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The use of biomarkers (catalase and malondialdehyde) in marine pollution monitoring: Spatial variability

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Abstract
In this study, two different oxidative biomarkers; catalase (CAT) and malondialdehyde (MDA) were evaluated for their influence on site-pollution status in the brown mussel *Perna perna* collected from four stations along the Moroccan Atlantic coast. The oxidative stress biomarkers showed statistically significant differences inthe polluted sites when compared to the control ones. Our data indicated that CAT activity and MDA concentration in the mussels collected at polluted sites were higher than that of the controls ($p < 0.05$). The oxidative stress biomarkers response obtained for January 2010 and December 2011, clearly demonstrate the potential presence of contaminants in site 4 and site 3 reflecting the intensity of pollution in these areas compared to the two other sites.

Keywords: biomarker, catalase, malondialdehyde, marine pollution, spatial variability.

1. Introduction
In polluted environments and especially in coastal waters, living organisms are often exposed to complex mixtures of chemical contaminants. These situations of bad environmental conditions have influences on the mechanisms of adaptation of animals. Because of the diversity and variability of the chemical threat, defense mechanisms exhibit considerable versatility and adaptability. Among the strategies that have been developed by organisms at the cellular level in the course of evolution to protect themselves from the toxic effects of metallic or organic compounds, the major ones are the antioxidant defense systems. The antioxidant systems protect cells against the deleterious effects of oxyradical generation by maintaining endogenous reactive oxygen species (ROS) at relatively low levels and attenuating the damages related to their high reactivity. A range of antioxidant defensemechanisms are present in bivalve molluscs, including Catalase (CAT). CAT is a major component of the antioxidant system that catalyzes the dismutation of H_2O_2 to H_2O and O_2. This enzyme can also act as peroxidase, for which several organic substances, especially ethanol, can act as a hydrogen donor. It occurs in almost all aerobically respiring organisms and is localized in peroxisomes [1].
An impaired capability to neutralize ROS can anticipate the appearance of oxidative damages at cellular level. The alteration of membrane phospholipids through lipid peroxidation is considered to be one of the primary key events in oxidative damage [2]. Malondialdehyde (MDA) reflected membrane degradation in a variety of pathological conditions [3, 4]. It has been proposed to appraise the health status of exposed species [5].
The present work was aimed to evaluate in the brown mussel (*Perna perna*) the response of two oxidative stress biomarkers, CAT activity and MDA level, in order to assess the marine environment quality in the Grand Casablanca area.

2. Materials and methods
2.1. Reagents
Hydrogen peroxide (H_2O_2), Thiobarbituric acid (TBA), and Tetramethoxypropane (TMP) were obtained from Sigma (Saint Quentin Fallavier, France). Bovine serum albumin (BSA) and Coomassie brilliant blue (CBB) were purchased from Genome Biotechnologies (Casablanca, Morocco).
2.2. Sampling sites and mussel handling
Ten specimens of adult blue mussels (*Perna perna*), were collected at low tide, from four sites (S) of Moroccan Atlantic coast (Fig. 1): S1 (33°38' N, 7°33' W) and S2(33°41' N, 7°27' W), both impacted by pollution (the

first is located in Aïn Sebaâ beach, the second is located in Mohammedia beach), and S3(33° 46' N, 7° 17' W) and S4(33°51' N, 7°02' W), both used as references (the third is located in Mansoria beach, the fourth is located in Skhirat beach). All specimens arrived at the laboratory on ice, and stored at -80 °C until analysis.

2.3. Preparation of homogenate fractions and biochemical analyses

Prior to biochemical analysis, the mussels were dissected and the total soft body tissue was sampled and was ground in in phosphate buffer (100 mM, pH 7.4). The homogenate obtained was centrifuged at 9000×g for 30 min at 4°C. The supernatant S9 (n=5) of each site was removed and used to determine catalase activity and MDA level. CAT activity was measured by Aebi method [6], byfollowing the decrease of absorbance at 240 nm due to H_2O_2 consumption. The reaction takes place in 100 mM phosphate buffer, pH 7.4 containing 500 mM H_2O_2. MDA was estimated according to the method described by Sunderman[7]with use of TMP as a standard. The reaction was determined at 532 nm, using TBA as reagent. The quantity of proteins was determined according to the Bradford[8] method, at 595 nm using Coomassie Blue as a reagent.

2.4. Statistical analyses

The results for biomarker measurements were investigated by the use of a parametric one-way analysis of variance (ANOVA) and level of significance was set at $p < 0.05$. Statistical analysis was performed using the software Statigraphics Centurion XVI (version 16.1.17).

3. Results and discussion

The oxidative stress biomarker responses obtained for each site studied are presented in Fig. 2 – 5. In general, the biomarker responses obtained for January 2010 showed generally a similar pattern to that of December 2011.

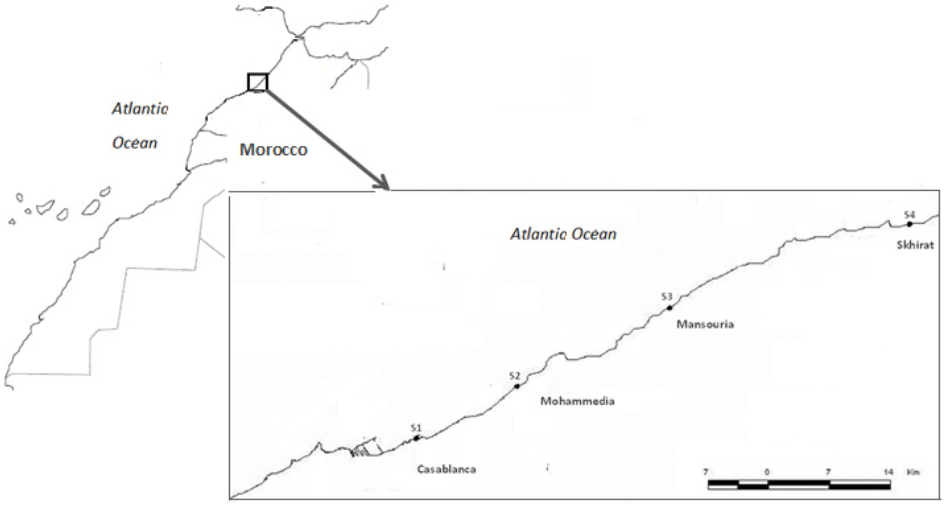

Figure 1: Map showing the different sampling sites along Moroccan Atlantic coast.

Measurements from sites S1 and S2 showed significant increased ($p < 0.05$) CAT activity compared to mussels from S3 and S4 (CAT activity was expressed as µmol/min/mg proteins)(Fig. 2 and 3). As shown in Fig. 4 and 5, a higher and significant ($p < 0.05$) accumulation of MDA was also registered in *Perna perna* collected at S1 and S2 when compared to specimen sampled from S3 and S4 (MDA content was expressed as nmol/mg proteins).

Our results of CAT activity in mussels from S1 and S2 showed an increase values, compared to mussels from S3 and S4. Increased activities of CAT have already been reported in several fish and invertebrate species [9, 10]. Indeed, the same increases in CAT activity were observed in *Perna perna* mussels [11-13] originating from stations of the Agadir Bay (South-Western Morocco) contaminated by PAHs and metals such as Fe, Zn, Cd, and Cu. It is well known that organic compounds are possible sources of oxidative stress which can bring changes in antioxidant enzyme activities. However, the antioxidant enzyme activities may also be induced by heavy metals [14], particularly iron and copper, which can be considered as an important prooxidant, because of their involvement as catalysts for •OH production via Haber-Weiss and Fenton reactions [15].

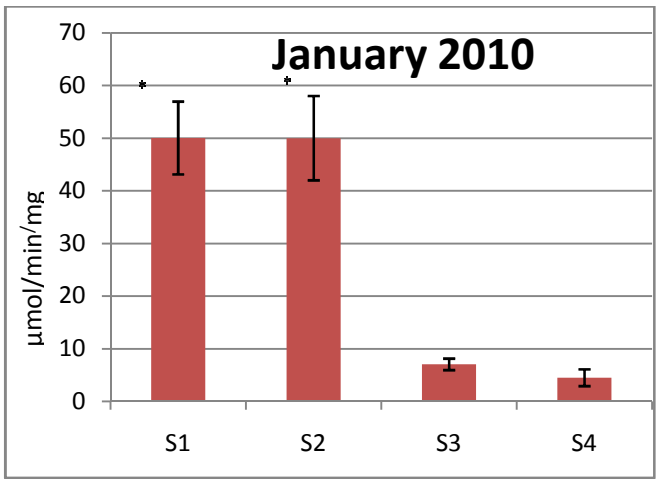

Figure 2: Determination of CAT activity (µmol/min/mg proteins) in*Perna perna* collected along the Moroccan Atlantic coast(Mean values ± SD, n = 5) in January 2010.

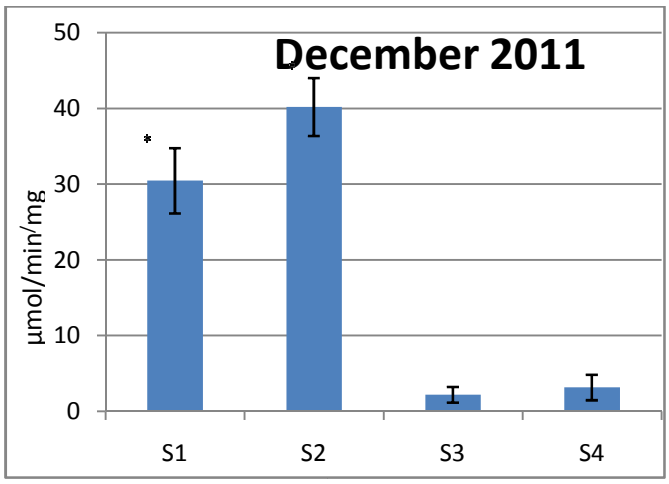

Figure 3: Determination of CAT activity (µmol/min/mg proteins) in*Perna perna* collected along the Moroccan Atlantic coast(Mean values ± SD, n = 5) in December 2011.

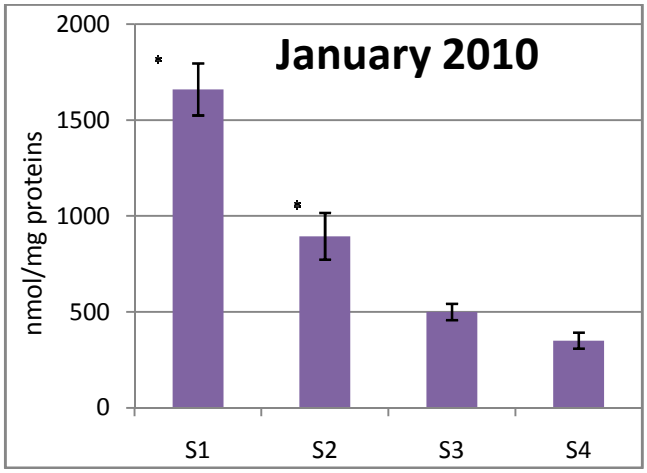

Figure 4: Determination of MDAlevel (nmol/mg proteins) in*Perna perna* collected along the Moroccan Atlantic coast(Mean values ± SD, n = 5) in January 2010.

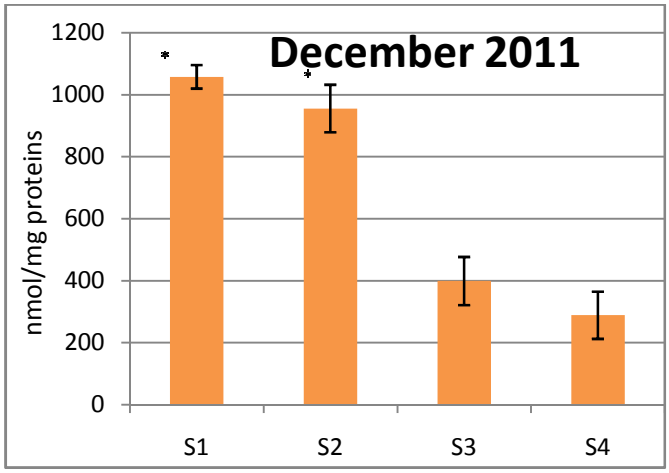

Figure 5: Determination of MDA level (nmol/mg proteins) in *Perna perna* collected along the Moroccan Atlantic coast(Mean values ± SD, n = 5) in December 2011.

The overall significance of such responses was reflected by an increased capability to neutralize peroxyl or hydroxyl radicals, thus indicating a more integrated unbalance of oxyradical metabolism [16, 17]. Considering that the induction of antioxidant enzymes represents a protective response to eliminate ROS resulting from contamination exposure, it has been hypothesized that such increase may be related to adaptations to contaminant induced stress [18, 19].

Our results for MDA showed a consistent trend for higher levels in polluted sites S1 and S2, whereas smaller increases were detected in site S3 and S4. Increased concentration of lipid peroxidation was observed in mussels exposed in polluted areas when compared to less polluted sites [20, 21]. In Morocco areas, an increase in the MDA concentration has also been observed in *Perna perna* (Agadir Bay) tissues exposed *in situ* to organic and metal compounds [11-13]. Several studies have evidenced that lipid peroxidation increases in tissues of different species of aquatic organisms, as result of being exposed to environmental pollutants [22-24]. It is known that lipid peroxidation in mussel can be stimulated via oxidation of polyunsaturated fatty acids, not only by various inorganic cations such as Cu, Cd, Ag and Hg [25, 26] but also by PCBs and PAHs [27, 28]. So the observed increase of MDA level may be attributed to the presence of contaminants in the environment.

Conclusion

CAT and MDA are considered by many scientists as important and sensitive biomarkers of oxidative stress, revealing biological effects on the redox status of the marine organisms [29, 30]. Our data for increases in CAT activity and in MDA level, demonstrate a ‘‘disturbance’’ from pollutants in the S1 and S2, reflecting the intensity of pollution in this area.
Variations of oxidative stress responses in different seasonal periods were indicated, confirming the influence of natural factors in modulating the oxidative status of mussels. Therefore prior to the correct use of CAT and MDA it is essential to know their seasonal variations in the studied mussels. We should also integrate physiological factors and physico-chemical indicators in future studies. Theses parameters, which contribute to the interpretation of pollution biomarkers variations, have already proved their effectiveness in previous environmental studies.

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