Total TEQ values calculated for samples from the Bizerte Lagoon varied from 3.1 to 53.7 ng g^{-1} dw with a mean value of 10.6 ng g^{-1}. The maximum value of total TEQ was found at station S1 situated right next to the Bizerte commercial harbor indicating a local source of

contamination. Mean values of relative BaPeq doses in total TEQ, decreased in the following order: BaP (65.4 %) > DahA (18.7 %) > BbF (10.4 %) > BaA (7 %) > BkF (0.4 %) > Chr (0.1 %) (Fig. 5). Similar contributions of BaP (43.6–70 %) were found in other studies in different environments (Savinov et al. 2003; Qiao et al. 2006; Ünlü et al. 2009), confirming the importance of using BaP as an indicator of carcinogenic PAHs in sediments.

In comparison to other studies, the total TEQ values in sediments of the Bizerte Lagoon were lower than the values detected in the Coastal lagoon, Northern Adriatic, Italy (Guerra 2012) and close to the ones found at the coastal lagoons in central Vietnam (Giuliani et al. 2008).

Conclusions

In the present study, analysis of surface sediment samples from 18 sites along the Bizerte Lagoon showed that the highest levels of PAHs were found at station S1. Four- to five-ring PAHs were predominant in sediments. Comparison of the concentration range with a world-wide survey of PAH concentrations in sediments ranked PAH contamination in Bizerte Lagoon 's sediments as poorly to moderately polluted. PAH source identification showed that grass, wood, and coal combustion were the primary PAH sources in most sediments of the Bizerte Lagoon. The sediment quality guidelines assessed the surface sediment of the Bizerte Lagoon to be within minimal effect ranges for benthic organisms. To better understand the importance of organic matter and particle geochemistry in the distribution of PAHs in

sediments, further detailed work is required. The results of this study would be helpful for understanding the levels, distribution, biological effects, and sources of PAHs in Bizerte Lagoon sediments, which can provide information for protecting water resources and human health of lagoon and/or coastal areas in Tunisia.

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