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RESEARCH ARTICLE

Biomarkers responses and polybrominated diphenyl ethers and their methoxylated analogs measured in *Sparus aurata* from the Lagoon of Bizerte, Tunisia

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Abstract

This study aimed to the examination of the levels and effects of organobromine compounds (polybrominated diphenyl ethers: PBDEs and methoxylated brominated diphenyl ethers: MeO-PBDEs), in *Sparus aurata* native to the Lagoon of Bizerte. For that, different biomarkers of exposure (somatic indices, superoxide dismutase, and catalase activities) and effect (malondialdehyde level, histopathologic alterations, and DNA damage) as well as pollutant levels were measured in specimens collected from this impacted ecosystem and the Mediterranean Sea as a reference site. Bizerte Lagoon PBDE fish levels were higher than the Mediterranean Sea, whereas MeO-PBDEs were higher in the reference site. Fish from Bizerte Lagoon presented a higher hepatosomatic index, lower catalase and superoxide dismutase activity, higher level of malondialdehyde, and higher percentage of DNA tail in comparison to fish from the reference area. The histological study of the liver indicated substantial lesions in fish from the polluted site. The results showed strong positive correlations between the concentrations of the PBDE or MeO-PBDE and the MDA and DNA tail % levels and negative correlations for the activities of enzymes of SOD and CAT. Consequently, these findings could suggest a potential link between exposure to these pollutants and the observed biomarker responses in the Bizerte Lagoon seabream. Taken together, these results highlight the importance of biomarker selection and the selected sentinel fish species as useful tools for biomonitoring of aquatic pollution.

Keywords *Sparus aurata* · PBDEs · MeO-PBDEs · Biomarkers · Bizerte Lagoon · Tunisia

Introduction

The structural resemblance of the polybrominated diphenyl ethers to polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/DFs) caused a lot of concern in recent years about their persistence in the environment, biomagnification, and bioaccumulation, as well as their harmful effects on wildlife and humans health (Cruz et al. 2020). Methoxylated brominated diphenyl ethers having a structure similar to the PBDEs have been extensively studied due to their toxic properties (thyroid hormone homeostasis disruptor, neurotoxic, oxidative phosphorylation disruptor, and estradiol synthesis alteration) (Ameur et al. 2020) and exhibit a high biomagnification aptitude than PBDEs in the marine food chain (Barhoumi et al. 2020).

In situ studies about PBDEs toxicity on fish are scarce and inexistent for MeO-PBDEs. In fact, previous in situ studies conducted on barbel from the Ebro River basin

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(NE, Spain) (Raldúa et al. 2008) and laboratory ones carried out on turbot (*Psetta maxima*) (Barja-Fernández et al. 2013), freshwater fish *Carassius auratus* (Feng et al. 2013), zebrafish (Usenko et al. 2015; Zezza et al. 2019), and *Sparus aurata* (Espinosa Ruiz et al. 2019) showed that the accumulation of PBDEs on fish species leads to the formation of free radicals inducing oxidative stress and causing histological alterations on the liver and other metabolic organs also as genotoxic effects. Concerning the MeO-PBDEs, there are no field or laborotaory studies about their genotoxic, oxidative, and histopathological effects on fish. There are only two laboratorory works realized on Maize (*Zea mays* L.) (Xu et al. 2015) and on *Daphnia magna* (Liu et al. 2018).

Biomarkers have become a valuable tool in recent environmental assessments for predicting contaminants in environmental monitoring programs (Osman et al. 2010; Osman 2012; Badr et al. 2014; Khallaf et al. 2018). In fact, field studies have showed the usefulness of the use of antioxidant defense system, namely represented by the catalase (CAT) and the superoxide dismutase (SOD), the lipid marker of oxidative stress represented by the malondialdehyde (MDA), histological and genotoxic biomarkers on liver, and other tissues of fish species in assessing the health of aquatic systems. These studies had been conducted on species having a similar trophic level value of the studied fish species in this work as white seabream (*Diplodus sargus*) from Northwest Atlantic (Ferreiraa et al. 2008); *Zosterisessor ophiocephalus* from the Venice Lagoon, Italy (Pascoli et al. 2011); *Heterotis niloticus* from Lekki Lagoon, Lagos, Nigeria (Akinsanya et al. 2020); *Channa punctatus* from canal in India (Javed et al. 2016b); *Arius thalassinus* from the Red Sea coast of Yemen Republic (Saleh and Marie 2016); *Aequidens metaethe* from Ocoa River, Villavicencio, Meta, Colombia (Corredor-Santamaría et al. 2019); *Solea senegalensis* from Huelva estuary (SW Spain) (Oliva et al. 2012); *Solea solea* from Tunisia coastline (Jebali et al. 2013); *Anguilla anguilla* from freshwater wetland ecosystem (Pateira de Fermentelos, Portugal) (Ahmad et al. 2006); *Sciades herzbergii* from Amazon estuaries (Nunes et al. 2020); mullet (*Mugil cephalus*) and flounder (*Platichthys flesus*) from River Douro Estuary, Portugal (Ferreira et al. 2005); and Mugil cephalus and Dicentrarchus labrax from Bizerte Lagoon (Ben Ameur et al. 2011).

In environmental monitoring, the selection of a bioindicator is a crucial factor. We chose to study the possible impact of pollutants on *Sparus aurata* using morphological, biochemical, molecular, and histological biomarkers on *Sparus aurata*. We selected this species as a bioindicator for numerous reasons. The gilthead seabream (*Sparus aurata*) is a valuable commercial fish species. Moreover, since this species is suitable for human consumption, it is primordial to protect it from being exposed to pollutants that could affect the health, particularly the reproduction function of this species (Piccinetti et al. 2012). Furthermore, this species is a sentinel fish for environmental monitoring programs since, as a demersal species sentinel fish and because of its native sandy coastal habitat, it can be exposed to pollutants via water column and sediment and due to its wide tolerance to environmental conditions and distribution throughout the entire Mediterranean Sea (Cretì et al. 2010).

This work puts forward to evaluate the responses of morphological (CF, HSI) and biochemical biomarkers (CAT and SOD activities and MDA levels) and molecular and histological biomarkers in wild seabream liver originating from Bizerte Lagoon due to the exposition to PBDEs and MeO-PBDEs. This study represents a continuation of the studies into combining chemical and biological measures to evaluate the Bizerte Lagoon's environmental quality. To reach this goal, we realized (i) measurements of organobromine compounds concentrations in seabream muscle, (ii) determination of environmental parameters of water (temperature, pH, dissolved oxygen, salinity, turbidity, conductivity, SPM, NO_3^-, NO_2^-, and PO_4^{3-} levels), (iii) measurements of fish morphometric parameters (length, weight), (iv) evaluation of biomarkers of exposure (somatic indices, SOD and CAT activities) and effect (MDA level, histopathologic alterations, and DNA damage in seabream liver), (v) analysis of the correlation analysis between the studied chemicals and the biomarkers.

Materials and methods

Study area and sampling

Bizerte Lagoon, a Mediterranean lagoon extended on 15 km^2 is of economic importance due to different human activities installed on the lagoon coasts (fishing, aquaculture, …). In June 2010, thirty fish from the Bizerte Lagoon and thirty samples from the reference location at 7 km north of the lagoon in the Mediterranean Sea were collected alive (Fig. 1). In addition to fish samples, three water samples from the each investigated site were collected. With reference to previous studies conducted to analyze the polybrominated diphenyl ethers, the Mediterranean Sea was selected as a reference area. The authors of these studies found low levels of contaminants in the Mediterranean Sea (Ben Ameur et al. 2011, 2013; Ameur et al. 2015). Instantly, the fish were sacrificed by exsanguination, and their size and weight were determined. The liver was removed then weighed and a part was fixed using 10% formalin for the analysis of histopathology. The rest of the liver was cut into 2 pieces (to study the oxidative stress and to measure the DNA integrity) and held at − 80° C after being frozen in liquid nitrogen until the start of analyses. Finally, muscle samples for PBDE and MeO-BDE analyses were obtained and held at − 20° C until they were freeze-dried and homogenized with a mixer.

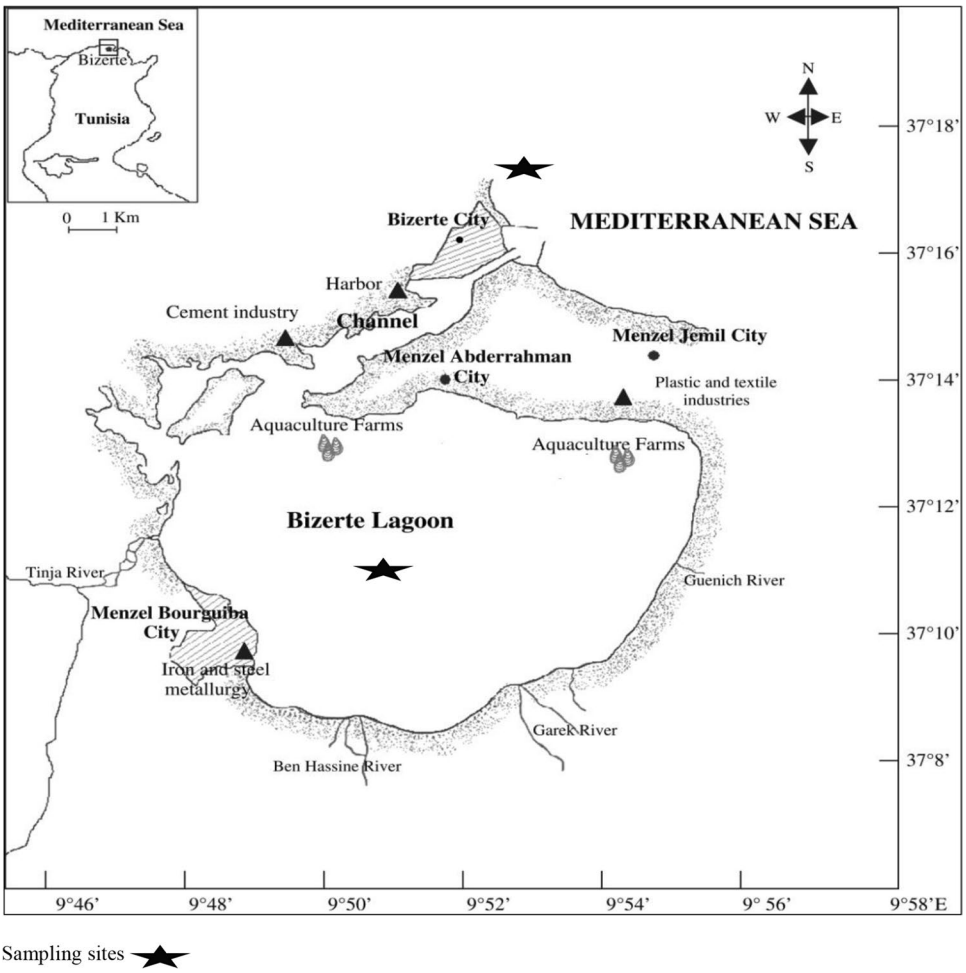

Fig. 1 Map indicating the sampling locations: the Bizerte Lagoon and the Mediterranean Sea

General water quality parameters

Water physicochemical characteristics comprising pH, temperature, conductivity, salinity, and dissolved oxygen were determined in situ using a WTW 315i/SET pH meter and a WTW 315i/SET conductivity meter.

For nutrient analysis, water samples (1000 mL) undergo filtration using Whatman GF/F filters and the collected filtrates were conserved (20 °C) in vials previously rinsed with acid until analysis. NO_3^- NO_2^- concentrations measurement was carried out by spectrophotometric method according to the method outlined by Wood et al. (1967), whereas PO_4^{3-} concentrations were assessed using the method proposed by Murphy and Riley (1962). For the estimation of the amounts of suspended particulate matter (SPM), samples of water (1 L) were filtered using paper filters (0.45 µm). The paper filters were then dried for 12 h at 105 °C. To determine the amount of SPM in the samples, the weight difference was calculated (Onderka and Pekárová, 2008).

Chemical analysis

In the present study, 40 standard mixtures of the PBDE congeners were used (Guelph, ON, Canada) and are the same used in the survey carried out by Ameur et al. (2020), 8 MeO-PBDE standard mixture (5-MeO-BDE-47, 6-MeO-BDE-47, 4′-MeO-BDE-49, 2′-MeO-BDE-68, 5′-MeO-BDE-99, 5′-MeO-BDE-100, 4′-MeO-BDE-101, and 4′-MeO-BDE-103) and the $^{13}C_{12}$-BDE-209. Tetra-BDE-77, hepta-BDE-181, and 13C-BDE-209 were used as internal standards. Merck (Darmstadt, Germany) provided all of the required solvents for the study of the compounds under investigation.

The target compounds were analyzed according to the method described by Ameur et al. (2020).

The analysis of the studied chemicals in the extracts was performed using a gas chromatograph (Agilent 7890C) coupled to a mass spectrometer (Agilent 5975A Network) operating in negative chemical ionization mode (NCI) and the reagent gas was the $NH4^+$. The adopted operating conditions for the instrument were as specified by Barón et al. (2014).

The samples were checked for quality assurance according to the method presented by Barón et al. (2014). The absence of analytes of concern was shown by procedural blanks. The confirmation criteria for the quantification and detection of the studied chemicals were those adopted by El Megdiche et al. (2017).

The used method yields recovery percentages ranging from 56 to 90% for the examined compounds. Relative standard deviations (RSD) were below 10% for all studied compounds, demonstrating that the reproducibility of the method is satisfactory. A multi-level calibration curve was conducted to quantify the examined chemicals, and good linearity ($R^2 > 0.995$) was found. The limits of detection (LODs) of the apparatus varied from 0.07 to 0.74 ng g^{-1} lipid weight (lw) for PBDEs and from 0.1 to 0.2 ng g^{-1} lw for MeO-PBDEs. The limits of quantification (LOQs) of the instrument fluctuated from 0.23 to 2.50 ng g^{-1} lw for PBDEs, and from 0.3 to 0.6 ng g^{-1} lw for MeO-PBDEs. The measurement of the LODs was determined as 3 times the signal to noise ratio and the LOQs were defined as 10 times the signal to noise ratio as described by El Megdiche et al. (2017).

Biomarker analysis

Individual weight and length of the studied fish species were measured and the livers were weighed and dissected. The condition factor (CF) and the hepatosomatic index (HSI) were determined according to the method used by Ben Ameur et al. (2012).

The method outlined in the previous study (Ben Ameur et al. 2012) was used to conduct the histopathological analysis. The histological alterations were identified, based on the works of Mumford et al. (2007) and Wolf et al. (2015).

The activity of Catalase was determined in the liver as reported by Ben Ameur et al. (2012).

SOD activity was assessed in the liver according to the previous method (Ben Ameur et al. 2012).

The method proposed by Ben Ameur et al. (2012) was used to measure MDA levels.

The alkaline single cell gel electrophoresis (SCGE) was applied to assess the DNA integrity (Ben Ameur et al. 2012).

Statistics

All given values are averages with standard deviations. Data were tested for normality and homogeneity of variances using Kolmogorov–Smirnoff and Levene's tests, respectively. Comparisons of water quality parameters were performed by the non-parametric Kruskal–Wallis test, since variables did not meet the normality assumption. To evaluate differences for polluant levels and biomarkers values across sites, the two-way ANOVA test with multiple comparisons (Tukey HSD test for post hoc comparisons) was used at a 5% significant level after verifying normal distribution and equal variance. The SPSS software (SPSS 10.0 for Windows, SPSS Inc.) was used to conduct all of the tests.

The STATISTICA 7.1 software from StatSoft (Maison Alfort, France) was used to determine the relationships between parameters using parametric Pearson's bivariate correlation analysis.

Results

Environmental parameters

Table 1 shows the environmental characteristics measured in the surface water at the two studied stations. The comparison between these two areas revealed no significant variations for temperature, pH, dissolved oxygen, salinity, turbidity, conductivity, SPM, and $PO4^{3-}$levels. However, important increases were registered for nitrate and nitrite levels between the two investigated areas ($p < 0.05$).

Fish biometrics

For both study sites, the weight and length measurements of the studied fish species were homogenous. The average length of fish samples is 23.8 ± 1.0 cm and 25.50 ± 1.32 cm in the Bizerte Lagoon and the Mediterranean Sea, respectively. The average weight is 193.3 ± 21.3 g and 229.27 ± 7.15 g in seabream from Bizerte Lagoon and the Mediterranean Sea, respectively.

The CF was 1.44 ± 0.10 g/cm^3 and 1.40 ± 0.21 g/cm^3 respectively for fish from Bizerte Lagoon and the Mediterranean Sea. No statistically significant differences were

Table 1 Physicochemical properties of water samples collected from the Bizerte Lagoon (BL) and the Mediterranean Sea (MS) (mean ± SD, $n = 3$)

Parameter	BL	MS
Temperature (°C)	29.7 ± 0.20	32.4 ± 0.05
pH	7.2 ± 0.08	8.7 ± 0.05
Dissolved oxygen (mg/L)	8.2 ± 0.09	7.3 ± 0.07
Salinity (PSU)	35.2 ± 0.05	40.5 ± 0.08
Turbidity (NTU)	2.6 ± 0.03	2.8 ± 0.01
Conductivity (ms cm^{-1})	9.5 ± 0.02	12.5 ± 0.04
SPM (mg/L)	25.7 ± 0.10	18.7 ± 0.20
Nitrate (µg/L)	175.2 ± 0.08*	88 ± 0.06
Nitrite (µg/L)	28.25 ± 0.10*	12 ± 0.08
$PO4^{3-}$ (µg/L)	58.2 ± 0.15	45 ± 0.25

*Indicates significant differences at $p < 0.05$

SD, standard deviation

observed regarding the CF values between the two studied areas ($p > 0.05$).

The HSI was 2.15 ± 0.02 and 1.09 ± 0.001 in seabream collected from Bizerte Lagoon and the Mediterranean Sea, respectively. The HSI was significantly higher in Bizerte Lagoon than in the Mediterranean Sea ($p < 0.05$).

The values of the lipid content, presented as the weight percentage of the muscle tissue, were relatively higher in Bizerte Lagoon. The lipid % was 0.87 ± 0.12 and 1.42 ± 0.31 in seabream collected from Bizerte Lagoon and the Mediterranean Sea, respectively. No significant difference between lipid % values was found between the two investigated sites ($p > 0.05$).

Chemical analyses

Table 2 summarizes the arithmetic means and the concentration ranges for PBDEs and MeO-BDEs in the muscle of seabream.

The mean concentrations of ΣPBDEs and ΣMeO-PBDEs in Bizerte Lagoon samples were 74.3 and 123.2 ng/g lw. Those for the Mediterranean Sea samples were 34.9 and 123.2 ng/g lw.

It was found that the mean concentrations of ΣPBDEs measured in the muscle of samples from the Bizerte Lagoon were two times higher than those detected in the reference site samples. ΣMe0-PBDEs in samples from the Mediterranean Sea were two times higher than those from the Bizerte Lagoon. The comparison between the two studied areas showed statistically significant differences in the concentrations of ΣPBDEs ($p = 0.04$) and ΣMeO-PBDEs ($p = 0.03$).

PBDE-28, -47, -99, -100, -153, -154, -183, and -209 congeners accounted respectively for 3%, 94%, 6%, 17%, 13%, 18%, 29%, and 2% of total PBDEs detected in the fish samples from Bizerte Lagoon, and for 2%, 69%, 4%, 13%, 10%, 14%, 21%, and 1% respectively for fish samples from the Mediterranean Sea.

Regarding the MeO-PBDEs, 6-MeO-BDE-47, 2-MeO-BDE-68, 5-MeO-BDE-47, 4-MeO-BDE-49, and 4-MeO-BDE-103 accounted respectively for 63%, 28%, 6%, 1%, and 1% of total Meo-PBDEs detected in the seabream samples collected from Bizerte Lagoon. In the Mediterranean Sea, they contributed for 69%, 26%, 4%, 1%, and 1% respectively.

6-MeOBDE-47 and 2-MeOBDE-68 were the dominant congeners, accounting together for 91% and 95% of the ∑MeO-PBDE concentration respectively in seabream from the Bizerte Lagoon and the Mediterranean Sea.

Table 2 PBDE and MeO-PBDE concentrations (ng g^{-1} lw) in *Sparus aurata* from Bizerte Lagoon (BL) and the Mediterranean Sea (MS). Values are given as mean ± SD ($n = 30$)

Compound	BL			MS		
	Mean	Range	FD (%)	Mean	Range	FD (%)
BDE-28	2.2 (2.1)	0.4–8.2	100	0.6 (0.1)	0.6–0.7	100
BDE-47	33.8 (7.6)	23.6–50.0	100	18.0 (0.3)	17.7–18.1	100
BDE-99	3.7 (4.4)	nd–13.3	25	3.3 (1.0)	2.5–4.4	100
BDE-100	1.3 (2.5)	nd–7.4	42	1.1 (0.6)	0.5–1.7	100
BDE-153	8.4 (8.9)	0.3–27.6	100	2.5 (1.5)	0.7–3.7	100
BDE-154	10.8 (10.7)	0.3–31.6	100	3.5 (1.3)	2.1–4.3	100
BDE-183	12.9 (8.9)	0.3–24.0	100	5.5 (0.1)	5.4–5.6	100
BDE-209	1.1 (2.9)	nd–10.4	42	0.4 (0.1)	0.3–0.4	100
∑PBDEs	74.3 (33.2)	31.7–153.2		34.9 (1.6)	30.3–37.0	
6-MeO-BDE-47	77.72 (18.23)	66.90–130.86	100	206.65 (21.09)	190.17–230.41	100
2′-MeO-BDE-68	35.04 (10.34)	24.18–48.72	100	78.74 (2.08)	76.77–80.91	100
5-MeO-BDE-47	7.72 (8.02)	nd–16.87	83	11.41 (0.63)	10.98–12.13	100
4-MeOBDE-49	1.18 (2.66)	nd–9.20	33	2.07 (3.59)	nd–6.22	33
5-MeOBDE-100	0.06 (0.13)	nd–0.38	17	0.05 (0.09)	nd–0.16	33
4-MeOBDE-103	0.92 (1.39)	nd–4.27	50	1.93 (1.88)	0.23–3.94	67
4-MeOBDE-101	0.56 (0.83)	nd–2.33	42	0.45 (0.09)	0.39–0.56	100
ΣMeO-PBDEs	123.20 (34.30)	95.91–214.49		301.30 (22.05)	279.00–323.10	

nd, not detected, and assumed as 0 for the calculation of total PBDE and total MeO-PBDE values; *FD*, frequency of detection

Values in parentheses represent the standard deviation

List of the 40 studied BDE congeners: BDE-1, BDE-2, BDE-3, BDE-7, BDE-8, BDE-10, BDE-11, BDE-12, BDE-13, BDE-15, BDE-17, BDE-25, BDE-28, BDE-30, BDE-32, BDE-33, BDE-35, BDE-37, BDE-47, BDE-49, BDE-66, BDE-71, BDE-75, BDE-77, BDE-85, BDE-99, BDE-100, BDE-116, BDE-118, BDE-119, BDE-126, BDE-138, BDE-153, BDE-154, BDE-155, BDE-166, BDE-181, BDE-183, BDE-190, and BDE-209

The comparison of the MeO-PBDE concentrations registered in the seabream muscle collected from Bizerte Lagoon and the Mediterranean Sea showed statistically significant differences ($p < 0.05$).

In the present study, the 6-MeO-BDE-47 and 2′-MeO-BDE-68 ratio was calculated for all samples. This ratio varied between 0.37 and 0.58 (mean value = 0.45) for samples of fish from the Bizerte Lagoon and between 0.34 and 0.41 (mean value = 0.38) for samples of fish from the Mediterranean Sea. No statistically significant correlation was registered for the BDE-47 when compared to the 6-MeO-BDE-47 levels in seabream from Bizerte Lagoon (rs = 0.13, $p > 0.05$) in this work.

Biomarkers

Figures 2 and 3 show the measures of the enzymatic activities, peroxidation of lipids, and percentage tail DNA in *Sparus aurata* liver from the two investigated sites. The values of the enzymatic activities in Bizerte Lagoon ranged from 2.03 to 3.57 (mean = 2.65) nmol/min/g tissue for CAT and from 7967 to 8018 (mean = 8007) U/g tissue for SOD, respectively. In the Mediterranean Sea, these enzymatic activities varied from 4.51 to 5.00 (mean = 4.83) nmol/min/g tissue and from 23,753 to 23,801 (mean = 23,791) U/g tissue, respectively.

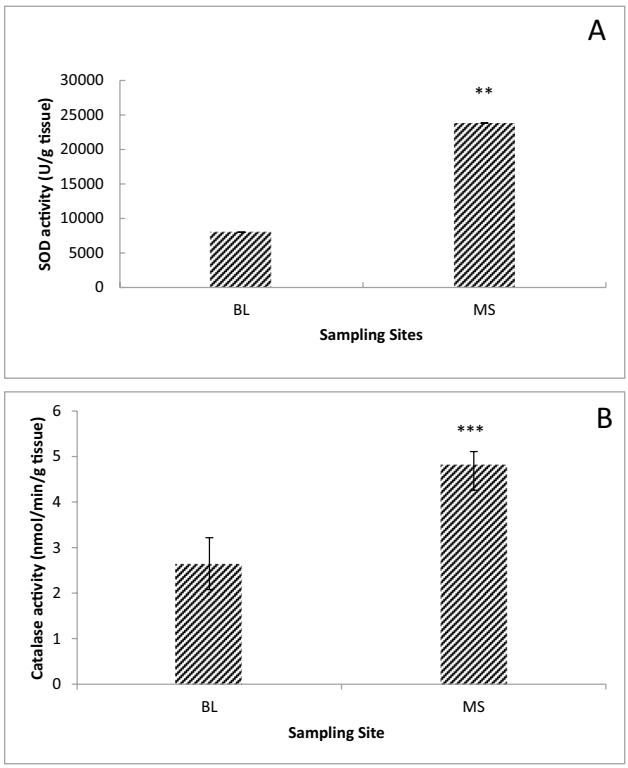

Fig. 2 Activities of antioxidant enzymes in liver of *Spaus aurata* obtained from Bizerte Lagoon (BL) and from the Mediterranean Sea (MS). Values are stated in terms of means ± SD ($n = 30$) (**A** CAT; **B** SOD. $^{**}p < 0.01$; $^{***}p < 0.001$)

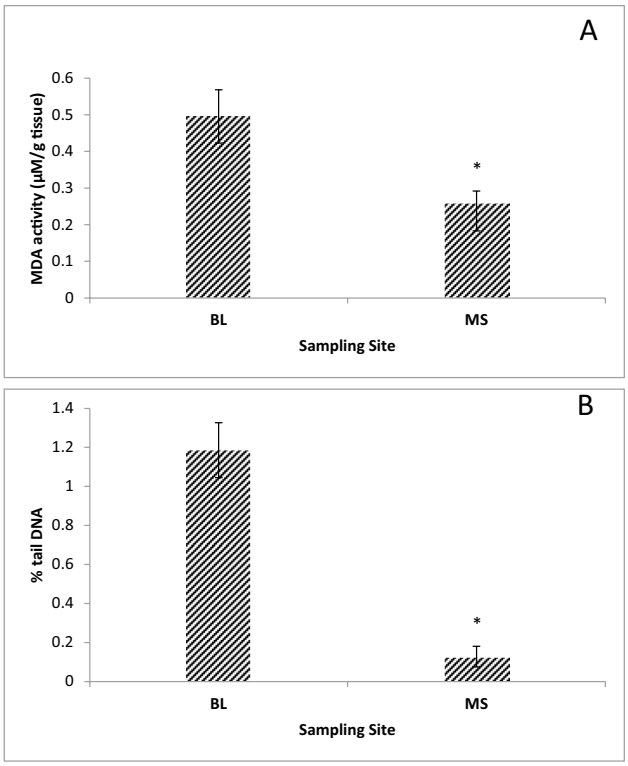

Fig. 3 The peroxidation of lipids and the damage of DNA in liver of *Sparus aurata* obtained from Bizerte Lagoon (BL) and from the Mediterranean Sea (MS). Values are stated in terms of means ± SD ($n = 30$) (**A** MDA activity; **B** DNA damage expressed as tail length (%), $^{*}p < 0.05$)

The MDA levels ranged from 0.42 to 0.70 (mean = 0.50) µM/g tissue and from 0.24 to 0.30 (mean = 0.26) U/g tissue, respectively, in the Bizerte Lagoon and the Mediterranean Sea samples.

The tail DNA values in the liver samples ranged from 0.99 to 1.37% (mean = 1.19%) and from 0.09 to 0.17% (mean = 0.13%) respectively for the Bizerte Lagoon and the Mediterranean Sea fish samples.

The impacts of pollutants on the system of enzymatic defense are shown in Fig. 2. In fish samples from Bizerte Lagoon, the activities of the catalase (Fig. 2A) and the SOD (Fig. 2B) were observed to be significantly lower than those registered into Mediterranean Sea samples (CAT: $p < 0.01$; SOD: $p < 0.001$) with 66.3% and 45.2% decreases respectively for CAT and SOD activities between the two sites.

Figure 3 demonstrates the damaging effects of pollutants. In fact, the MDA activity showed a significant increase (48.35%) in fish species from Bizerte Lagoon (Fig. 3A). Moreover, an important increase in DNA damage (% tail DNA = 89.21%) was observed in samples from Bizerte Lagoon (Fig. 3B). The comparison between the two studied

sites showed a statistically significant difference in DNA damage and lipid ($p < 0.05$).

The liver histological alterations detected in the *Sparus aurata* are shown in Fig. 4. In fact, 70% of the analyzed specimens from the Bizerte Lagoon showed evidences of liver histological alterations. Normal hepatocytes were observed in the liver samples from the reference site (Fig. 4A) whereas, in the Bizerte Lagoon fish liver, congestion of blood vessel, pyknotic nuclei, hemorrhage, and melanomacrophage centers were significant (Fig. 4B).

Correlation analyses

Table 3 presents the correlation analysis between the studied chemicals and the biomarkers. The results showed important positive correlations between the activities of the CAT and SOD, MDA values, ∑PBDEs, and ∑MeO-PBDEs as well as individual congeners. Concerning MDA level and DNA tail percentage, significant negative correlations were revealed between the values of these two biomarkers and ∑PBDEs and ∑MeO-PBDEs as well as individual congeners. However, the results did not show any correlation between the studied biological markers and the environmental parameters.

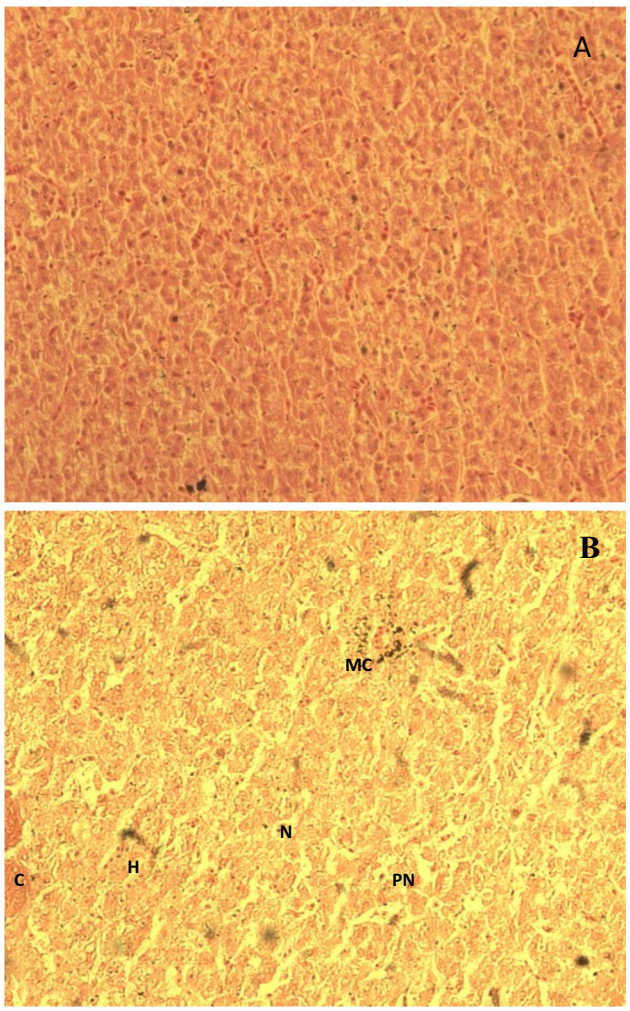

Fig. 4 Histology of *Sparus aurata* liver, collected from the Mediterranean Sea (**A**) ($n = 30$), exhibiting normal hepatic tissue without pathological abnormalities and the Bizerte Lagoon (**B**) ($n = 30$), revealing hepatic tissue with pathological modifications: necrotic liver tissue (N), melanomacrophage center (MC), congestion of blood vessels (C), pyknotic nuclei (PN) and hemorrhage (H). HE × 20

Discussion

In the present study, different biological and chemical markers were used to study the levels of exposure and the effects of organobromine contaminants in *Sparus aurata* living in the Bizerte Lagoon. The results presented a preliminary overview of the ecosystem quality and the population health status. To our knowledge, this is the first study that combined simultaneous evaluation of the biological and chemical markers to assess pollution in a wild Mediterranean fish (*Sparus aurata*) from the Bizerte Lagoon.

The physical and chemical parameters could affect the responses of biological markers and the distribution of contaminants in the living organisms (Barhoumi et al. 2014). The temperature variation could affect the binding capacity and the catalytic efficiency of the enzymes (Barhoumi et al. 2014) and also impacts the oxygen balance in tissues. Moreover, the hypo-osmotic stress could impact the biochemical responses (Barhoumi et al. 2014). In our study, the absence of significant correlations between physicochemical parameters and biomarkers is similar to the results reported by Barhoumi et al. (2014).

Chemical concentrations in the studied fish species showed variability between the investigated sites. This is most likely due to localized environmental and anthropogenic influences. The high levels of PBDEs detected in Bizerte Lagoon could be related to the growth of the population and the development of the industry. The major sources are local wastewater discharges and effluents from plastic and textile product factories (Ben Ameur et al. 2013; Ameur et al. 2020).

The patterns of the PBDE congeners were very similar in the two study areas. Among the eight detected congeners, the BDE-47 was the predominant congener in all analyzed samples. This finding matched the general pattern observed in other investigations and showing the dominance of the congener BDE-47 in fish samples (Labandeira et al. 2007; Ben Ameur et al. 2011, 2013; Barhoumi et al. 2014; Mekni et al. 2020).

The BDE-99 and BDE-100 congeners showed a low detection percentage in fish samples collected from the studied sites. The low percentage detection of the BDE-99 and BDE-100 congeners was in line with previous studies

Table 3 Pearson's correlation coefficients among the different biological (CF, HSI), biochemical (CAT, SOD, MDA), molecular (DNA tail), chemical (∑PBDEs, ∑POPs), and environmental parameters ($n = 30$)

	CF	HSI	CAT	SOD	MDA	DNA tail
CF	1					
HSI	0.03	1				
CAT	0.35	0.30	1			
SOD	0.42	0.42	0.85	1		
MDA	0.28	0.36	0.90**	0.72**	1	
DNA tail	0.18	0.38	0.75*	0.88*	0.65*	1
T	−0.15	−0.50	−0.24	−0.34	0.18	0.15
pH	0.67	0.44	−0.31	−0.28	0.21	0.11
DO	−0.28	0.41	−0.27	−0.32	0.10	0.06
Sal	0.15	0.34	−0.15	−0.20	0.25	0.15
Turb	0.07	0.29	−0.15	−0.18	0.05	0.05
Cond	0.10	0.20	−0.15	−0.21	0.15	0.09
SPM	0.04	0.31	0.23	0.27	0.09	0.13
NO_3^-	0.06	0.43	0.35	0.38	0.13	0.09
NO_2^-	0.09	0.50	0.44	0.30	0.10	0.12
PO_4^{3-}	0.10	0.34	0.40	0.15	0.13	0.15
∑PBDEs	0.07	0.60*	−0.65*	−0.75*	0.95*	0.98*
∑MeO-PBDEs	0.05	0.75*	−0.70*	−0.80*	0.94*	0.85*
BDE-47	0.11	0.78*	−0.62*	−0.70*	0.84*	0.92*
BDE-99	0.07	0.65*	−0.57*	−0.65*	0.64*	0.45*
BDE-100	0.08	0.60*	−0.31	−0.36*	0.48*	0.51*
BDE-153	0.06	0.70*	−0.41*	−0.56*	0.54*	0.72*
BDE-209	0.15	0.75*	−0.53*	−0.72*	0.85*	0.95*
6-MeO-BDE-47	0.05	0.68*	−0.42*	−0.65*	0.95*	0.90*

*Significant correlation ($p < 0.05$)

**Significant correlation ($p < 0.01$)

carried out in the Bizerte Lagoon and showed the absence of these congeners in marine organisms (Barhoumi et al. 2014, Ben Ameur et al. 2011, 2013, 2017; Ameur et al. 2020).

Based on the detected PBDE congeners, in addition to the PBDE congener percentage in each commercial PBDE mixture, the identified congeners in *Sparus aurata* could result from the use of the commercial Penta-BDE, Octa-BDE, and Deca-BDE formulations (El Megdiche et al. 2017). The possible source of PBDEs might be deposition (or leaching) from high temperature and photo degradation of commercial products of deca-BDEs such as in textiles in upholstery, electronic circuit boards, mattresses, and TVs used (Kofi et al. 2018). It could possibly come from the deposition from products containing penta-BDE formulation as additives such as textiles and polyurethane foams; pyrolytic/thermally degraded residual deposits from deca-BDE formulations such as in textiles, electronic circuit boards, mattresses, and TVs used (Kofi et al. 2018); and finally from leaching/residual deposits from penta/octa-mix BDE formulations such as in polyurethane foams and acrylonitrile–butadiene–styrene (ABS raisins) used in plastics for some household electric devices such as computers and housing appliances.

The mean concentrations of the PBDE congeners registered in fish samples from the Bizerte Lagoon were similar to those obtained in *Anchoa mitchilli*, *Paralichthys lethostigma*, *Paralichthys lethostigma Centopristis philadelphica*, and *Cynoscion nothus* from Georgia waters in America (77.5 ng g^{-1} lw; Sajwan et al. 2008); in *Pseudorhombus jenynsii*, *Pomatomus saltator*, *Acanthopagrus australis*, *Monocanthus chinensis*, *Mugil cephalus*, and *Girella tricuspidata* from the Harbour of Sydney in Australia (24–115 ng g^{-1} lw; Losada et al. 2009); in *Anguilla anguilla* from freshwater bodies in Flanders in Belgium (94 ng g^{-1} lw; Malarvannan et al. 2015); in *Squalius keadicus* from Evrotas river basin in Greece (9.32–116 ng g^{-1} lw; Giulivo et al. 2017); and in *Salmo trutta fario*, *Salmo marmoratus*, *Cottus gobio*, *Thymallus thymallus*, and *Squalius cephalus* from Italy Adige river basin (18.6–187 ng g^{-1} lw; Giulivo et al. 2017). However, the present study showed relatively low PBDE congener mean concentrations in fish samples when compared to those recorded in *Omoxis annularis* and *Lepomis macrochirus* from Hardley Lake in America (1600 ng g^{-1} lw; Dodder et al. 2002); in *Lateolabrax japonicus* and *Mugilogobius abei* from Tokyo Bay in Japan (130 ng g^{-1} lw; Mizukawa

et al. 2009); in *Oncorhynchus mykiss*, *Prosopium williamsoni*, *Ictalurus punctatus*, *Cyprinus carpio*, *Catostomus macrocheilus*, *Platichthys stellatus*, *Micropterus dolomieui*, and *Catostomus commersoni* from Yakima River in the USA (960 ng g^{-1} lw; Johnson and Olson 2001); in *Anguilla anguilla* from Gironde Estuary in France (24–237 ng g^{-1} lw; Tapie et al. 2011); in *Cyprinus carpio* from Anoia and Cardener River in Spain (29–744 ng g^{-1} lw; Labandeira et al. 2007); in channel catfish, flathead catfish, carp, white bass, and striped bass from Virginia watersheds in the USA (7200 ng g^{-1} lw; Hale et al. 2001); in *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella*, *Aristichthys nobilis*, *Carassius auratus*, and *Cyprinus carpio* from Yangtze River in China (140 ng g^{-1} lw; Xian et al. 2008); in *Cyprinus carpio*, *Abramis brama*, *Sander lucioperca*, and *Silurus glanis* from Italy Po river (94.9–821 ng g^{-1} lw; Luigi et al. 2015); in *Oncorhynchus mykiss*, *Squalius cephalus*, and *Barbus barbus* from Slovenia Sava river basin (11.9–461 ng g^{-1} lw; Giulivo et al. 2017); and in *Cirrhinus molitorella*, *Tilapia nilotica*, and *Hypostomus plecostomus* from Pearl River Delta (6.9–690 ng g^{-1} lw; Sun et al. 2016). The levels found in this study were higher than the ones measured in *Pseudosciaena crocea* and *Pampus argenteus* from Chinese coastal waters (3.04 ng g^{-1} lw; Xia et al. 2011), China Pearl river (3.88–59.8 ng g^{-1} lw; Sun et al. 2015), and in *Boreogadus saida* from eastern Svalbard (3.55 ng g^{-1} lw; Wolkers et al. 2004).

High proportion of the 6-MeO-BDE-47 could reflect the presence of algae which is the major source of the MeO-PBDEs, but also high levels of the 2′-MeO-BDE-68 could be associated with the presence of sponges which is the primordial origin of the MeO-PBDEs (Vetter 2006). According to the 6-MeO-BDE-47 and 2′-MeO-BDE-68 ratio values, the fish samples from the studied areas might receive MeO-PBDEs principally from algae (Vetter 2006).

Due to the absence of human activity–related sources of the MeO-PBDEs, the concentration differences of these chemicals could be associated with the differences in algae distribution, marine sponge, and other aquatic species capable of MeO-PBDEs' synthesis.

The absence of statistically significant correlation between BDE-47 and 6-MeO-BDE-47 suggests that 6-MeO-BDE-47 could be a BDE-47's metabolism product in fish, and/or may have other marine sources. This result is in line with the one explained in a study conducted in northeastern Australia where the authors did not register high levels of the PBDEs in aquatic animals (mammals) regardless of the presence of the 6-MeO-BDE-47 with high amounts. Because of the high correlation registered between the 6-MeO-BDE-47 mean concentrations and the 2′-MeO-BDE-68 mean concentrations in samples of fish from Bizerte Lagoon ($rs = 0.84$, $p < 0.05$), these two compounds could accumulate from the same sources.

The mean concentrations of the MeO-PBDEs registered in Bizerte Lagoon fish (123.20 ng g^{-1} lw) are comparable to the levels registered in *Thunnus thynnus* from the Mediterranean Sea (150 ng g^{-1} lw) (Pena-Abaurrea et al. 2009), in *Clupea harengus membras* from the Baltic Sea (97.0 ng g^{-1} lw) (Dahlberg et al. 2016), and Southeast Asia fishmeal (97.0 ng g^{-1} lw) (Li et al. 2018). However, these concentrations were higher than the registered levels in *Coilia sp.* from the Yangtzer River Delta (9.10 ng g^{-1} lw) (Su et al. 2010), in the Atlantic salmon (2.98 ng g^{-1} lw) (Sinkkonen et al. 2004), in Baltic Sea salmon (5.18 ng g^{-1} lw) (Sinkkonen et al. 2004), in *Chromis crusma* from the Coast of Concepcion (Chile) (15.7 ng g^{-1} lw) (Barón et al. 2013), in US fishmeal (8.95 ng g^{-1} lw) (Li et al. 2018), in China fishmeal (23 ng g^{-1} lw) (Li et al. 2018), in European fishmeal (5.97 ng g^{-1} lw) (Li et al. 2018), and in *Pseudorhombus jenynsii* from Sydney Harbor (25.8 ng g^{-1} lw) (Losada et al. 2009). The mean concentrations recorded in Bizerte Lagoon fish were lower than those in *Solea solea* from the Mediterranean Sea (325 ng g^{-1} lw) (Ben Ameur et al. 2013).

The comparisons of the PBDE levels recorded in *Sparus aurata* collected from the Bizerte Lagoon during this study were higher than those obtained in fish species sampled from this same site, namely *Mugil cephalus*; lower than those in *Dicentrarchus labrax*, which were sampled in November 2009; and lower than those in *Solea solea* sampled in December 2010 (Ben Ameur et al. 2011, 2013). Concerning the MeO-PBDEs, their levels in the studied fish species collected from the Bizerte Lagoon are similar to those found in *Mugil cephalus* and lower than those measured in *Dicentrarchus labrax* and *Solea solea* (Ben Ameur et al. 2011, 2013).

The toxic effects of the environmental contaminants have been assessed using different types of biomarkers (Linde-Arias et al. 2008). The toxic effects could be assessed by different parameters that involve whole-tissue organisms mainly at higher levels of organization (Olivares et al. 2010). It has been found that integrating different biomarkers to assess the biological impacts of pollutants in the environment is very useful than using a single biological marker mainly when assessing the health condition of the sentinel species (Linde-Arias et al. 2008; Frenzilli et al. 2008). Overt sickness or before death, biological changes are the responses of the organisms to stress. For an early warning and detection of possible later serious consequences, it is mandatory to understand these changes of biological responses (Linde-Arias et al. 2008). In field searches, it is recommended to use the CF and HSI as morphological parameters to evaluate the health condition of fish species and the toxicity of the contaminants (Barhoumi et al. 2014). The results of this study showed that no statistically significant difference was registered for the CF in fish specimens obtained from the two studied areas, which could be explained by the low number of the studied samples that were intentionally selected to

prevent changes in biochemical responses due to size effects. The measurements of the metabolic activities and energetic reserves of the liver implicated the determination of the HSI. It has been demonstrated that the HSI would increase with increased amounts of chemical pollution (Slooff et al. 1983).

Bagnasco et al. (1991) showed that the CF of individuals is associated with reproductive status and food quality. In our study, the CF was similar in fish samples from the two studied sites. This result is comparable to that found in our previous studies (Ben Ameur et al. 2012; Ameur et al. 2015) carried out on two fish species having a similar trophic level to *Sparus aurata* and which are successively *Mugil cephaus* (CF = 0.94 in BL and 1.19 in MS) and *Dicentrarchus labrax* (CF = 0.98 in BL and 1.06 in MS) and also to the study conducted by Tsangaris et al. (2011). This result indicates that the seabream samples collected from the Bizerte Lagoon may adapt to insufficient food sources, even if polluted water would deprive fish of sufficient nutrition.

Regarding the obtained HSI values in analyzed samples from the two investigated areas, several studies showed variable trends in fish from contaminated sites: decreased index values (Napierska et al. 2009), increased index values (Corsi et al. 2003; de la Torre et al. 2007), and no changes in the index values (Barhoumi et al. 2014). The results of the present study demonstrated increased HSI values in seabream from the Bizerte Lagoon. This result could be explained by an augmentation in the number and size of the cell (hyperplasia or hypertrophy) (van der Oost et al. 2003). Moreover, the variations in CF and HSI might be associated with the impacts of pollutants on fish wellbeing. At the same time, these parameters could be influenced by other factors classified as non-pollutants which could not give detailed data on pollutant responses. But they could be used as preliminary biomarkers of screening to specify toxic effects of contaminants in fish. The obtained result is similar to that obtained in the study conducted by Chen et al. (2018) in which zebrafish were exposed to a penta-BDE mixture (DE-71).

It has been shown that molecular and biochemical markers are effective techniques for detecting potential and particular environmental effects (Olivares et al. 2010). Antioxidant enzymes are usually used as oxidative stress biomarkers, but their responses to contamination may vary according to several factors: enzymes, species, single or mixed contaminants, field situations… (Livingstone 2001). Previous studies on fish species reported non-similar data (lower, higher, or no change in the enzymatic activities of the antioxidant) between contaminated areas and non-contaminated areas (van der Oost et al. 2003).

Similar findings concerning the obtained results for the oxidative stress biomarkers in this study were recorded in the *Mugil cephalus* and *Dicentrarchus labrax liver* (Ben Ameur et al. 2012) sampled from Tunisia, in the liver of the *Mugil cephalus* collected from the Ennore Estuary in India (Padmini and Usha Rani 2009; Padmini et al. 2009), in the *Dicentrarchus labrax liver* collected from the Aveiro Lagoon in Portugal (Maria et al. 2009), and liver of *B. Bocagei* (Francisco et al. 2013a, b) collected from the Vizela River in Portugal. The significant correlation between these two enzymatic biomarkers and the two studied pollutant levels lead to the hypothesis that the organobromine compounds accumulated in these tissues could be potentially redox-active causing a lack of equilibrium between the generation of ROS (reactive oxygen species)/free radicals and fish's antioxidant defenses and is thought to be affected by oxidative stress. Oxidative stress may cause cell and tissue damages and consequently could lead to the activation of the antioxidant defense mechanisms (Tabrez and Ahmad 2011). The type and the concentration of the toxic compound could determine the increase or the inhibition of the enzyme antioxidant activities.

In comparison to fish from the Mediterranean Sea (reference site), the current study revealed reduced SOD and CAT activity in the *Sparus aurata* liver from Bizerte Lagoon, showing antioxidant enzyme depletion.

The samples of the *Mugil cephalus* collected from a contaminated estuary in India (Padmini and Usha Rani 2009; Padmini et al. 2009), the *Dicentrarchus labrax* from the Aveiro Lagoon in Portugal (Maria et al. 2009), and the *Hanna punctatus* from Sumera reservoir in India (Padmini et al. 2009) have also been reported to have low CAT and SOD activities (Javed et al. 2016a). However, previous works in gray mullet and sardine revealed that fish from contaminated environments have increased CAT and SOD activities (Rodriguez-Ariza et al., 1993; Peters et al. 1994). High production of ROS could induce the antioxidant enzymes as an antioxidative stress defense mechanism. These enzymes could be inhibited by a systems' deficiency which suggests toxicity (Tsangaris et al. 2011). The exposure to harmful compounds induced an enzymatic response that could be represented by a bell-shaped curve starting with an increased enzymatic activity followed by a decreased activity caused by an increase in catabolic rate and/or direct harmful chemical inhibition (Ben Ameur et al. 2012; Viarengo et al. 2007). Consequently, the decreased enzyme activities in Bizerte Lagoon fish can be attributed to the lack of ability to adapt to oxidative stress, most likely as a result of high amounts of pollution exposure. The low enzymatic activities could be linked also to exposure to pollution for a lengthy period, and numerous studies have demonstrated a similar response (Bainy et al. 1996; Lenartova et al. 1997; Padmini et al. 2009).

ROS are toxic constituents known to cause several types of cell damages mainly the peroxidation of lipids, the alteration of lysosomes, and the damage of the DNA (Pampanin et al., 2005). The peroxidation of lipids is one of the very studied mechanisms of cellular injury in animal species. This process is also considered a bioindicator of oxidative destruction in tissues and cells. For that, the MDA

assessment is commonly applied to detect the peroxidation of lipids (Pampanin et al., 2005) since the MDA could interact with the protein amino groups producing adducts from different types (Pampanin et al., 2005). The liver of fish collected from the contaminated area had an important rise in MDA content. Similar results of augmentation in MDA level have been shown by Ameur et al. (2012) and Javed et al. (2016a,b). As in the case of enzymatic biomarkers, the detection of a significant positive correlation between MDA level and PBDEs and their methoxylated analogs could indicate that these organobromine compounds are responsible for the increase of this lipid peroxidation biomarker.

The comparison with the reference specimens (Mediterranean Sea), fish captured from the Bizerte Lagoon, had significantly more DNA damage. Our findings are consistent with prior studies that have shown an increase in damages detected in DNA molecules from fish species collected in the polluted sites (Flammarion et al. 2002; Winter et al. 2004; Nogueira et al. 2010; Ben Ameur et al. 2012; Ameur et al. 2015; Javed et al. 2016a, b; Dalzochio et al. 2018). The impact of xenobiotics with high reactivity tracking the metabolism of the liver could be one reason for the high amounts of DNA damage reported. It is worth noting that the DNA damage detected by the comet assay was accompanied by a depletion of CAT and SOD activities in liver fish from the contaminated area. These two enzymes' activity is required to eliminate free radicals (superoxide radical anion and H_2O_2), which are precursors to the hydroxyl radical, a reactive oxygen species that causes DNA damage (Halliwell and Gutteridge 1999). The detection of a significant positive correlation between DNA tail % and PBDEs and their methoxylated analogs could indicate that these organobromine compounds are responsible for the increase of this genotoxic biomarker.

The irritant effects in different organs could be rapidly detected through histopathology (Johnson et al. 1993). In living organisms, the main organ for the excretion of harmful substances, metabolism, and detoxification of xenobiotics is the liver. This organ can break down hazardous substances; however, high levels of these toxic compounds could affect its regulating mechanism and cause different damages to the structure (Brusle and Gonzalez 1996). The major changes registered in Bizerte Lagoon fish samples were hemorrhage, pyknotic nuclei, and necrosis areas. Similar damages were observed in fish from contaminated sites by PBDEs (Raldúa et al. 2008).

The presence of melanomacrophage centers in specimens captured from the polluted site is in accordance with those found in the previous study (Ameur et al. 2015). These central melanomacrophage accumulations are responsible for the storage of foreign or hazardous substances and their intensity and frequency are thought to be a useful biological indicator of the pollution and degradation of the environment (Couillard and Holdson 1996; Manera et al. 2000).

However, even if the present study has shown a positive correlation between biological indicators and the levels of organobromines, the registered effects in seabream from the Bizerte Lagoon could be also associated with pollutants from other sources. Several studies conducted in the Bizerte Lagoon revealed the presence of many pollutants known as responsible for oxidative stress induction, histopathological alterations, and genotoxicity such as PAHs, TBT, metals, DTTs, and PCBs. Many anthropogenic activities are installed on coastal areas of the Bizerte Lagoon such as industrial activities and urbanization. Consequently, the runoff and the discharges of industrial and urban wastes both direct and indirect result in the lagoon contamination by several toxic pollutants, namely organo-chlorinated pesticides (Ben Ameur et al. 2013; Mhadhbi et al. 2019), heavy metals (Yoshida et al. 2004), halogenated aromatics compounds (e.g., PCBs) (Ben Ameur et al. 2013), polycyclic aromatic hydrocarbons (PAHs) (Trabelsi and Driss 2005), organotins (Abidli et al. 2011), and organophosphate flame retardants (Mekni et al. 2020).

Conclusion

The present work intended to evaluate the contamination levels in the Bizerte Lagoon using a battery of biomarkers in *Sparus aurata*. We reported the concentration of the target organobromine compounds in fish muscle and their repercussions on morphological, biochemical, and molecular biomarkers, as well as histopathological changes.

The findings revealed that fish from a contaminated environment showed clear evidence of stress. The response of biomarkers could be associated with the bioaccumulation of contaminants, according to linear correlation analysis.

The current work demonstrated that an integrative methodology that relies on biomarkers from fish species is an effective and sensitive method for assessing the aquatic environmental health state.

The current study might be highly valuable as a guideline for future PBDE and MeO-PBDE pollution surveillance programs along the coasts of Tunisia.

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Author contribution * Walid Ben Ameur conceived of the presented study.

* Yassine El Megdiche and Takoua Mhadhbi contributed to sample collection and preparation.

* Walid Ben Ameur carried out the chemical compounds extraction from samples with help from Soukaina Ennaceur.

* Walid Ben Ameur carried out the chromatographic analysis of the studied compounds with help from Sihem Ben Hassine.

* Walid Ben Ameur carried out the biomarker analysis with help from Ali Annabi and Joaquin de Lapuente.

* Walid Ben Ameur wrote the manuscript with support from Mohamed Ridha Driss, Miquel Borràs, and Ethel Eljarrat.

* All authors provided critical feedback and helped shape the research, analysis, and manuscript.

* All authors discussed the results and contributed to the final manuscript.

Data availability All data generated or analyzed during this study are included in this published article.

Declarations

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