The effects of total hydrocarbons on the polychaete *Perinereis cultrifera* (Grübe, 1840): Impacts on morphometric aspects and biochemical markers

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Abstract. Following the development of urban and industrial centers, petrochemical products have become a widespread class of contaminants. This paper aimed to investigate the effects of petrochemical contamination in wild populations of annelid polychaetes *Perinereis cultrifera* along the Algerian east coasts, based on biomarkers response. Worms were collected from three sites: Skikda, Annaba, and El Kala, the latter being considered a reference site. The biomarkers selected in this work were: (1) the acetylcholinesterase activity (AChE), as a neurotoxicity marker; (2) glutathione S-transferase (GST), as phase II enzyme; (3) oxidative stress was evaluated using catalase activity (CAT); and (4) lipid peroxidation using malondialdehyde levels. Furthermore, the concentrations of total hydrocarbons and abiotic parameters were quantified in seawater at each site. The results showed differences between sites compared with the reference samples; seaworms from polluted sites showed higher neurotoxicity, oxidative stress responses and lipid peroxidation values. Principal component analysis (PCA) produced significant groupings that revealed correlative relationships (both positive and negative) between neurotoxicity/oxidative stress variables and Total Petroleum Hydrocarbons (TPHs) and physicochemical parameters. The overall results suggest that the multi-marker approach confirms that worms from Annaba and Skikda have been exposed to a highly polluted environment. Seaworms from Skikda had the highest values, indicating a high contamination status.

Key Words: Algerian eastern coast, bioaccumulation, morphometry, oxidative stress, polychaetes.

Introduction. The Mediterranean Sea is a semi-enclosed basin where waters are slowly renewed (fifteen years for deep waters) (Zaghden et al 2016). In the past few decades, the increasing economic development on its coastline has resulted in major environmental changes (Barhoumi et al 2014). In the 1988-2008 period, Algeria's population has increased by 50% and about 45% of this population is concentrated on a very narrow strip of the littoral, especially in the industrial and harbor zones (Grimes et al 2010). The development of urbanization, industrialization, and population has led to environmental pollution in some coastal regions. Every year, large amounts of wastewater from industrial, agricultural, and municipal sources are discharged into the sea, which is the main receiving end for pollutants (Danion et al 2011). Most of these pollutants accumulate in the tissues of marine organisms and cause physiological problems and genetic disorders in marine species (Chen et al 2012).

Invertebrates, in particular worms, are essential vectors of contaminants from environmental compartments (water, food, or sediment) to higher trophic levels (Cattaneo et al 2009), like wading birds and economically important species. Polychaetes such as *Perinereis cultrifera* (Grübe, 1840) are commonly used in ecotoxicological studies because of their high sensitivity towards organic contamination (Bouraoui et al 2009); they contribute to the biogeochemical cycles of nutrients and contaminants during bioturbation activity due to their close interaction with the environment (Scaps 2002); they are easy to procure due to their abundance (Suriya et al 2012). These characteristics make polychaetes a suitable class for environmental studies under a broad set of abiotic conditions. Thus, polychaetes are good candidates for ecotoxicological studies (Cong et al 2011).

Considering the putative presence of contaminants such as total petroleum hydrocarbons (TPH), chronic exposure to this combination of chemical classes is likely to result in oxidative stress, metabolic alterations, and changes in the cholinergic neurotransmission mechanisms (Oliva et al 2012). TPH can enter the environment through accidents, from industries, or as by-products from commercial or private uses (Cai et al 2021). When TPHs directly enter waters through spills or leaks, some fractions float and form thin surface films. Other heavier fractions will accumulate in the sediment at the bottom, which may affect fish and other organisms (Olaji et al 2014).

According to Lam & Gray (2003), biomarkers are relatively effective in revealing overall toxicities of complex mixtures, particularly those that are at a high level of a biological organization such as physiological biomarkers relating to the growth or reproduction of individual organisms. The natural factors and the biology of a species needs to be known to interpret ecotoxicological data. The abundant literature on energetics in polychaetes provides a convenient basis (Durou et al 2007).

Therefore, in the present study, three sites (El Kala, Annaba, Skikda) located on the Algerian eastern coast were selected because of the distinct nature of the pollution sources present. The present study aimed to establish a seasonal assessment of the marine environment quality and a putative contamination gradient between the three sites. For this purpose, a morphometric study of seaworms was performed. The bioavailability and the bioaccumulation of total hydrocarbon, and the biochemical responses of *P. cultrifera* were assessed and related to seasons and sites. The overall results improve our knowledge on the influence of oil pollution, monitoring the potential impact of anthropogenic activities on the water quality of the Algerian east coast.

Material and Method

Sampling sites. The studied area extends along the eastern coast of Algeria, from El Kala to Skikda. As presented in Figure 1, three sampling sites were selected concerning the main identified pollution sources. Site 1 was El Morjan beach, El Kala (S1: 36°53'52.7"N; 8°26'10.7"E). It served as a reference site for the study, because of its remoteness from any polluting source. The site was classified as a biosphere reserve by UNESCO in 1990. Site 2 was St. Cloud Beach, Annaba (S2: 36°55'00.5"N; 7°46'05.8"E). St. Cloud beach is located near a commercial harbor, which is characterized by the presence of urban and anthropogenic pollutants. Site 3 was Bikini beach, Skikda (S3: 36°53'54.5215"N; 6°52'48.229"E) and it was characterized by the presence of pollutants, such as total hydrocarbons, by the implementation of the petrochemical complex, and has more human activities.

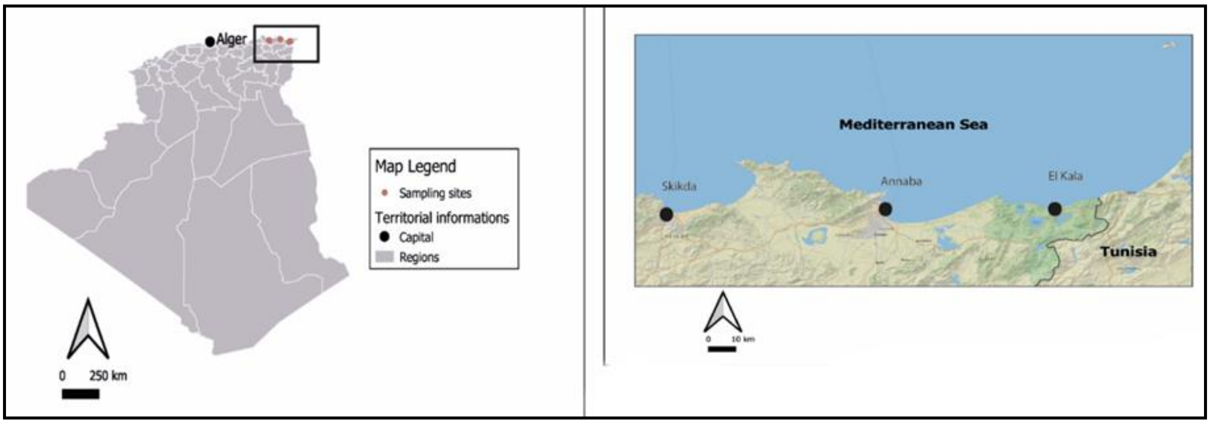

Figure 1. Location of the sampling sites along the eastern coast of Algeria (black dots).

Collection and preparation of samples. Coastal waters and *P. cultrifera* samples were collected monthly From January to July 2017 from each of the three studied sites (1050 individuals). Identification and description of species were basically focused on the count and shape of paragnath from all areas of the everted pharynx. The species were identified using the Fauvel species identification guide (Fauvel 1924). In algal-covered hard bottom, the worms prefer the low intertidal zone and extend down into the sublittoral (Scaps et al 2002). In consequence, the intertidal and shallow sublittoral hard bottoms were sampled methodically by scraping algae and looking for individuals. The fresh weight, the length, and the number of setigers of each individual were measured. The specimens were immediately washed at each site with seawater to eliminate encrusted organisms and transported in cooler boxes to the Laboratory of Applied Animal Biology (Badji Mokhtar University, Annaba, Algeria). Upon arrival, seaworms were inspected. Thirty individuals per month and site were used for biometric characterization.

Seawater characterization. Three samples of seawater from each site were collected from 3 m from the seafront, at a depth of 30 cm. 3 samples were corrected each month, from each site. For the determination of hydrocarbon, samples were collected in glass bottles transported in cooler boxes at to 4°C, to avoid contamination and sample development. According to Chapman (1996), basic hydrological parameters of surface coastal waters were monitored simultaneously at the sampling sites to provide information on the global water quality. The three samples of seawater were collected at each site while sampling. Physico-chemical parameters (temperature, pH, salinity, dissolved oxygen, and conductivity) were measured using a multiparameter (Thermo Scientific Orion StarMeter; 5 Star) to provide a preliminary assessment of the water quality.

Determination of total hydrocarbons. Samples were collected in 1.5 L glass containers, acidified to a pH of 2, and stored at 4°C. The analysis was carried out in the laboratory using an OCMA 220 type infrared spectrometer (OIl Content Analyzer). The instrumental analysis of TPHs was carried out with UV-VIS, using AFNOR T90-114 (1994) standards. Quantification was performed at 310 and 360 nm, as excitation and emission wavelengths, respectively, after Rodier et al (2009). The concentration in hydrocarbons was calculated taking into account the volumes of water and solvent given by the following equation (Rodier et al 2009):

$$X=(50\times A\times 50)/[(m2-m1)\times 20]$$

Where: X - concentration in hydrocarbons; m1 - mass of the empty container; m2 - mass of the container full of water; A - absorbance (OD).

Bioaccumulation assessment (BAF). Bioaccumulation often occurs when a chemical is absorbed and retained in an organism through all routes of exposure in the natural ecosystem (Akinola et al 2019). The bioaccumulation factor (BAF) in organisms was determined using the next equation (Akinola et al 2019):

Bioaccumulation factor = concentration of TPH in *P. cultrifera* tissue (mg kg^{-1})/ concentration of TPH in the water (mg L^{-1})

Ecological hazard assessment (EHA). High levels of ecological hazard occur when the concentrations of chemical substances in an aquatic environment are above the permissible limits. The EHA was therefore calculated using the following equation (Akinola et al 2019):

Ecological Hazard Quotient (EHA) = concentration in the environment (mg L^{-1})/ recommended limit (mg L^{-1})

According to the Executive Decree No. 06-141 of April 19, 2006, defining the limit values for discharges of industrial liquid effluents, published in the official journal of the Algerian Republic No. 26, the maximum limits of hydrocarbons must not exceed 10 mg L^{-1}.

Biomarkers. Individuals were homogenized in a solution of 38.08 mg EGTA, 0.1 mL TritonX100, 5.845 g NaCl and 80 mL Tris-HCL buffer pH 7 for AChE, and in 0.1 M phosphate buffer pH 6 for glutathione S-transferase (GST) and catalase (CAT), at 4°C. Homogenates were centrifuged at 9000×g for 15 min at 4°C for AChE activity and 13000×g for 30 min at 4°C for GST and CAT activities. The supernatant of each sample was stored at -20°C. Acetylcholinesterase activity was determined according to Ellman et al (1961) and results were expressed as nanomoles (AtChl) hydrolyzed per minute per mg protein. (GST) activity was determined according to Habig et al (1974), and results were expressed as micromoles produced per minute per mg protein. (CAT) activity was determined according to Greenwald (1985) and results were expressed as micromoles H_2O_2 consumed per minute per mg protein. The malondialdehyde (MDA) was assayed according to the method of Draper & Hadley (1990). The method is based on the colorimetric measurement of the reaction between thiobarbituric acid (ATB) and (MDA), and results were expressed as nanomoles per mg of protein. The total protein content in the homogenate was determined according to Bradford (1976), at 595 nm using Bovine Serum Albumin as standard.

Statistical analysis. Data was expressed as mean (± standard deviation) and checked for variance homogeneity by Levene's test and for distribution normality by Kolmogorov-Smirnov or Shapiro-Wilk's test, depending on the number of samples. Significant differences were assessed by one-way or two-way analysis of variance (ANOVA) followed by the post-hoc Tukey test, if the conditions were met, or with non-parametric tests: U-Mann-Whitney. The principal component analysis (PCA) was applied to establish relationships among total hydrocarbon concentrations and abiotic parameters. A multivariate analysis was performed to compare the biomarker responses, hydrocarbon levels, and abiotic parameters. According to PCA, after using a KMO test (Kaiser-Meyer-Olkin Measure of Sampling Adequacy) (KMO=0.65) and Bartlett's test of sphericity with significance (p=0.000) we could confirm a strong use of the PCA. The significance level was at p<0.05. All the statistical analyses were performed using SPSS v. 20 software (SPSS Inc., Chicago IL, USA).

Results and Discussion

Morphometric parameters. The mean fresh weight of individuals collected from the three study sites increased from January to April, when the highest values were recorded (0.21±0.065 g; 0.097± 0.01 g and 0.13±0.047 g for El Kala, Annaba and Skikda, respectively), then it decreased to July (0.13±0.07 g, 0.07±0.06 and 0.089±0.05 g for El Kala, Annaba and Skikda, respectively) (Figure 2a). The mean fresh weight was significantly higher in El Kala compared to Annaba and Skikda throughout the studied period (Figure 2a). There was a significant difference between worms from the three sites (p=0.000).

The mean length of individuals collected from the three study sites increased from February to May (4.1±0.78 cm, for El Kala, 3.52±0.82 cm for Annaba, and 3.48±0.49 cm for Skikda) then it decreased (Figure 2b). The mean length was significantly higher in El Kala compared to Annaba and Skikda throughout the studied period, except in July. There was no significant difference between individuals collected in Annaba and Skikda, excepting March and May (Figure 2b).

The mean number of setigers of individuals collected from the three study sites increased from February to April (59.06±17.37; 35.4±13.05 and 45.2±9.09 setigers for El Kala, Annaba and Skikda, respectively). It decreased to July (35.5±12.22; 35.93±11.52 and 31.1±5.8 setigers for El Kala, Annaba and Skikda, respectively). The mean number of setigers was significantly higher in El Kala compared to Annaba and Skikda in March, April, and May, but no significant differences were observed in the other months (Figure 2c). The mean number of setigers was not significantly higher in Annaba compared to Skikda throughout the studied period.

Seawater characterization. The physico-chemical parameters were measured *in situ* throughout the study at the three study sites. We did not observe any significant differences between the studied sites for seawater temperature, pH, and salinity (Table 1). For dissolved oxygen (DO), we observed a significant difference between the study sites (p=0.004) (Table 1). DO at El Kala was significantly higher than in Annaba and Skikda. No significant difference was observed between Annaba and Skikda.

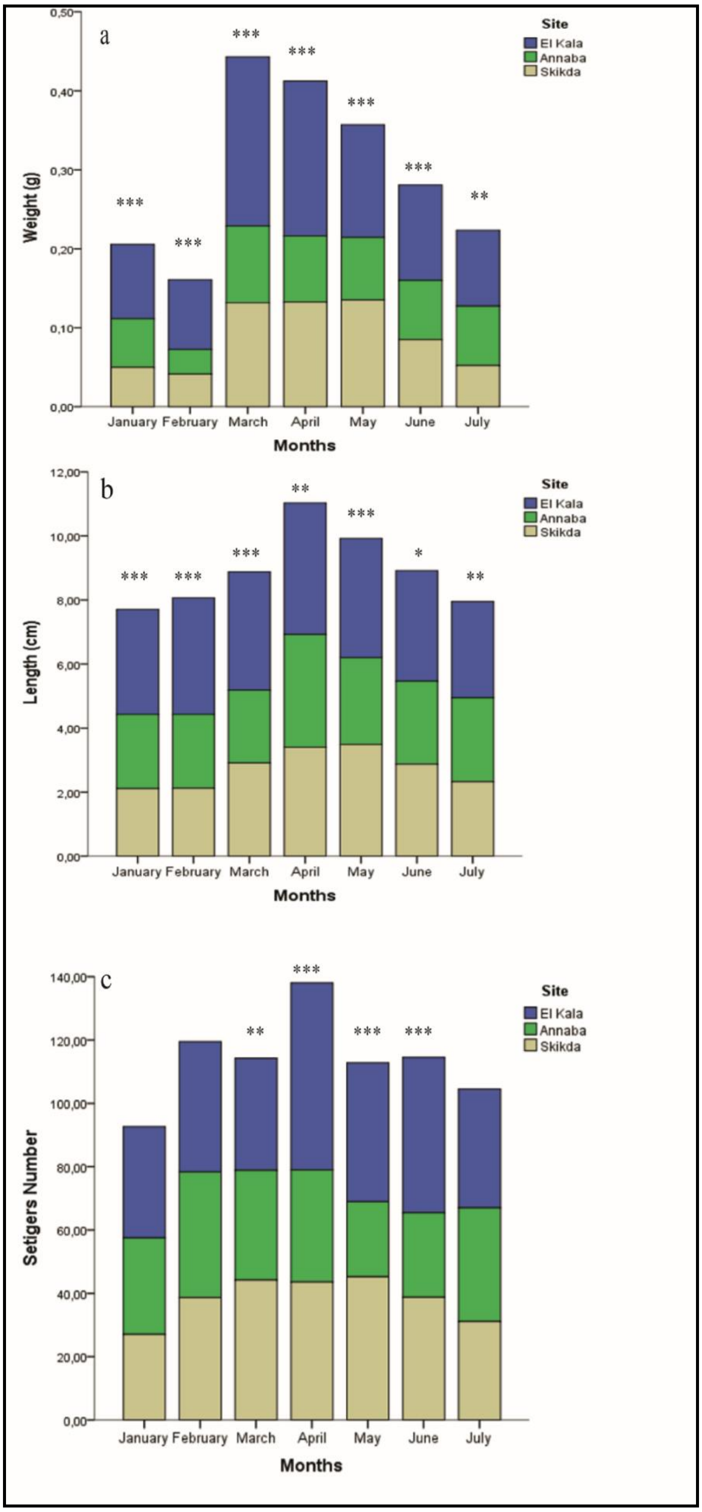

Figure 2. Monthly variations of morphometric parameters for individuals collected in El Kala, Annaba and Skikda from January to July 2017; a - mean fresh weight (g); b - mean length (cm); c - mean number of setigers; * - p<0.05; ** - p<0.01; *** - p<0.001.

Determination of total hydrocarbons. Samples collected at Skikda presented the highest total hydrocarbons concentrations. There was a significant difference between concentrations of total hydrocarbons in the three study sites, both in organisms and water (p=0.000) (Table 1).

Table 1

Water physicochemical parameters for the study sites and total hydrocarbon concentrations

Water parameters		*Site*		
		El Kala	*Annaba*	*Skikda*
pH	Mean	7.97	8.14	8.21
	SD	0.18	0.20	0.20
Temperature (degrees Celsius)	Mean	20.24	21.86	20.21
	SD	6.20	6.07	5.04
Salinity (ppt)	Mean	40.67	38.93	39.26
	SD	3.93	3.32	0.66
Dissolved Oxygen (mg L^{-1})	Mean	10.5***	6.86	6.90
	SD	2.62	1.76	1.08
Conductivity (ms cm^{-1})	Mean	47.60	53.21	51.22
	SD	5.76	6.07	4.58
THC in water	Mean	1.87***	2.68***	5.52***
	SD	0.14	0.20	0.16
THC in organisms	Mean	0.42***	1.25***	3.23***
	SD	0.05	0.04	0.05

Note: each data point represents mean±standard deviation (n=3); THC - total hydrocarbon concentration; * - p<0.05; ** - p<0.01; *** - p<0.001.

Relationship between TPH in water and P. cultrifera. A Shapiro-Wilk test of independent samples for hydrocarbons in water and *P. cultrifera* at 95% confidence level is presented in Table 2. The test reveals a significant relationship between TPH in *P. cultrifera* and water, suggesting the rejection of the null hypothesis. The relationship between the presence of hydrocarbons in *P. cultrifera* and water suggests that the chemicals were derived exogenously.

Table 2

Relationship between total hydrocarbon concentration in water and *Perinereis cultrifera* (Shapiro-Wilk's test)

Total (n)		*63*
Most extreme differences	Absolute	0.964
	Positive	0.964
	Negative	0.338
Signification		0.05

Bioaccumulation assessment (BAF). The factor of bioaccumulation of total hydrocarbons in the worms in the three study sites was significantly different (p=0.000) throughout the months, as presented in Figure 3. It can be observed that the reference site El Kala had the lowest values compared to the other two sites, Annaba and Skikda. The maximum values were observed at Skikda (0.61), and the lowest at El Kala (0.17).

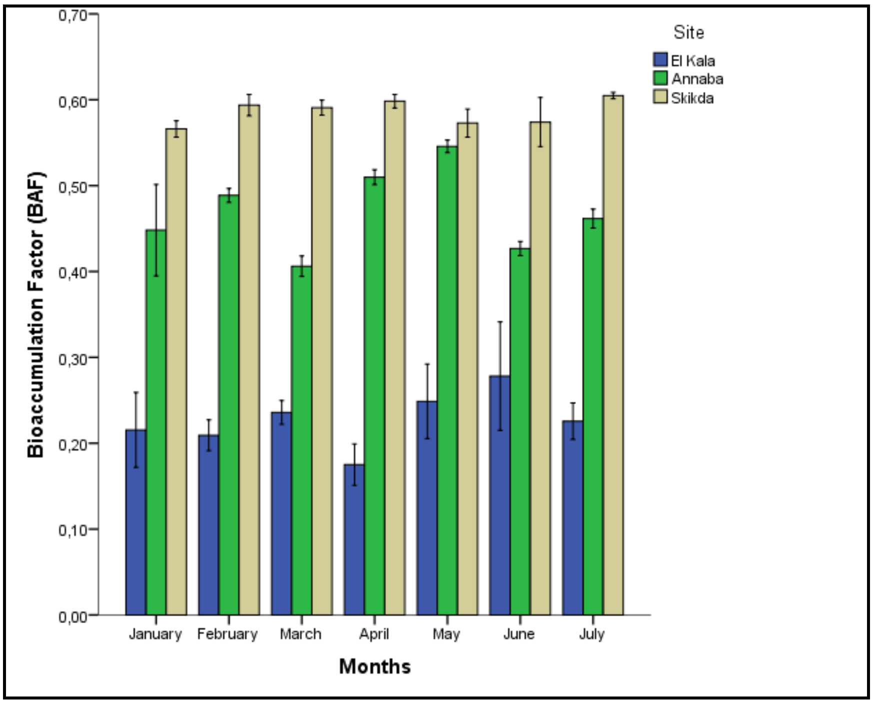

Figure 3. Bioaccumulation factor (BAF) in *Perinereis cultrifera* during the study period at the three sites.

Ecological hazard assessment (EHA). The ecological risk indices of TPH are presented in Figure 4. The results show a very high significant difference between the sites (p=0.000). All the hydrocarbons examined at the Skikda site presented significant ecological risks throughout the months, as they were very close to the tolerable range of 1. The highest risk values were recorded in January and May (0.68) in Skikda, and the lowest in May (0.16), in El Kala.

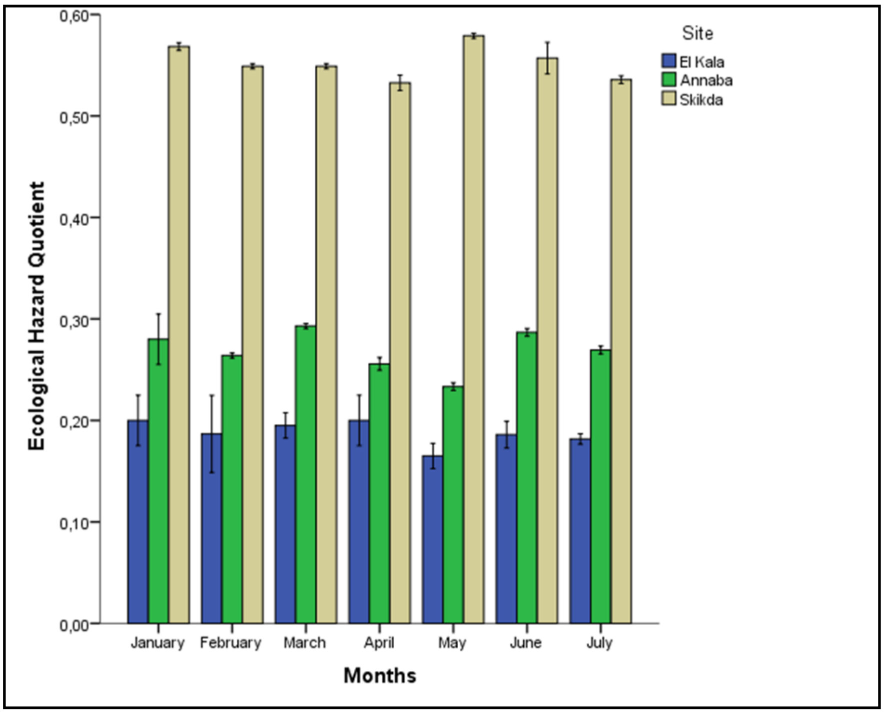

Figure 4. Ecological hazard assessment of hydrocarbons from *Perinereis cultrifera* in the three study sites.

AChE activity. AChE activity remained more or less stable from February to April in each site (Figure 5a). The higher values of AChE activity were observed in June and July, at the three study sites (32.72±0.13, 24.27±0.14 and 17.96±0.17 nmol min^{-1} mg^{-1} protein for individuals collected in El Kala, Annaba and Skikda, respectively), while the lower values were observed in March (12.64±0.18, 8.94±0.15 and 7.64±0.22 nmol min^{-1} mg^{-1} protein for individuals collected in El Kala, Annaba and Skikda, respectively). AChE activity was significantly higher in El Kala compared to Annaba and Skikda throughout the studied period (Figure 5a). AChE activity was significantly higher in Annaba compared to Skikda throughout the studied period.

GST activity. GST activity remained more or less stable. The lowest values were at El Kala. GST activity increased in early summer (Figure 5b). The higher values of GST activity were observed in July at the three study sites (6.39±0.09, 8.68±0.11 and 9.50±0.11 µmol min^{-1} mg^{-1} protein for individuals collected in El Kala, Annaba and Skikda, respectively). The lowest values were observed in May (2.43±0.07, 4.65±0.18 and 8.31±0.14 µmol min^{-1} mg^{-1} protein for individuals collected in El Kala, Annaba and Skikda, respectively). GST activity was significantly lower in El Kala compared to Annaba and Skikda throughout the studied period (Figure 5b). GST activity was significantly higher in Skikda compared to Annaba throughout the studied period.

CAT activity. We did not observe a seasonal variation of CAT activity at the three studied sites (Figure 5c). CAT activity was significantly higher in Skikda compared to Annaba and El Kala, and in Annaba compared to El Kala (Figure 5c).

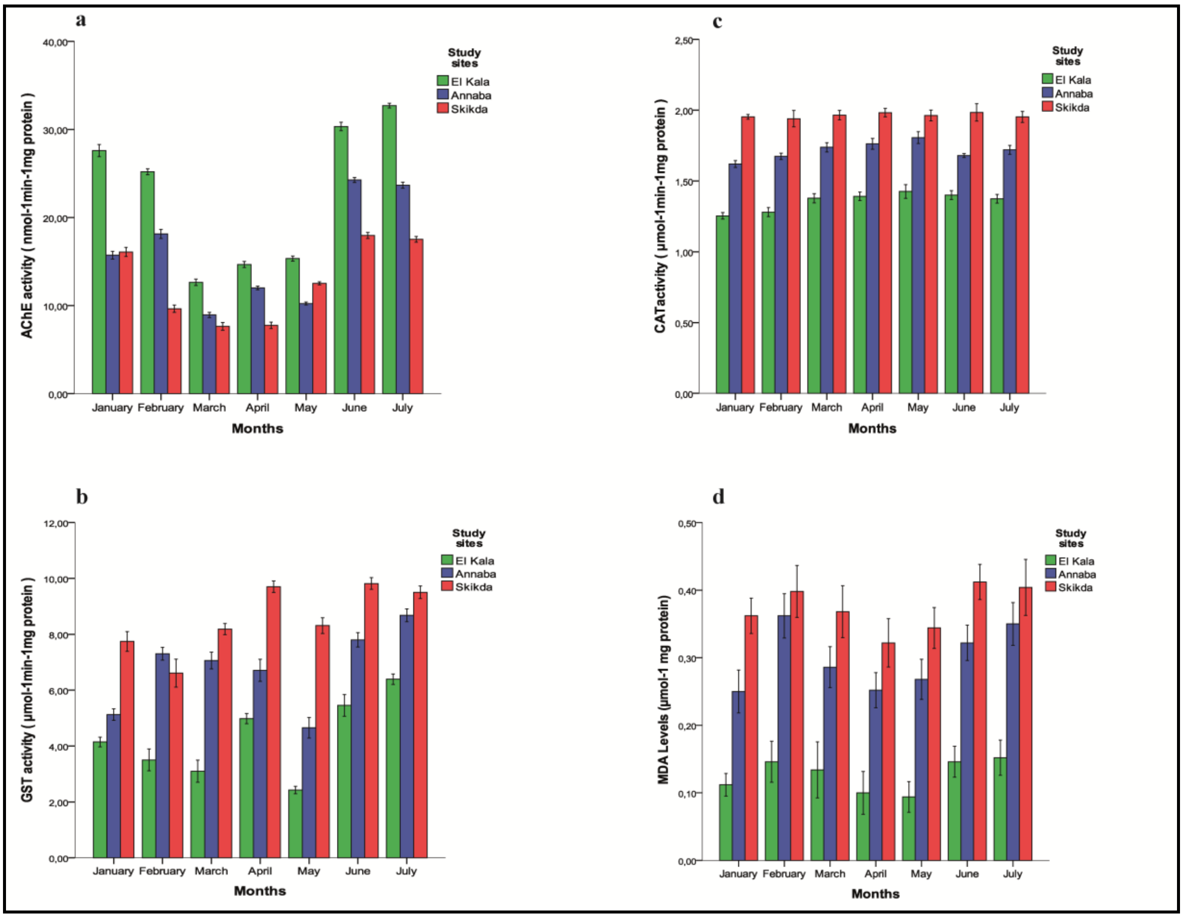

Figure 5. Monthly variations of biochemical markers of individuals according to sampling sites from January to July 2017; a - acetylcholinesterase activity (nmol min^{-1} mg^{-1} protein) ; b – glutathione S-transferase (µmol min^{-1} mg^{-1} protein) ; c - catalase activity (µmol min^{-1} mg^{-1} protein); d - malondialdehyde levels (µmol $^{-1}$ mg protein). Each data point represents mean ± standard deviation (n=5).

MDA levels. The MDA levels results revealed significant differences among polychaetes from the three sites (one-way ANOVA: $p<0.05$), being statistically higher in organisms from Skikda and Annaba when compared to El Kala (Figure 5d).

Relationships among biomarker's responses, total hydrocarbons, and abiotic parameters. In our study, two main components described 67.009% of the variation (Figure 6). Each component is described according to the dominant group of variables. The first principal component accounting for 44.109% of the variances was associated with hydrocarbon content, as well as an increase in biomarker activities. Only AChE and DO were represented by the second component. Correlations were established to assess the relationships between total hydrocarbon contents in samples of water and organisms and biochemical responses in *P. cultrifera* (Figure 6).

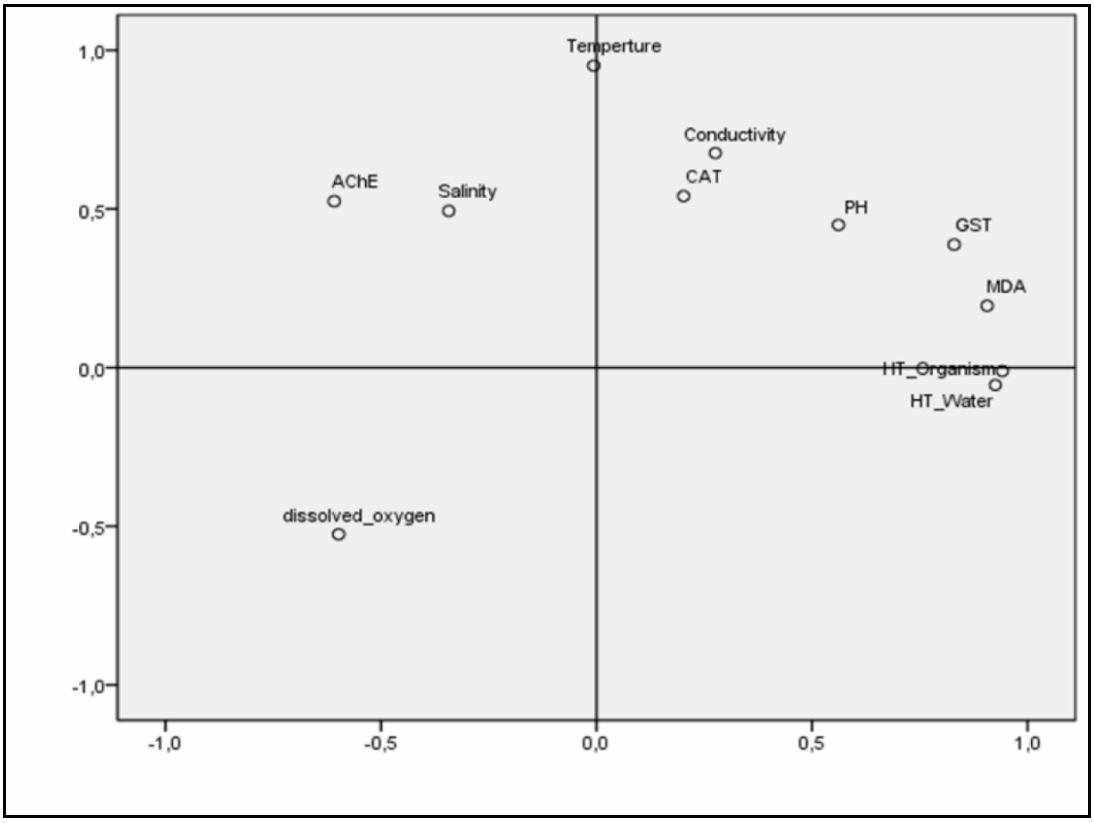

Figure 6. Relationship among biochemical, geochemical, and abiotic parameters via a representation on the correlation circle using a principal component analysis (PCA).

The results showed that total hydrocarbons were strongly correlated to GSTs and MDA activities, and negatively correlated with AChE activity. We observed a negative correlation between pH and DO. The position of stations and variables in a biplot is presented in Figure 7. Skikda site differs from the two other sites with its highest levels of TPH being associated with increased biological alterations. El Kala had the highest values for DO and AChE activity.

Due to their life-history characteristics and the relatively rapid response to pollution, polychaetes are frequently used to evaluate the impact of environmental disturbances in aquatic systems (Maranho et al 2014). However, almost no information is available on their capacity to reflect toxicity effects induced by the presence of pollutants in aquatic systems (Maranho et al 2014). For this reason, the present study evaluated the effects induced by total hydrocarbons on *P. cultrifera*. These compounds are widely distributed in coastal regions, and contamination by hydrocarbons is increasing yearly due to anthropogenic activities (Feknous et al 2017).

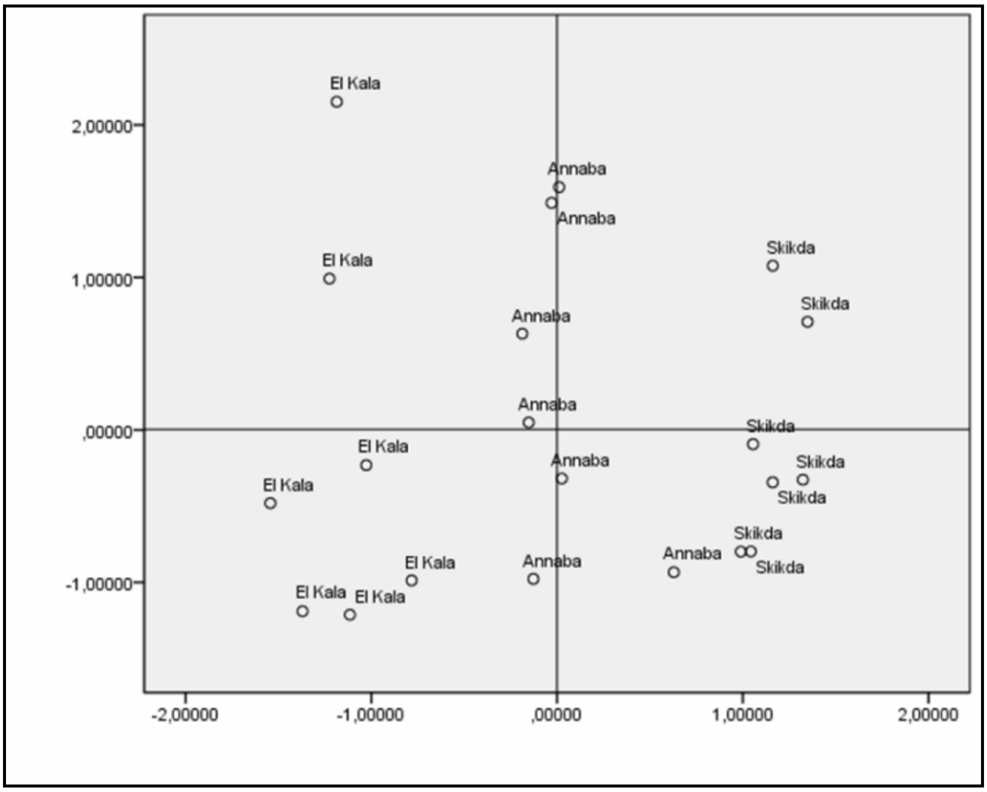

Figure 7. Principal component analysis correlation biplot based on position of sites and variables.

In the present study, the overall physico-chemical parameters were within the same range of values for all three sites. These values were similar to those reported in previous studies performed in El Kala, Annaba and Skikda (Guemouda et al 2014). We noticed a decrease in the DO level in water samples collected from sites with pollutants (Annaba and Skikda). DO is consumed during heterotrophic oxidation of the organic material, and respiration by aquatic fauna and flora. All measured total hydrocarbon concentrations in water and species clearly showed a higher level in the Skikda site compared with the other sites. We observed that Annaba had higher values compared to El Kala. These results are similar to those obtained by Guemouda et al (2014) and Saker (2007). The major alterations found in exposed organisms could result from the presence of total hydrocarbon with high toxicity.

The examined organisms indicated a lower level of bioaccumulation when compared with the standard limit. This could be explained by the low chemical concentration in water. The studied species does not possess the potential to metabolize the chemical substance in the aquatic ecosystem (Arnot & Gobes 2006).

The mean fresh weight, length, and the number of setigers decreased in individuals collected from the polluted sites. Previous studies using the same species showed that the mean fresh weight of worms collected in a polluted site was lower than those of organisms collected in reference sites (Zoubeida et al 2015). The length of animals followed the same principle in our study. The only difference in our work compared with these studies was in the number of setigers, as presented in Figure 2c, with a non-significant difference.

The use of biomarkers integrative approach could provide some additional information that cannot be obtained otherwise, like from a chemical analysis of pollutant concentrations, and they can integrate the effects of mixtures of chemicals over long exposure periods, without analyzing many different chemicals (Lionetto et al 2019).

AChE is involved in nerve impulse termination, and its inhibition is an established biomarker of neurotoxicity caused by exposure to a wide range of contaminants other than pesticides, such as metals, treated effluents and TPHs (Ghedira et al 2009). Several studies showed that petroleum contaminants generally inhibit AChE activity. Moreover, studies on invertebrates and fish reported this enzyme's inhibition after the exposure to fuel oil or PAHs. An AChE inhibition was observed in *Mytilus galloprovincialis* and *Mytilus edulis* after an oil spill and in individuals of Nile tilapia (*Oreochromis niloticus*) (De Lima et al 2013). Our obtained results in terms of AChE activity are in agreement with previous works and have shown an overall inhibition of this parameter in *P. cultrifera* individuals from the two contaminated sites. We suggest that Annaba and Skikda caused significant AChE inhibition, which is characterized by higher concentrations of total hydrocarbons compared with the reference site El Kala. Thus, it is thus possible to associate exposure to the predominant contaminants measured in our samples with the inhibition of the main cholinesterase in our test species, which can be considered as a clear indication of neurotoxicity. However, issues like ecological representability of the test species may be considered. Among the few studies that focused on the Algerian east coast, the report of Feknous et al (2017) demonstrated the occurrence of contamination in this area. However, the study did not assess the profile contamination of our specific sampling sites and was not aimed at using biomarkers assays with polychaetes species. Daas et al (2011) presented a report on the application of a classification scale based on biochemical markers on worms of the species *P. cultrifera* along the Algerian east coasts, which preceded the works of Guemouda et al (2014) and Zoubeida et al (2015).

Regarding biochemical responses, the significant differences in GST activity in Skikda and Annaba worms, compared to those from El Kala are similar to those found in the same species in other contaminated sites (Banni et al 2009). This result reflects the greater detoxification activity of this species, triggered by the exposure to TPH. It is also consistent with other studies on estuarine species and it establishes the characteristic of induction of GST to nereid contaminants (Elumalai et al 2007). Moreover, the metabolism of polychaetes regarding TPHs should also be considered as a potential GST inducer of phase II detoxification enzyme.

CAT is a fundamental antioxidant enzyme that takes part in the defense against oxidative stress, since it controls the amount of hydrogen peroxide (H_2O_2), and results from normal and xenobiotic-induced metabolic processes (Ferreira et al 2005). The contribution of hydrocarbon pollutants to the establishment of oxidative conditions cannot be discarded. A significant positive correlation of CAT activity with anthracene concentration in sediment was observed in *Pomatoschistus microps* fish species (Vieira et al 2008). *Hediste diversicolor* exposed to increasing sub-lethal concentrations of TPH also had higher concentrations of CAT (Catalano et al 2012). This can suggest that TPH may induce oxidative stress requiring the activation of catalase activity.

The level of MDA is widely used as an indicator of oxidative stress in invertebrates. Therefore, chemical changes in lipids and alterations in the activity of antioxidant enzymes may confirm the presence of induced oxidative stress in organisms (Carregosa et al 2014). The increased MDA levels in seaworms from Skikda and Annaba reflects the inability of the antioxidant defenses to overcome the pro-oxidant charge directly or indirectly induced by the high levels of pollutants in the biotope. We found a similarity with previous works on nereid species from the polluted estuary (San Giuliano, iItaly), where higher MDA levels were found in a station close to the mainland industrial site of Porto Marghera compared to other stations (Nesto et al 2010). The seasonal variability of this "oxidative damage" endpoint from historical multi-polluted estuarine sites is evident and should be considered to avoid misinterpretation in terms of biomonitoring.

Thus, monitoring pollution in the Algerian eastern coast area using a scale of classification based on biochemical markers in sentinel organisms like worms (e.g., of the species *P. cultrifera*, or other autochthonous polychaete species) may contribute to a better comprehension of the real toxicological risk in the investigated sites. Until today, the number of studies concerning this area of the Mediterranean Sea is still scarce and demands the adoption of new testing frameworks, encompassing the large biodiversity in the area.

Conclusions. This study suggests that *P. cultrifera* can be used as a good biological indicator for petroleum hydrocarbon pollution in water. Therefore, it is important to note that the responses of biochemical biomarkers depend on the analyzed tissue and sampling sites, and on the need for a judicious selection of an adequate species to serve as a test organism. This study also underlined the establishment of potential causal relationships between biomarkers and total petroleum hydrocarbon. These relationships were more pronounced in industrial areas. It is recommended that the addition of sites along with a wider selection of sizes and a greater number of worms would give considerable insight into the relationships between biomarkers, worm weight, and TPH concentrations in the environment. Finally, additional work is still needed to formulate and conduct a continuous monitoring program, and ensure that the concentrations of total petroleum hydrocarbons are within the baseline levels established in the present study.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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