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### Measure of environmental stress biomarkers in *Donax trunculus* (Mollusca, Bivalvia) from the gulf of Annaba (Algeria)

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#### ABSTRACT

The aim of our study was to test various biomarker responses in an edible mollusc, *Donax trunculus* (Mollusca, Bivalvia) associated with environmental pollution. The biomarkers selected were the antioxidant enzymes catalase (CAT), the phase II detoxifying enzyme glutathione S transferase (GST) and the neurotoxicity marker acetylcholinesterase (AChE). Samples were collected monthly from two sites located over the gulf of Annaba (North-East of Algeria): the first site El Battah is far away from the sources of pollution while the second Sidi Salem is contaminated by various sources. The results demonstrated decrease in the activity of AChE in *D. trunculus* collected from Sidi Salem compared to that from El Battah site, while an increase was found in GST and CAT activity in Sidi Salem samples comparatively with those of El Battah. In conclusion, the overall results suggest that an alteration in the activity of AChE with an induction of GST and CAT activity during the observed period reflects the presence of certain prooxidative compounds that can lead to oxidative stress in *Donax trunculus* at Sidi Salem site.

**Keywords:** *Donax trunculus*, Gulf of Annaba, Biomarkers, CAT, AChE, GST.

#### INTRODUCTION

The presence of toxic agents in the ecosystems has increased in recent decades, especially in aquatic environments [1]. Environmental monitoring programmes including both chemical analyses of seawater for contaminant levels and the measurements of a battery of biomarkers are recommended [2, 3]. Biomarkers are presently becoming part of the health assessment and management of aquatic ecosystems in addition to the more traditional water chemical analyses [4, 5]. Marine bivalves molluscs are recognized as bioindicator organisms in environmental pollution studies [6-10], Various molluscs are used as bioindicators of physiological and cellular alterations caused by pollution [11,12,13,14], organic contaminants, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and pesticides [15]. These

contaminants induce biotransforming enzymes that catalyze phase I or II reactions [16, 17]. The AChE activity has been used as a biomarker of exposure to several chemicals such as organophosphate and carbamate insecticides in aquatic environments [18], but by other contaminants such as metals, synthetic detergents, some components of fuel oils and algal toxins [19, 20],

Antioxidant defence systems, present in all aerobic cells, neutralize chemical reactive intermediates produced by endogenous pathways and/or xenobiotic metabolism [21]. Their response is non-specific to a kind of contaminants and therefore antioxidant parameters represent biomarkers of interest, considering the complexity of pollution in aquatic ecosystems [22]. Here, we selected two of these biomarkers to estimate changes in the redox status: catalase (CAT), anti-oxidant enzyme, widely used as a marker involved in the primary defence against oxidative damage [23], while glutathione S-transferases (GST) play a key role in the cellular detoxification and excretion of a variety of xenobiotic compounds in bacteria, plants, and animals [24-26].

The aim of this spatial variation study was to use the above mentioned biomarkers (AChE, CAT, GST) to investigate the influence of site quality on health status of a population of mollusk, *Donax trunculus*, this bivalve has been previously used as a sentinel species in environmental assessment [11,13, 27, 28, 29, 30, 31]. This mollusc, living in the sediment and largely distributed in West-African and West European Atlantic and Mediterranean coasts, exhibits the same characteristics as other mussels in that they are suspension feeders as well as potential bioaccumulators [32]. This species are collected in 2010 respectively from a polluted and a relatively clean site in Annaba gulf, Sidi Salem and El Battah respectively.

## MATERIALS AND METHODS

### 2.1. Presentation of sampling sites

The gulf of Annaba is located in the east of Algeria. It is limited by the Rosa Cap (8° 15 ' E and 36° 38 ' N) in the East and by the Garde Cap (7° 16 ' E and 36° 68 ' N) in the West. El Battah site (36°50 ' N - 8° 50 ' E), is located about 30 km to the East of Annaba far from any human activities, and is considered as a relatively clean site. Sidi Salem site (36° 50 ' N - 7° 47 ' E), located about 1 km to the East of Annaba city, receiving industrial and domestic wastewater, and considered as the polluted area (Fig 1).

### 2.2. Samples Collection

Mollusc bivalve (*D. trunculus*) with the same shell length ( $26 \pm 1$  mm) were collected monthly in 2010, from two sampling sites (El Battah and Sidi Salem) and transferred to the laboratory.

### 2.3. Biochemical procedure

The mantle of each mussel species was dissected during the same day until biomarker analyses. Determinations of AChE activity were performed using a method described by [33] with the use of acetylthiocholine (ASCh) as substrate. The activity rate was measured as change in OD/min at 412 nm (ext. coeff.  $13,6 \text{ mM.cm}^{-1}$ ). Activity was expressed as  $\mu\text{mol/min/mg protein}$ . CAT activities were assayed as described by [34]. The decrease in absorbance at 240 nm, caused by the consumption of hydrogen peroxide  $\text{H}_2\text{O}_2$  (500Mm). The reaction takes place in phosphate buffer (0.1 M; pH 7.4). CAT activity was expressed as  $\mu\text{mol/min/mg protein}$  (ext. coeff.  $40 \text{ M.cm}^{-1}$ ). GST activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate in a final reaction mixture containing 1 mM CDNB and 5 mM reduced glutathione [35]. The activity rate was measured as change in optical density (OD/min) at 340 nm (ext. coeff.  $9.6 \text{ mM. cm}^{-1}$ ),

and the final activity was expressed as  $\mu\text{mol}/\text{min}/\text{mg}$  protein. The protein content was evaluated [36] using serum albumin as standard (BSA, Sigma).

#### 2. 4. Statistical analysis

The normality of data was verified using the Kolmogorov-Smirnov test, and the homogeneity of variances was checked by Levene's test. Data are presented as mean $\pm$ standard error of the mean. Comparison of mean values between sites was estimated by Student's t-test. The effects of time, sampling sites were made using two-way analysis of variance (ANOVA). A significant difference was assumed when  $p < 0.05$ . All statistical analyses were performed using MINITAB Software (Version 14, PA State College, USA).

## RESULTS

### 3. 1. Change in acetylcholinesterase activity

Measurements of monthly AChE activity in mantle of mussels (*D. trunculus*) from two sampling locations (EB and SS) are presented in Figure. 2. AChE activities ranged from 16,24 at Sidi Salem to 64,93 U/mg proteins at El Battah and were generally higher at EB comparatively to SS; the differences between the two sites were significant in March, May, November and December ( $p < 0.01$ ) and June, July, August and September ( $p < 0.001$ ), The two-way ANOVA revealed a significant ( $p < 0.001$ ) effects of both site ( $F = 98.11$ ;  $df = 1, 96$ ) and months ( $F = 136.44$ ;  $df = 11, 96$ ) (Table 1).

### 3. 2. Change in Catalase activity

Catalase activities in mussels collected monthly from two different sites are presented in figure.3. CAT activities ranged from 0,141 at Sidi Salem to 2,49 U/mg proteins at El Battah and were generally lower in Donax specimens collected from El Battah than that in samples from Sidi Salem site. Significant differences between the sampling sites were recorded in February and May ( $P < 0,05$ ) and March, June, August ( $p < 0.001$ ). These results were confirmed by ANOVA two-way, a significant ( $p < 0.001$ ) effects of both site ( $F = 18.85$ ;  $df = 1, 96$ ) and months ( $F = 124.05$ ;  $df = 1, 96$ ) were observed (Table 2).

### 3. 3 Change in glutathione S-transferase activity

The results of monthly *Glutathione S-transferase* activities in mantle of mussels are presented in Fig. 4. GST activity ranged between 1,46 at El Battah to 12,95 at Sidi Salem UM/mg proteins and overall higher in natural mussels from polluted site than those from reference site with significant differences in February, September and December ( $p < 0.05$ ) and August, October ( $p < 0.01$ ) and April, May ( $p < 0.001$ ). Significant effects ( $p < 0.001$ ) of site ( $F = 23.30$ ;  $df = 1,96$ ) and months ( $F = 85.79$ ;  $df = 1,96$ ) were determined by ANOVA tow-way test (Table 3).

**Table 1: Monthly variation of specific activity of AChE ( $\mu\text{M}/\text{mn}/\text{mg}$  of protein) in *D. trunculus* from two sites of Annaba gulf: two-way ANOVA.**

Sources of variation	DF	SS	MS	Fobs	P
Site	1	1391.1	1391.1	98.11	0.000***
Month	11	21279.9	1934.5	136.44	0.000***
Interaction site/ month	11	3569.4	324.5	22.89	0.000***
Residual error	96	1361.2	14.2	-	-
Total	119	27601.6	-	-	-

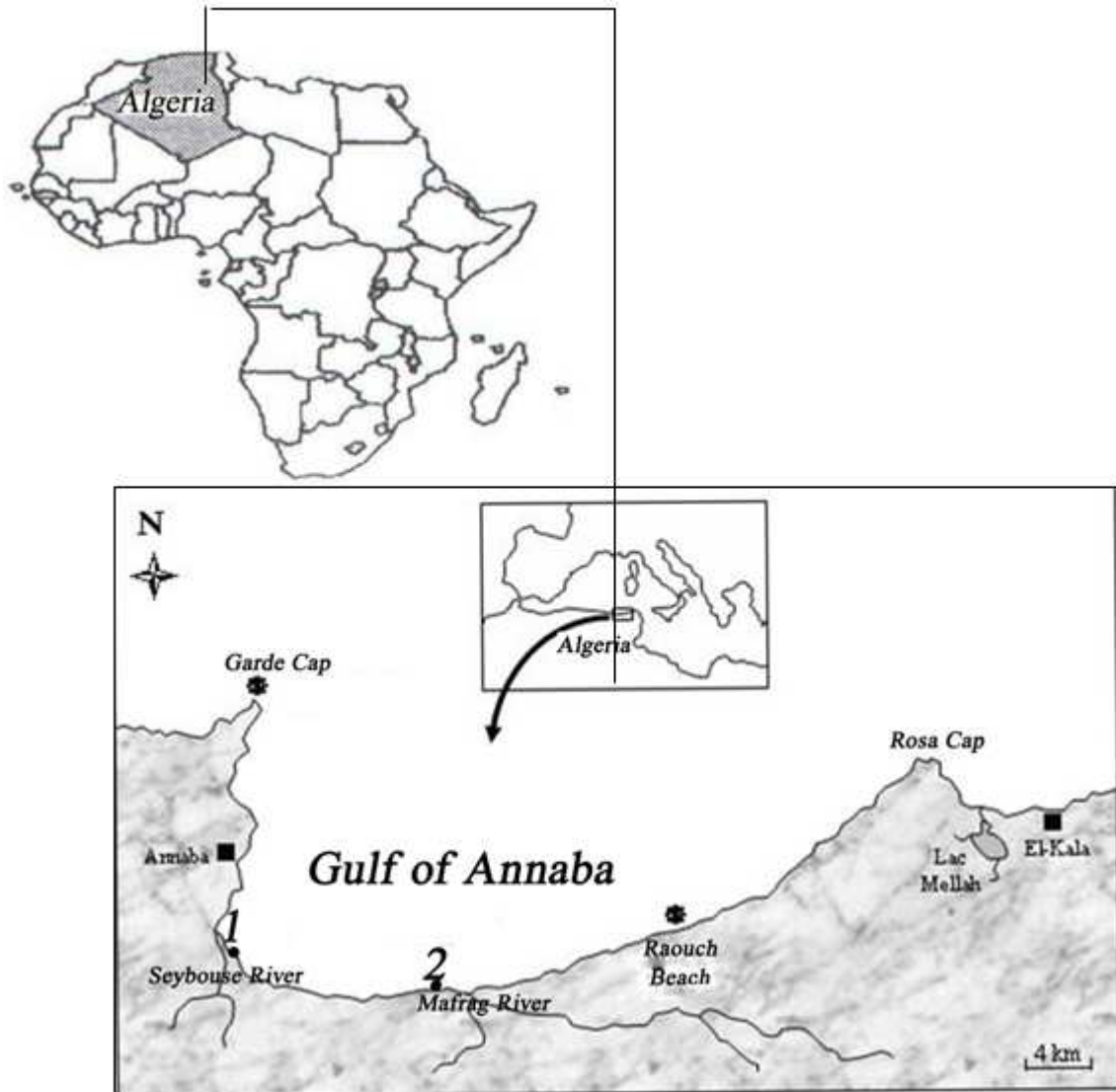


Figure 1. Localisation géographique des sites d'échantillonnage (1: Sidi Salem, 2: El Battah).

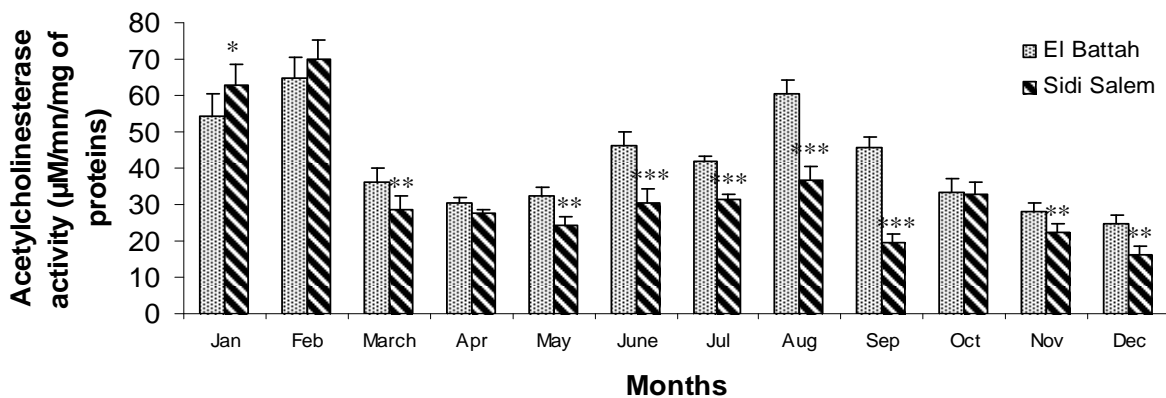


Fig 2. Monthly variation of specific activity of Acetylcholinesterase ( $\mu\text{M}/\text{mn}/\text{mg}$  of protein) in *D. trunculus* from two sites of Annaba gulf during 2010 (mean  $\pm$  SD; n= 5).

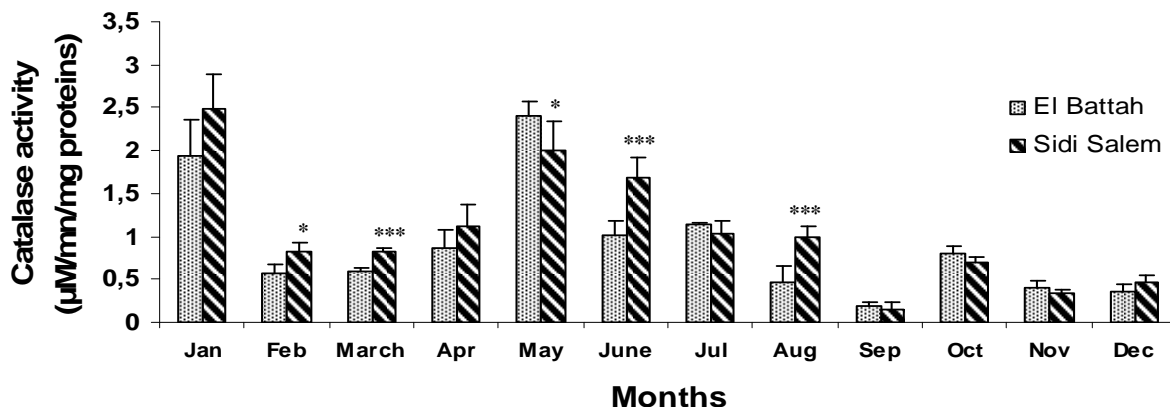


Fig 3. Monthly variation of specific activity of Catalase ( $\mu\text{M}/\text{mn}/\text{mg}$  of protein) in *D. trunculus* from two sites of Annaba gulf during 2010 (mean  $\pm$  SD; n= 5).

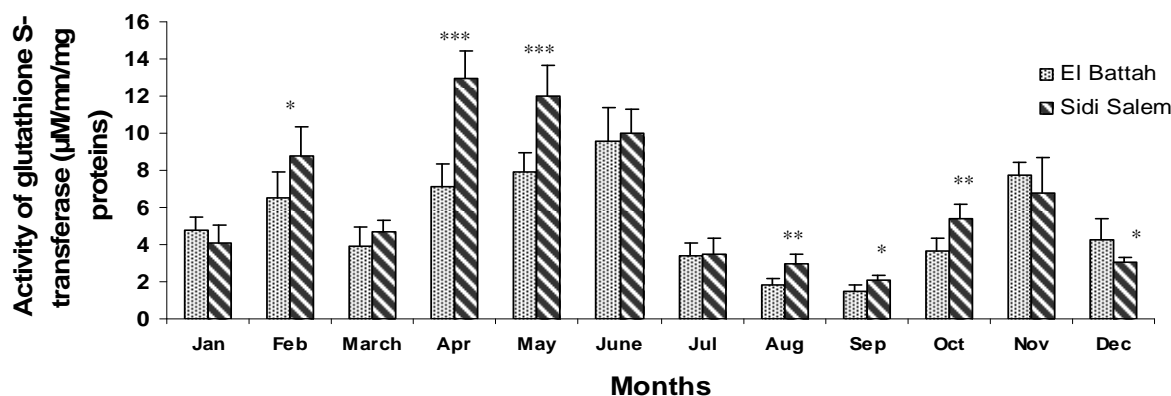


Fig 4. Monthly variation of specific activity of glutathione S-transferase ( $\mu\text{M}/\text{mn}/\text{mg}$  of protein) in *D. trunculus* from two sites of Annaba gulf during 2010 (mean  $\pm$  SD; n= 5).

Table 2: Monthly variation of specific activity of CAT ( $\mu\text{M}/\text{mn}/\text{mg}$  of protein) in *D. trunculus* from two sites of Annaba gulf: two-way ANOVA.

Sources of variation	DF	SS	MS	Fobs	P
Site	1	0.6683	0.6683	18.85	0.000***
Month	11	48.3866	48.3866	124.05	0.000***
Interaction site/ month	11	2.885	2.885	7.4	0.000***
Residual error	96	3.4041	3.4041	-	-
Total	119	55.3440	-	-	-

Table 3: Monthly variation of specific activity of GST ( $\mu\text{M}/\text{mn}/\text{mg}$  of protein) in *D. trunculus* from two sites of Annaba gulf: two-way ANOVA.

Sources of variation	DF	SS	MS	Fobs	P
Site	1	0.8912	0.8912	23.30	0.000***
Month	11	36.0862	3.2806	85.79	0.000***
Interaction site/ month	11	2.5222	0.2293	6.00	0.000***
Residual error	96	3.6711	0.0382	-	-
Total	119	43.1706	-	-	-



## DISCUSSION

Pollution of air [37] and water [38-41] is one of the areas of major concern to environmentalists. Coastal and estuarine environments are subjected to several forms of disturbance, amongst which chemical pollution associated with industrial production and high levels of urbanisation are both of major concern [42]. The Gulf of Annaba is a recipient of a large amount of contaminants from urban, agricultural, harbor and industrial activities [43, 44]. Different animals in aquatic ecosystems have developed various ways of protection from changing environmental conditions and pollution [45]. Among the biological tools recommended for marine pollution monitoring, biomarkers have been successfully incorporated in the assessment of the quality of the coastal environment during last years [45-47]. One of the key functions of biomarkers is to provide early warning signals of significant biological effects [48]. Aquatic organisms, especially marine bivalves, exhibit a variety of changes in enzymatic antioxidant defences after exposure to pollutants with oxidative potential [49, 50]. In the present study, we have used the activities of AChE an important enzyme that hydrolyzes the neurotransmitter acetylcholine (ACh), the antioxidant enzyme CAT, the activity of the GST, an enzyme of the phase II of biotransformation process to evaluate their utility as biological tools for pollution monitoring in the Gulf of Annaba.

Temporal evolution of AChE activity in the mantle of *D. trunculus*, showed significant inhibition of AChE activities in bivalves from Sidi Salem compared to El Battah populations confirming previous observations [29]. This suggests that potential pollutants like pesticides may be highly at Sidi Salem which is located proximity to industrial zone that produced phosphoric fertilizers and pesticides such organophosphates. Indeed, due to the large number of studies dealing with pesticides, the inhibition of AChE used as a biomarker of exposure to organophosphorus and carbamate pesticides is well-documented for aquatic biota [51]. Recent studies have shown that other types of pollutants such as heavy metals, surfactants and PAHs may also inhibit AChE activity [52-54]. Indeed, Sidi Salem was more exposed to metallic pollution as compared to El Battah [28]. Moreover; various heavy metals were detected in the sediments represented by Fe, followed by Zn and Cu [55]. Some authors reported that AChE activity can be modulated by trace metals (Cd, Cu, Hg, Zn) or natural factors (seawater temperature, biotoxins or cyanobacteria in mussel tissues) [51-54].

Increased catalase activity rates were attributed to elevated levels of exogenous hydrogen peroxide  $H_2O_2$  which is the main cellular precursor of the hydroxyl radical ( $HO\cdot$ ), a highly reactive and toxic form of ROS [55]. The high mean annual catalase activity observed in *D. trunculus* in the contaminated site (Sidi Salem) by comparison with the reference site (El Battah) implies that organisms have been exposed to an oxidative stress certainly due to the presence of pesticides and heavy metals such as Zn, Cu, Pb and Cd in Sidi Salem site [28]. The presence of heavy metals in our environment has been of great concern because of their high toxicity to organisms [56]. Several organic contaminants, such as pesticides and fertilizers, increased CAT activity [57-59]. On the other hand, the values of CAT showed temporal changes in the two sampled sites. These biological responses can be modulated also by seasonal changes of both environmental and biological factors, potentially influencing responsiveness and sensitivity to pollutants [52]. The effect of endogenous and environmental factors on the response of CAT has been previously described [60-63]. According to the results presented here, such trends in CAT activities can be found in mussels at polluted sites according to the levels and duration of pollutant exposure [64- 66].

GST is an important phase II enzyme that catalyzes the conjugation of reduced glutathione (GSH) to cellular components damaged by ROS attack, leading to their detoxication [67]. The GST activity, as a biomarker of defence, participates also in anti-oxidative defenses [68] and can be triggered by some pollutants [69, 70]. [71] reported induction of some GST isoenzymes by substrates such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenols (PCBs). GST response to toxic chemicals follows a similar bell-shaped trend as CAT [72], while GST inhibition has been indicated as more a specific response to chemical challenge [73]. So also, in the present study, significantly higher activities of these enzymes were recorded in bivalves from the multi-contaminated Sidi Salem site in comparison with the reference site (El Battah). In the field, the presence of complex chemical mixture including pesticides and heavy metals such as Zn, Cu, Pb and Cd [28] may explain the variability in GST activity. In a previous study [29] reported that GST activity was higher in samples collected in Sidi Salem than in those collected in El Battah. This enzyme is the most sensitive biomarker and its activity has been shown to increase in the whole organism or particular organs (gills, digestive gland) as a function of the xenobiotic concentration [74].

### CONCLUSION

This paper reports the results on some biochemical markers (AChE, CAT, GST) measured in the mantle of *D. trunculus* an useful sentinel for environmental monitoring. As a result, changes and differences between the two sites in biomarkers levels may be related to the intensity and the duration of the stress detected at Sidi Salem as compared to El Battah.

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