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Acute toxicity of cadmium on *Donax trunculus*: acetylcholinesterase, glutathione S-transferase activities and pattern of recovery

Soumeya Belabed and Noureddine Soltani*

Laboratory of Applied Animal Biology, Department of Biology, Faculty of Sciences, University Badji Mokhtar of Annaba, Annaba, Algeria
**noureddine.soltani@univ-annaba.org*

ABSTRACT

Pollution by heavy metals is a serious problem due to their toxicity and ability to accumulate in the biota. The present study was undertaken to investigate the acute toxicity of cadmium, a heavy metal widely detected in the aquatic environment due to natural effects and anthropogenic activities. Thus, we evaluate the toxicity of this metal on acetylcholinesterase (AChE) and glutathione S-transferase (GST) activities in the marine bivalve Donax trunculus L. (Mollusca, Bivalvia). Cadmium was added in the rearing water at a concentration corresponding to 96-h LC50 prealably determined. The activities of GST and AChE were determined in the mantle at different exposure times (0, 24, 48, 72 and 96h). The results show a significant decrease ($p< 0.001$) in AChE activity and a significant increase ($p< 0.05$) in GST activity as compared with controls. In a second series of experiment, exposed animals were thereafter transferred to clean water up to 4 days to assess the recovery pattern. The data obtained suggested that D. trunculus was able to overcome relatively rapidly the stress induced by cadmium.

INTRODUCTION

Pollution of aquatic environments by heavy metals is a world-wide problem due to the persistency and continuing accumulation of metals in the environment [1]. The occurrence of heavy metals in the environment mainly results from anthropogenic activities [2].Trace metals are important persistent pollutants in aquatic ecosystems world-wide and are especially prevalent in freshwater, estuarine and coastal marine ecosystems exposed to high degrees of urban pressure [3,4,5]. The concentration of heavy metals in natural environment depends on both natural and anthropogenic factors, which may play an important physiological role, but, also may impose a toxic effect on biosensors [6]. Morever, heavy metals can bioconcentrate and bioaccumulate in the food chain and contribute to chronic toxicity [7]. Consequently, evaluating the ecological and ecotoxicological risks linked to trace metal contamination is becoming a major issue [8]. Such heavy metal like cadmium (Cd), is a non essential element to living organisms in nature, which can cause highly toxicity [9]. Most of the cadmium in the marine environment is estimated to come from anthropogenic sources, mainly as industrial effluent [10]. Marine invertebrates represent an integrant part of aquatic ecosystem and for this reason they are essential keys to evaluate its health [11], they can bioaccumulate, biomagnificate or biotransfer certain metals to concentrations high enough to bring about harmful effects [12, 13]. The suspension-feeder bivalve *Donax trunculus* (Bivavia: Donacidae) is largely distributed in West-African, European and Mediterranean coasts and has been previously used as a sentinel species in environmental assessment [14, 15, 16, 17]. [18,19]. This species was found in higher densities in the sand beaches of the Annaba gulf in Algeria [20]. Growth and population dynamics [21] and the reproductive cycle of *D. trunculus* in Annaba gulf have been examined [22]. Their habitats are exposed to several pollutants from different sources [23]. Biomarkers are now generally accepted as useful tools in monitoring programs for the assessment of the impact on

marine ogranisms and ecological health of pollutants and anthropogenic activities [24, 25, 26]. Indeed, these organisms are protected against oxidative stress by several defense mechanisms with antioxidant enzymes such as glutathione S-transferase, a family of enzymes with a key role in the general biotransformation of xenobiotics and endogenous substances [27]. Acetylcholinesterase activity is considered as an exposure biomarker to organophosphate and carbamate pesticides [28], and also to other contaminants such as metals, synthetic detergents, some components of fuel oils and algal toxins [29,30,31,32].The present study was undertaken in order to estimate the 96-h LC50 value of cadmium, a heavy metal widely detected in the aquatic environment, and to investigate its acute toxicity on AChE and GST activities, and to study the recovery pattern in an edible species used as a sentinel organism *D. trunculus*.

MATERIALS AND METHODS

2.1. *Animals and experimental conditions*

Donax trunculus (Linnaeus, 1758) were collected from El-Battah beach (36° 50'N- 8° 50'E).

The sandy beach of El-Battah was chosen as a relatively clean site, located approximately 30 kilometers to the Est of Annaba city (Algeria), far from any source of pollution. After collection, bivalves (27.48 ± 2.28 mm) were transported to the laboratory and acclimatized during 48 h before exposure in 50-liter glass aquaria. Exposed and control bivalve were reared in aquaria containing seawater (temperature: 17.68 ± 0.14 °C, salinity: 34.0 ± 2.25 g /L, pH: 8.28 ± 0.27, dissolved oxygen: 3.05 ± 0.06 mg/L) and sand which come from El-Battah site and 100 individuals in each aquarium.

2.2. *Chemical and toxicity test*

The concentrations of cadmium used in this study were 5, 7.6 and 10 mg/L. Three replications of 100 individuals per dose, were used. The duration of experiment was 96h. The mortality percentages in the different treatments were corrected in accordance [33] and analysed by probit analysis [34]. The LD_{50} and LD_{90} values (i.e., the dose causing mortality/effects in 50 and 90% of the treated animals, respectively) together with corresponding 95% confidence limits (95% CL) were calculated [35].

2.3. *Enzyme essays*

D. trunculus were exposed to LC_{50} concentration for 96 h of cadmium determined previously. Bivalve that survived after 96 h of exposure were transferred into a medium not contaminated (considered as day 0) up to 4 days to study the recovery pattern of environmental biomarkers. In each experiment, untreated bivalve were also used as controls. At appropriate time, animals were sampled from control and treated series during the exposure (96 h) and recovery (4 days) periods. Each mantle was dissected and stored until biomarker analysis. The AChE activity was estimated accordingly to [36], using acetylthiocholine as a substrate. The activity rate was measured as change in optical density (OD/min) at 412 nm. GST activity was determined using 1-chloro-2,4-dinitrobenzene (CDNB) as suubstrate according to [37]. The activity rate was measured as change in optical density (OD/min) at 340 nm. The final avtivity of AChE and GST was expressed as µM/min/mg protein. Protein content was quantified by the Coomassie Blue method [38], using Bovine Serum Albumin (BSA) as standard.

2.4. *Statistical analysis*

Results are presented as means ± standard deviation (SD). Data from bioassays subjected to analysis of variance after angular transformation of corrected mortality percentages. When the analysis of variance was significant ($p<0.05$), mean values obtained were separated by Least Significant Difference test (LSD). Differences between control and exposure groups were determined by Tukey's test. In the other experiments, the comparison of mean values was made by Student's *t*-test. A significant difference was assumed when $p < 0.05$. All statistical analyses were performed using MINITAB Software (Version 15, Penn State College, PA, USA).

RESULTS

3.1 *Toxicity tests*

Figure 1 shows the relation between the cadmium concentration and the mortality rate of *Donax trunculus*. Percentage of mortality at 96 h was 75% in 5mg/L and 83% in 7.6mg/L of Cd. *D. trunculus* had 92% mortality in 10mg/L. The results obtained from acute statistic toxicity experiments of cadmium upon *D. trunculus* were evaluated by using Finney's Probit Analysis. The lethal concentrations estimated after a 96-h exposure together with their corresponding 95% confidence limits were: LC50= 2.59 mg/L (2.25-2.97) and LC90= 9.25 mg/L (8.04-10.63).

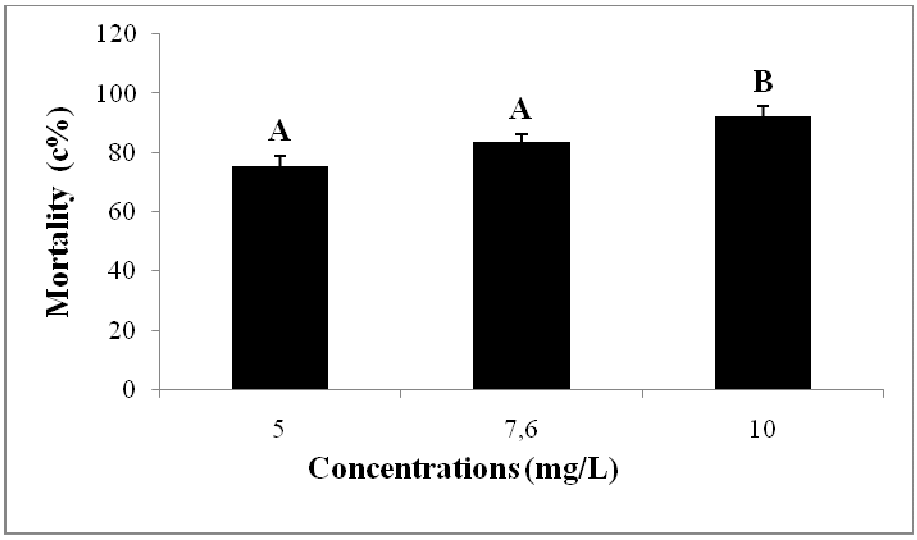

Fig 1. Acute toxicity of cadmium on *D. trunculus*: mortality (%) recorded after a 96-H exposure as function the concentration (means ± SD; values affected with a different letter are significantly different at p<0.05).

3.2. *Change in glutathione S-transferase activity*

The acute studies comprised of daily exposure to the metal at 2.59 mg/L for 96 h followed by a recovery studies up to 4 days. The results relating to the effect of cadmium on the GST activity are presented in figure 2. The exposed bivalves exhibited significant induction in GST activity. The increase in GST activity is significant ($p < 0.05$) at 24, 48 h, 72 h ($p< 0.01$) and at 96 h ($p < 0.001$) of exposure. The values recorded increased until 96 h of exposure to reach a maximum of 4.67 ± 1.00 µM/min/mg proteins. These results were confirmed by ANOVA two-way, a significant ($p < 0.001$) time ($F = 7.13$ df= 4, 33) and treatment ($F = 43.78$, df= 1, 33) effects, and a significant ($p < 0.01$) time-treatment interaction ($F = 4.08$, df= 4, 33) were observed. The Tukey's test revealed that there was a significant difference in GST activity between controls and Cd-treated series.

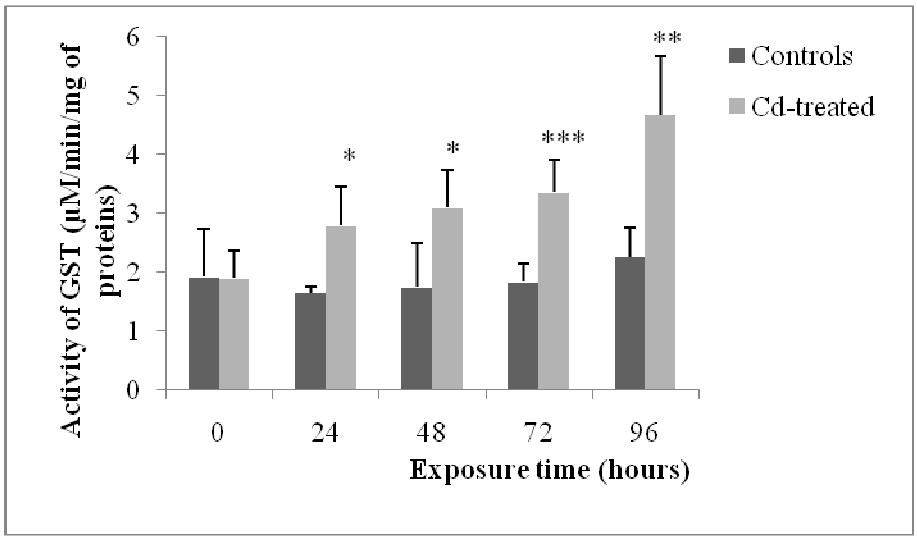

Fig 2. Activity of GST (µM/mn/mg of proteins) on *D. trunculus* exposed to cadmium at LC_{50} (m ± SD ; n=4-5) (*: significant difference at p < 0.05; **: significant difference at p < 0.01; *: significant difference at p < 0.001).**

3.3. *Change in acetylcholinesterase activity*

After a 24h exposure to Cd, data show that the rate of AChE activity decreased significantly in dose dependent manner in treated bivalves compared with the control (Fig 3). Variance analysis showed that the inhibition of AChE activity was very highly significant ($p < 0.001$) at the end of exposure to achieve a minimum of 10.19±1.17 µM/min/mg proteins.

Two way ANOVA revealed a significant ($p < 0.001$) effects of treatment ($F = 60.87$, df= 1, 40) and exposure time ($p< 0.01$) ($F = 4.61$, df= 4, 40), and a significant ($p < 0.001$) time - treatment interaction ($F = 7.02$, df= 4, 40). The Tukey's test revealed that there was a significant difference in GST activity between controls and Cd-treated series.

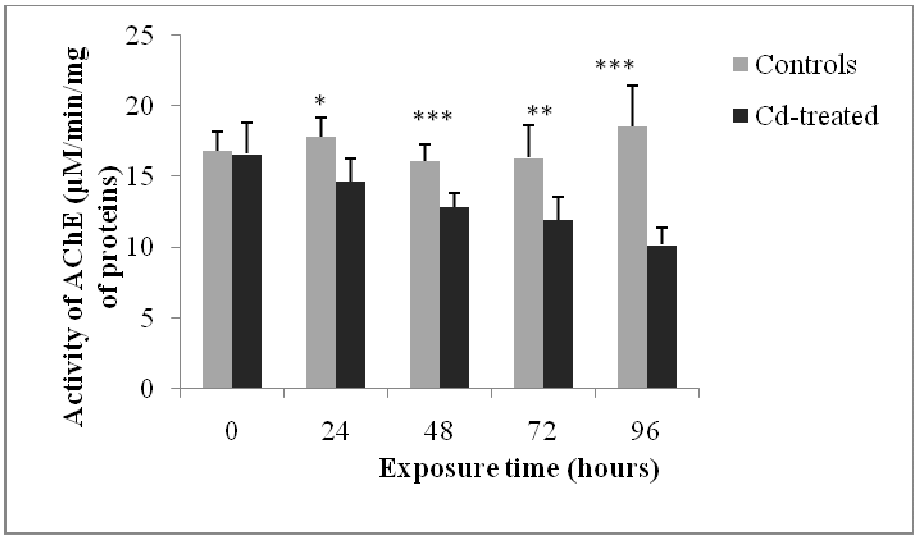

Fig 3. Activity of AChE (µM/mn/mg of proteins) on *D. trunculus* exposed to cadmium at LC_{50} (m ± SD ; n=4-5). (*: significant difference at p < 0.05; **: significant difference at p < 0.01; *: significant difference at p < 0.001).**

3.4. *Recovery study*

Bivalves were exposed to LC_{50} of cadmium for 96 h (day 0) then transferred to clean water, when AChE and GST activities were measured at different intervals of day 0, 2 and day 4 (Figs 4 & 5). At day 2, the exposed bivalves exhibited significant decrease in GST activity. In contrast, there was a very significant difference ($p < 0.01$) in GST amounts between Cd-treated series and controls at day 0 and 2, which was gradually restored to the control levels by day 4 (Fig 4). AChE activity remained relatively lower compared to control during the recovery period, followed by an significant decrease ($p < 0.05$) at day 2 (Fig 5). On the other hand, at the end of depuration period (4 days) there was no significant ($p > 0.05$) difference between control and treated series, which indicates that the mollusc bivalve *D. trunculus* have the ability to overcome the stress of toxicant.

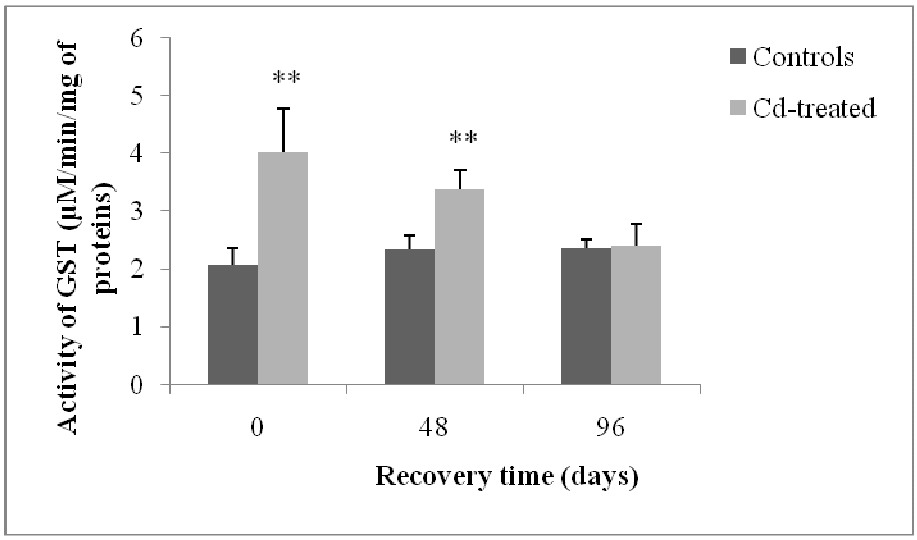

Fig 4. Activity of GST (µM/mn/mg of proteins) on *D. trunculus* exposed to cadmium at LC_{50} for 96 h and its recovery response (m ± SD ; n=4-5). (*: significant difference at p < 0.05; **: significant difference at p < 0.01; *: significant difference at p < 0.001).**

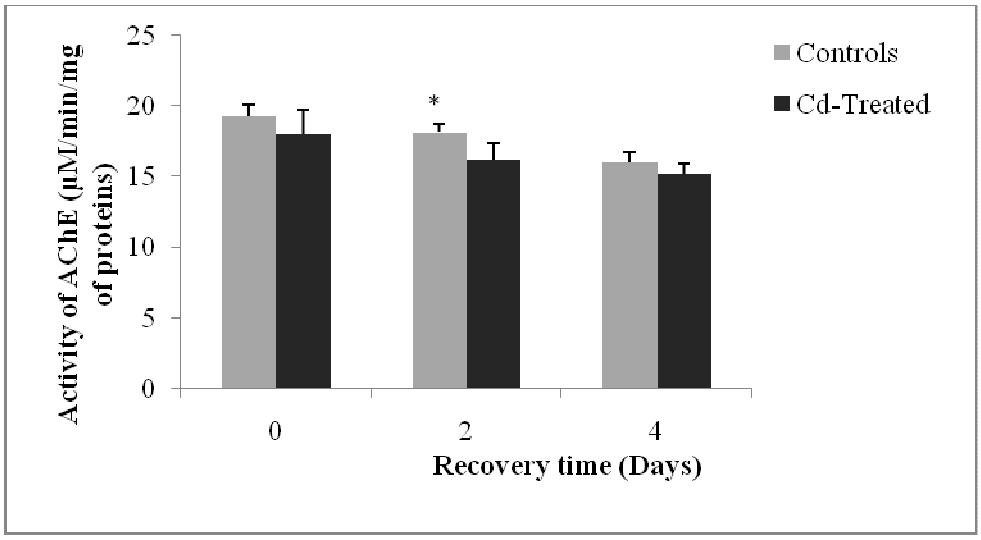

Fig 5. Activity of AChE (µM/mn/mg of proteins) on D. trunculus exposed to cadmium at LC_{50} for 96 h and its recovery response (m ± SD ; n=4-5). (*: significant difference at p < 0.05; **: significant difference at p < 0.01; *: significant difference at p < 0.001).**

DISCUSSION

Antioxidant defense system, which is generally ubiquitous in animal species and different tissues types, is found widely in aquatic organisms. When subjected to metal contamination, bivalves are subjected to oxidative damage, increasing the cellular concentration of Reactive Oxygen Species (ROS). Under elevated metal levels, they are also able to activate their antioxidant systems in order to eliminate ROS, inducing the activity of antioxidant enzymes [39]. The present investigation presents the effect of Cd exposure to bivalve *D. trunculus*, we tested the responses of biochemical biomarkers such as GST activities, an enzyme of the phase II of biotransformation process, and the activity of AChE, an important enzyme in the maintenance of normal nerve function [40].

Induction of GST activity has been used as a biomarker of exposure to xenobiotics, that catalyze the conjugation of variety of electrophilic substrate to the thiol group of GSH, producing less toxic forms [41]. Numerous studies reported raised GST activities in diverse aquatic species in response to environmental or laboratory exposure to xenobiotics [42, 43]. However, the activity of antioxidant enzymes such as CAT, SOD, and GST, can vary depending on the intensity and duration of the chemical stress applied to the organism in addition to the susceptibility of the exposed species [44].In the present work, GST activity of *D. trunculus* was increased in a time-dependent manner at all of the exposed concentrations of Cd, indicating that Cd stress can induce the ROS generation and interfere with the antioxidant enzymatic defense system in bivalves [45]. Additionally, some studies suggested that the oxidative stress induced by the heavy metals results in an increase in ROS, stimulating an increase in antioxidant enzyme activity [46]. It has been shown that antioxidant defense systems protect cells from Cd-induced toxicity [47]. GST plays a critical role in mitigating oxidative stress in all life forms [48, 49], and GST activity also has been widely used as a biomarker to detect stress [50, 51]. As an antioxidant enzyme, GST activities had either a significant increase or decrease with different patterns according to the exposed elements or exposure conditions [39]. In fact, GST activity of *D. trunculus* increased upon Cd exposure, coinciding with our study. Similar results were obtained in previous investigations of polychaetes *Nereis diversicolor* [39] and *Laeonereis acuta* [52] exposed to Cd. The induction of GST activities was also reported in several fish species: *G. affinis* treated with cadmium [53], with halofenozide [54] or with diflubenzuron and flucycloxuron [55], in *Oreochromis niloticus* exposed to diazinon [56] and in *O. mossambicus* exposed to monocrotophos [57] indicating ongoing detoxification mechanisms. [58] showed that with Cd contamination *Ruditapes philippinarum* increased the activity of GST. On the other hand, a decrease in the GST activity was reported in mussels *Mytilus galloprovincialis* exposed to benzo[a]pyrene [59, 60]. It was also shown that the population of *D. trunculus* inhabiting in a polluted site had a lower activity of GST than that living in an unpolluted site [61]. GST 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 [62].

Acetylcholinesterase (AChE) activity is considered of great interest in evaluating the effects of exposure to neurotoxic compounds in aquatic animals [63].It is an enzyme involved in the synaptic transmission of nerve impulses and is inhibited by neurotoxic compounds [64]. However,the responsiveness of AChE to other chemicals including metals has also been reported [65,66]. Several studies showed the potential use of this enzyme activity as a

useful biomarker for detecting general physiological stress in aquatic organisms caused by exposure to contaminants [67]. The results of present study showed important inhibition of AChE activity after the exposure of *D. trunculus* to cadmium compared to controls. The significant responses indicated that the AChE activity decreased as function the exposure time to reach a minimum until the end of treatment (96h).This inhibition may be the result of a neurotoxic effect due to cadmium toxicity. Moreover, A correlation was reported between heavy metal pollution and decreases in AChE activity in *D. trunculus* from industrialised areas and harbour sectors in the gulf of Annaba [68, 23, 69, 19, 70]. Similar observations have been reported in Silver Catfish *Rhamdia quelen* exposed to Cadmium [71], in *G. affinis* exposed to FCX [72] and to chlorpyrifos [73]. [74] found significant reduction in AChE activity of *M. galloprovincialis* exposed to copper, and in the blue mussel *M. edulis* exposed to azamethiphos[75], as well as in the zebra mussels *Dreissena polymorpha* exposed to chlorpyrifos and terbutilazine[76]. The inhibition of AChE by different metals and PHC indicated that lead, cadmium and copper are the most predominant inhibitor [77]. Moreover, AChE activity is extremely variable between species [28].

When *D. trunculus* were removed from cadmium exposure and transferred to clean water, recovered rapidly AChE and GST activities after 4 days. In the recovery period, GST and AChE levels of mollusc exposed to cadmium for 4 days were similar to the control value. This metal induced an oxidative stress [78]. Subsequently, this rapidly stimulated the antioxidant defences as evidenced by changes in biomarkers measured during the treatment and the recovery period. However, these enzymes reacted during this period, GST decreased its activity, possibly indicating a compensatory response against the toxic. In contrast, AChE increased during the recovery period, indicating metal toxicity. Increased GST activity in *Rhamdia quelen* after cadmium exposure was observed and the recovery period of 7 days are needed [71]. GST activity in *G. affinis* exposed to DFB and FCX required periods of 1 and 2 days respectively of recovery [72]. The activity of GST showed in both species *Ruditapes decussatus* and *Ruditapes philippinarum*, a decrease from environment condition to 7 days of depuration [79]. In *Rhamdia quelen* exposed to cadmium, a period of 14 days was necessary to recover AChE activity [71] while *G affinis* exposed to FCX, recovered AChE activity after 4 and 8 days in clean water according to concentration [72]. The recovery was influenced by time of exposure and type of toxic used.

In conclusion, our results indicate that the mollusk bivalve *D. trunculus* can be used as a bioindicator for acute exposure to cadmium. This metal stimulated rapidly the antioxidant system as evidenced by an increase in GST activities The decreases in AChE activity also suggested a neurotoxic action of Cd . The recovery pattern showed that *D. trunculus* have the ability to overcome rapidly the stress induced by treatment.

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