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Monitoring pollution in East Algerian coasts using biochemical markers in the polychaete annelid *Perinereis cultrifera*

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ABSTRACT

The aim of this study was to assess the marine environment quality along the east Algerian coasts using an approach based on biomarkers in the polychaete Perinereis cultrifera. Individuals were collected from three sites (Skikda, Annaba and El-Kala) based on the level of pollution. The non urbanized site of El-Kala was considered as a healthy reference site. The biomarkers selected during this study were the activities of acetylcholinesterase as neurotoxicity marker, glutathione S-transferase as phase II enzyme and catalase as oxidative stress marker. The results show differences between sites compared with the reference samples. This approach confirms that individuals from Skikda and Annaba have been submitted to highly polluted environment. Individuals collected from Skikda show the highest reduction of the mean fresh weight, length and number of setigers as well as the highest inhibition or induction of enzyme activities indicating a highly contamination status.

Keywords: *Perinereis cultrifera*, Pollution, Biomonitoring, Biomarkers, Eastern Algeria.

INTRODUCTION

Polychaete annelids are well represented in marine environments and constitute a significant percentage of the total biodiversity and abundance of benthic macrofauna. Polychaetes are the dominant macrofauna within fine sediments [1] and are commonly used in toxicological studies [2, 3]. The polychaete *P. cultrifera* (Nereididae) was described for the first time by Grube (1840) from the Adriatic Sea. It occurs along the north-western coasts of Europe and the Mediterranean. This species has also been described in the Indian and Pacific Oceans [4, 5, 6]. According to the geographical location of the populations, mode of reproduction differs largely [7]. Reproduction in the English Channel and the Atlantic is of an epitokous type [8, 9, 10, 11] as in the Mediterranean Sea at Salammbô near Tunis, at Annaba on the Algerian Mediterranean coast near the Tunisian border and in the Venice Lagoon in Italy [12, 13, 14]. However, on the west coast of Algeria in the Bay of Algiers, the reproduction has been described as atokous [15, 16], as on the Moroccan Atlantic coast [17] and the Gulf of Marseille [18]. *P.cultrifera* is an intertidal poor disperser polychaete. It is reported to be a free-spawner. According to [19] *P. cultrifera* has a bentho-pelagic life cycle with a brief semi-pelagic phase. Eggs are large (egg diameter 350 µm), lecithotrophic and demersal. Hatching occurs at the 3-setiger erpochaete stage. Larvae exhibit a little developed ciliary crown and often crawl on the bottom. At the end of the semi-pelagic phase, animals become sedentary at the 4-setiger erpochaete stage. Then

erpochaeta loose their ciliary crown and thus are completely benthic. The juvenile, benthic worm of 10 or 11 segments has the same life style as the adult.

The pollution of marine environments by the vast number of xenobiotics has increased during the last decade as a direct consequence of a wide variety of anthropic activities [20 21]. Such contamination represents a serious threat to the overall health of aquatic ecosystems [22]. The multibiomarker approach appears to be effective in estimating the toxicity of complex mixtures [23, 24, 25, 26, 27]. Acetylcholinesterase (AChE) is an enzyme essential to the correct transmission of nerve impulses. An inhibition of this enzymatic activity has been used to detect and measure the biological effects of organophosphorus and carbamates in the marine environment [28]. Moreover, AChE may be also inhibited by heavy metals [29, 30, 31]. The glutathione-S-transferases (GSTs) are a multiple-enzyme family involved in phase II detoxification processes and are used as biomarkers of organochlorine pesticides and PCBs pollution in invertebrates [28, 32, 33, 34]. Catalase (CAT) is an anti-oxidant enzyme that is used as a marker involved in the primary defense against oxidative damage [35].

The aim of this study was to test if the polychaete *P. cultrifera* could be used as a bioindicator in biomonitoring programs on the eastern coast of Algeria. In order to test this hypothesis we studied the effects of environmental pollution on AChE, GST and CAT activities. We also measured indicators of physiological (mean individual body weight, mean length and mean number of setigers) status.

MATERIALS AND METHODS

Sampling sites

Sampling sites were chosen because of their geographical locations in eastern Algeria (Fig. 1). Site selection was based on the level of pollution as well as ease of access to the study area and abundance of species. El-Kala (36°53'44" N; 08°26'35" E) is close to the Tunisian border (10 km). This site is part of a national park and is not urbanized, therefore, it was considered as the healthy reference site. Annaba (36°54'27" N; 7°45'26" E) is located about 80 km from the Tunisian border. This site is exposed to the pollution by pesticides and/or heavy metals released from the FERTIAL factory and the port activities [36]. Skikda (36°45'0" N; 06°49'60" E) is located about 180 km from the Tunisian border. This site is near from Stora port that is characterized by an intense maritime traffic. This site is exposed to the pollution by PAH due to the presence of an important petrochemical complex.

Seawater temperature, pH, dissolved O_2 and salinity were measured with a Multiparameter-Oxymeter (Multi 340 i/SET) at each site monthly from January 2011 to December 2011.

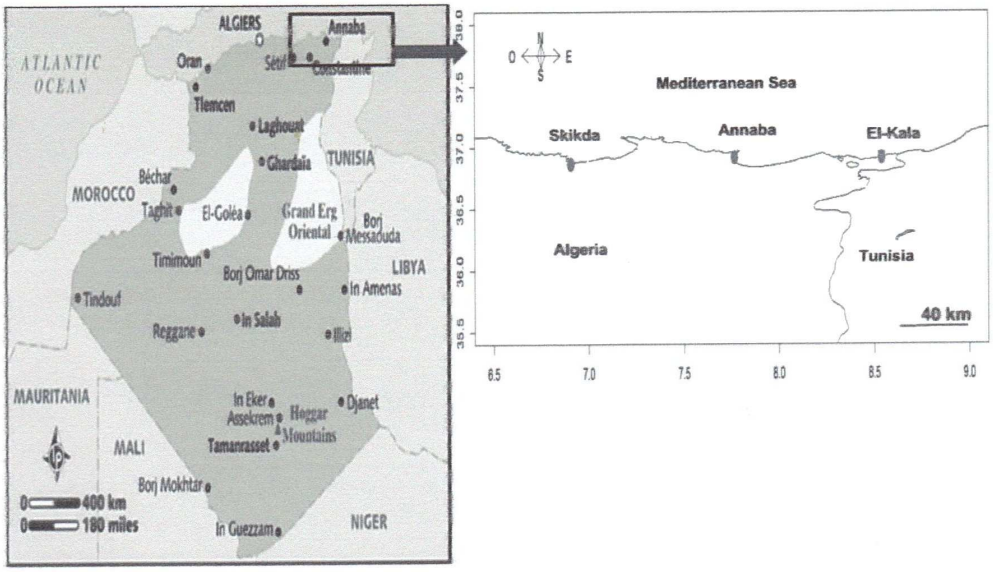

Fig. 1. Location of the different sampling sites along the east Algerian coast: El-Kala, Annaba, Skikda

Collection of individuals
Individuals were collected monthly from January to December 2011. They were found within Rhodophyceae, in algal-covered hard bottoms. They occur low in the intertidal zone and extend down into the sublittoral; in consequence, the intertidal and shallow sublittoral hard bottoms were sampled methodically by scraping algae and looking for individuals [14]. The fresh weight, the length and the number of setigers of each individual were measured.

Biochemical analyses
Individuals were homogenized at 4°C in a solution containing 38.08 mg EGTA, 0.1 ml Triton X 100, 5.845 g NaCl and 80 ml Tris/HCl buffer pH 7 for AChE and in 0.1 M phosphate buffer pH 6 for GST and CAT. Homogenates were then 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. Total protein content in the homogenate was determined according to Bradford [37] at 595 nm using Bovine Serum Albumin as standard.

Acetylcholinesterase activity
AChE activity was determined according to Ellman et al. [38]. Reaction mixture contained 0.1 M sodium phosphate buffer (pH 7.5), 8 mM 2.4- dinitroochiocyanatebenzene and the stock cytosolic solution containing acetylcholinesterase fractions. After pre-incubation, the reaction was started by addition of 8.25 mM acetylthiocholine (AtChl) as substrate. AChE activity was determined by kinetic measurement at 420 nm. Results were expressed as nanomoles (AtChl) hydrolyzed per minute per milligram protein.

Glutathione S-transferase activity
Glutathione S-transferase (GST) activity was determined according to Habig et al. [39] using 1-chloro-2,4-dinitrobenzene as substrate and glutathione (1 and 4 mM final concentration, respectively) in 100 mM sodium phosphate buffer, pH 7.5. All GST activity assays were realized in conditions of linearity with respect to incubation time. Results were expressed as micromoles produced per minute per milligram protein.

Catalase activity
Catalase (CAT) activity was determined according to Clairbone [40] measuring the rate of enzymatic decomposition of H_2O_2 determined as absorbance decrements at 240 nm. The assay mixture consisted of 750 µl of sodium phosphate buffer (0.1 M, pH 7.5 and 25°C), 200 µl solution of 0.5 mM H_2O_2 and 50 µl of cytosolic fraction. Results were expressed as micromoles H_2O_2 consumed per minute per milligram protein.

Statistical analysis
The results were expressed as means ± standard deviation (S.D). The normality of the distribution was tested using the Shapiro-Wilk test. To assess multiple comparisons, a parametric one-way analysis of variance (ANOVA) was performed on data with a Tukey's test. Statistical significance was defined at the $p \leq 0.05$ level. Statistical analysis was performed using Minitab (2000) Statistical Software version 13.31.

RESULTS

Abiotic parameters
The physico-chemical parameters were measured *in situ* throughout the duration of the study at the three study sites. We did not observed any significant differences between the study sites for seawater temperature, pH, dissolved O_2 and salinity (Fig. 2).

Indicators of physiological status
Mean fresh weight
The mean fresh weight of individuals collected from the three study sites increased from January to April (0.306 ± 0.001; 0.248 ± 0.004 and 0.191 ± 0.005 g for El-Kala, Annaba and Skikda respectively), then it decreased to August (0.098 ± 0.002; 0.085 ± 0.001 and 0.074 ± 0.004 for El-Kala, Annaba and Skikda respectively) and slightly increased again to December (Fig. 3).

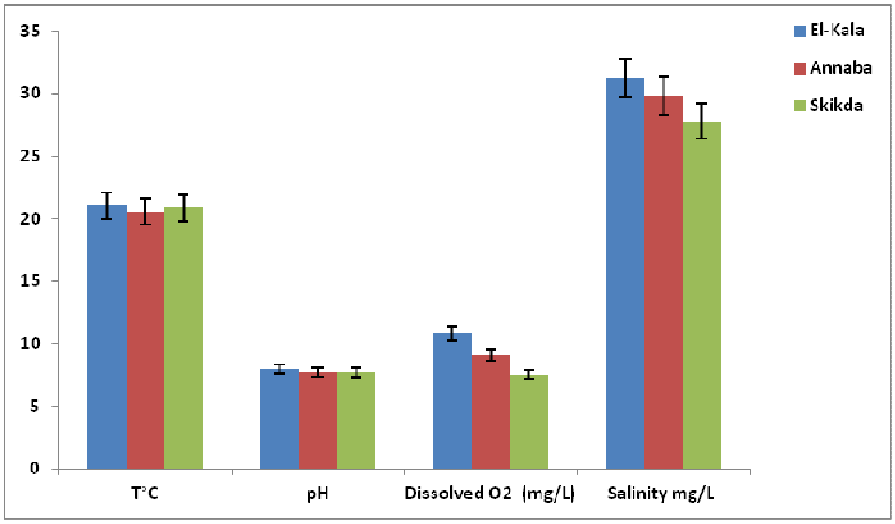

Fig. 2. Seawater mean physico-chemical parameters from El-Kala, Annaba and Skikda during 2011. Each data point represents mean ± standard deviation (n=12)

The mean fresh weight was significantly higher in El-Kala compared to Annaba and Skikda throughout the studied period excepted in February for individuals collected from El-Kala and Annaba (Fig. 3). The mean fresh weight was significantly higher in Annaba compared to Skikda throughout the studied period excepted in September.

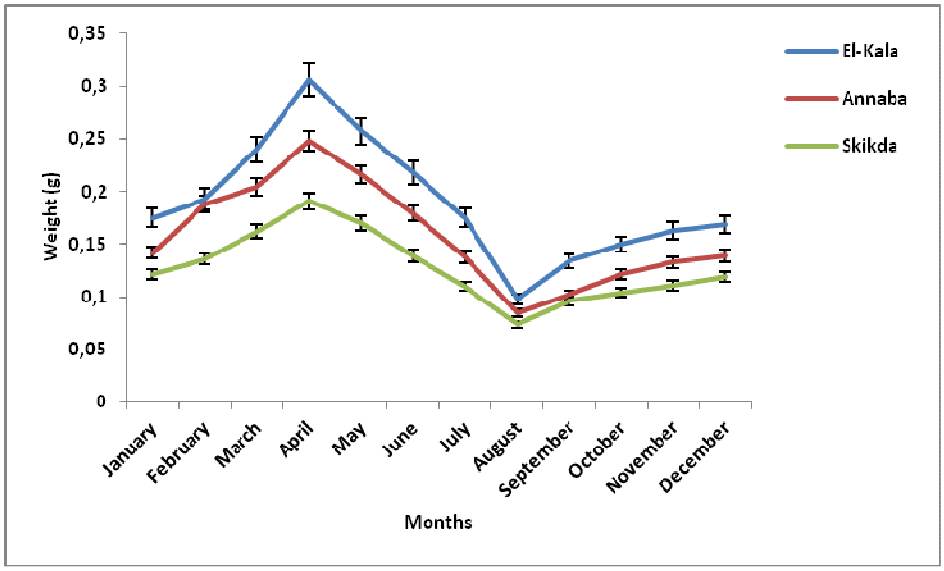

Fig. 3. Monthly variations of the mean fresh weight (g) for individuals collected in El-Kala, Annaba and Skikda from January to December 2011
Each data point represents mean ± standard deviation (n=3)

Mean length

The mean length of individuals collected from the three study sites increased from February to May (51.33 ± 1.52; 47 ± 1 and 42 ± 1 mm for El-Kala, Annaba and Skikda respectively) then it decreased to August (30 ± 1; 27 ± 1 and 21.33 ± 1.52 mm for El-Kala, Annaba and Skikda respectively) and slightly increased again to December (Fig. 4).

The mean length was significantly higher in El-Kala compared to Annaba and Skikda throughout the studied period excepted in March, August and October for individuals collected from El-Kala and Annaba, and in July for

individuals collected from the three sites (Fig. 4). The mean length was significantly higher in Annaba compared to Skikda throughout the studied period excepted in June.

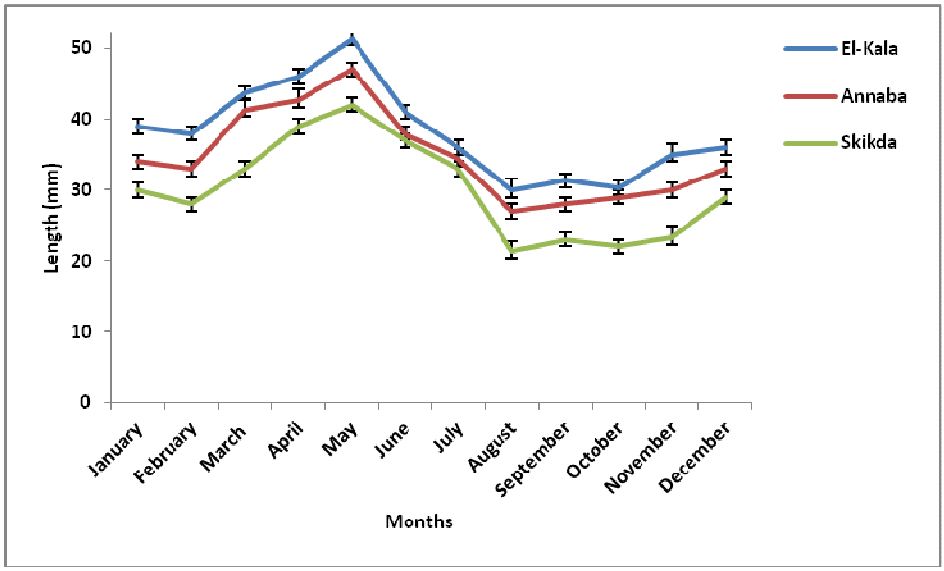

Fig. 4. Monthly variations of the mean length (mm) for individuals collected in El-Kala, Annaba and Skikda from January to December 2011

Each data point represents mean ± standard deviation (n=3)

Mean number of setigers

The mean number of setigers of individuals collected from the three study sites increased from January to April (75.66 ± 4.93; 67 ± 2 and 54.33 ± 1.52 setigers for El-Kala, Annaba and Skikda respectively) then it decreased to August (36.33 ± 1.52; 32.33 ± 1.52 and 24.66 ± 2.51 setigers for El-Kala, Annaba and Skikda respectively) and slightly increased again to December (Fig. 5).

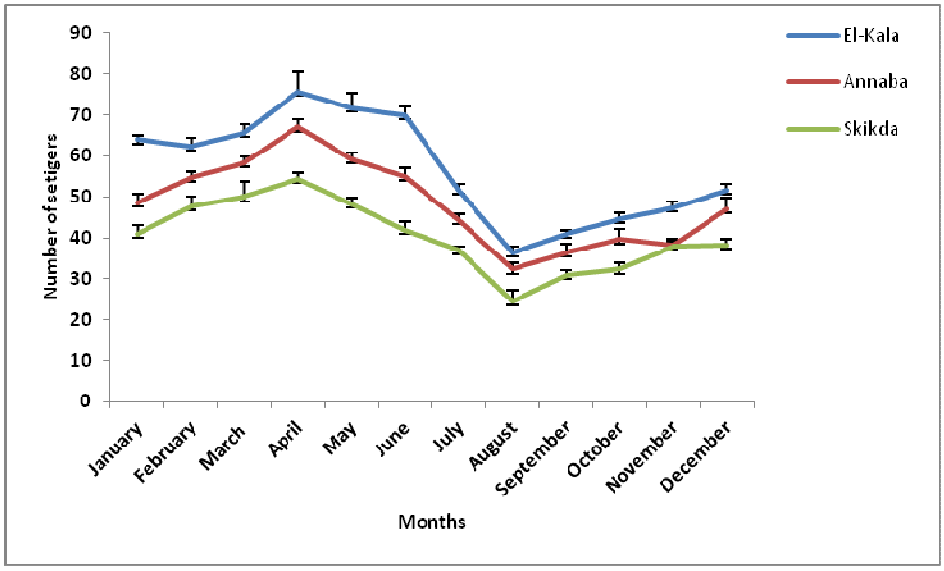

Fig. 5. Monthly variations of the mean number of setigers for individuals collected in El-Kala, Annaba and Skikda from January to December 2011

Each data point represents mean ± standard (n=3)

The mean number of setigers was significantly higher in El-Kala compared to Annaba and Skikda throughout the studied period excepted in August and December for individuals collected from El-Kala and Annaba (Fig. 5). The mean number of setigers was significantly higher in Annaba compared to Skikda throughout the studied period excepted in November.

Biomarkers

AChE activity

We observed seasonal variations of AChE activity at each sites. Globally, AChE activity was maximal in spring then it decreased in summer and then remained more or less stable (Fig. 6). The higher values of AChE activity were observed in April at the three study sites (34.00 ± 0.07; 28.38 ± 1.86 and 25.60 ± 0.76 nmol $min^{-1}mg^{-1}$ protein for individuals collected in El-Kala, Annaba and Skikda respectively) while the lower values were observed in August (22.58 ± 0.67; 19.47 ± 0.83 and 14.69 ± 1.21 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 excepted in September where the difference was not significant between El-Kala and Annaba (Fig. 6). AChE activity was significantly more important in Annaba compared to Skikda throughout the studied period excepted in October.

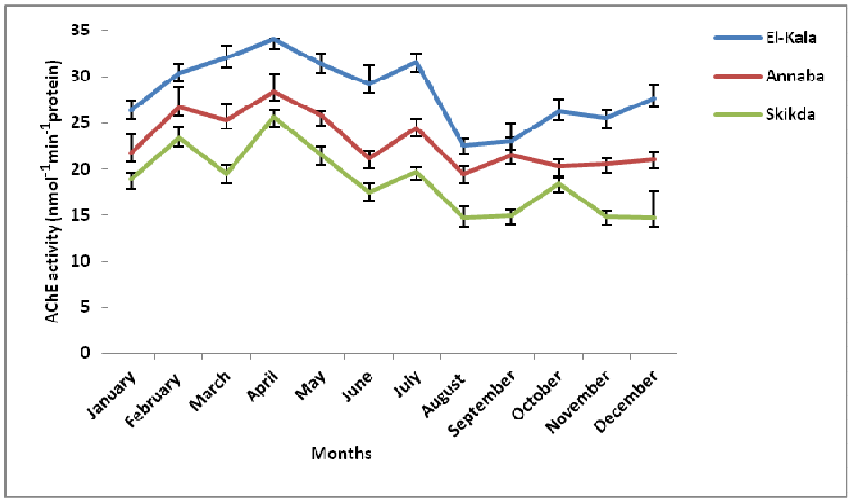

Fig. 6. Monthly variations of AChE activity expressed as $nmol^{-1}min^{-1}mg$ protein for individuals collected in El-Kala, Annaba and Skikda from January to December 2011

Each data point represents mean ±standard deviation (n=3)

GST activity

We observed seasonal variations of GST activity at each sites. Globally, GST activity was maximal in spring then it decreased in summer. In Skikda and Annaba GST activity increased in late summer (September) and then remained more or less stable while GST activity remained low at El-Kala (Fig. 7). The higher values of GST activity were observed in April at the three study sites (6.54 ± 1.3; 8.84 ± 0.93 and 9.77±0.7 µmol $min^{-1}mg^{-1}$ protein for individuals collected in El-Kala, Annaba and Skikda respectively) while the lower values were observed in August (4.52 ± 0.75; 5.11 ± 2.49 and 6.67 ± 1.06 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 (Fig. 7). GST activity was significantly more important in Skikda compared to Annaba throughout the studied period excepted in May and June.

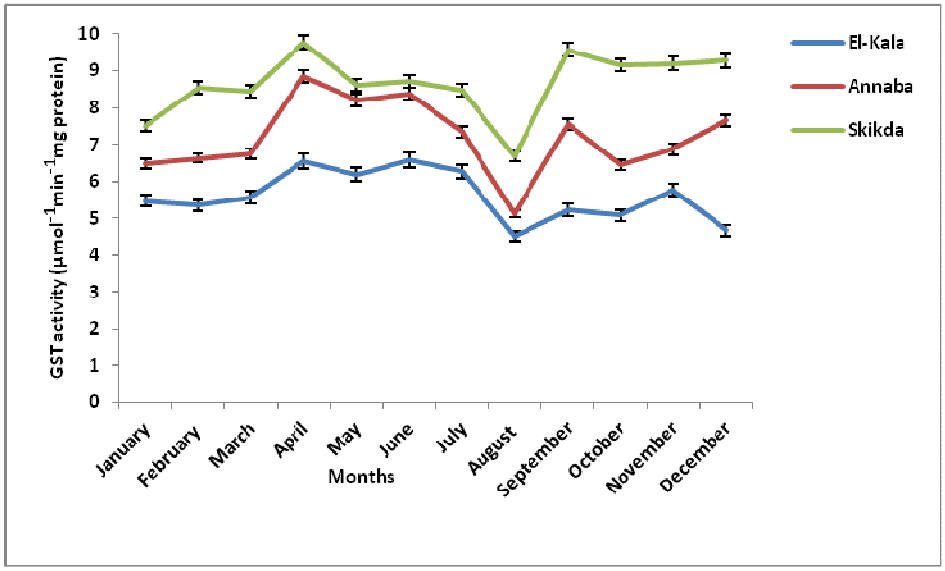

Fig. 7. Monthly variations of GST activity expressed as µmol^{-1}min^{-1}mg protein for individuals collected in El-Kala, Annaba and Skikda from January to December 2011
Each data point represents mean ± standard deviation (n=3)

CAT activity

We did not observed seasonal variations of CAT activity at the three studied sites (Fig. 8). CAT activity was significantly higher in Skikda compared to Annaba and El-Kala and in Annaba compared to El-Kala (Fig. 8).

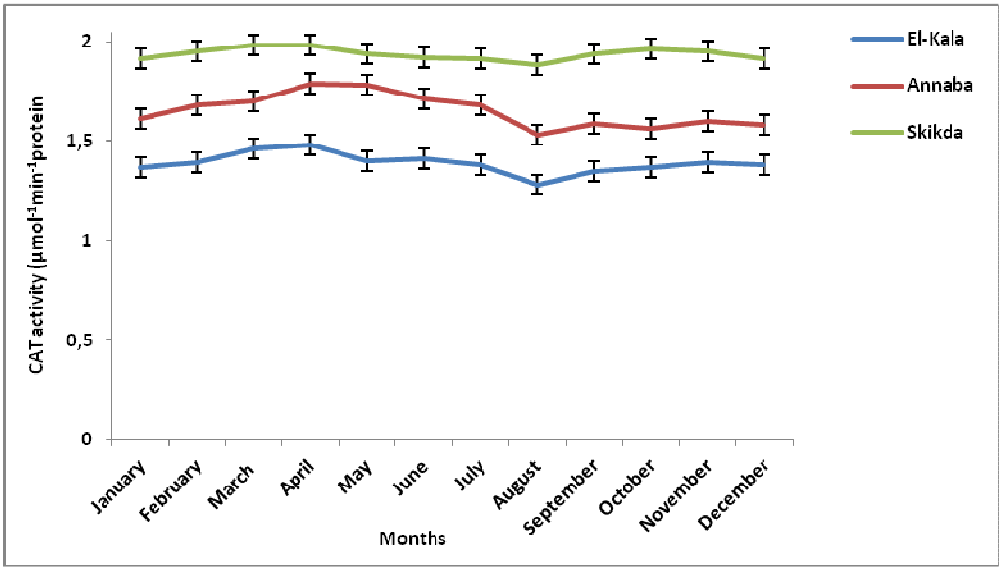

Fig. 8. Monthly variations of CAT activity expressed as µmol^{-1}min^{-1}mg protein for individuals collected in El-Kala, Annaba and Skikda from January to December 2011
Each data point represents mean ± standard deviation (n=3)

DISCUSSION

The Mediterranean area has been classified by PNUE as one of the five regions of the world where environmental problems are the most severe [41], while the Mediterranean Sea is ranked among the seven most threatened seas [42]. During the last years, Algeria has experienced a significant urban, industrial and agricultural development, threatening the quality of the marine environment. Currently scientists and environmental managers consider more specific concepts in the biomonitoring of the marine environment based on the study of the biological response by

measuring biomarkers in bioindicator species of pollution [43]. The inhibition or induction of biomarkers *in vivo* is a good tool to assess environmental exposure and potential effects of xenobiotics on organisms [44, 45, 46, 47, 48].

The evaluation of the marine ecosystem quality could be done by the study of its abiotic and biotic components [49]. In fact, water temperature, salinity, pH and dissolved oxygen are important components of water quality. We only noticed a slight non significant decrease of the dissolved oxygen level in water samples collected from sites receiving pollutants (Annaba and Skikda). Dissolved oxygen is consumed during the heterotrophic oxidation of organic matter and respiration by aquatic flora and fauna. Ali and Soltan [50] have reported an increase of water temperature, a decrease of the dissolved oxygen and a neutral pH (due to alkaline effluents), in water samples taken from the polluted sites of Nile River. The same authors have highlighted that the increase of water temperature (caused by pollution) may be responsible for the reduction of dissolved oxygen concentrations, because oxygen is less dissolved in warm water.

Our investigation highlights a relationship between the gradient of altered conditions and the variation of the studied physiological and biochemical parameters in the worm *P. cultrifera*. In fact, the mean fresh weight, length and number of setigers decreased in individuals collected from altered sites. In another studies using the same species, Daas et al. [51] showed that the mean fresh weight of females collected in a polluted site was lower than that of females collected in a reference site but that the mean oocyte diameter of females collected from the two sites showed no difference.

We found an inhibition of AChE activity in individuals taken from altered sites. This inhibition may be the result of a neurotoxic effect by exposure to pollutants. Cholinesterase activities are known to be inhibited in the presence of some pesticides [28, 52] and several studies have used AChE inhibition to evaluate the biological impact of organophosphate and carbamate pesticides [53]. Some studies have also demonstrated that AChE may be inhibited by heavy metals in various organisms [29, 30, 54]. PCBs induce cytochrome P450 (phase I) enzymes and the biotransformation products (including reactive electrophiles) could play a role in AChE enzyme inhibition [55]. According to Leiniö and Lehtonen [27] there is an increasing evidence of the potential use of AChE activity in ‘‘stress screening’’.

Inhibition of AChE activity from polluted sites have also been reported in two marine invertebrates *Nereis diversicolor* and *Patella vulgata* collected from Tangier’s Bay (Morocco) [56] and in the blue mussel *Mytilus edulis* and the female eelpout *Zoarces viviparous* from the southwestern Baltic Sea [57]. Ricciardi et al. [58] have highlighted comparable results in zebra mussel *Dreissena polymorpha* taken from polluted locations of the Lake Maggiore (northern Italy). Canty et al. [59] also reported an inhibition of AChE activity in the mollusc *M. edulis* exposed to the organophosphate pesticide azamethiphos. Analogous disturbances were shown also in clams *Tapes philippinarum* taken from contaminated sites of Venice lagoon [60].

Moreover, we showed an increase of GST activity in worms sampled from altered sites. These findings are similar to those obtained for the worms *N. diversicolor* collected from polluted estuary of the Seine [61] and from the Oued Souss (Bay of Agadir, Morocco) before implantation of wastewater treatment [62]. GST is a phase II enzyme involved in the metabolism of lipophilic organic contaminants such as PAHs and PCBs detected at comparatively high levels in the Seine estuary [61]. In addition, this enzyme plays a role in cellular protection against oxidative stress which can be triggered by pollutants such as metals, PCBs and PAHs [63].

Finally, we noticed an increase of CAT activity in worms sampled from altered sites. This may be related to an induction of its biosynthesis, for protection against oxidative stress resulting from the xenobiotics metabolism. In fact, CAT is an antioxidant enzyme that facilitates the breakdown of hydrogen peroxide thus preventing cell damage by reactive oxygen species (ROS), although its inducibility under field conditions is more controversial [64]. Our findings are similar to those obtained for the worms *N. diversicolor* collected from the Oued Souss (Bay of Agadir, Morocco) before implantation of wastewater treatment [62]. Douhri and Sayah [56] also reported an increase of CAT activity in two marine invertebrates *Nereis diversicolor* and *Patella vulgata* taken from altered sites of Tangier’s Bay (Morocco) and Lionetto et al. [65] in mussels *Mytilus galloprovincialis* collected from polluted sites in the Italian coastline. Such effects were also seen in the mussels *Perna viridis* and the clams *Ruditapes philippinarum* of the Hong Kong coastline [66], and in the clam *Ruditapes decussatus* sampled from Tunisian polluted marine ecosystems [67]. In contrast, Da Silva et al. [68] have reported a lack of the CAT activity change in the oyster *Crassostrea rhizophorae* exposed to an acute exposure to diesel.

CONCLUSION

Our study reports the use of physiological parameters and enzymatic biomarkers, in *P. cultrifera* for coastal biomonitoring. The multi-marker approach confirms there is an obvious correlation between contamination of the sites by xenobiotics and the studied physiological and biochemical parameters in this species. We can conclude that the biomarkers' responses can reflect the pollution degrees of the sites and predict the ecological effects of xenobiotics. So, they can be used as biomarkers of pollution.

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