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# CADMIUM EXPOSURE ON *Donax trunculus* (MOLLUSCA BIVALVIA) LETHALITY PARAMETERS AND BIOMARKERS RESPONSES

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## ABSTRACT

Cadmium is a non-essential heavy metal in the biological system. In this study, we have highlighted to evaluate the effect toxicity of cadmium chloride (CdCl<sub>2</sub>) against Marine Bivalve *Donax trunculus* L. (Mollusca, Bivalvia) under laboratory conditions. The acute bioassay tests at various gradient concentrations of cadmium (5, 7.6, and 10 mg/L) were performed to estimate lethal concentrations 50 (LC<sub>50</sub> Concentration estimated that produces a 50% mortality in a test population over a specific period) and 90 (LC<sub>90</sub>) for 24, 48, 72, and 96 hours of exposure. 96h-LC<sub>50</sub> and 96h-LC<sub>90</sub> values are: 2.59 and 9.25 mg/L respectively. In addition, the sublethal concentrations (LC<sub>10</sub> and LC<sub>25</sub>) at acute exposure (96 h) which are: 0.72 mg/L and 1.32 mg/L respectively, and their fiducial limits were investigated. The results obtained indicate that cadmium causes mortality in the species studied with a Concentration-response relationship. Thus, specimens of this marine bivalve were experimentally exposed to CdCl<sub>2</sub> at concentrations corresponding to 96h-LC<sub>10</sub> and 96h-LC<sub>50</sub>, to evaluate the effect of toxicity on catalase (CAT) and acetylcholinesterase (AChE) activities, followed by a recovery period during 4 days. Results showed that Cd inhibited AChE activity suggesting neurotoxicity disturbances. In addition, we observed a stimulation of the detoxification system as shown by an increase in catalase activity. Additionally, the transfer of individuals exposed to CdCl<sub>2</sub> in untreated water showed a gradual restoration of the initial state. The data obtained from this study will provide useful information for understanding the toxicological process of CdCl<sub>2</sub> in *Donax trunculus*.

## KEYWORDS:

Toxicity, Cadmium, Biomarkers, *Donax trunculus*, Mortality

## INTRODUCTION

Aquatic systems are highly endangered by pollutants due to a variety of chemical compounds from the constant increase in anthropogenic activities.

Such activities are considered a threat to aquatic life; these are altering the natural interactions to the extent that they are polluting seawater and threatening marine life [1]. Among the various environmental pollutants due to human activities, the use of fertilizer, industrial mining, smelting, and production of municipal waste [2], are the most common contaminants present in the aquatic system [3]. Heavy metals are naturally present in the environment, and some are even essential to the proper functioning of many biological processes. They are also potentially accumulated in the marine environment as well as in organisms [4, 5]. Indeed, these metal contaminations do not undergo any biological or chemical degradation; they are easily bio-concentrated, biomagnified in food chains [6], and are mainly toxic for terrestrial and marine species [7, 8]. However, high levels of those known as non-essential trace elements can be toxic for aquatic organisms; this is the case for lead and cadmium [9], [10]. Many of the mussel's samples resulted in contamination, therefore potentially posing a considerable health risk to consumers [11]. In this respect, contaminants affecting the quality of marine waters are classified by environmental agencies according to the level of toxicity [1]. Cadmium (Cd) is characterized as a toxic heavy metal that accumulates in biological systems [12]. Although toxic, cadmium is widely used in industrial activities [13], and its concentrations have reached 42 µg/L in coastal waters [14]. In these environments, benthic organisms are frequently subject to cadmium pollution [15]. Some effects of Cd, even at low concentrations, have been demonstrated to produce cytotoxicity, oxidative stress, and severally effects on the immune system from marine organisms [16, 17, 18, 19]. Therefore, the need to understand the patterns of exposure and the toxic effect is of great importance [20, 21]. Various experimental studies have shown that oxidative damage induced by ROS has an important role in metal toxicity [22]. Likewise, new findings on the potential accumulation of pathogenic bacterium "*Salmonella* spp" associated with neurotoxicity and oxidative stress in the wedge clam *Donax trunculus* have been provided [23].

Indicators of oxidative stress are enzymes of the antioxidant defense system, that play a key role in detoxification by eliminating free radicals and

protecting the cell, and are used as assessment tools to measure the impacts of environmental stress such as catalase (CAT, EC 1.11.1.6) [24,25]. Catalase is implied in the defense of the cell against the toxic effects of hydrogen peroxide by catalyzing its decomposition to water and oxygen, it is a biomarker of oxidative stress [26]. Thus, it shows sensitivity to various contaminants [27, 28]. Acetylcholinesterase activity (AChE, EC 3.1.1.7) is widely used as a biomarker of exposure and even effects to some pesticides including organophosphate and carbamate [29, 30]. Damage to the nervous system is measured by cholinesterase activity [25]. Thenceforth, AChE inhibition induces neurotoxicity due to the acetylcholine building up in the area of the synapse, which causes constant stimulation of the nervous system, which leads to tetanic paralysis and mortality [31, 32]. The activity of this enzyme can also be selectively inhibited by other Pollutants such as metals [33, 34].

*Donax* species are recently being used to monitor the ecological status of coastal areas [35, 36], as *Donax trunculus* L. (Mollusca, Bivalvia) has been used widely in ecotoxicology and marine pollution studies [37, 38, 39]. In the gulf of Annaba (Algeria), the quality of hydro-systems is altered by the quantity and diversity of effluents released into the natural environment. Also, previous studies have shown that the effluents are enriched in heavy metals, particularly cadmium, with rates that vary significantly depending on the season and the site [40, 41, 42, 43]. Moreover, the changes in antioxidant defense systems reveal the perturbation of the physiological states in clams [44].

The present study aims to evaluate the acute toxic effect of cadmium chloride in marine bivalve, *Donax trunculus*. CdCl<sub>2</sub> is added to the reared water at different concentrations (5, 7.6 and 10 mg/L) to estimate the lethal concentrations (LC<sub>10</sub>, LC<sub>25</sub>, LC<sub>50</sub>, LC<sub>90</sub>) and to evaluate under laboratory conditions its effect on catalase and acetylcholinesterase activities during an acute exposure (24, 48, 72 and 96 hours) and recovery periods of 4 days (transfer to uncontaminated water). *D. trunculus* was chosen in our study for its wide potential tolerance to high concentrations of heavy metals [41, 42], in particular cadmium, and also for its natural resistance to difficult conditions in the Gulf of Annaba (Algeria) [45, 46, 47].

## MATERIALS AND METHODS

**Sample collection and rearing.** Specimens of *Donax trunculus* (Linnaeus, 1758) were collected from El-Battah Beach (northeast of Algeria) (36 ° 50 'N, 8 ° 50' E), it was chosen as a relatively clean site [46], located approximately 30 km east of Annaba city (Algeria), far from any source of pollution. The experiments were carried out during the spring

corresponding to the reproduction period [48]. After collection by using a hand scraper, bivalves (26.20 ± 2.19 mm shell length) were immediately transported to the laboratory in plastic buckets with natural seawater and they were acclimatized for 2 days in an ambient condition before exposure in 50-l glass aquaria equipped with air pumps (Nirox X5). After acclimation, Exposed and controlled bivalves (without distinction of sex) were reared in aquaria containing seawater and sandy bottom material from the El-Battah site. One hundred individuals were placed in aquaria filled with 25 L of seawater (4.0 individuals/L) and exposed to Cadmium chloride (CdCl<sub>2</sub>) for 96 h, counterpart jointly with a control group kept in clean seawater. Three repeats per treatment were used. The physicochemical parameters of seawater were recorded using a multiparameter (Multi 340 I, Germany) during the study period. Physico-chemical characterization of seawater during exposure (such as: temperature: 16.37 ± 1.50 °C; salinity: 26.50 ± 2.60 g/l; pH 9.00 ± 1.64; dissolved oxygen: 4.80 ± 0.20 mg/l) and recovery periods (such as: temperature: 16.60 ± 4.00°C; salinity: 28.80± 9.76 g/l; pH 7.74 ± 0.47; dissolved oxygen: 4.233 ± 0.65 mg/l) was estimated. Each individual of *D. trunculus* is considered dead when completely immobile and remaining on the surface of the sand, and no movement is observed.

**Acute toxicity bioassay.** The metal was added to the seawater as cadmium chloride (CdCl<sub>2</sub>: BIO-CHEM Chemopharma, Hemi pentahydrate, USA). Acute toxicity (96 hours of exposure) bioassay experiment was done by exposing one hundred individuals to each of the concentrations of 5, 7.6 and 10 mg/L after each exposure period of 24, 48, 72, and 96 hours. Three replications per dose were used. The mortality percentages in the different treatments were corrected in accordance [49] and analyzed by probit analysis [50]. The LC<sub>10</sub> and LC<sub>25</sub> values (lethal concentration inducing 10 and 25% mortality), and The LC<sub>50</sub> and LC<sub>90</sub> values (lethal concentration causing mortality effects in 50 and 90% of the treated animals, respectively) together with corresponding 95% confidence limits (95% CL) were calculated [51].

**Determination of catalase and acetylcholinesterase activities.** *D. trunculus* were exposed to LC<sub>10</sub>, and LC<sub>50</sub> concentrations for 96 h of cadmium chloride determined previously (96h-LC<sub>10</sub> = 0.72 mg/L; 96h-LC<sub>50</sub> = 2.59 mg/L). The biochemical assay was performed on individuals with 26.20 ± 2.19 mm representing the most abundant size. Measurements were made in control (Unexposed bivalves) and Cd-treated samples during 0, 24, 48, 72, and 96 h exposure followed by a period of recovery (0, 2, and 4 days).

Catalase (CAT, EC 1.11.1.6) activity was measured by the method of [52] and the absorbance was

read at 240 nm, by the variation in optical density resulting from the dismutation of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). The samples are homogenized in 1 ml phosphate buffer (50 mM, pH 7). The homogenate is centrifuged at 15,000 rpm for 10 min and the supernatant is used as an enzyme source. The CAT activity was expressed as  $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}\text{protein}$ . The protein content was evaluated according to [53] using serum albumin as standard (BSA, Sigma) and the absorbance was measured at 595 nm wavelengths.

Acetylcholinesterase (AChE, EC 3.1.1.7) activity in the homogenates was determined following the procedure of [54], by measuring the increase in absorbance of the sample at 412 nm in the presence of 1 mM acetylthiocholine as substrate and 0.1 mM 5,5'-dithiobis-2-dinitrobenzoic acid (DTNB). The tissue was ground in Tris buffer 0.1 M, pH 7.5. The homogenate obtained was centrifuged at 9000g for 20 min. The supernatant was removed and used to determine AChE activity. The AChE activity was expressed as  $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}\text{protein}$ .

**Statistical analysis.** Results are presented as means  $\pm$  standard deviation (SD). Data from bioassays were 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 the 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 16, Penn State College, PA, USA).

## RESULTS AND DISCUSSION

Results obtained on individuals of *D. trunculus* exposed at different concentrations of  $\text{CdCl}_2$  (5, 7.6, and 10 mg/L) show that mortality increased with the concentration and length of time of exposure. Mortality percentage was recorded every 24 h during the 96 h experimental duration. The results obtained from acute statistic toxicity experiments of cadmium, upon *D. trunculus* were evaluated by using [50] Probit Analysis. After treatment, the rate of mortality observed after 96 h reach 90 % (10 mg/L). However, in controls (Unexposed bivalves), the recorded mortality rate is lower (3 %). This confirms that the high mortality rate could be entirely due to cadmium toxic effects. Our results are similar to those of [55] obtained in the same species after 48 and 96 hours of exposure to cadmium. Other reports suggested that the bacterial genus seems to induce high levels of mortalities up to 87% more than chemical compounds such as heavy metals, hydrocarbons, and

pesticides in mussels [23].

$\text{LC}_{50}$  and  $\text{LC}_{90}$  values and their 95% confidence intervals from the toxicity tests are presented in Table 1. Lethal concentrations of 50 and 90 are 13.61 mg/L and 30.55 mg/L at 24 h, compared to 2.59 mg/L and 9.25 mg/L at 96 h, respectively. Toxicity tests revealed a dose-response relationship for different periods of exposure. With the increase of metal exposure concentration, the Cd concentrations significantly increased in a concentration-dependent manner ( $P < 0.05$ ) [24]. Mortality in the control was on average 3 %, and it never exceeded 10 % for all tests [56]. Lethal concentrations 50 were recorded for the different species after 96 h hours of exposure: *Emerita sp.* 1.35 mg/L, *Donax spiculum* 1.8 mg/L, *Perna Viridis* 2.5 mg/L, *Sabellaria clandestinus* 2.8 mg/L, *Modiolus Carvalho* 5.6 mg/L, and *Modiolus sp.* 9.2 mg/L [57]. It is apparent that the toxic effect of cadmium in the invertebrate aquatic mainly concerns several physiological parameters that take a definite species-specific pattern and experiment conditions [57, 58, 59]. Further, cadmium exhibits toxicity to *D. trunculus*, which varies with sex, concentration, and duration of exposure. Indeed, cadmium-induced significant mortality in cadmium-treated females compared to males after acute exposure (96 h). These results suggest a relative sensitivity of females that accumulate more reserves and mobilize more energy during reproduction [43]. Other species have also been widely to estimate  $\text{LC}_{50}$  following cadmium exposure. [60] Found out that the 96h- $\text{LC}_{50}$  in Mollusks *Cerithedia cingulata* and *Modiolus philippinarum* was 9,19 mg/L and 0,22 mg/L respectively. Moreover, the 96h- $\text{LC}_{50}$  value reported in freshwater ostracod *Stenocypris* exposed for four days in laboratory conditions to cadmium was 13.15  $\mu\text{g/L}$  [61]. In addition, with the increase in Cd concentration, an increase in mortality was observed. The  $\text{LC}_{50}$  value in adult palaemonid shrimp *Palaemon macrrodactylus* for 24 h, 48 h, 72 h, and 96 h was 2.60 mg/L, 0.88 mg/L, 0.49 mg/L, and 0.37 mg/L respectively [24].

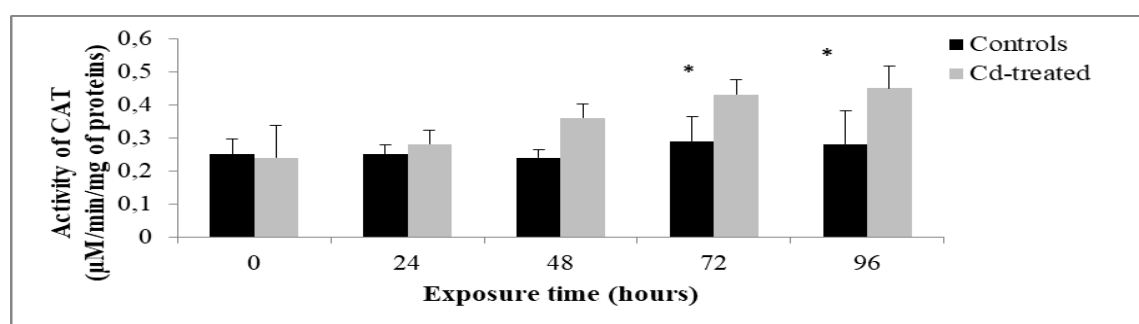
Lethal concentrations 10 ( $\text{LC}_{10}$ ) and 25 ( $\text{LC}_{25}$ ), determined from the regression line are 0.72 mg/L and 1.32 mg/L, respectively (Table 2). Our results are following the results obtained by [62] on a crustacean *Echinogammarus meridionalis*, that they indicate after 96 h of exposure to cadmium an  $\text{LC}_{10}$  of 44.15  $\mu\text{g/L}$  (confidence interval: 33.76 - 63.39). The study carried out by [63] on a Bivalve Mollusk, *Perna Viridis* after 48 hours of exposure to cadmium revealed an  $\text{LC}_{25}$  of 0.21 mg/L (Confidence interval: 0.14 - 0.32), while it is 50  $\mu\text{g/L}$  in *Bathymodiolus azoricus* after 24 days of exposure to cadmium [64]. Also, in the Bivalve Mollusk, *Mytilopsis sallei* the  $\text{LC}_{10}$  is 0.135 mg/L and an  $\text{LC}_{25}$  of 0.298 mg/L after 96 hours of exposure [65]. These differences are probably due to different species used, age, size of the organism, test methods, and water quality that may affect the toxicity [66, 67].

**TABLE 1**  
Lethal concentrations of 50 and 90 (mg/L; LC) of CdCl<sub>2</sub> for *D. trunculus* and their fiducial limits.

Exposure time (Hours)	Regression line	Slope	LC <sub>50</sub> IC [95%]	LC <sub>90</sub> IC [95%]
24	Y = 3,65 X + 0,861	1, 87	<b>13,61</b> (11,73 – 15,78)	<b>30,55</b> (26,33 – 35,43)
48	Y = 2,78 X + 2,24	2, 28	<b>9,83</b> (8,69 – 11,10)	<b>28,43</b> (25,15 – 32,12)
72	Y = 1,84 X + 3,84	3, 47	<b>4,27</b> (3,71 – 4,91)	<b>21,23</b> (18,46 – 24,41)
96	Y = 2,32 X + 4,04	2,69	<b>2,59</b> (2,25 – 2,97)	<b>9,25</b> (8,04 – 10,63)

**TABLE 2**  
Lethal concentrations of 10 and 25 (mg/L; LC) of CdCl<sub>2</sub> for *D. trunculus* and their fiducial limits.

Exposure time (Hours)	Regression line	Slope	LC <sub>10</sub> IC [95%]	LC <sub>25</sub> IC [95%]
24	Y = 3,65 X + 0,861	1, 87	<b>6,06</b> (5,22 – 7,02)	<b>8,89</b> (7,66 – 10,31)
48	Y = 2,78 X + 2,24	2, 28	<b>3,40</b> (3,00 – 3,84)	<b>5,62</b> (4,97 – 6,35)
72	Y = 1,84 X + 3,84	3, 47	<b>0,85</b> (0,73 – 0,97)	<b>1,83</b> (1,59 – 2,10)
96	Y = 2,32 X + 4,04	2,69	<b>0,72</b> (0,62-0,82)	<b>1,32</b> (1,14-1,51)



**FIGURE 1**

CAT activity (µM/min/mg of proteins) in the mantle of *D. trunculus* exposed to cadmium chloride at 96h-LC<sub>10</sub> (m ± SD; n=4). (\*: significant difference at p < 0.05; \*\*: significant difference at p < 0.01; \*\*\*: significant difference at p < 0.001).

The results relating to the effect (LC<sub>10</sub> and LC<sub>50</sub>) of cadmium chloride on CAT activity are presented in Figures 1 and 3. The activity of antioxidant-related enzyme CAT content was significantly influenced by Cd treatment. Exposed bivalves to the metal at 0.72 mg/L (96h-LC<sub>10</sub>) showed significant induction in CAT activity after Cd treatment. CAT activity increased significantly at 48 h (p < 0.01) duration of exposure compared to the controls. However, there was no significant difference (p > 0.05) at 24 h. The recorded values increased through 96 h of exposure to reach a maximum of 0.45 ± 0.066 µM/min/mg proteins against 0.28 ± 0.10 µM/min/mg proteins in controls. A two-way ANOVA revealed a significant effect of Treatment (F<sub>1,27</sub> = 17.56, p < 0.001) and for time (F<sub>4,27</sub> = 5.44, p < 0.01). In marine organisms, the measurement of biomarkers allows the detection of early biological effects of their exposure to toxic compounds from anthropogenic activities [34]. Due to their distribution, fate, and diversity, metals have a significant toxic effect at the sub-individual level [68]. Previous studies have used *D. trunculus* as a sentinel organism to assess the toxicity of cadmium [41, 42, 43, 69, 70]. [69] Have demonstrated that Cd concentrations, exposure time, and sex affect significantly proximate composition and minerals contents

in *D. trunculus*. Exposure to CdCl<sub>2</sub> at two concentrations (96h-LC<sub>10</sub> and 96h-LC<sub>25</sub>) may decrease dry matter, fat and metallic elements, and increase moisture, ash, and Cd contents [69]. Likewise, low-dose (LC<sub>10</sub> and LC<sub>25</sub>) exposure of Cd may activate the antioxidant system and increase the activity of antioxidant enzymes such as the contents of metallothioneins (Mts) [46, 70]. In the present study, Cadmium chloride exposure at a sublethal concentration (96h-LC<sub>10</sub>) affects the activity of the antioxidant enzyme, whose activity of CAT was significantly increased, which indicates damage to the antioxidant system in the Cd-treated series. In contrast, these findings differ from some studies that have proved that Cd exposure leads to significant inhibition of the activity of catalase (CAT) at 96h-LC<sub>50</sub> in shrimp *P. macrodactylus* [24], and in Marine Bivalve *M. galloprovincialis* [71]. [72] Suggested that fish *G. affinis* exposed to chlorpyrifos (organophosphate) to lethal concentration (LC<sub>50</sub>) causes the decreased levels of CAT and SOD activities. In *R. decussatus* exposed to different Cd concentrations (4, 40, and 100 g/L) for 28 days, a decrease in cytosolic and mitochondrial catalase activity was observed [73]. Antioxidant enzymes, such as catalase, belong to the cellular antioxidant system that neutralizes the toxicity of reactive oxygen species that are improved by several

classes of pollutants, including trace metals or organic compounds. Therefore, the CAT activity may play an important contribution to antioxidant protection, suggesting their potential use as biomarkers of effects such as oxidative stress resulting from Cd exposure in aquatic organisms. Individuals of *D. trunculus* that survived after 96 h of exposure (depuration period at 0 days) were transferred to uncontaminated water. CAT levels of unexposed bivalves remained unchanged during the experiment, followed by a decrease during the depuration period in the Cd-treated series (Figure 2). CAT activity decreased until the end of the depuration period (4 days), with similar CAT levels in both control ( $0.25 \pm 0.008 \mu\text{M}/\text{min}/\text{mg}$  proteins) and Cd-treated series ( $0.26 \pm 0.058 \mu\text{M}/\text{min}/\text{mg}$  proteins). During the whole experiment depuration, no significant differences were observed in CAT levels between control and treated ( $p > 0.05$ ). [64] Reported that CAT activity was increased in Mollusk *Bathymodiolus azoricus* after chronic exposure at 24 days to cadmium, although, during the recovery period this activity was restored after 7 days in uncontaminated water. On the other hand, the exposed fish exhibited significant inhibition in CAT activity, which was gradually restored to the control levels by day 16 [72].

The results of CAT activity during exposure at 96h-LC<sub>50</sub> and depuration periods are presented in Figures 3 and 4 respectively. Significant differences in CAT activity were found among *D. trunculus* during exposure and depuration periods. Furthermore, the results obtained show a significant increase ( $p < 0.05$ ) at 24, 72, and 96 hours of exposure in the treated series, which were progressively restored to control levels, there was no significant change observed in comparison to controls by day 4. Increased CAT activity suggests the toxic effect of cadmium. Also, results indicate that CAT activity increased in the mantle of *D. trunculus* with the increase in Cd concentrations. Moreover, CAT activity at 96 h of exposure was  $0.45 \pm 0.066 \mu\text{M}/\text{min}/\text{mg}$  proteins, and  $0.89 \pm 0.045 \mu\text{M}/\text{min}/\text{mg}$  proteins in *D. trunculus* treated by LC<sub>10</sub> and LC<sub>50</sub> respectively. Indicating that both concentrations (LC<sub>10</sub> and LC<sub>50</sub>) of cadmium chloride show an increase in activity of the antioxidant enzyme CAT. A two-way ANOVA revealed a significant effects of Treatment ( $F_{1,34} = 11.14$ ,  $p < 0.01$ ) and for time ( $F_{4,34} = 3.86$ ,  $p < 0.05$ ). Concerning depuration period, a significant induction ( $p < 0,05$ ) has been noted in treated series ( $0.51 \pm 0.10 \mu\text{M}/\text{min}/\text{mg}$  of proteins) after 2 days of recovery comparably with controls ( $0.71 \pm 0.14 \mu\text{M}/\text{min}/\text{mg}$  of proteins).

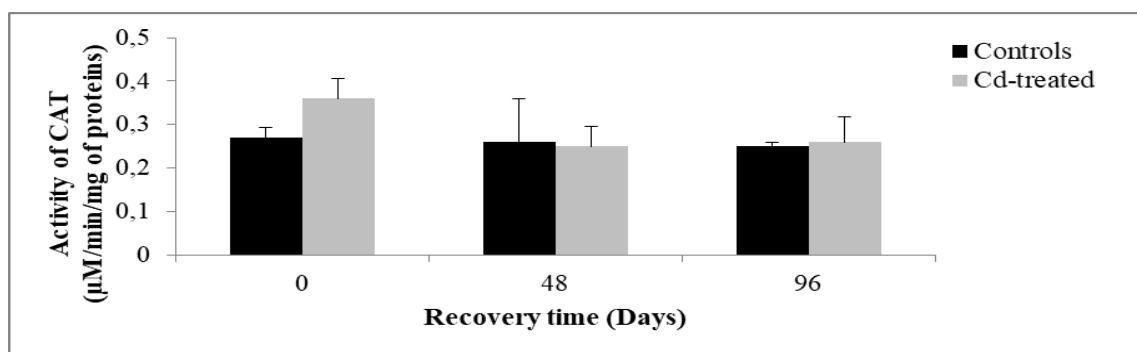


FIGURE 2

CAT activity ( $\mu\text{M}/\text{min}/\text{mg}$  of proteins) in the mantle of *D. trunculus* exposed to cadmium at 96h-LC<sub>10</sub> and its recovery response ( $m \pm \text{SD}$ ;  $n=4$ ). (\*: significant difference at  $p < 0.05$ ; \*\*: significant difference at  $p < 0.01$ ; \*\*\*: significant difference at  $p < 0.001$ ).

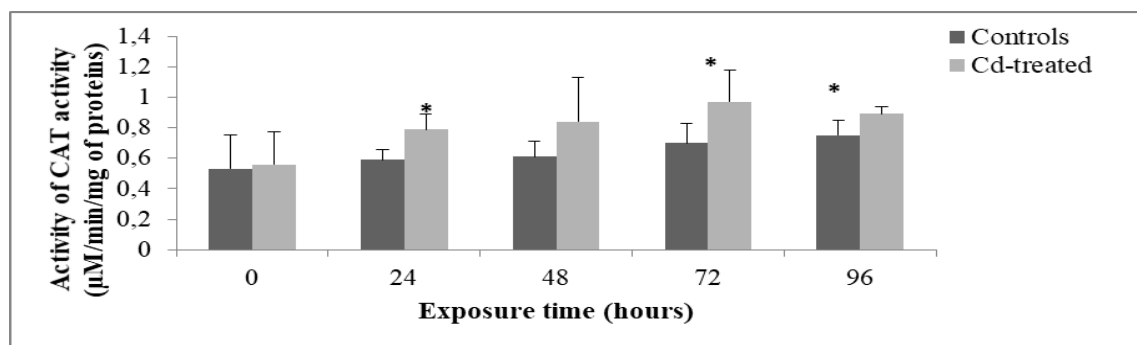


FIGURE 3

CAT activity ( $\mu\text{M}/\text{min}/\text{mg}$  of proteins) in the mantle of *D. trunculus* exposed to cadmium chloride at 96h-LC<sub>50</sub> ( $m \pm \text{SD}$ ;  $n=4$ ). (\*: significant difference at  $p < 0.05$ ; \*\*: significant difference at  $p < 0.01$ ; \*\*\*: significant difference at  $p < 0.001$ ).

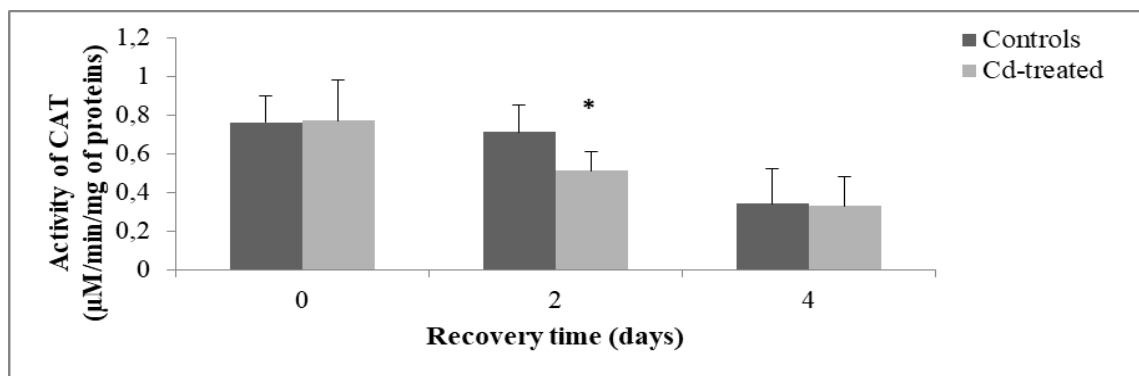


FIGURE 4

CAT activity ( $\mu\text{M}/\text{min}/\text{mg}$  of proteins) in the mantle of *D. trunculus* exposed to cadmium at 96h-LC<sub>50</sub> and its recovery response ( $m \pm \text{SD}$ ;  $n=4$ ). (\*: significant difference at  $p < 0.05$ ; \*\*: significant difference at  $p < 0.01$ ; \*\*\*: significant difference at  $p < 0.001$ ).

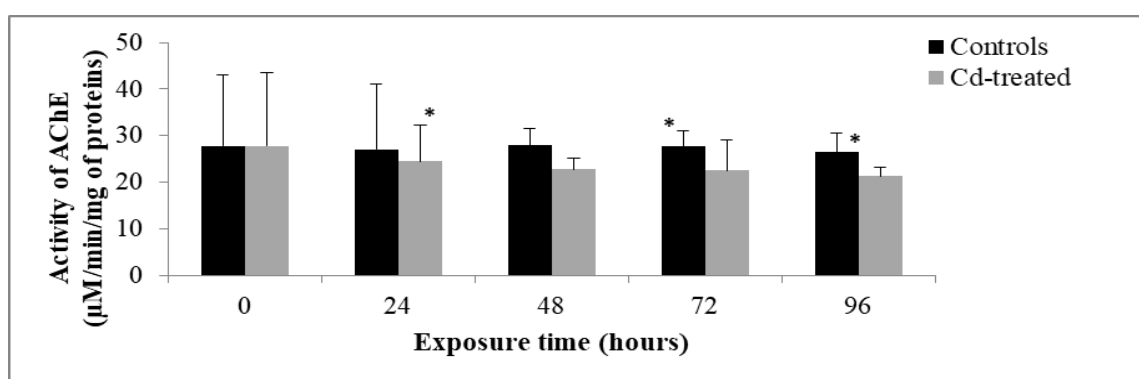


FIGURE 5

AChE activity ( $\mu\text{M}/\text{min}/\text{mg}$  of proteins) in the mantle of *D. trunculus* exposed to cadmium chloride at 96h-LC<sub>10</sub> ( $m \pm \text{SD}$ ;  $n=4$ ). (\*: significant difference at  $p < 0.05$ ; \*\*: significant difference at  $p < 0.01$ ; \*\*\*: significant difference at  $p < 0.001$ ).

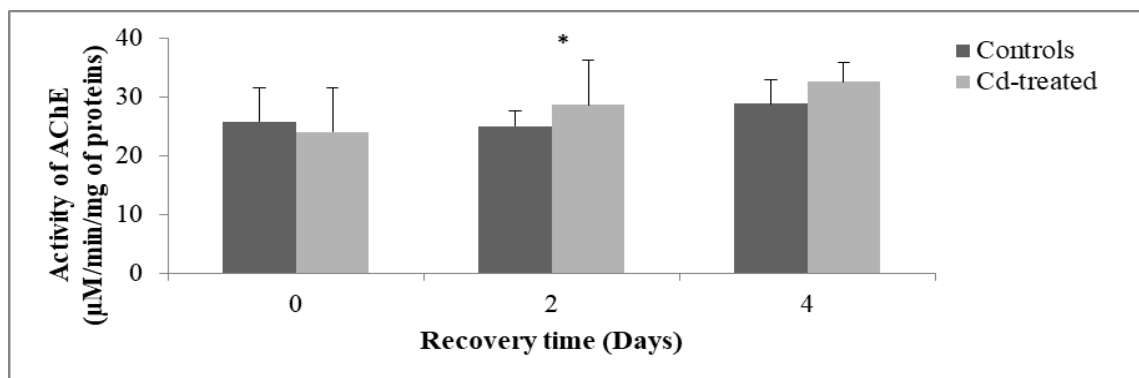


FIGURE 6

AChE activity ( $\mu\text{M}/\text{min}/\text{mg}$  of proteins) in the mantle of *D. trunculus* exposed to cadmium chloride at 96h-LC<sub>10</sub> and its recovery response ( $m \pm \text{SD}$ ;  $n=4$ ). (\*: significant difference at  $p < 0.05$ ; \*\*: significant difference at  $p < 0.01$ ; \*\*\*: significant difference at  $p < 0.001$ ).

The results obtained for the variation in AChE activity after Cd treatment (96h-LC<sub>10</sub>) are presented in Figure 5. Results show that the rate of AChE activity exhibited a significant decrease in a concentration-dependent manner ( $P < 0.05$ ). Increases in CAT activity ( $p < 0.01$ ) as well as an inhibition of AChE activity ( $p < 0.01$ ) were found in both concentrations (LC<sub>10</sub> and LC<sub>50</sub>). AChE activity in treaties was significantly reduced compared to controls ( $p > 0.05$ ) at

24, 72 h and 96 h. However, no significant difference was observed at 48 h ( $p > 0.05$ ). The lowest levels were recorded at the end of the experiment ( $21.35 \pm 1.84 \mu\text{mol}/\text{min}/\text{mg}$  proteins in *D. trunculus* treated against  $26.49 \pm 4.01 \mu\text{mol}/\text{min}/\text{mg}$  proteins in controls). A two-way ANOVA indicated a highly significant main effects of treatment ( $F_{1,29} = 10.31$ ;  $p < 0.001$ ), significant effect of time ( $F_{4,29}$ ;  $p < 0.01$ ). Several studies have found that AChE activity

exhibited significant reductions exposed to various concentrations of insecticides with mortality after 96 h exposure [29, 74]. It was widely demonstrated in toxicological experiments that the AChE response show inhibition in marine organisms. As reported in studies [75] a 50% inhibition of AChE activity in Marine Bivalve *M. galloprovincialis* exposed to 0.1 mg/L carbaryl (carbamate) or 0.2 mg/L methyl-parathion (organophosphate) for 24 h. The same findings are in accordance in freshwater fish *Poecilia reticulata* treated by dimethoate (organophosphate) [76] and in shrimp *Metapenaeus Monoceros* after 48 h of exposure to lethal concentrations of methyl parathion (organophosphate) and phosphamidon [77]. Similarly, significant inhibition in AChE activity of *D. trunculus* exposed for 96 h to CdCl<sub>2</sub> at LC<sub>50</sub> followed by a significant increase in its activity in the depuration period was reported previously [41]. However, it must be considered that AChE activity is inhibited by carbamate and organophosphate insecticides besides heavy metals in particular cadmium, which explains the reduction of this enzymatic activity, observed in the treated series. Recently, previous studies have reported a remains metallic contamination that caused an inhibition of AChE activity in marine bivalve *D. trunculus* [47, 78], and in *Ruditapes decussatus* [44]. AChE activity is used as a specific stress index in marine organisms [34].

Recovery is defined as a significant increase in AChE activity that occurs after exposure to cadmium chloride. Maximum recovery was in the Cd-treated bivalves *D. trunculus*, where the AChE level increased by  $23.98 \pm 7.56$   $\mu\text{mol}/\text{min}/\text{mg}$  proteins at 0 days to  $32.50 \pm 3.21$   $\mu\text{mol}/\text{min}/\text{mg}$  proteins at the end of duration reputation (4 days) (Figure 6). However, no significant differences in values were observed during depuration period at 4 days ( $p > 0.05$ ) between the two series (controls and treated). Further, a significant difference ( $p < 0.05$ ) has been recorded after longer days for 2 days of recovery. Restoration after exposure to the cadmium chloride was rapid in *D. trunculus*. In the current study, upon transfer to untreated water, AChE activity was restored to level within 4 days. This suggests that although Cd is being accumulated in the tissues of *D. trunculus* treated [42], the defense system is enough to detoxify the effect of Cd accumulated in the tissues [64]. A two-way ANOVA indicated a significant effect of time ( $F_{2,17}$ ;  $p < 0.01$ ). Inhibition and restoration of AChE activity would provide an understanding of AChE data in aquatic organisms and facilitate the use of AChE as a biomarker of exposure or even effect [29]. Acetylcholinesterase activity of the *P. australiensis* required 7 days for recovery [29, 79]. A significant decrease in AChE activity has been shown to require longer recovery periods of more than 4 days

## CONCLUSIONS

In conclusion, our investigation contributes to the knowledge of the mechanism of toxicity related to the use of marine organisms as sentinels of environmental quality. This study illustrated that the presence of cadmium chloride (CdCl<sub>2</sub>) in seawater leads to the mortality of a marine benthic *Donax trunculus* exposed at concentrations corresponding to LC<sub>10</sub> (0.72 mg/L) and LC<sub>50</sub> (2.59 mg/L) for 96 h, and impacted the biomarkers responses.

These data in the present study indicated that *D. trunculus* is sensitive to Cd, which might be used as a potential bioindicator species in biomonitoring programs and ecotoxicology Research. In *D. trunculus*, AChE activity showed a significant inverse correlation with catalase activity. These results indicate that cadmium levels cause dose-dependent toxicity by inhibiting neurotoxic biomarker AChE and induction of antioxidant enzyme CAT. All the results showed the most significant effects of cadmium on the analyzed biomarkers and underlie the potential negative impact of the pollutants on marine organisms. The recovery test aimed to observe the effect of lethal exposure of Cd on the survival of *D. trunculus* upon transfer to a clean environment. Our work has led us to conclude that *D. trunculus* establishes a relatively efficient detoxification system following Cd exposure.

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## REFERENCES

- [1] Karthikeyan, P., Marigoudar, S.R., Mohan, D., Sharma, K.V. and Murthy, M.R. (2021) Prescribing sea water quality criteria for arsenic, cadmium and lead through species sensitivity distribution. *Ecotoxicology and Environmental Safety*. 208, 111612.
- [2] Hu, Y. (2016) Early generation of nitric oxide contributes to copper tolerance through reducing oxidative stress and cell death in hullless barley roots. *Journal of Plant Research*. 129(5), 963-978.

- [3] Andreu, V., Gimeno-García, E., Pascual, J.A., Vazquez-Roig, P. and Picó, Y. (2016) Presence of pharmaceuticals and heavy metals in the waters of a Mediterranean coastal wetland: potential interactions and the influence of the environment. *Science of the Total Environment*. 540, 278-286.
- [4] Yi, X., Chi, T., Liu, B., Liu, C., Feng, G., Dai, X., Zhang, K. and Zhou, H. (2019) Effect of nano zinc oxide on the acute and reproductive toxicity of cadmium and lead to the marine copepod *Tigriopus japonicus*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 222, 118-124.
- [5] Laouati, I., Rouane-Hacene, O., Derbal, F. and Ouali, K. (2021) The mussel caging approach in the assessment of trace metal contamination in southern Mediterranean coastal waters: a multi-biomarker study. *Environmental Science and Pollution Research*. 28(44), 63032-63044.
- [6] Mishra, S., Bharagava, R.N., More, N., Yadav, A., Zainith, S., Mani, S. and Chowdhary, P. (2019) Heavy metal contamination: an alarming threat to environment and human health. In *Environmental biotechnology: For sustainable future*. Springer, Singapore. 103-125.
- [7] IPCS, International Programme on Chemical Safety. (1992) Cadmium: environmental aspects. *Environmental Health Criteria*. 134.
- [8] Storelli, M.M., Barone, G., Storelli, A. and Marcotrigiano, G.O. (2008) Total and subcellular distribution of trace elements (Cd, Cu and Zn) in the liver and kidney of green turtles (*Chelonia mydas*) from the Mediterranean Sea. *Chemosphere*. 70(5), 908-913.
- [9] Yang, T., Cheng, H., Wang, H., Drews, M., Li, S., Huang, W., Zhou, H., Chen, M.C. and Diao, X. (2019) Comparative study of polycyclic aromatic hydrocarbons (PAHs) and heavy metals (HMs) in corals, surrounding sediments and surface water at the Dazhou Island, China. *Chemosphere*. 218, 157-168.
- [10] Wang, X., Zhao, L., Xu, H. and Zhang, X. (2018) Spatial and seasonal characteristics of dissolved heavy metals in the surface seawater of the Yellow River Estuary, China. *Marine Pollution Bulletin*. 137, 465-473.
- [11] Abderrahmani, K., Boulahdid, M., Bendou, N., Guenachi, B., Hacene, O.R., Masino, F. and Montevecchi, G. (2021) Partitioning of trace elements in the tissues of Mediterranean mussels (*Mytilus galloprovincialis*) sampled from industrial sites along the Algerian coast. *Marine Pollution Bulletin*. 173, 113006.
- [12] Zhang, H. and Reynolds, M. (2019) Cadmium exposure in living organisms: A short review. *Science of the Total Environment*. 678, 761-767.
- [13] Jarup, L. and Akesson, A. (2009) Current status of cadmium as an environmental health problem. *Toxicology and Applied Pharmacology*. 238(3), 201-208.
- [14] Soares, S.S., Martins, H., Gutiérrez-Merino, C. and Aureliano, M. (2008) Vanadium and cadmium in vivo effects in teleost cardiac muscle: metal accumulation and oxidative stress markers. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 147(2), 168-178.
- [15] Li, Y.F., Luo, X.X., Fan, Y.Q., Pan, J.F. and Cheng, F.L. (2008) Bioavailability of cadmium in different fractions of sediment by *Ruditapes philippinarum*. *Ecol. Environ.* 17(3), 909-913.
- [16] Guardiola, F.A., Cuesta, A., Meseguer, J., Martínez, S., Martínez-Sánchez, M.J., Pérez-Sirvent, C. and Esteban, M.A. (2013) Accumulation, histopathology and immunotoxicological effects of waterborne cadmium on gilthead seabream (*Sparus aurata*). *Fish & Shellfish Immunology*. 35(3), 792-800.
- [17] Guardiola, F.A., Dioguardi, M., Parisi, M.G., Trapani, M.R., Meseguer, J., Cuesta, A., Cammarata, M. and Esteban, M.A. (2015) Evaluation of waterborne exposure to heavy metals in innate immune defences present on skin mucus of gilthead seabream (*Sparus aurata*). *Fish & Shellfish Immunology*. 45(1), 112-123.
- [18] Lee, M.C., Puthumana, J., Lee, S.H., Kang, H.M., Park, J.C., Jeong, C.B., Han, J., Hwang, D.S., Seo, J.S., Park, H.G., Om, A.S. and Lee, J.S. (2016) BDE-47 induces oxidative stress, activates MAPK signaling pathway, and elevates de novo lipogenesis in the copepod *Paracyclops nana*. *Aquatic Toxicology*. 181, 104-112.
- [19] Morcillo, P., Esteban, M.Á. and Cuesta, A. (2016) Heavy metals produce toxicity, oxidative stress and apoptosis in the marine teleost fish SAF-1 cell line. *Chemosphere*. 144, 225-233.
- [20] Hellström, L., Elinder, C.G., Dahlberg, B., Lundberg, M., Järup, L., Persson, B. and Axelsson, O. (2001) Cadmium exposure and end-stage renal disease. *Am. J. Kidney Dis.* 38, 1001-1008.
- [21] Mason, R.P. (2013) *Trace Metals in Aquatic Systems*. 1st ed. John Wiley-Blackwell.
- [22] Ma, Y., Rivera-Ingraham, G., Nommick, A., Bickmeyer, U. and Roeder, T. (2020) Copper and cadmium administration induce toxicity and oxidative stress in the marine flatworm *Macrostomum lignano*. *Aquatic Toxicology*. 221, 105428.
- [23] Chahouri, A., Agnaou, M., El Hanaoui, M., Yacoubi, B., Moukrim, A. and Banaoui, A. (2022) Assessment of seasonal and spatial variation responses of integrated biomarkers in two marine sentinel bivalve species: Agadir Bay (Southern of Morocco). *Marine Pollution Bulletin*. 174, 113179.

- [24] Zhang, C., Jin, Y., Yu, Y., Xiang, J. and Li, F. (2021) Cadmium-induced oxidative stress, metabolic dysfunction and metal bioaccumulation in adult palaemonid shrimp *Palaemon macrodactylus* (Rathbun, 1902). *Ecotoxicology and Environmental Safety*. 208, 111591.
- [25] Rebecchi, D., Palacio-Cortés, A.M., Richardi, V.S., Beltrão, T., Vicentini, M., Grassi, M.T., Da Silva, S.B., Alessandere, T., Hasenbein, S., Connon, R. and Navarro-Silva, M.A. (2021) Molecular and biochemical evaluation of effects of malathion, phenanthrene and cadmium on *Chironomus sancticaroli* (Diptera: Chironomidae) larvae. *Ecotoxicology and Environmental Safety*. 211, 111953.
- [26] Jebali, J., Banni, M., Almeida, E.A.D. and Boussetta, H. (2007) Oxidative DNA damage levels and catalase activity in the clam *Ruditapes decussatus* as pollution biomarkers of Tunisian marine environment. *Environmental Monitoring and Assessment*. 124(1), 195-200.
- [27] Livingstone, D.R., Lemaire, P., Matthews, A., Peters, L., Bucke, D. and Amlap, W.R. (1993) Prooxidant, anti-oxidant and 7-Ethoxyresorufin-O-deethylase (EROD) activity responses in liver of Dab (*Limanda limanda*) exposed to sediment contaminated with hydrocarbons and other chemicals. *Mar. Pollut. Bull.* 26(11), 602-606.
- [28] Labrot, F., Ribera, D., Denis, M.S. and Narbonne, J.F. (1996) In vitro and in vivo studies of potential biomarkers of lead and uranium contamination: lipid peroxidation, acetylcholinesterase, catalase and glutathione peroxidase activities in three non-mammalian species. *Biomarkers*. 1(1), 21-28.
- [29] Kumar, A., Doan, H., Barnes, M., Chapman, J.C. and Kookana, R.S. (2010) Response and recovery of acetylcholinesterase activity in freshwater shrimp, *Paratya australiensis* (Decapoda: Atyidae) exposed to selected anti-cholinesterase insecticides. *Ecotoxicology and Environmental Safety*. 73(7), 1503-1510.
- [30] Bocquené, G., Galgani, F. and Truquet, P. (1990) Characterization and assay conditions for use of AChE activity from several marine species in pollution monitoring. *Marine Environmental Research*. 30(2), 75-89.
- [31] Colovic, M.B., Krstic, D.Z., Lazarevic-Pasti, T.D., Bondzic, A.M. and Vasic, V.M. (2013) Acetylcholinesterase inhibitors: pharmacology and toxicology. *Current Neuropharmacology*. 11(3), 315-335.
- [32] Shugart, L.R. (2007) Principles of Ecotoxicology, by CH Walker, SP Hopkin, RM Sibly, DB Peakall. *Ecotoxicology*. 16(6), 483-483.
- [33] De la Torre, F.R., Ferrari, L. and Salibián, A. (2002) Freshwater pollution biomarker: response of brain acetylcholinesterase activity in two fish species. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 131(3), 271-280.
- [34] Lionetto, M.G., Caricato, R., Giordano, M.E., Pascariello, M.F., Marinosci, L. and Schettino, T. (2003) Integrated use of biomarkers (acetylcholinesterase and antioxidant enzymes activities) in *Mytilus galloprovincialis* and *Mullus barbatus* in an Italian coastal marine area. *Marine Pollution Bulletin*. 46(3), 324-330.
- [35] Tenjing, S.Y., Narasimhaiah, N. and Ciabotti, C. (2020) Seasonal concentrations of heavy metals and environmental parameters with reference to the Indian largest wedge clam, *Donax scortum* (Linnaeus, 1758). *Indian Journal of Geo-Marine Sciences*. 49(10), 1627-1635.
- [36] Edward, F.B., Yap, C.K., Ismail, A. and Tan, S.G. (2009) Interspecific variation of heavy metal concentrations in the different parts of tropical intertidal bivalves. *Water, Air and Soil Pollution*. 196(1), 297-309.
- [37] Fishelson, L., Bresler, V., Manelis, R., Zuk-Rimon, Z., Dotan, A., Hornung, H. and Yawetz, A. (1999) Toxicological aspects associated with the ecology of *Donax trunculus* (Bivalvia, Mollusca) in a polluted environment. *Science of the Total Environment*. 226(2-3), 121-131.
- [38] Zeichen, M.M., Agnesi, S., Mariani, A., Maccaconi, A. and Ardizzone, G.D. (2002) Biology and population dynamics of *Donax trunculus* L. (Bivalvia: Donacidae) in the South Adriatic coast (Italy). *Estuarine, Coastal and Shelf Science*. 54(6), 971-982.
- [39] Özden, Ö., Erkan, N. and Deval, M.C. (2009) Trace mineral profiles of the bivalve species *Chamelea gallina* and *Donax trunculus*. *Food Chemistry*. 113(1), 222-226.
- [40] Beldi, H., Gimbert, F., Maas, S., Scheifler, R. and Soltani, N. (2006) Seasonal variations of Cd, Cu, Pb and Zn in the edible mollusc *Donax trunculus* (Mollusca, Bivalvia) from the gulf of Annaba, Algeria. *African Journal of Agricultural Research*. 1(4), 85-90.
- [41] Belabed, S. and Soltani, N. (2013) Acute toxicity of cadmium on *Donax trunculus*: acetylcholinesterase, glutathione S-transferase activities and pattern of recovery. *European Journal of Experimental Biology*. 3(2), 54-61.
- [42] Belabed, S. and Soltani, N. (2018) Effects of cadmium concentrations on bioaccumulation and depuration in the marine bivalve *Donax trunculus*. *Euro-Mediterranean Journal for Environmental Integration*. 3(1), 1-5.
- [43] Merad, I. and Soltani, N. (2017) Sublethal effects of cadmium on energy reserves in the edible Mollusk *Donax trunculus*. *J. Entomo. Zool. Studies*. 5(1), 100-105.

- [44] Bejaoui, S., Rabeh, I., Telahigue, K., Tir, M., Chetoui, I., Fouzai, C., Nechi, S., Chelbi, E., EL Cafsi, M. and Soudani, N. (2020) Assessment of oxidative stress, genotoxicity and histopathological responses in the digestive gland of *Ruditapes decussatus* collected from northern Tunisian lagoons. *Scientia Marina*. 84(4), 403-420.
- [45] Sifi, K. and Soltani, N. (2019) Seasonal changes of two biomarkers of oxidative stress (LDH, MDA) in the edible mollusc *Donax trunculus* (Mollusca: Bivalvia) from the Gulf of Annaba (Algeria): correlation with carbohydrate and lipid contents. *Molluscan Research*. 39(1), 44-52.
- [46] Rabei, A., Hichami, A., Beldi, H., Bellenger, S., Khan, N.A. and Soltani, N. (2018) Fatty acid composition, enzyme activities and metallothioneins in *Donax trunculus* (Mollusca, Bivalvia) from polluted and reference sites in the Gulf of Annaba (Algeria): Pattern of recovery during transplantation. *Environmental Pollution*. 237, 900-907.
- [47] Amira, A., Merad, I., Almeida, C.M.R., Guimaraes, L. and Soltani, N. (2018) Seasonal variation in biomarker responses of *Donax trunculus* from the Gulf of Annaba (Algeria): implication of metal accumulation in sediments. *Comptes Rendus Geoscience*. 350(4), 173-179.
- [48] Hamdani, A., Soltani, N. and Zaidi, N. (2020) Growth and reproduction of *Donax trunculus* from the Gulf of Annaba (Northeast Algeria) in relation to environmental conditions. *Environmental Science and Pollution Research*. 27(33), 41656-41667.
- [49] Abbott, W.S. (1925) A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18(2), 265-267.
- [50] Finney, D.J. (1971) (3 rd edn) Cambridge University Press London. Probit analysis Published.
- [51] Swaroop, S., Gilroy, A.B., Uemura, K. and World Health Organization. (1966) Statistical methods in malaria eradication. World Health Organization. Geneva. 51, 1.
- [52] Claiborne, A.L. (1985) Catalase Activity. In *CRC Handbook of Methods for Oxygen Radical Research*. CRC Press.
- [53] Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 72(1-2), 248-254.
- [54] Ellman, G.L., Courtney, K.D., Andres Jr, V. and Featherstone, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*. 7(2), 88-95.
- [55] Neuberger-Cywiak, L., Achituv, Y. and Mizrahi, L. (1989) The ecology of *Donax trunculus* Linnaeus and *Donax semistriatus* Poli from the Mediterranean coast of Israel. *Journal of Experimental Marine Biology and Ecology*. 134(3), 203-220.
- [56] Cusimano, R.F., Brakke, D.F. and Chapman, G.A. (1986) Effects of pH on the toxicities of cadmium, copper, and zinc to steelhead trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences*. 43(8), 1497-1503.
- [57] Mohan, C.V., Menon, N.R. and Gupta, T.R.C. (1984) Acute toxicity of cadmium to six intertidal invertebrates. *Fishery Technology*. 21(1), 1-5.
- [58] Eisler, R. (1971) Cadmium poisoning in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) and other marine organisms. *Journal of the Fisheries Board of Canada*. 28(9), 1225-1234.
- [59] Chiffolleau, J.F., Auger, D. and Chartier, E. (1999) Fluxes of selected trace metals from the Seine estuary to the eastern English Channel during the period August 1994 to July 1995. *Continental Shelf Research*. 19(15-16), 2063-2082.
- [60] Ramakritinan, C.M., Chandurvelan, R. and Kumaraguru, A.K. (2012) Acute Toxicity of Metals: Cu, Pb, Cd, Hg and Zn on Marine Molluscs, *Cerithedia cingulata* G. and *Modiolus philippinarum* H. *Indian Journal of Geo-Marine Sciences*. 41(2), 141-145.
- [61] Shuhaimi-Othman, M., Yakub, N., Ramle, N.A. and Abas, A. (2011) Toxicity of metals to a freshwater ostracod: *Stenocypris major*. *Journal of Toxicology*. 2011.
- [62] Pestana, J.L.T., Ré, A., Nogueira, A.J.A. and Soares, A.M.V.M. (2007) Effects of Cadmium and Zinc on the feeding behaviour of two freshwater crustaceans: *Atyaephyra desmarestii* (Decapoda) and *Echinogammarus meridionalis* (Amphipoda). *Chemosphere*. 68(8), 1556-1562.
- [63] Yin, X.H., Wu, Q.J., Li, X.F., Zhang, Y.J. and Xu, B.Y. (2008) Sublethal effects of spinosad on *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Crop Protection*. 27(10), 1385-1391.
- [64] Company, R., Serafim, A., Cosson, R.P., Fiala-Médioni, A., Camus, L., Serrão-Santos, R. and Bebianno, M. J. (2010) Sub-lethal effects of cadmium on the antioxidant defence system of the hydrothermal vent mussel *Bathymodiolus azoricus*. *Ecotoxicology and Environmental Safety*. 73(5), 788-795.
- [65] Devi, V.U. (1996) Bioaccumulation and metabolic effects of cadmium on marine fouling bivalve, *Mytilopsis sallei* (Recluz). *Archives of Environmental Contamination and Toxicology*. 31(1), 47-53.

- [66] McCahon, C.P. and Pascoe, D. (1988) Use of *Gammarus pulex* (L.) in safety evaluation tests: culture and selection of a sensitive life stage. *Ecotoxicology and Environmental Safety*. 15(3), 245-252.
- [67] Ebrahimpour, M., Alipour, H. and Rakhshah, S. (2010) Influence of water hardness on acute toxicity of copper and zinc on fish. *Toxicology and Industrial Health*. 26(6), 361-365.
- [68] Tlili, S. and Mouneyrac, C. (2021) New challenges of marine ecotoxicology in a global change context. *Marine Pollution Bulletin*. 166, 112242.
- [69] Kroini, H., Hamdani, A., Soltani, N., Zaidi, N. and Sleimi, N. (2021) Effects of cadmium exposure on proximate analysis and metallic element contents of wedge clam (*Donax trunculus*). *Applied Ecology and Environmental Research*. 19(6), 5103-5128.
- [70] Merad, I., Bellenger, S., Hichami, A., Khan, N.A. and Soltani, N. (2018) Effect of cadmium exposure on essential omega-3 fatty acids in the edible bivalve *Donax trunculus*. *Environmental Science and Pollution Research*. 25(19), 18242-18250.
- [71] Gonçalves, J.M., Rocha, T., Mestre, N.C., Fonseca, T.G. and Bebianno, M.J. (2020) Assessing cadmium-based quantum dots effect on the gonads of the marine mussel *Mytilus galloprovincialis*. *Marine Environmental Research*. 156, 104904.
- [72] Kavitha, P. and Rao, J.V. (2008) Toxic effects of chlorpyrifos on antioxidant enzymes and target enzyme acetylcholinesterase interaction in mosquito fish, *Gambusia affinis*. *Environmental Toxicology and Pharmacology*. 26(2), 192-198.
- [73] Geret, F., Serafim, A., Barreira, L. and Bebianno, M.J. (2002) Effect of cadmium on antioxidant enzyme activities and lipid peroxidation in the gills of the clam *Ruditapes decussatus*. *Biomarkers*. 7(3), 242-256.
- [74] Fulton, M.H. and Key, P.B. (2001) Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. *Environmental Toxicology and Chemistry: An International Journal*. 20(1), 37-45.
- [75] Mora, P., Michel, X. and Narbonne, J.F. (1999) Cholinesterase activity as potential biomarker in two bivalves. *Environmental Toxicology and Pharmacology*. 7(4), 253-260.
- [76] Frasco, M.F. and Guilhermino, L. (2002) Effects of dimethoate and beta-naphthoflavone on selected biomarkers of *Poecilia reticulata*. *Fish Physiology and Biochemistry*. 26(2), 149-156.
- [77] Srinivasulu Reddy, M. and Ramana Rao, K.V. (1988) In vivo recovery of acetylcholinesterase activity from phosphamidon and methylparathion induced inhibition in the nervous tissue of *penaeid prawn* (*Metapenaeus monoceros*). *Bulletin of Environmental Contamination and Toxicology*. 40(5), 752-758.
- [78] Amamra, F., Sifi, K., Kaouachi, N. and Soltani, N. (2019) Evaluation of the impact of pollution in the gulf of Annaba (Algeria) by measurement of environmental stress biomarkers in an edible mollusk bivalve *Donax trunculus*. *Fresen. Environ. Bull.* 28(2), 908-915.
- [79] Abdullah, A.R., Kumar, A. and Chapman, J.C. (1994) Inhibition of acetylcholinesterase in the Australian freshwater shrimp (*Paratya australiensis*) by profenofos. *Environmental Toxicology and Chemistry: An International Journal*. 13(11), 1861-1866.

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