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Effects of Metal Sulfates on Catalase and Glutathione-S-transferase of Marine Gastropod: *Osilinus turbinatus*

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ABSTRACT

The aim of this study is to identify a biological responses potentially used as an alarm signal for metal pollution in marine environment.
Several samples of gastropod *Osilinus turbinatus* were taken in north of Algeria, they were exposed to different metal sulfates concentrations ($CdSO_4$, $ZnSO_4$ and $CuSO_4$) under controlled laboratory conditions. After this treatment, the variation of two shield biomarkers was evaluated: the glutathione-S-transferase activity (GST) and catalase activity (CAT). Metal sulfates contamination didn't cause a significant increase in glutathione-S-transferase activity. On the other hand, catalase activity has slightly increased among samples infected with these pollutants.

KEYWORDS: *Osilinus turbinatus*, metal sulfates, biomarkers, glutathione-S-transferase, catalase.

INTRODUCTION

Biomonitoring is now recognized as an essential tool programs monitoring aquatic environment quality. In general, biological monitoring measures disturbance effects on biological communities in place [01].

In recent years, more and more studies have focused on biomarkers evaluation in marine organisms to detect environment heavy metals presence. In mediterranean marine biomonitoring context, several biomarkers have been studied in different organisms [02]. However, we found little ecotoxicological studies on *Osilinus turbinatus*.

Our job is to assess catalase (CAT) and glutathione-S-transferase (GST) changement in *Osilinus turbinatus* exposed to different metal sulfates concentrations under laboratory controlled conditions. Catalase (CAT) and glutathione-S-transferase (GST) activities were used in monitoring programs of aquatic environments to provide early signals of environmental troubles.

Catalase (CAT) is an enzyme found throughout animal kingdom. It is part of antioxidant system [03]. Glutathione-S-transferase (GST) is an enzyme present both in vertebrates and invertebrates. GST catalyzes glutathione conjugation with xenobiotics and endogenous substances [04, 05].

MATERIALS AND METHODS

Gastropods collection

Osilinus turbinatus gastropod individuals were collected from the rocks, at the coast. Sampling was done before the laying period which coincides with summer season, we collected only adult individuals with a diameter ≥ 13 mm [06]. Samples were then transported to the laboratory in aeration tanks containing sea water.

Sampling was carried out at any point of an exempt industrial or urban rejection website, this site is about 1000 m from Ain Defla beach and 20 Km from the Oran city.

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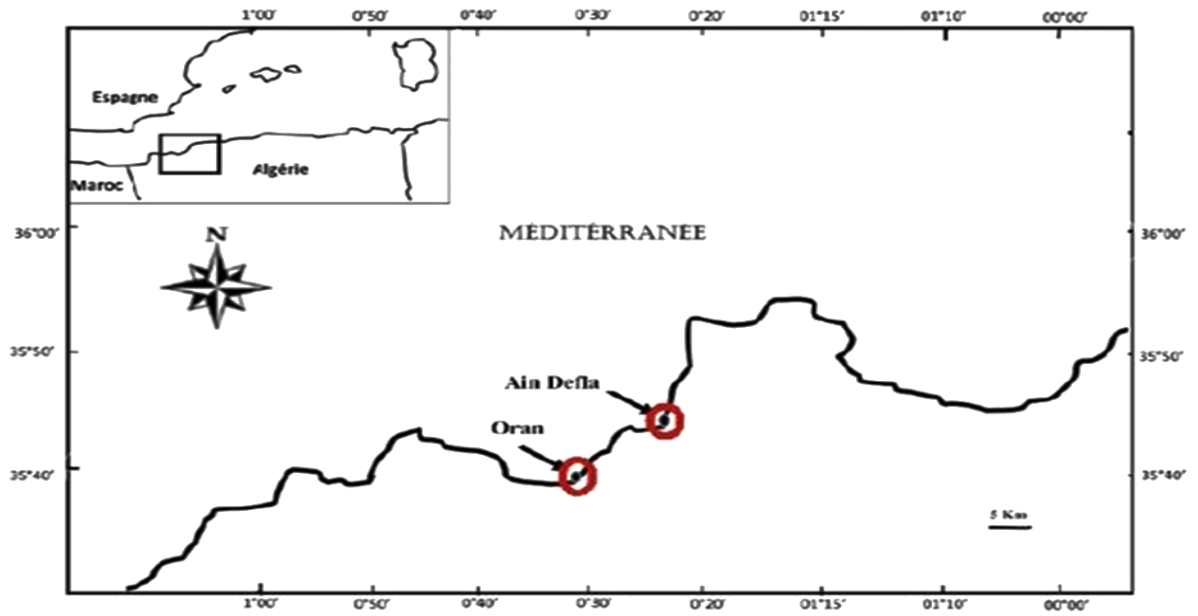

Fig. 01: Sampling site location (Ain Defla) on the Algerian west coast

Gasteropods exposion to metal sulfates

CAT and GST activity was assessed by snails exposing for 96 h at concentrations of the following metal sulfates:

- $CdSO_4$: 1 and 2 $mg.l^{-1}$.
- $ZnSO_4$: 3 and 4 $mg.l^{-1}$.
- $CuSO_4$: 0.1 and 0.15 $mg.l^{-1}$.

Zinc, cadmium and copper sulfates were diluted in sea water with a salinity of 36 $g.l^{-1}$. Nine animals were used for each dilution. Controls were held in tanks containing pure sea water. Ponds were continuously aerated with pumps during the experimental period. Bioassays were conducted in a acclimatized room at 20 ± 1 °C and a photoperiod of 16 h light and 8 h of dark [07].

Dead individuals were recognized by their stillness, and their release foot outside of the shell.

It should be noted that chosen concentrations in this study are lower than lethal concentration (LC50) in recorded species [08].

Biochemical analyses

Digestive glands were crushed and homogenized in 0,1 M phosphate buffer at pH = 7 with a suspension ratio of 1/4 (Tissue weight in grams / ml added in buffer volume). Obtained homogenate was centrifuged at 9000 g for 20 min at a temperature of 4 °C. Supernatant called S9 used to determined CAT and GST activity.

Protein concentrations were determined according to the Bradford (1976) method using bovine serum albumin (BSA) as standard [09]. Catalase activity was measured by the method using hydrogen peroxide as substrate water (Claiborne, 1985) [10]. GST activity was measured according to the technique using the DCNB (dichloronitrobenzene) as substrate [11].

Statistical analysis

Statistical analysis was performed using the software STATISTICA (Statsoft STATISTICA version 6.1.478.0). One-way analyses of variance (ANOVA) were used to compare means calculated. The mean and standard error of the mean are presented. The significance level for all statistical tests was set at $p < 0.05$. Average represented with the same small letter in a histogram indicate that they do not differ significantly.

RESULTS AND DISCUSSIONS

1 - Catalase activity

Tab. 01: Changes in catalase activity in the digestive gland of O. turbinatus exposed to metal sulfates (SO_4Cd, $ZnSO_4$, $CuSO_4$)

	Cd SO_4		Zn SO_4		Cu SO_4	
Metal sulfates on centration (mg l^{-1})	1	2	3	4	0.1	0.15
CAT Activity (nmol min^{-1} mg^{-1} protein)	23,4 ± 1,8	25,5 ± 1,9	11,5 ± 0,7	12,1 ± 0,9	33,9 ± 2,1	35,1 ± 2.9

CAT activity in control sample is estimated to be 4.6 ± 0.4 nmol min-1 mg-1 protein.

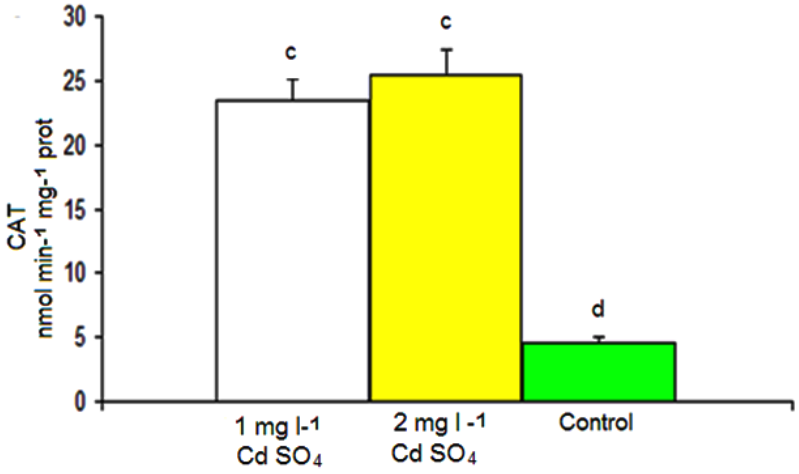

Fig. 02: Catalase activity change in *O. turbinatus* digestive gland exposed to $CdSO_4$

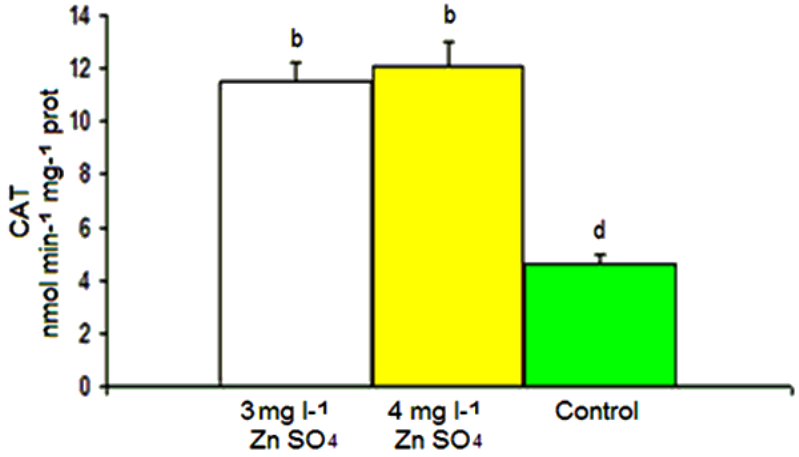

Fig. 03: Catalase activity change in *O. turbinatus* digestive gland exposed to $ZnSO_4$

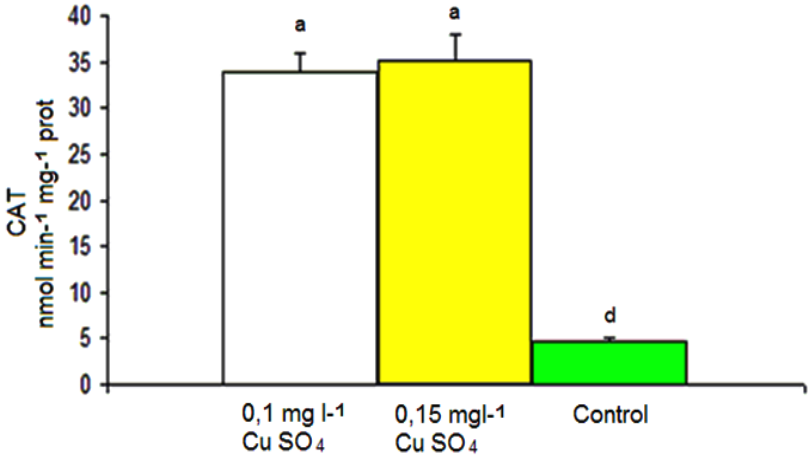

Fig. 04: Catalase activity change in *O. turbinatus* digestive gland exposed to $CuSO_4$

Obtained results show a significant difference in catalase response between individuals exposed to metal sulphates and control individuals. These results demonstrate an effective response to the CAT to the metal contamination in *O. turbinatus* . The increase in catalase activity lead the struggle of the body against reactive oxygen species (ROS).

Some metals susceptible to cyclical changes in their oxidation state (eg, Copper and Iron) can generate reactive oxygen species production such as O2-, HO • and O2 • intracellularly [12]. These oxygen free radicals are highly reactive due to their unstable electronic structure as they have an unpaired electron. These derivatives

in cell can lead to membrane lipid peroxidation, thiol groups oxidation of certain enzymes or coenzymes, to nucleic acids alteration [13].

To control the oxidizing action of reactive oxygen species, organisms have many antioxidant enzymes including catalase [14].

We note that laboratory: *Perna viridis* mold exposure to Aluminium, Lead and Cadmium caused an increase of catalase [15].Due to contamination by Cadmium, catalase activity was significantly increased in the murex *Hexaplex trunculus* [16].

2- Glutathione-S-transferase activity

Tab. 02: Glutathione-S-transferase activity variation in *O. turbinatus* digestive gland exposed to metal sulfates (SO_4 Cd, Zn SO_4, Cu SO_4)

	Cd SO_4		Zn SO_4		Cu SO_4	
Metal sulfates concentration (mg l^{-1})	1	2	3	4	0.1	0.15
GST activity (nmol min^{-1} mg^{-1} protein)	09,05 ± 0,7	10,12 ± 0,8	09,32 ± 1,1	10,9 ± 0,6	10,11 ± 0,1	09,87 ± 1,23

GST activity in control sample is estimated to be 09,37 ± 0 ,67 nmol min-1 mg-1 protein.

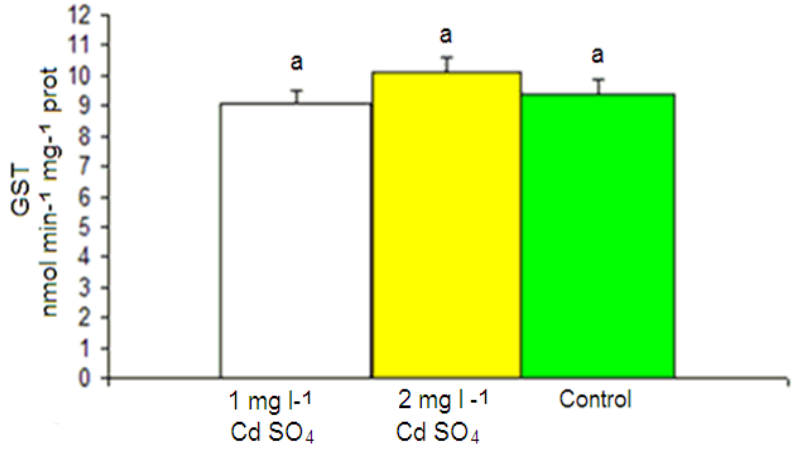

Fig. 05: Glutathion-S-transferase activity change in in *O. turbinatus* digestive gland exposed to $CdSO_4$

Fig. 06: Glutathion-S-transferase activity change in *O. turbinatus* digestive gland exposed to $ZnSO_4$

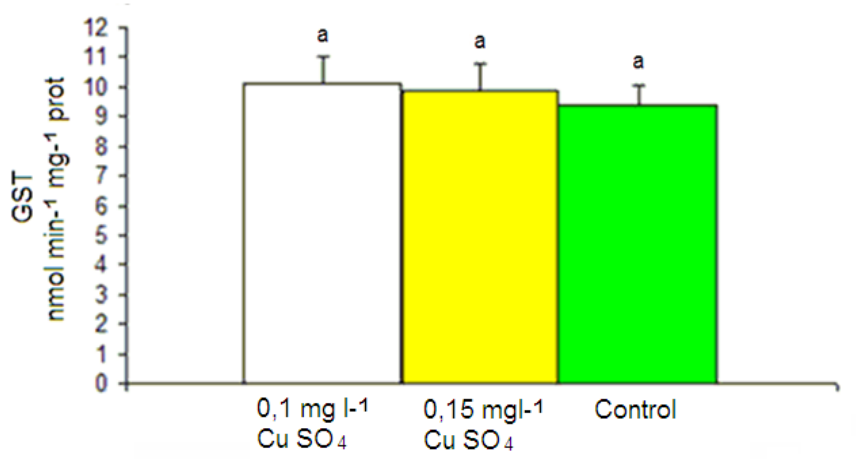

Fig. 07: Glutathion-S-transferase activity change in *O. turbinatus* digestive gland exposed to $CuSO_4$

No significant GST activity variation was observed among individuals exposed to metal sulphates and control individuals. Our results show that *O. turbinatu* does not favor the GST to fight against metal pollution. Instead, the species favors CAT to control heavy metals action, and possibly superoxide dismutase (SOD), glutathione peroxidase (GPX) and metallothionein (MT). These were used as a biomarker of metal contamination in *Mytilus galloprovincialis* [17].

We note that laboratory: no induction of GST activity was observed in *Mytilus galloprovincialis* molds exposed to Copper [18]. GST activity inhibition has been reported in shells St Jacques *Adamussium colbecki* contaminated by copper and cadmium [19]. An increase of GST was observed following exposure to Cadmium, Copper and Zinc in *Perna perna* mussel [20].

CONCLUSION

Enzyme activities measurements have different profiles. Unlike glutathione S-transferase activity, which showed no significant change in our experiments conducted in laboratory, catalase activity increased significantly in metal sulfates contaminated individuals. Catalase activity Increase can be regarded as a defense mechanism to control heavy metals action may cause adverse effects at cellular and subcellular level in *O. turbinatus*.

A thorough study of abiotic parameters influence on catalase activity in *O. turbinatus* pave the way for an *in situ* catalase activity use as biomarker of metal pollution.

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