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ORIGINAL RESEARCH PAPER

OXIDATIVE STRESS BIOMARKERS IN MUSSELS SAMPLED FROM FOUR SITES ALONG THE MOROCCAN ATLANTIC COAST (BIG CASABLANCA)

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Abstract: Catalase (CAT) activity and malondialdehyde (MDA) level in whole bodies of the mussel *perna perna*, collected from four stations along the Moroccan Atlantic coast (Big Casablanca area), were monitored to evaluate stress effects on mussels collected from the selected sites. The oxidative stress biomarkers showed statistically significant differences at the polluted sites when compared to the control ones. In general, our data indicated that CAT activity and MDA concentration are a higher and significant ($p < 0.05$) in mussels collected at polluted site when compared to specimen sampled from control ones. In conclusion, the oxidative stress biomarkers response obtained for October 2010 and 2011, clearly demonstrate the potential presence of different contaminants in Site 4 and Site 3 reflecting the intensity of pollution in these areas.

Keywords: *catalase, malondialdehyde, marine pollution, mussels, oxidative stress biomarkers, perna perna*

INTRODUCTION

The characterization of the quality of marine systems requires specially designed biological methods for assessing the health status. Biomarkers are known to be useful tools for measuring environmental health [1 - 2].
Antioxidants represent the cellular defense mechanisms which counteract toxicity of reactive oxygen species (ROS), these mechanisms have been extensively investigated in sentinel organisms such as marine mussels [3 - 4].
Among these antioxidants biomarkers are Catalase (CAT), a well-known antioxidant enzyme, which converts H_2O_2 into water. The biological importance of CAT is more evident from various studies due to the fact that H_2O_2 is the main cellular precursor of the hydroxyl radical (HO^-) which is a highly reactive and toxic form of ROS (Reactive oxygen species) leading to oxidative damage to basic biological molecules.
Biomarkers of toxicity, such as malondialdehyde (MDA), have been proposed to appraise the health status of exposed species [5]. MDA reflected membrane degradation in a variety of pathological conditions [6]. The alteration of membrane phospholipids through lipid peroxidation is considered to be one of the primary key events in oxidative damage [7].
Oxidative stress usually characterizes chemically induced toxicity [8]. An increase in MDA levels can thus relate to degradation of an environmental site.
In the present study, we measured oxidative stress response in the mussel *perna perna* as an indicator of environmental disturbances. Bivalve molluscs, particularly marine mussels, have been used as indicator organisms in many studies to monitor environmental pollution in coastal waters [3, 9] due to their wide distribution, sedentary lifestyle, tolerance to a large range of environmental conditions and because they are filter feeders with very low metabolism which allows the bioaccumulation of many chemicals in their tissues [10].
The aim of this work was to use the response of two oxidative stress biomarkers, CAT activity and MDA level, in the brown mussel *Perna perna* to assess the marine environment quality in the Big Casablanca area.

MATERIAL AND METHODS

Reagents

Hydrogen peroxide (H_2O_2), thiobarbituric acid (TBA), and tetramethoxypropane (TMP) were obtained from Sigma (Saint Quentin Fallavier, France). Bovine serum albumin (SAB) and Coomassie blue were purchased from Genome Biotechnologies (Casablanca, Morocco).

Studied areas

For this study, four stations are selected attending to various degree of human impact. The Site 1 (ST1) constitutes the site furthest away from the polluting industrial activities established on the coastal fringe Casablanca-Mohammedia. ST1 is located in beach of the south, Skhirat which is characterised by a total prohibition of human activities. The

Site 2 (ST2), which is selected due to the absence of contamination sources, is located in the north of Mansoria beach. ST1 and ST2 are characterized by an important density of mussels and the high faunistic and floristic richnesses of the site are well marked. On the contrary the Site 3 (ST3) is located approximately 7 km in South of Mohammedia beach. The Site 4 (ST4) is located in Aïn Sebaâ beach. Due to intense human activities, the ST3 and ST4 are constantly subjected to contamination. In addition, ST3 and ST4 have low biodiversity of intertidal organisms, indicating high levels of pollution pressure.

Sampling

Ten mussels from each site were sampled during October 2010 and 2011. Following collection, the adult mussels were placed in thermally insulated boxes previously filled with water from the sampling site and immediately transported to the laboratory and stored at -80 °C until analysis.

Biochemical analyses

Whole soft tissues from each specimen (n = 5 for each station) were dissected out and immediately homogenized (1:3) in phosphate buffer 100 mM, *p*H 7.4. Homogenates were then centrifuged at 9000 G at 4 °C for 30 min. After centrifugation, supernatants were collected and immediately used for the determination of CAT activity and MDA concentration. CAT activity was measured following the decrease of absorbance at 240 nm due to H_2O_2 consumption [11]. The reaction takes place in 100 mM phosphate buffer, *p*H 7.4 containing 500 mM of H_2O_2. Results of CAT activity were expressed as µmol/min/mg proteins. MDA concentration was estimated according to the method described by Sunderman [12] with use of Tetramethoxypropane (TMP) as a standard. The reaction was determined at 532 nm, using TBA (Thiobarbituric Acid) as reagent. MDA content was expressed as nmol/mg proteins. Total protein was determined by Bradford's method [13] using bovine serum albumin (BSA) as standard.

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 $p < 0.05$.

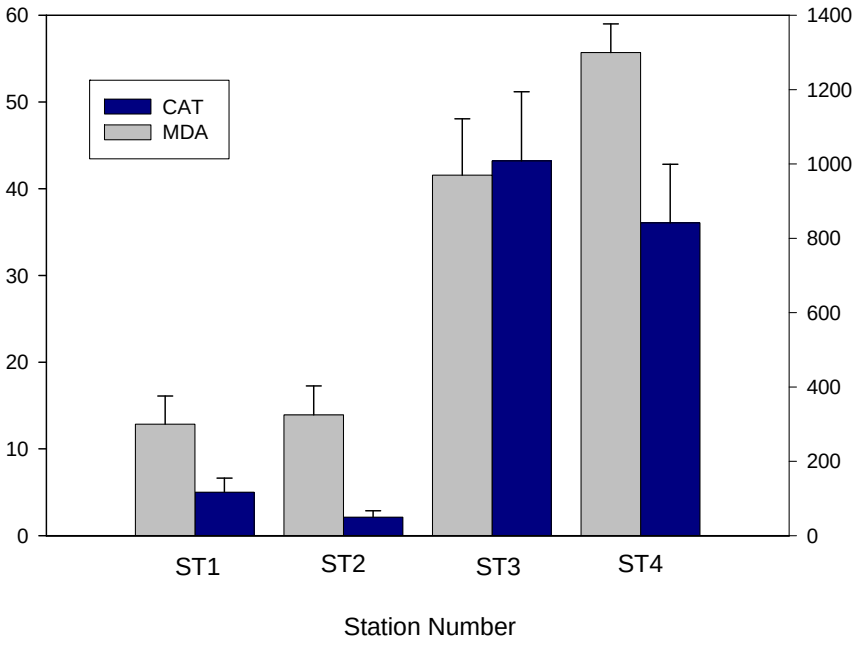
RESULTS AND DISCUSSION

The oxidative stress biomarker responses obtained for each site studied are presented in Figure 1. In general, the biomarker responses obtained for October 2010 showed generally a similar pattern to October 2011.

The results relative to the CAT activity are reported in Figure 1. Measurements from sites ST4 and ST3 showed significant increased ($p < 0.05$) CAT activity in October 2010 with value up to 36.06 and 43.2 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ protein, in ST4 and ST3 respectively, compared to mussels from sites ST2 and ST1 **(**2.14 and 5.01 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ protein, respectively**)**. Our results for CAT in October 2011 showed a

higher level in site 3 ($p < 0.05$) with 40.3 µmol·min^{-1} ·mg^{-1} protein, whereas the mussels from the site 4 had much lower levels of CAT **(**8.01 µmol·min^{-1} ·mg^{-1} protein**)**.

a: October 2010



b: 0ctober 2011

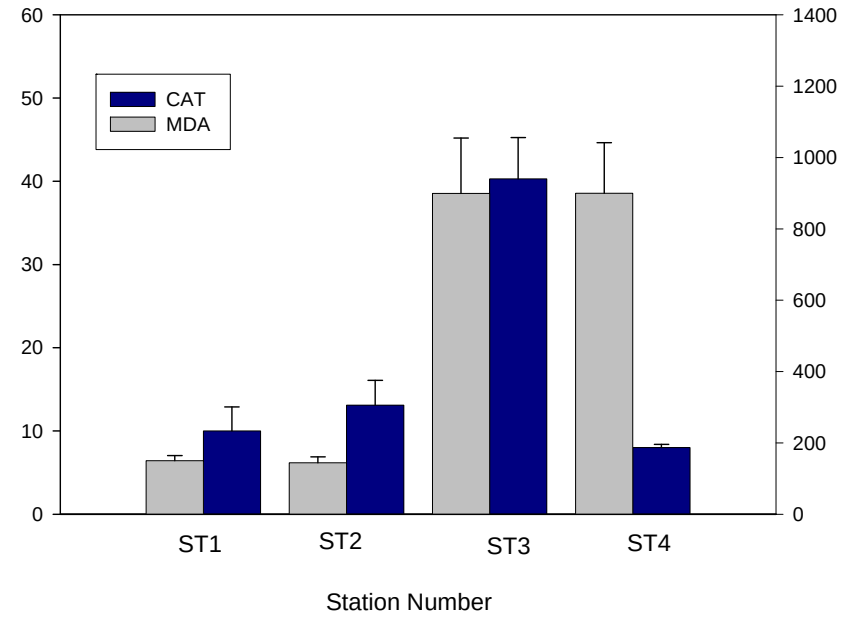


Figure 1. *CAT activity and MDA level in* Perna perna *collected from the studied areas*

As shown in Figure 1, a higher and significant ($p < 0.05$) accumulation of MDA in October 2010 and 2011was registered in *Perna perna* collected at ST4 and ST3 when compared to specimen sampled from ST2 and ST1.

Antioxidants represent the cellular defense mechanisms which counteract toxicity of reactive oxygen species (ROS), these mechanisms have been extensively investigated in sentinel organisms such as marine mussels [3 - 4]. CAT, as the first line of antioxidant defense, is very responsive to increasing levels of contaminant stimulated ROS production. The present study illustrated that mussels collected at ST4 and ST3 in October 2010 presented significantly higher levels of CAT activity in the whole soft body than those collected at ST2 and ST1. Previous studies showed that antioxidant enzymes [3, 14-15] also demonstrated higher activity values in response to contaminants. 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 [14, 16]. The presence of organic and metal contaminants is a possible source of oxidative stress and could induce variations in antioxidant enzyme activities [17]. Higher variability of different antioxidant enzyme in populations of aquatic organism from polluted and unpolluted areas has been observed [16, 18]. However, the induction of antioxidant enzyme activity due to the presence of high levels of contaminants in the environment should not be considered as being a general rule, since a considerable variation of responses has been found among different species, following exposure to single or complex mixture of contaminants [16]. For example, under laboratory conditions, some authors have reported a decrease in antioxidant enzyme activities following a short-term exposure of mussels to several pollutants [19, 20]. Others suggested that if mussels were under intense pollution degree, antioxidants appear to be overwhelmed and the antioxidant enzyme activities show a progressive decrease up to a severe depletion [18, 21]. Therefore the inhibition of the CAT activity in October 2011 observed in ST4 compared with reference confirms the presence of an oxidative stress that may affect mussels. Other studies have demonstrated that when invertebrates are subjected to oxidative stress, a series of defense mechanisms start to protect the organism [18, 22]. Nevertheless, when this stress increases, an inhibition of enzyme activity, such as CAT, has been found [18]. This inhibition/decrease in antioxidant defenses indicates the difficulty that the organisms have in defending against oxidative stress.

The toxicity effect of ROS can produce various damages to the cell, such as DNA damage, lipid peroxidation and lysosomal alteration [3, 23 - 24]. Lipid peroxidation is a well-known mechanism of cellular injury in vertebrates and invertebrates, and is an indicator of an oxidative damage in cells and tissues. Therefore, measurement of MDA is widely used as an indicator of lipid peroxidation [25]. An increase in MDA concentration is found in ST4 and ST3. 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].

Significantly higher MDA levels were found in mussels collected at ST4, and ST3, despite the high activity levels of CAT. Nevertheless the anti-oxidant enzyme was unable to prevent the deleterious effects on the lipid membranes (as reported in some cases by Pellerin-Massicotte [26]. This may be due to the fact that low levels of contaminant-stimulated ROS can have a significant toxic effect, particularly upon the

cell membrane and DNA, even when antioxidant enzymatic defenses are responding [20]. According to Regoli [23], when the antioxidant defenses are overwhelmed by the generation of ROS, oxidative damage (lipid peroxidation, protein degradation, enzyme inactivation) and oxidative stress occur.

CONCLUSIONS

The aim of this work was to study two oxidative stress biomarker responses in *perna perna* collected from four stations along the Moroccan Atlantic coast (Big Casablanca area). A higher CAT activity, with a higher induction of MDA levels were observed in mussels collected from site 3 and site 4. The oxidative stress biomarker responses obtained for October 2010 and 2011, clearly demonstrate the potential presence of different contaminants in Site 3 and Site 4 reflecting the intensity of pollution in these areas.

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