BIOACCUMULATION AND BIOMARKER RESPONSES CAUSED BY ORGANOCHLORINE PESTICIDES IN BOGUE *BOOPS BOOPS* (LINNEAUS, 1758) FROM THE BAY OF ORAN, ALGERIA

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Abstract. In the present study we investigated the concentration of organochlorine pesticides (OCPs) in the gills and muscles as well as the levels of Thiobarbituric acid-reactive substances (TBARS), Catalase, GlutathioneS-Transferase (GST) and Acetylcholinesterase (AChE) activity in the liver and muscles of the bogue *Boops boops* (Linneaus,1758) fish that were caught in the bay of Oran (Algeria). For the purpose of evaluating the toxicological risk in the abovementioned area. The concentrations of total Dichlorodiphényltrichloroéthane (∑DDT) was present at highly significant levels ($P>0.001$) compared to other OCPs, thus displayed in the following descending order: ∑DDTs > Lindane > Heptachlor. These OCPs had largely bioaccumulated in the gills compared to muscles. The PCA projection reported significant positive correlations between the concentrations of Lindane, DichloroDiphenyldichloroEthylene (DDE), DDD, DDT, and Heptachlor in gills and muscles. Seasonal variations of (OCPs) showed the highest average concentrations in the muscle and gills of fish collected mainly in Winter and Autumn ($p < 0.001$). The lowest concentrations were noted during the Spring and Summer periods. Significant negative correlations existed between the CAT, TBARS, GST and Lindane, ∑DDTs, and Heptachlor. The AChE (in liver and muscles) showed significant negative correlations with total DDT, with Correlation coefficient values $R = -0.67$, $R = -0.64$, respectively (significant $R > 0.5$). This study underlines that the bay of Oran suffers from chemical defilement.

Keywords: *acetyl cholinesterase, SPE/GC/MS, Boops boops, DDT, organochlorine pesticides, Oran Bay*

Introduction

In the first place the pesticides increase agricultural production by a large margin, yet they also run the risk of deleterious impact on aquatic biodiversity (El Nahas et al., 2017; Clasen et al., 2018). Thus, it is feared that watershed runoff from agricultural areas may be a source of contamination of water bodies, resulting in the destruction of aquatic resources, including fish (Kaczyński et al., 2017).

Organochlorine pesticides are substances classified as Persistent Organic Pollutants and banned for use for 30 years, known by the scientific community for their harmful effects on human health and the ecosystem.

As a result of their hydrophobicity, organochlorine pesticides are most typically found in association with soil particles when they infiltrate the marine environment by means of run-off, rainfall, and air (Tierney et al., 2013).

Moreover, the Fish are exposed to pollutants in the environment via gills, skin and through their diet. The bioaccumulation detected in fish organs is considered a health index for aquatic bodies (Clasen et al., 2018). In this respect they can serve as useful indicators of the risks facing the ecosystem (Lemarchand et al., 2012; Kaczyński et al., 2017; Henríquez-Hernández, 2017; Buah-Kwofie et al., 2018; Taiwo, 2019).

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As well as the pesticides can accumulate in concentrations that are a thousand times higher in marine environments than on soil. They then enter the food chain and amass in the fatty tissues of fish, crustaceans, and other animals. Untreated domestic and industrial waste water and effluents marked a significant source of chemical pollution for maritime life on the western coast of Algeria (Gareche, 2014; Rouane-Hacene et al., 2018). This shift in water composition is certainly of detrimental impacts, some of which are at the level of cells, metabolism, and DNA, not limited to the critters living in these locations, but also extending to human health (Bocchetti and Regoli., 2006; Benali et al., 2015; Clasen et al., 2018).

Contemporary Algeria is in a phase of "environmental transition" synchronous to that of its "economic transition". Indeed, the country is facing health problems and non-communicable diseases, whose risk factors and determinants are now identified (Mansouri and Regabi, 2019).

The Algerian west coast is unimmune to pollution, and as one of the Mediterranean's most environmentally vulnerable locations, it is increasingly targeted by a variety of nuisances.

Oran's coast suffers from high levels of urban and industrial pollution, as it is continuously exposed to discharges of untreated wastewater, therefor running the risk of contamination caused by heavy metals, polyaromatic hydrocarbons, and pesticide compounds (Taleb et al., 2009; Gareche, 2014; Rouane-Hacene et al., 2018; Belhoucine, 2012; Remili et Kerfouf, 2013; Belhoucine et al., 2015; Benali et al., 2015; Rouane-Hacene et al., 2018; Laredj, 2018; Kaddour et al., 2021; Tabeche et al., 2021).

Due to the worrying nature of the compounds, persistent organochlorine insecticides have been the subject of much research. Various works describe the mechanisms and factors governing the bioaccumulation of those pollutants (Lemarchand et al., 2012; Wang et al., 2013; Karaca et al., 2014; El Nahas et al., 2017; Henríquez-Hernández et al., 2017; Buah-Kwofie et al., 2018; Nejatkhah Manavi, et al., 2018).

Secondly, the use of biomarkers has become a crucial tool in modern environmental assessment, as they can help predict pollutants involved in the biomarker enzyme monitoring process, which can be used to identify possible environmental contaminations before the occurrence of irreversible ramifications on the health of aquatic organisms (Benali et al., 2015; Amri et al., 2017; Clasen et al., 2018; Vieira et al., 2019).

The present work is part of a problematic of evaluation of the state of health of the waters of Algerian Western coast using a biological model based on the research and identification of pesticides in a teleost fish: the Boops boops (Linnaeus, 1758) which is widespread in the Oran Bay. Along with that, to satisfy the requirement for information on the concentrations of these chemicals in this teleost fish, which served as a quantitative indicator of contamination. This choice was motivated by their great persistence in the environment, their ability to accumulate in the fatty tissues of living organisms and to spread along the food chain. The potential toxicity to ecosystems and human health of this family of persistent organic pollutants (POPs) is a global concern (RNO, 1993; ESMASPA/CEE/WHO, 2002). We also evaluated the impact of these pollutants on enzymatic biomarkers in the liver and muscle of this species, in particular the level of thiobarbituric acid reactive substances (TBARS), biomarkers of oxidative stress, catalase, Glutathione-S-Transferase (GST) activity and acetylcholinesterase (AChE). In this respect to ensure that bogue eating does not put the public's health at risk.

Materials and methods

Study location

The Algerian coastline, an integral part of the Mediterranean, endures numerous pollutants, such as the continuous discharges of water and urban waste, which are completely pumped into the sea without any prior treatment, due to the lack of processing plants (Kaddour et al., 2021; Tabeche et al., 2021). In addition, this coastline is bathed by waters of Atlantic origin of which circulation appears to be very turbulent around this coast. These turbulences would cause the spread of nonpoint source pollution, which in turn infiltrate through the various levels of the food chain Millot (1989). concluding that the environmental degradation of the west coast of Algeria has reached alarming levels Ghodbani and Berrahi-Midoun (2013).

The bay of Oran represents an extremely large basin that is open to the Mediterranean and is located in the Northwest of Algeria (*Fig. 1*). This latter stretches to about 180 km, with a width measuring from 20 to 25 km (Belhoucine, 2012; Belhoucine et al., 2022). The western coastal bank of Oran undergoes a galloping population growth, coupled with chaotic urbanization, and intense port activity related to several industrial and agricultural pursuits, which, as a result, have an impact on the environment around the coast, in the form of biological and physicochemical contamination of marine waters (Boutiba et al., 2003; Kerfouf et al., 2010; Belhoucine et al., 2015; Benali et al., 2015; Bouhadiba et al., 2017).

Figure 1. *Location of the study area: Oran Bay*

Sample collection and processing

In order to carry out the inventory of the contamination of the bogue by organochlorine compounds and to have an estimate of the levels of this contamination in a representative and relevant way, an adapted sampling is necessary. To this end, our sampling was carried out in accordance with the technical recommendations recommended by the QUASIMEME (1992) program (Quality Assurance of Information for Marine Environmental Monitoring in Europe

The sampling campaign took place over a period of 12 months between October 2018 and September 2019, sampling was carried out once each month in groups of around 15 fish. In total we collected 180 specimens of bogue (*Boops boops*) (Linnaeus, 1748) for all sexes and ages from the fishing ports. The samples were processed the same day at the laboratory. After the biometric measurements, the fish were dissected in order to remove the liver, gills and muscles, then were transported to the laboratory in coolers for later storage at +4 °C until analysis.

Extraction and analysis of POCs

Analyzing the wide range of pesticides in the matrix of fish is a complex and challenging task. Despite advances made in Chromatographic Separation and dynamic development of instrumental methods (Mass Spectrometry Techniques), sample preparation is still one of the keystones in the analytical protocol, considering how essential effective sample treatment is for achieving authentic findings. In our study, we used SPE/GC/MS (Solid Phase Extraction coupled with gas chromatography/Mass Spectrometry) to screen for pesticides in muscles, and gills according to the “MET-LFSAL-404” extraction screening protocol, as advised by the Federal Safety Agency of the Food Chain of the Cork Laboratory (2013). whose protocol is as follows: Gills and muscles were ground for homogenization. The extraction was done by sonication and agitation. The purification of the extract was done in three steps: centrifugation with SIGMA 2-16 KL centrifuge, freezing of the supernatant and finally passage of the sample was done on SPE Columns Interchim C18-S-500/3 cartridges.

Instrumental analyses

The target chemicals were quantified using gas chromatography (GC) - brand Perkin Elmer Clarus 500 conjoined with mass Clarus’ 500 type detector (MS). An HP-5 MS capillary column (length: 30 m, and 250 µm inside diameter). The oven temperature was set from 60° to 140 °C with a level of 15 °C/min; ramp 2: from 150 to 300 °C with a level of 5 °C/min, finally hold for 10 min. Mass scanning was programmed from 40 to 500 (m/z). Helium gas was used with a flow rate of 20 ml/min. The optimal chromatographic conditions are: Splitless injection of a 2 µL sample was performed with Interface temperature: 280°, Source temperature of the electrical current: 200 °C, electrical intensity: 447 V, Fractionation:70 ev, Acquisition mode: SIM (m/z).

Protocol of validation and qualification

Guaranteeing the carrying out of an undisputed quality of measurement, parallel to the analysis of the real samples, blanks demonstrate the absence of interference with the compounds sought. The accuracy and reliability of the analysis were estimated by analyzing the standard solution of organochlorine pesticide AB No.1 prepared in accordance to meticulous EU regulations No. 2015/830, batch No. A 0135696, stock number 32291 certified from the RESTECK Company.

Biochemical parameters

Protein was quantified through the Coomassie Blue method, according to (Bradford, 1976), Lipid peroxidation (LPO) was estimated through TBARS (thiobarbituric acid reactive substances) assayed in the liver and muscles, according to Buege et Aust (1976). Catalase (CAT) activity was inspected through ultraviolet spectrophotometry (Nelson et Kiesow, 1972) Glutathione Stransferase (GST) was probed following the method of Habig et al. (1974). AchE activity was measured in the fish’s brain and muscles, according to Ellman et al. (1961). The enzymatic assays were carried out by spectrophotometer UV-visible of mark AGILLENT. Instruments that measure the absorbance of ultraviolet and/or visible light of a sample; they are based on the Beer-Lambert law, which states that the concentration of a solute is proportional to the absorbance of the sample.

Statistical analysis

Experimental data were initially tested for normality and homogeneity of variance, to meet statistical requirements. Data were expressed as ± Standard of Error (SD). Statistical analysis of data was performed using parametric and non-parametric tests: results are analyzed by 2-factor ANOVA test followed by Fisher’s LSD test for multiple comparisons and determination of significance levels. All statistical tests were performed using Statistica Version 10 software. Principal Component Analysis (PCA) was used to discriminate the month, as a function of POCs and Biomarker responses. Tests were performed at a 0.05 level of significance.

Results

Pesticide contamination

The concentrations of contaminants were calculated in relation to the fresh weight (F.W) of muscle and gills of the bogue.

The average concentrations of organochlorine pesticides in gills and muscles of bogue fish for all sexes and ages from the bay of Oran are respectively recorded in (*Fig. 2*).

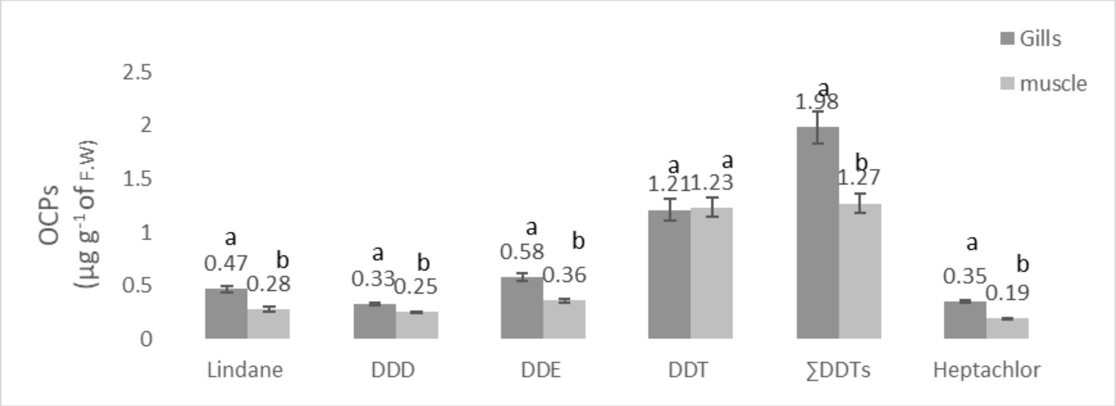

Figure 2. *Average levels of OCPs evaluated in muscle and gills (µg g^{-1} of F.W) of bogue from Oran Bay (n = 5, mean ± SD). The different letters (a, b) designate significant differences between OCPs in organ. (Test post Hoc, Fischer’s LSD test (P < 0.05))*

The investigated OCPs (P, p′-DDT, p, p′-DDD, p, p′-DDE, Lindane and Heptachlor) were detected in muscle and gills. These OCPs in the gills were significantly higher than that in the muscle ($p \leq 0.001$) (*Fig. 2*).

Our results showed that the sum of DDT(∑DDTs) were the dominant substances, with the average concentrations ranging from 1.98 ± 0.15 µg g^{-1} of F.W in the gills as compared to Lindane and heptachlor, which had levels of 0.47 ± 0.03 and 0.35 ± 0.01 µg g^{-1} of F.W, respectively.

At the muscle level of the bogue of Oran Bay, there was once more a significant accumulation of DDTs with a means concentrations ranging from 1.27 ± 0.09 µg g^{-1} of F.W compared to Lindane and Heptachlor with corresponding concentrations of 0.28 ± 0.02 and 0.19 ± 0.01 µg g^{-1} of F.W (*Table 1*).

The classification of pesticides according to their level of detection in the two organs leads to the following scheme: ∑DDTs > lindane > Heptachlor (*Fig. 2*).

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***Table 1.** Average levels of OCPs (µg g^{-1} of F.W) (DDT DDD, DDE Lindane, Heptachlor) evaluated in muscle and gills of bogue from Oran Bay. (n = 5, mean ± SD)*

	Lindane	**DDD**	**DDE**	**DDT**	**∑DDTs**	**Heptachlor**
Gills	0.47 ± 0.03	0.33 ± 0.01	0.58 ± 0.04	1.21 ± 0.10	1.98 ± 0.15	0.35 ± 0.01
Muscle	0.28 ± 0.02	0.25 ± 0.01	0.36 ± 0.02	1.23 ± 0.09	1.27 ± 0.09	0.19 ± 0.01

Figure 2 represents the Average concentration of OCPs evaluated in muscle and gills (µg g^{-1} of F.W) of bogue for both sexes from Oran Bay measured monthly during the period October 2018/September 2019.

Our results showed significant seasonal variations of the OCPs in the two organs of the bogue fished in the bay of Oran. For gills, lindane (ANOVA 2, F value = 4.51, *p* value < 0.0001), DDD (ANOVA 2, F value = 3.65, *p* value < 0.0001), DDE (ANOVA 2, F value = 11.58, *p* value < 0.0001), DDT (ANOVA 2, F value = 20.47, *p* value < 0.00001), Total DDT (ANOVA 2, F value = 19.63, *p* value < 0.00001) and Heptachlor (ANOVA 2, F value = 2.47, *p* value < 0.00001) (*Table 2*). For the Muscle, lindane (ANOVA 2, F value = 2.06, *p* value = 0.04 > 0.05), DDD (ANOVA 2, F value = 1.89, *p* value = 0.06 < 0.05), DDE (ANOVA 2, F value = 2.36, *p* value = 0.02 > 0.05), DDT (ANOVA 2, F value = 9.19, *p* value < 0.00001), Total DDT (ANOVA 2, F value = 11.68, *p* value < 0.00001) and Heptachlor (ANOVA 2, F value = 4.80, *p* value < 0.00001) (*Table 2*).

***Table 2.** ANOVA base (post hoc test (Fischer's LSD test) for the comparison of OCPs in the organs of bogue from Oran Bay*

Organs	**POCs**	**Df**	**Mean**	**F value**	***p* value**
Gills	Lindane	11	0.47	04.51***	0.0001
	DDD	11	0.33	03.65***	0.0001
	DDE	11	0.58	11.85***	0.000
	DDT	11	1.21	20.47***	0.000
	∑DDT	11	1.98	19.63***	0.000
	Hpt	11	0.35	02.41*	0.01
Muscle	Lindane	11	0.28	02.06*	0.04
	DDD	11	0.25	01.89 ns	0.06
	DDE	11	0.36	02.36*	0.02
	DDT	11	1.23	11.68***	0.000
	∑DDT	11	1.27	11.68***	0.000
	HPT	11	0.19	04.80*	0.018

Df: Degrees of freedom, ∑DDTs = Total DDT, HPT: Heptachlor, *: significant ($p < 0.05$); **: ($p < 0.001$); ***: ($p<0.000$); ns: not significant ($p > 0.05$); F: Fischer's LSD test for the homogeneity of variances

Seasonal variations of organochlorine pesticides (OCPs)

In Oran Bay, seasonal variations of OCPs showed the highest average concentrations in the organs of bogue collected mainly in winter and autumn. The lowest concentrations were noted during the spring and summer. The lowest concentrations were noted during the spring and summer period (*Table 3*).

The analysis (mean ± standard deviation (SD)) of concentrations of pesticides found in *Boops boops* organs recorded during the different seasons, are grouped together in *Table 3*.

***Table 3.** Seasonal variations in the average concentrations of pesticides found in Boops boops organs (muscle and gills) (µg/g W.F) from Oran Bay*

Organs	Seasons	Lindane (mean ± SD)	DDD (mean ± SD)	DDE (mean ± SD)	DDT (mean ± SD)	Total DDT (mean ± SD)	HPT (mean ± SD)
Muscle	Autumn	0.287±0.079	0.193±0.050	0.366±0.091	0.865±0.143	1.477±0.171	0.154±0.058
	Winter	0.324±0.068	0.280±0.064	0.423±0.101	0.919±0.133	1.620±0.177	0.191±0.030
	Spring	0.157±0.008	0.112±0.072	0.168±0.044	0.289±0.0934	0.568±0.152	0.076±0.060
	Summer	0.228±0.063	0.228±0.0185	0.308±0.050	1.018±0.133	1.543±0.098	0.185±0.0261
Gills	Autumn	0.453±0.080	0.249±0.065	0.663±0.212	1.494±0.382	2.406±0.504	0.113±0.049
	Winter	0.6278±0.107	0.4293±0.086	0.773±0.100	1.604±0.214	2.807±0.228	0.378±0.063
	Spring	0.254±0.071	0.226±0.084	0.249±0.073	0.438±0.097	0.913±0.2097	0.239±0.016
	Summer	0.403±0.051	0.295±0.046	0.450±0.074	0.973±0.139	1.721±0.171	0.333±0.062

Our results of monthly variations show that the highest concentrations of Total DDT in gills and muscle (3.63 ± 0.25) (1.86 ± 0.18) (µg g^{-1} of F.W) respectively are observed during January (winter). The lowest concentrations of Total DDT in gills and muscle (0.66 ± 0.12) (0.40 ± 0.18) (µg g^{-1} of F.W) respectively are observed during the month of April (*Fig. 4*).

For Lindane, the highest concentration found in the gills was observed during the month of December (0.75 ± 0.18) while in the muscle (0.37 ± 0.08) was noted during the month of January. The minimum concentrations were noted during the months of May and April (spring) respectively (0.28 ± 0.06) and (0.14 ± 0.01) (µg g^{-1} of F.W) (*Fig. 3*).

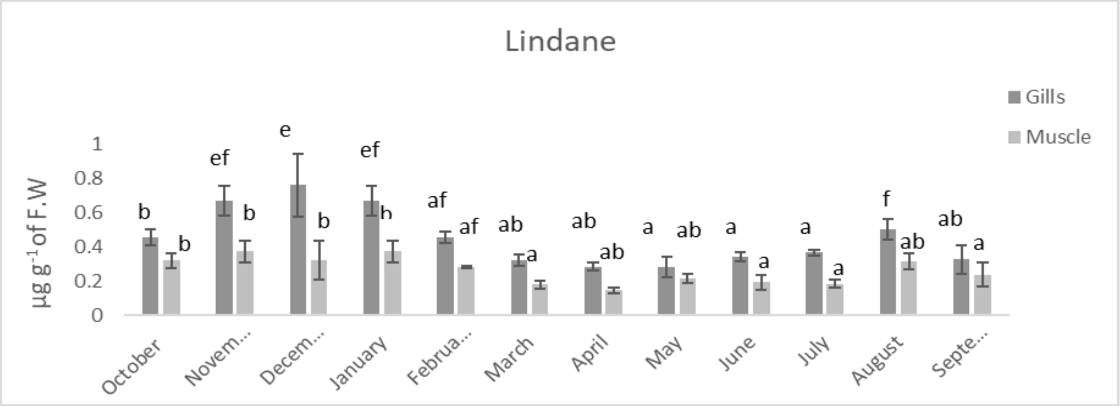

***Figure3.** Monthly variations of Lindane levels evaluated in muscle and gills (µg g^{-1} of F.W) of bogue from Oran Bay (n = 5, mean ± SD). The different letters (a, b, e, f) designate significant differences between OCPs in organ and month (Fischer's LSD test (P < 0.05))*

The results of base ANOVA for the comparison of the level of OCPs of the bogue fished in the Oran Bay measured monthly present a highly significant difference ($p < 0.001$) between November, December and January (autumn and winter seasons)

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compared to the months March, April, May, June (spring and summer season) (*Figs. 3, 4* and *5* ,*Tables 4 and 5*).

Figures 3, 4, and *5* represent monthly variations of OCPs evaluated in muscle and gills (µg g^{-1} of F.W) of bogue for all sexes and ages from Oran Bay measured monthly during the period October 2018/September 2019.

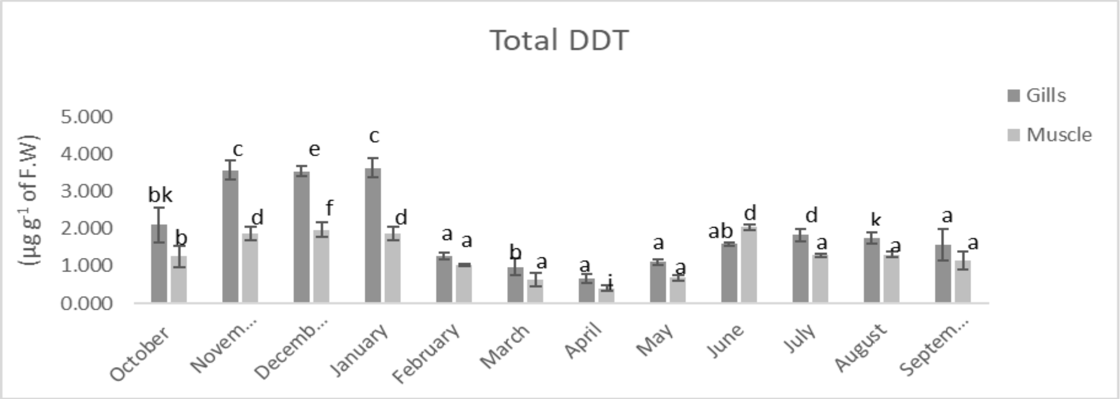

Figure 4. *Average levels of Total DDT, (n = 5, mean ± SD) evaluated in muscle and gills (µg g⁻¹ of F.W) of bogue from Oran Bay. The different letters (a, b, c, e, f, d, k) designate significant differences between OCPs in organ and month (Fischer's LSD test (P < 0.05))*

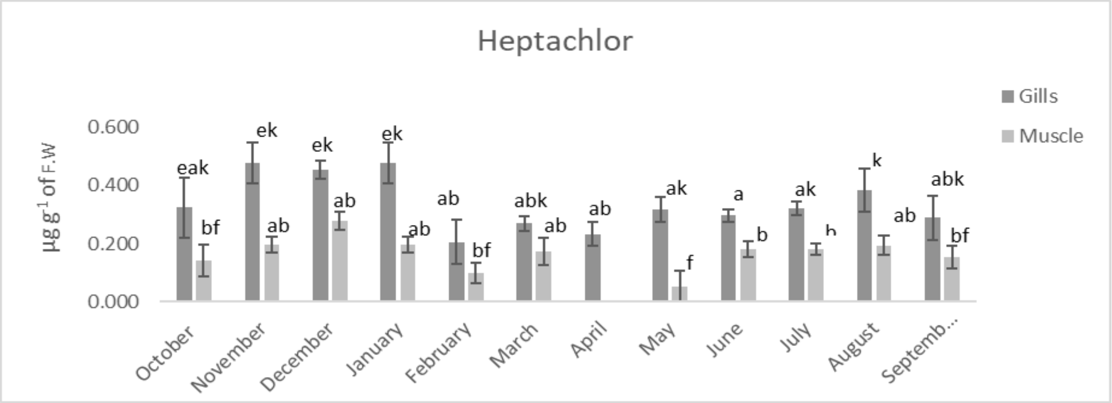

Figure 5. *Average levels of Heptachlor, (n = 5, mean ± SD) evaluated in muscle and gills (µg g⁻¹ of F.W) of bogue from Oran Bay. The different letters (a, b, c, e, f, d, k) designate significant differences between OCPs in organ and month (Fischer's LSD test (P < 0.05))*

Figures 6, 7, and *8* present the average levels of DDT and its derivate DDD and DDE evaluated in muscle and gills (µg g^{-1} of F.W) of bogue from Oran Bay.

Our results showed that DDT presents a highly significant difference compared to the DDE and DDD. The p, p′-DDE which is the first metabolite of DDT presents a highly significant difference compared to the DDD ($p \leq 0.001$).

The ratio DDE + DDD/∑DDTs in the muscle and gills is the same and it was ranged from 0.46 to 0.48 < 0.5, respectively.

The results of base ANOVA for the comparison of the seasonal variations for the average levels of POC_s in gills and muscles of the bogue fished in the Oran Bay were grouped together in *tables 4 and 5*.

Table 4. *The results of the post hoc (Fischer's LSD test) of the seasonal variations for the average levels of POC$_s$, (n = 5, mean ± SD) evaluated in gills (µg g^{-1} of F.W) of bogue from Oran Bay*

Organs	**POC$_s$**	**Comparison**	**F value**	***p* value**	**Df**
Gills	Lindane	Autumn - Spring	4.78	0.0041**	5
		Autumn - Summer	3.16	0.025*	5
		Autumn - Winter	2.79	0.04*	5
		Spring - Summer	32.40	0.006**	5
		Spring - Winter	8.74	0.00***	5
		Summer - Winter	5.09	0.00***	5
	DDD	Autumn - Spring	8.52	0.00***	5
		Autumn - Summer	4.55	0.005**	5
		Autumn - Winter	2.50	0.06^{ns}	5
		Spring - Summer	0.93	0.47^{ns}	5
		Spring - Winter	3.50	0.01**	5
		Summer - Winter	1.69	0.17^{ns}	5
	DDE	Autumn - Spring	19.43	0.00***	5
		Autumn - Summer	8.22	0.001***	5
		Autumn - Winter	16.95	0.00***	5
		Spring - Winter	18.50	0.00***	5
		Summer - Winter	9.55	0.00***	5
	DDT	Autumn - Spring	46.24	0.00***	5
		Autumn - Summer	12.07	0.00***	5
		Autumn - Winter	23.99	0.00***	5
		Spring - Summer	3.80	0.01*	5
		Spring - Winter	27.31	0.000***	5
		Summer - Winter	9.74	0.03*	5
	∑DDT	Autumn - Spring	28.53	0.00***	5
		Autumn - Summer	9.67	0.00***	5
		Autumn - Winter	21.43	0.00***	5
		Spring - Winter	45.08	0.000***	5
		Summer - Winter	14.75	0.000***	5
	HPT	Autumn - Spring	3.04	0.03*	5
		Autumn - Summer	1.40	0.25^{ns}	5
		Autumn - Winter	3.20	0.025*	5
		Spring - Winter	3.50	0.017*	5
		Summer - Winter	2.27	0.080^{ns}	5

Df: Degrees of freedom, ∑DDTs = Total DDT, HPT: Heptachlor, *: significant ($p < 0.05$); **: ($p < 0.001$); ***: ($p<0.000$); ns: not significant ($p > 0.05$); F: Fischer's LSD test for the homogeneity of variances

Our results showed that ∑DDTs in gills and muscles of the bogue caughted in the Oran Bay present a highly significant difference ($p < 0.001$) among the four seasons: autumn, winter, spring and summer (*Tables 4 and 5).*

***Table 5.** The results of the post hoc (Fischer's LSD test) of the comparison of the seasonal variations for the average levels of POC$_s$, (n = 5, mean ± SD) evaluated in muscle ($\mu g\ g^{-1}$ of F.W) of bogue from Oran Bay*

Organ	POC$_s$	Comparison	F value	*p* value	Df
Muscle	Lindane	Autumn -Spring	1.98	0.12^{ns}	5
		Autumn-Summer	1.08	0.39^{ns}	5
		Autumn - Winter	1.16	0.35^{ns}	5
		Spring - Summer	1.73	0.165^{ns}	5
		Spring - Winter	5.15	0.0028**	5
		Summer - Winter	2.73	0.044*	5
	DDD	Autumn -Spring	2.98	0.037*	5
		Autumn-Summer	2.23	0.08^{ns}	5
		Autumn - Winter	1.93	0.1^{ns}	5
		Spring - Summer	2.0058	0.12^{ns}	5
		Spring - Winter	1.84	0.15^{ns}	5
		Summer - Winter	0.87	0.51^{ns}	5
	DDE	Autumn -Spring	5.89	0.001***	5
		Autumn-Summer	1.40	0.25^{ns}	5
		Autumn - Winter	1.24	0.32^{ns}	5
		Spring - Summer	2.05	0.10^{ns}	5
		Spring - Winter	2.91	0.03*	5
		Summer - Winter	0.91	0.49^{ns}	5
	DDT	Autumn -Spring	10.22	0.00***	5
		Autumn-Summer	2.25	0.08^{ns}	5
		Autumn - Winter	5.26	0.022*	5
		Spring - Summer	24.78	0.00***	5
		Spring - Winter	30.61	0.00***	5
		Summer - Winter	6.86	0.00***	5
	∑DDT	Autumn -Spring	16.47	0.00***	5
		Autumn-Summer	3.23	0.0025**	5
		Autumn - Winter	7.78	0.00***	5
		Spring - Summer	23.46	0.00***	
		Spring - Winter	34.46	0.00****	5
		Summer - Winter	7.312	0.0002***	5
	Heptachlor	Autumn -Spring	8.03	0.000****	5
		Autumn-Summer	1.99	0.00****	5
		Autumn - Winter	3.14	0.028	5
		Spring - Summer	5.89	0.001***	5
		Spring - Winter	5.33	0.00***	5

F: Levene's test for the homogeneity of variances ,Df: Degrees of freedom, ∑DDTs = Total DDT, HPT: Heptachlor, *: significant ($p < 0.05$); **: ($p < 0.001$); ***: ($p<0.000$); ns: not significant ($p > 0.05$); F: Fischer's LSD test for the homogeneity of variances

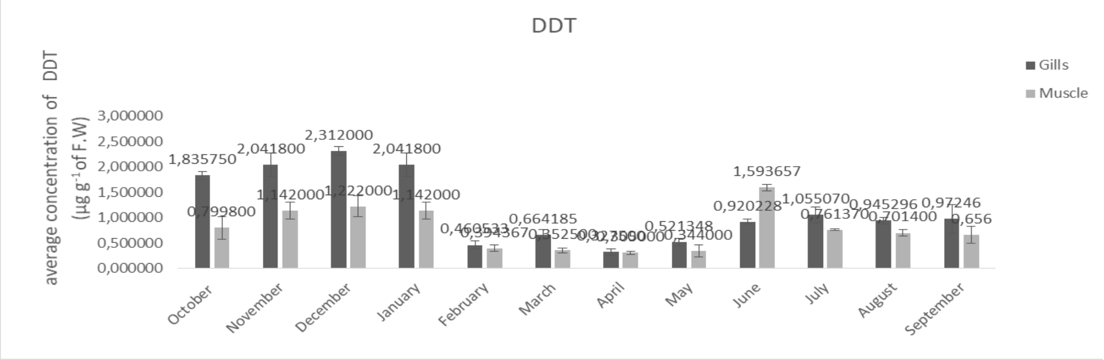

Figure 6. *Average levels of DDT (µg g^{-1} of F.W) evaluated in muscle and gills of bogue from Oran Bay*

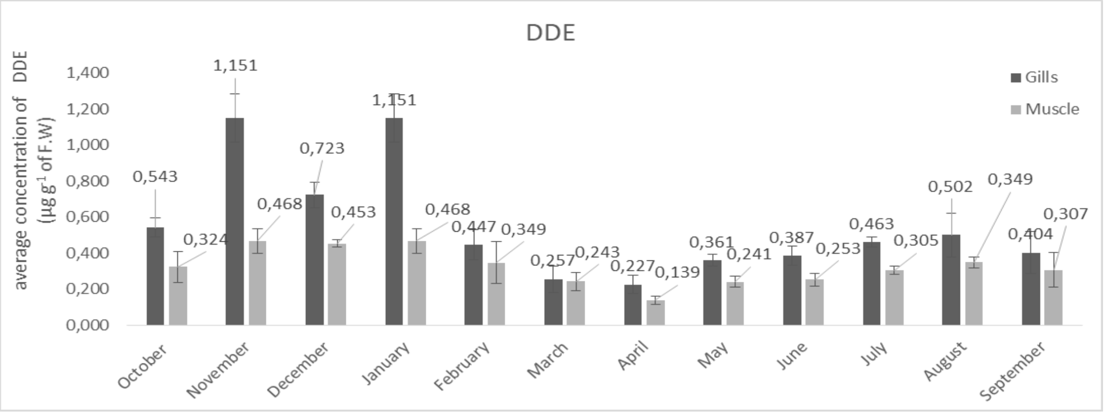

Figure 7. *Average levels of DDE, (n = 5, mean ± SD) evaluated in muscle and gills (µg g^{-1} of F.W) of bogue from Oran Bay*

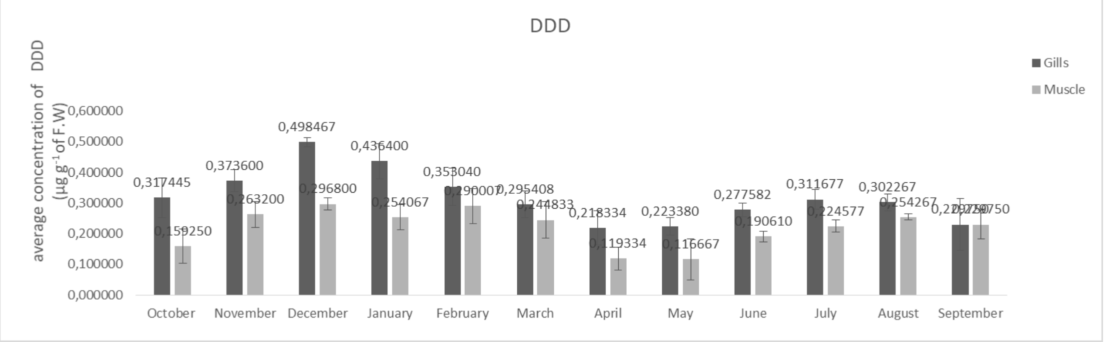

Figure 8. *Average levels of DDD, (n = 5, mean ± SD) evaluated in muscle and gills (µg g^{-1} of F.W) of bogue from Oran Bay*

Biomarker assessment

The TBARS level and enzymatic activities in the muscle and liver of *Boops boops* fish, from the bay of Oran were provided (*Fig. 9*).

Bogue liver and muscle showed monthly changes in CAT, GST, and AchE activity as well as TBARS levels (*Figs. 9*, *10*, *11*, and *12*).

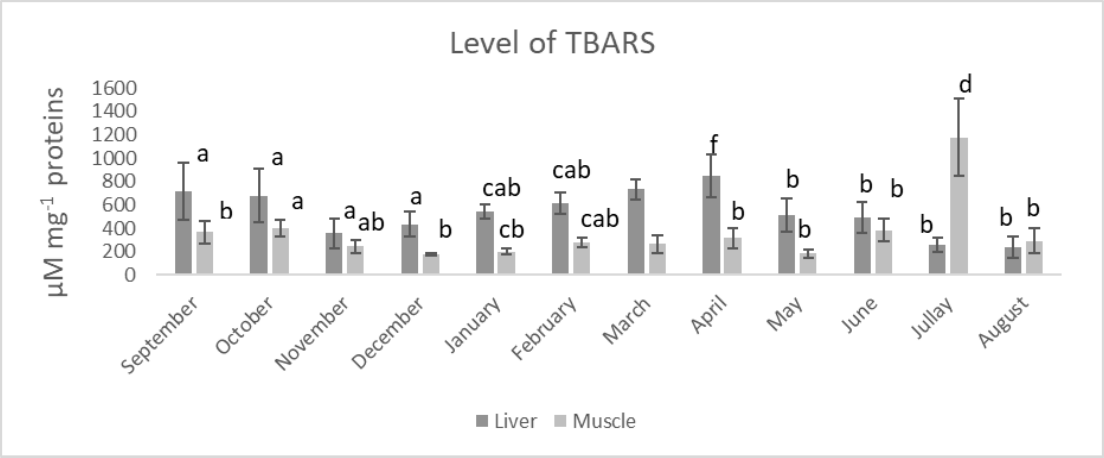

Figure 9. *TBARS level (µM mg^{-1} proteins) in muscle and liver of Boops boops fish, from Oran Bay. The different letters (a, b, c, e, f, d, k) designate significant differences between Level of TBARS in organ and month (Fischer's LSD test (P < 0.05))*

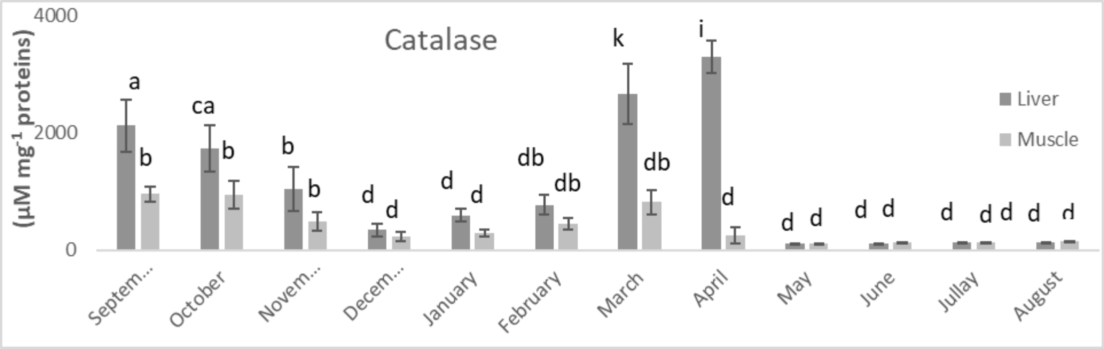

Figure 10. *Catalase activity (µM mg^{-1} proteins)***,** *in muscle and liver of Boops boops fish from Oran Bay. The different letters (a, b, c, d, k, i) designate significant differences between level of catalase in organ and month, (Fischer's LSD test (P < 0.05))*

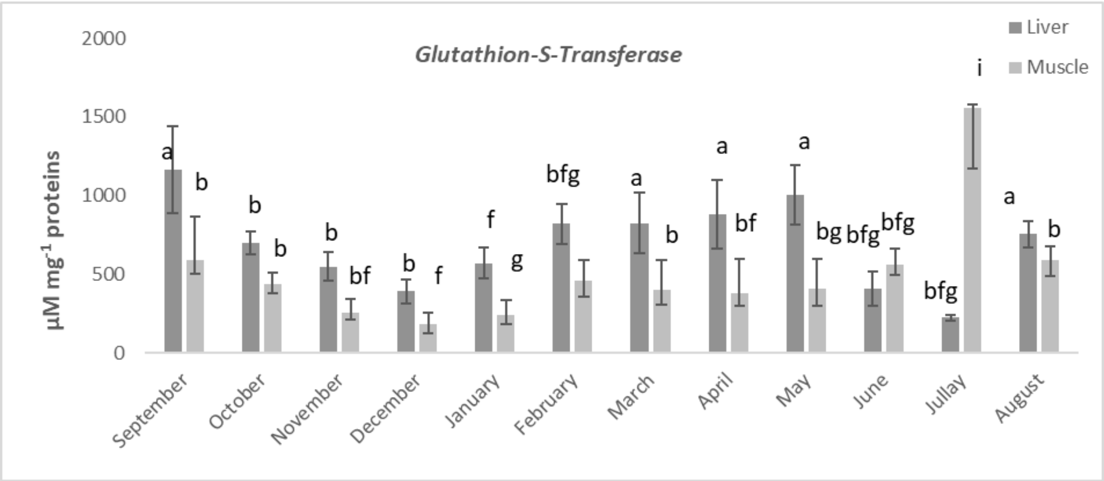

Figure 11. *Glutathione-S-Transferase activity (µM mg^{-1} proteins)***,** *in muscle and liver of Boops boops fish from Oran Bay. The different letters (a, b, c, d, g, f, i) designate significant differences between level of catalase in organ and month (Fischer's LSD test (P < 0.05))*

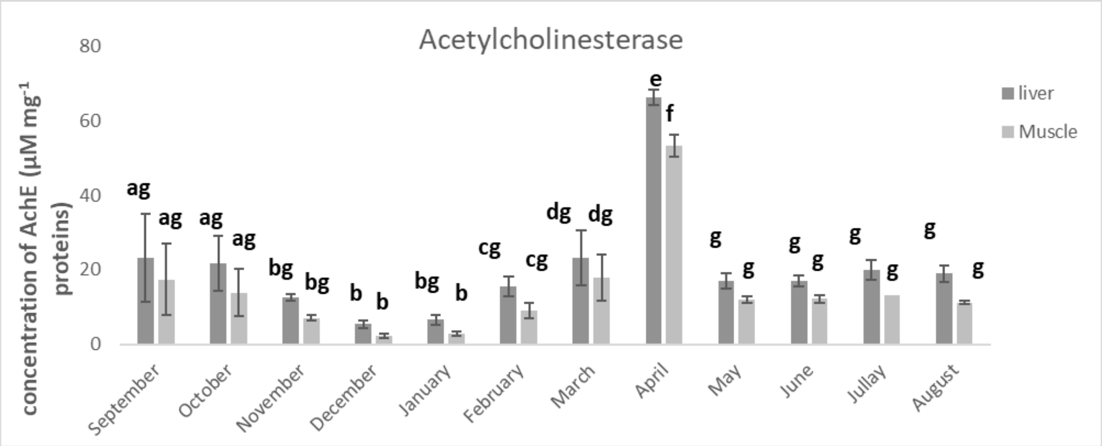

Figure 12. *Acetyl cholinesterase activity (µM mg^{-1} proteins)****,*** *in muscle and liver of Boops boops fish from Oran Bay. The different letters (a, b, c, e, f, d, g) designate significant differences between level of AChE in organ and month (Fischer's LSD test (P < 0.05))*

Except for the month of July, we can see a significant increase in the level of TBARS and GST activity in muscle versus liver. In addition, a significant increase in the activity of CAT, GST, and AchE as well as in the level of TBARS in the liver compared to the muscle throughout the duration of the study.

We performed a variance analysis of average levels of OCPs, (Lindane, DDE, DDD, DDT, ∑DDTs, and Heptachlor) in (gills and muscle) of bogue, as well as the average levels of CAT, TBARS, GST, and AchE in (liver and muscle), according to Levene's F test for the homogeneity of variances.

The factors month and the organ had a highly significant impact on the rate of all OCPs ($p < 0.001$), as well as on the activity and rate of the other parameters (CAT, TBARS, AchE).

This analysis revealed that the interaction between the two factors (months and organs) were significant: all OCPs ($p < 0.001$), as well as other parameters CAT, TBARS, AchE ($p < 0.001$). While having no effect on the activities of GST, AchE, and the amount of DDD (*Table 6*).

As indicated in *Table 7,* there were significant negative correlations between CAT, TBARS, and GST (muscle and liver) measurements and total DDT and Lindane

The results of our work highlighted a significant positive association between TBARS levels and GST activity in liver tissue and flesh of bogue (R = 0.96, R = 0. 94 respectively).

The results of the projection of the variables and the individuals in the two-dimensional PCA space are presented (*Fig. 13*). Two main components: fact.1 and fact.2 have been identified, representing 63.55% and 17.25% respectively of the total variance (87%).

The projection reveals that fact.1 is negatively correlated with CAT and AchE, while having positively correlated with all of the organochlorine micropollutants (Lindane, DDE, DDD, DDT, Total DDT, and HPT) identified in bogue (gills, muscles) and (CAT, GST, TBARS and AchE) of Oran Bay.

As shown by the projection, fact.2 has a positive correlation with AchE, but a negative correlation with lindane, DDE, DDD, DDT, Total DDT, HPT, CAT, GST, and TBARS.

Table 6. *Illustration of results from the analysis of variances (Test F, ANOVA) for the average concentrations of OCPs in gills and muscles as well as the average activity of (CAT, TBARS, GST, and AChE) in the liver and muscle of the bogue fish from Oran Bay*

	F (month)	**F (organs)**	**F (month × organs)**
Lindane	8.75**	42.57**	1.77**
DDD	5.691**	19.89**	1.563 ^{ns}
DDE	16.77**	36.09**	2.97**
DDT	10.40**	14.03**	6.88**
∑DDTs	21.89**	42.30**	2.37**
HPT	8.71**	24.08**	2.86**
CAT	19.92**	61.63**	9.92**
TBARS	8.72**	1.26**	0.03**
GST	9.26**	0.40 ^{ns}	0.16 ^{ns}
AchE	21.11**	12.05**	0.16 ^{ns}

∑DDTs = Total DDT, HPT: Heptachlor, CAT: catalase, GST: Glutathione-S-transferase, AchE; Acetyl choline Esterase; *: significant ($p < 0.05$); **: ($p < 0.001$); ns: not significant ($p > 0.05$), F: Levene's test for the homogeneity of variances.

Table 7. *The correlation coefficients between the levels of organochlorine pesticides found in the gills, muscles and the rate of TBARS, the activity of CAT, GST, and AChE in liver, and muscle of the bogue, Oran Bay*

	Lindane G	**Lindane M**	**DDE G**	**DDE M**	**DDD G**	**DDD M**	**DDT G**	**DDT M**	**∑DDTs G**	**∑DDTs M**	**HPT G**	**HPT M**
CAT L	-0.46	**-0.58***	-0.48	**-0.64***	**0.71***	**-0.39**	**-0.47**	0.27	**-0.61***	**-0.76***	**-0.56***	-0.34
CAT M	-0.43	**-0.55***	-0.46	**-0.63***	**-0.72***	**-0.38**	**-0.49**	0.28	**-0.61***	**-0.75***	**-0.59***	-0.32
TBARS L	-0.43	**-0.59***	-0.47	**-0.64***	**-0.77***	**-0.36**	**-0.60***	0.14	**-0.66***	**-0.74***	**-0.63***	-0.35
TBARSM	-0.41	**-0.58***	-0.42	**-0.63***	**-0.72***	**-0.39**	**-0.52***	0.20	**-0.60***	**-0.70***	**-0.58***	-0.34
GST L	-0.48	**-0.58***	**-0.51***	**-0.64***	**-0.76***	**-0.36**	**-0.55***	0.22	**-0.66***	**-0.79***	**-0.61***	-0.35
GST M	-0.46	**-0.58***	-0.48	**-0.64***	**-0.71***	**-0.39**	-0.47	0.27	**-0.61***	**-0.76***	**-0.56***	-0.34
AchE L	-0.48	**-0.65***	**-0.65***	**-0.72***	**-0.61***	**-0.80***	**-0.71***	-0.48	**-0.67***	**-0.67***	**-0.67***	-0.45
AchE M	-0.46	**-0.63***	**-0.62***	**-0.72***	**-0.57***	**-0.84***	**-0.66***	-0.45	**-0.63***	**-0.64***	**-0.64***	-0.44

∑DDTs = Total DDT, HPT: Heptachlor, CAT: catalase GST: Glutathione-S-transferase, AchE: Acetyle choline Esterase, L: liver, M: muscle, G: gills. Correlation factor '*' > 0.5

The data distribution is strongly influenced by the monthly aspect, as shown by the projection of the observations onto the fact2 axis. November, December, and January all displayed statistically significant positive correlations with Lindane, DDD, DDE, DDDT Total, and HPT, as well as extremely strong negative correlations ($p < 0.001$) with AchE (*Fig. 13*). This latter, as measured in the liver, had a significant positive correlation with that in muscles (R = 0. 99).

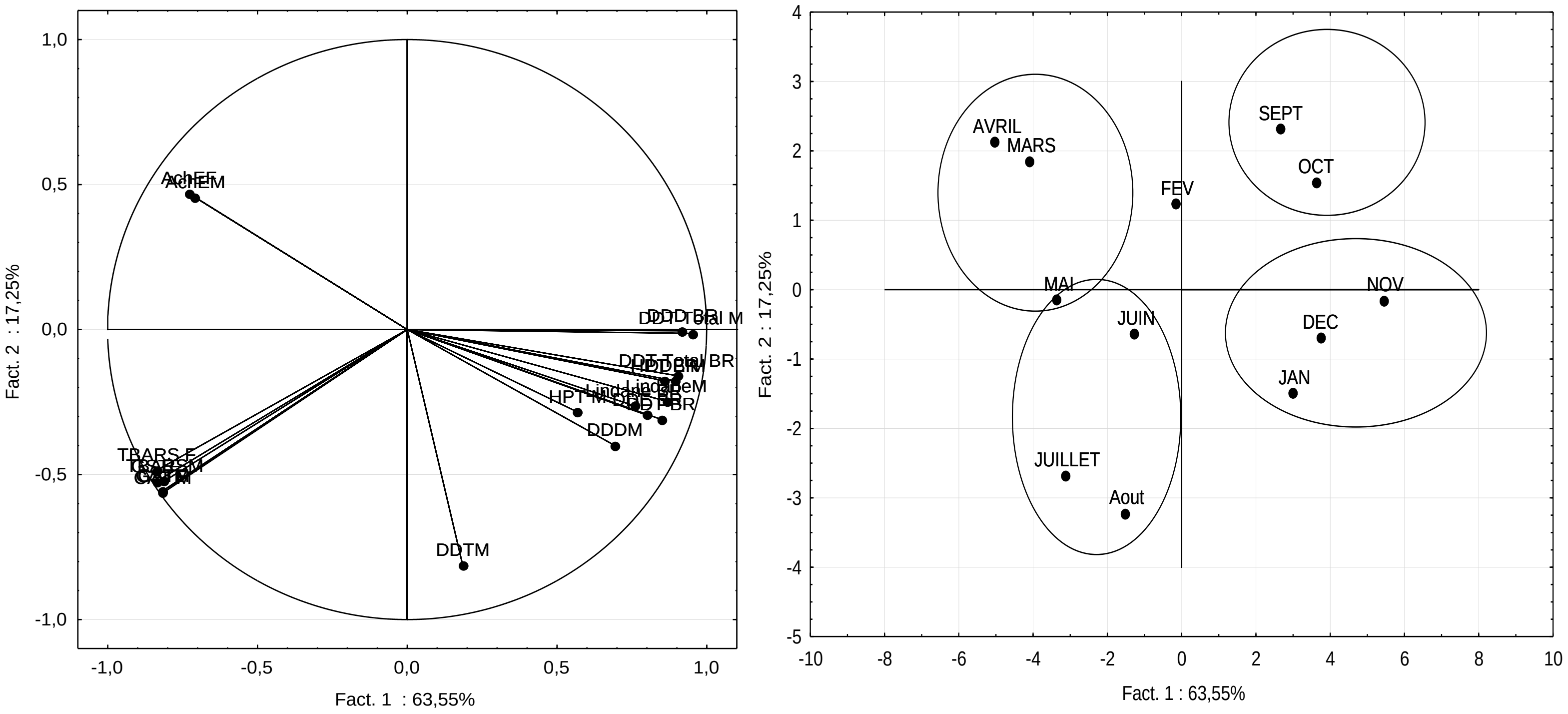

Figure 13. *Principal component analysis of the two main factors (F1 vs. F2) produced by the levels of POCs measured in the gills, muscles and the biomarkers activities, as measured in the liver and muscle of the bogue fished from the bay of Ora*

Discussion

Pesticide contamination

The POCs studied (DDT, DDD, DDE, Lindane and Heptachlor) were detected in both organs: muscle and gills of the bogue, suggesting that those POCs are currently used in agricultural areas. Similar results were obtained by this group of authors (Boutiba et al., 1996; Brahim Tazi (1998); Henríquez-Hernández et al., 2016; Nejatkha Manavi et al., 2018; Bartalini et al., 2020).

In addition, these rates found in our sub-samples (gills and muscles) indicate that the bogue is a good bioaccumulator of pollutant molecules from the degradation caused by organochlorine pesticides (OCPs). These findings may be related to the characteristics of each pollutant and the fish. Taking that into account, the bioaccumulation process of xenobiotic elements in aquatic organisms depends on the level of contamination of the environment, which is closely related to the segregation coefficient specific to each substance (Belhoucine et al., 2015; Rouane-Hacene et al., 2018; Tabeche et al., 2021).

Considering that Organochlorine pesticides, taken as a whole, are renowned for their extreme resistance to destruction in the environment, and their high lipophilicity, their persistence remains very high, especially in the adipose tissues of organisms (Juc, 2008; Nejatkha Manavi et al., 2018) and their lipophilic nature makes them bioaccumulative, raising concerns of widespread contamination of the food chain (Karaca et al., 2014).

The organochlorines found in gills were significantly higher compared to what is in the muscle ($p \leq 0.001$). We find that the gills are the site of transfer of contaminants to fish, especially organic contaminants which have a low water solubility toward this species. The results of this work tend to support the observations made by Mckim et al. (1985), Abarnou et al. (2000), Lazartigues (2010), and Classen (2018).

The classification of pesticides according to their level of detection in the two organs leads to the following diagram: DDT and its metabolites > lindane > Heptachlor. Similar results were found by Henríquez-Hernández et al. (2017) on wild bogue caught on the coasts of the Spanish Canary Islands, and so are those reported by Boutiba et al. (1996) and Brahim Tazi (1998) which they indicated the presence of these organochlorines in the bogue and the sardine (Sardina Pilchardus) around the coast of Oran.

The data recorded by Rouane-Hacene (2007) and Bouchaib et al. (2010) are practically similar to our results, which have shown that the amounts of DDT and its metabolites are the highest.

In contrast, a study of Lemarchand et al. (2012) and Nejatkhah Manavi et al. (2018) reported that Lindane is the most abundant compound compared to DDT.

These lofty concentrations of DDT may be due to their highest bioaccumulative properties, and to extensive past use and/or continued release into the aquatic environment (Henríquez-Hernández et al., 2017). A second possibility is the use of a legal pesticide: dicofol. However, dicofol may contain DDT as an impurity, since DDT is the starting compound for dicofol synthesis, which was recorded (Karaca et al., 2014; Nejatkhah Manavi et al., 2018). DDT can be metabolized into DDD under anaerobic or DDE in aerobic or oxidation environment (Wang et al., 2013). In Our result the p, p′-DDE, which is the first metabolite of DDT, shows a highly significant difference from DDD (P> 0, 001). This research is similar to those of Lemarchand et al. (2012), Wang et al. (2013), and Nejatkhah Manavi et al. (2018). Indeed, according to Henríquez-

Hernández et al. (2017) DDD and DDE are often found in fish because of their longer half-life (around 7 years) compared to the half-life of DDT (around 8 months). We also suggest that the presence of DDT and its derivatives (DDE, DDD) and the abundance of DDE indicate recent use of this compound.

The ratio DDE + DDD/∑DDTs, made it possible to have a global approach on the metabolism of DDT depending on the organs and to determine the state of its degradation. Its ratios in gills and muscles, ranging from 0.46 to $0.48 < 0.5$, respectively, might suggest that DDT degradation is not significant and does not differ among organs in the Oran Bay however, this ratios can indicate past or recent usage of DDT. Our study, allow us to suggest that a recent contamination has taken place.

(A ratio of > 0.5 signifies long-term biotransformation of DDT, while a ratio of < 0.5 may indicate recent entry of DDT (Wang et al., 2013).

The low levels of Lindane and Heptachlor found in our samples may be due to the prohibition of its use, or to their more soluble, less persistent formula, therefore accumulating less in aquatic organisms (Wang et al., 2013; Karaca et al., 2014; Lazartigues, 2018). However, the detection of these pollutants may provide an explanation for the continued illicit use of some legacy stocks of these chemicals in agricultural areas. However, according to our study the measured compounds were less than the maximum residue limits (MRLs) (FAO/WHO).

Agricultural wastewater seems to be one of the most important pollution sources in in the bay of Oran (Algeria). However, the absence of urban and industrial waste water treatments for many years caused an important environmental degradation. The principal inputs of fresh water to the coastal zone are the wastewater discharges from Oran city (Benzaoui et al., 2015; Tabeche et al., 2021).

Agricultural activities and anthropogenic discharges in Algeria’s west coast are also to blame for the high amounts of pollutants in fish. The data recorded by Taleb et al. (2009), Remili et Kerfouf (2013), Benzaoui et al. (2015), Belhoucine et al. (2015, 2022), Bouhadiba et al. (2017), Rouane-Hacene et al. (2018), Tabeche et al. (2021), and Kaddour (2021) are practically similar to ours. The former noted the presence of stress on the bay of Oran, which resulted from various forms of nuisance, industrial activities, agricultural activities, and anthropogenic tourism and massive urbanization, which generates increasing pollution.

The highest average concentrations of OCPs were found in the muscle and gills of bogue collected primarily in the winter and autumn, but during the spring and summer, the lowest amounts were documented. These findings contradict the study of Rouane-Hacene (2007) which noted high levels of total DDT during the summer season.

Seasonality has a significant influence on the distribution of the data, and it is one of the most important elements in the accumulation of pollutants. Crucially, pesticide enrichment throughout the wet and rainy season must have had some effect on the origin of the increase pollutants in the early flood run-off, which explains their high concentration in our target organs during the winter the results of this inquiry tend to support the observations of Amri (2017).

State of the art on the use of pesticides in our region

It should be noted that OCPs have been widely used in Algeria for several years (60, 70 and 80) in agriculture and are currently used illegally despite their prohibition (Law No. 87-17 of August 1, 1987, relating to plant health protection (OJ, 1995). Although they are banned from use in the United States and in Europe in 1972.

According to our surveys of farmers and professionals specializing in agricultural products in several cities in the region of Oran on the status and use of plant protection products in Western Algeria, and with the help of the National Institute of Plant Protection (Oran) (survey over 8 years from 2010 to 2018). we found that old stocks of unlabeled insecticides are exposed and sold through weekly markets. In addition, farmers and even some individuals can buy this pesticide to treat their seeds and eliminate certain pests, because it is very effective and cheaper than other insecticides that exist on the market and good agricultural practices (GAP) in the use of pesticides are almost never respected. In fact, given that the majority of them lack literacy, farmers frequently struggle with understanding the dosages and frequency of pesticide application. As a result, instances of overdose and repetitive usage of particular persistent pesticides are documented. As a result, dirt accumulation zones may develop (Bettiche, 2017). Moreover, the results of this inquiry tend to support the observations of Bettiche (2017), Bensaid et al. (2019), Zergui et al. (2019), Saadi et al. (2019), Mokhtari et al. (2019), and Chefirat et al. (2019).

DDT and lindane have been used in Algeria for the control of crop pests, for the eradication of certain vector-borne diseases and for the control of epidemics such as malaria and typhus. Knowing that in Algeria, malaria occupies the first place in the list of transmissible diseases to be declared; In the south of Algeria, 300 cases of malaria were declared between 1980 and 2007 (Hammadi et al., 2009; Bettiche, 2017).

Algerian legislation on pesticides and its place in the international context

The publication of Silent Spring in 1962 contributed significantly to the increase in public concern about the environmental damage caused by pesticides. Since then, their use has been regulated by several pieces of legislation harmonized by international bodies such as the Codex Alimentarius Commission, the European Union (EU) and the Organization for Economic Cooperation and Development (OECD). These legislations provide, among other things, the maximum residue limits (MRLs) and the acceptable daily intake (ADI) (Barka et al., 2019). Like developing countries, Algeria has established a pesticide management policy, whose legislative texts are grouped in the index of plant protection products, which lists the 400 products registered to date. International harmonization of MRLs improves protection of public health and the environment and facilitates trade (Barka et al., 2019).

Biomarker assessment

Antioxidant defense

Our findings show a very significant negative association ($P < 0.001$) between the presence of Lindane, Heptachlor, DDT, and its derivatives (muscles and gills) and the oxidative biomarker enzyme CAT and TBARS levels (muscles and liver) of the bogue. This could explain the oxidative damage caused by these organochlorine contaminants.

In our study, increased or decreased CAT activity and TBARS levels indicate an interruption of the normal oxidation process. Suggesting that the defense systems of the bogue are stimulated, these findings endorse ones arrived at earlier (Narra et al., 2017; Clasen et al., 2018; Teixeira et al., 2018). They proved the ability of organochlorines to induce oxidative stress in different organs, by generating free radicals and inducing lipid peroxidation.

The pattern of variations in CAT concentrations and TBARS levels are marked by a highly significant increase in liver compared to muscle. Our results clearly show that the liver is the organ of choice where these biomarkers are best expressed, since it is crucial for the detoxification of pollutants, and typically exhibits a higher sensitivity to the oxidative stress induced by pesticides. Karaca et al. (2014), Sinhorin et al. (2014), and Narra et al. (2017) reported similar results.

The monthly variations of the average concentrations of the antioxidant defense CAT and TBARS of the bogue from the bay of Oran had shown significant difference from one month to the other ($P < 0.001$). They are marked by a significant increase in the liver during the month of April compared to other months. This rise in CAT and TBARS activity in April may reflect a state of stress, that results in the production of antioxidant enzymes that induce the production of reactive oxygen species.

Because the bogue spawns primarily in spring (Bnina, 2015; Benhamou, 2017; Benomar, 2017) this increase can be attributed to precise factors. We can suggest that this increase is related to reproduction and/or to an increase in the availability of nutrients during this period, since its food is composed of sediments coated with algae, such food is more abundant and accessible during April and May in the western Mediterranean. Box et al. (2008); Taleb et al. (2009); Benali et al. (2015) reported the presence of aggressive algae that create toxic or repulsive secondary metabolites, which may in turn be a source of reactive oxygen species (ROS) in marine animals. These results corroborate the works of Amri et al. (2017).

In our study, reduced activity of CAT and level of TBARS (liver and muscle) in autumn and winter may be the result of the high presence of organochlorine pesticides found in the gills and muscles of the bogue during these periods. These results have previously been documented in a study by several authors (Sellami et al., 2012; Amri et al., 2017) who reported that prolonged exposure to large levels of pesticides can, in fact, cause damage to lipids, nucleic acids, and proteins involved in cellular defense including invasion of antioxidant defense.

In the current investigation, we found that the presence of DDT, its derivatives, and Lindane were negatively correlated with the enzyme activity of biotransformation (GST). The findings of this study generally support the observations of Fernández et al. (2010), Clasen et al. (2018), and Ozmen et al. (2008), who had pointed out in their study that the increase in GST activities is due to the presence of organochlorine contaminants such as DDT. These findings contradict with studies by Roche et al. (2007) that found no association.

Our investigations showed a significant positive correlation ($R = 0.94$) between the levels of TBARS and the biotransformation enzyme activity (GST). According to Park et al. (2009) GST is essential for protecting cells against toxins in living organisms, and has been employed as a biomarker of anthropic chemical exposure. Our observations suggest that lipid peroxidation is one of the mechanisms contributing to the rise in GST activity, which confirms previous works (Ozmen et al., 2008). However, Clasen et al. (2018) showed a negative correlation between GST activity and TBARS levels.

Biomarker of neurotoxicity (AChE)

With a correlation factor of 0.99 the AChE measured in the liver and muscle had a very strong correlation. We noted a very significant negative correlation ($p < 0.001$) between AchE (liver, muscle) and the presence of Lindane, DDD, DDE, DDDT Total and HPTE (gills, muscles). This gets in line with Teixeira et al. (2018).

According to Menéndez-Helman et al. (2012) with induction of its receptors brought on by the accumulation of acetylcholine which was begotten by this reduction, the neurotransmission mechanism is altered, resulting in behavior anomalies and even death.

The data distribution is strongly influenced by the month factor, as shown by the projection of the observations onto the fact2 axis. November, December, and January all displayed statistically significant positive correlations with Lindane, DDD, DDE, DDDT Total, and HPT, as well as extremely strong negative correlations ($p < 0.001$) with AchE (*Fig. 13*).

Knowing that the Bay of Oran can host many types of cholinesterase inhibitors (pesticides) carried by runoff water during rainy periods, when the contamination of coastal waters is more severe (Rouane-Hacene, 2007; Taleb et al., 2009; Benali et al., 2015); made the same observation.

These results hold that the high levels of pesticides in the winter season cause a strong inhibitory effect of AChE in the liver and muscle of the bogue. We can consider that the inhibition of AChE in the bogue could be a good biomarker of contamination by organochlorine pesticides (Roche et al., 2007; Clasen et al., 2018) and made the same observation.

Conclusion

The presence in living organisms of these organochlorine xenobiotics, which do not exist in the natural state, is indicative of aquatic pollution by certain human activities. These contaminants are well accumulated in the gills compared to the muscles, and are lower than the standards recommended by FAO/WHO, confirming that the bay of Oran is not spared from chemical adulteration.

In the present study DDE + DDD/∑DDTs ratios in gills and muscles ranged from 0.46 to $0.48 < 0.5$, respectively, suggesting recent contamination has occurred.

The results also show that the biomarkers CAT, TBARS, GST, and AchE levels are reliable tools for evaluating the effects of pesticide contamination to assess the ecotoxicological risk.

It is concluded that the inhibition of AChE in bogue could be a good biomarker of contamination by organochlorine pesticides.

The Oran littoral bogue does not seem to present a real danger in the immediate future for the consumer. However, the impact of these five substances combined remains scarcely studied, so the bioaccumulation of these micropollutants can cause a harmful effect in the long term on public health. we can conclude that this teleost fish B. boops is a good bio-accumulator of these organochlorine pesticides and can serve as an experimental model indicator of pollution by this type of organic pollutants in the area where it evolves.

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