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The effect of cadmium exposure on malonedialdehyde and reduced glutathione concentrations in several tissues of a bivalve mollusc (*Ruditapes decussatus)* fished from Mellah lagoon (North East of Algeria)

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Abstract

This study investigates the effect of cadmium exposure on the detoxification capacity in some tissues of sentinel species and edible Ruditapes decussatus (Mollusca, Bivalvia). Clams are caught and then immediately transferred to the laboratory breeding. Treatment with cadmium at 100 and 200 µg of $CdCl_2$ per litter of water lasts 21 days. We assessed content of protein, lipid, reduced Glutathione (GSH) and malonedialdehyde (MDA) in the digestive gland, gills, adductor muscles and mantle. Results show that cadmium exposure causes in the four tissues studied an increase of lipid and protein contents, particularly evident with the highest dose. The MDA levels of the tissues studied increase significantly following treatment with a dose-response manner, while the concentration of GSH is drastically reduced in the Cd-treated clams with two doses, thus demonstrating the positive tissue sensitivity to Cd. Moreover, among the tissues studied, it appears that the mantle is the most sensitive tissue to Cd exposure and that this tissue is more suitable for monitoring metal pollution.

Key words: Cadmium, *Ruditapes decussatus*, malondialdehyde, glutathione, mantle.

INTRODUCTION

The pollution of aquatic ecosystems by heavy metals is an important environmental problem [1, 2]. Cadmium (Cd), one of the most toxic metals, is a persistent contaminant that accumulates in the environment. Large amounts of this metal are released annually in various environmental compartments and may pose a significant threat to the ecosystem [3]. During the last decade, various studies have shown that sea water and sediments of industrialized coastal regions are considerably contaminated by heavy metals [2, 4]. The high concentration of Cd is extremely toxic to aquatic organisms and sublethal levels may significantly affect their physiology [5, 6]. Therefore, heavy metal contamination is still an environmental problem today in both developing and developed countries throughout the world [2]. The absorption of metals takes place in humans mostly via the intake of food. Molluscs and crustaceans are present in our diet, they are great bioaccumulators of metals even if they originate from sites in which the levels of such

contaminants are considered low [7] and could be considered 'potentially' dangerous for consumers [8].

The environmental risk assessment and ecotoxicological involve the use of biomarkers designed to highlight an early stage of pollution [9]. Many biochemical and cellular biomarkers have been studied in aquatic organisms, and particularly in fish and bivalve molluscs. These biomarkers include those that are specific to oxidative stress, recommended for biomonitoring the quality of the aquatic environment, including malondialdehyde (MDA) which is derived from lipid peroxidation of polyunsaturated fatty acids in cell membranes during oxidative stress [10, 11, 12] and reduced glutathione (GSH) involved in the antioxidant defence system [13].

Some studies about pollution and biomarkers were devoted to some bivalves such as the clam *Ruditapes decussatus* [10, 14, 15, 16] and the cockle *C. glaucum* which was validated in previous studies as a biomonitor organism showing correlation between site contamination and metal accumulation [17, 18, 19, 20]. The tissues studied are often the gills and digestive gland which are in direct contact with the pollutant [21] while few studies have examined the effects of heavy metals on tissues with metabolic activity (mantle or muscle).

The present work aims to characterize the biochemical response of *R. decussatus* to cadmium exposure in laboratory. Two biomarkers were used: malondialdehyde (MDA) and reduced glutathione (GSH) in several tissues (mantle, gills, digestive gland and adductor muscles) to assess the sensitivity of each tissue to Cd exposure.

MATERIALS AND METHODS

Studied area

Clams *Ruditapes decussatus* were collected at Laguna of El Mellah along the gulf of Annaba's (Algeria). The Laguna is located at 40 km to the city of Annaba (North East of Algeria) which have an important industrial complex.

Bivalve collection, treatment and dissection

R. decussates 34-37 mm (n=60) were collected near the low water level during February 2008. At the time of sampling, water temperature varied between 16 and 18°C. The clam's samples were immediately transferred to our laboratory and allowed to acclimate to laboratory conditions in fiberglass tanks (30L) filled with continuously aerated water. During the acclimation period, half of the water in each tank was renewed with water every 3 days.

After one week, clams were divided into three groups: Control (n=10) and treated-cadmium (100 and 200 $\mu g.l^{-1}$ of $CdCl_2$ for 21 days, n=10 for each dose).

After exposure period, clams were rapidly dissected; gills, mantle, adductor muscles and digestive gland were removed in duplicate and weighed. Protein and lipid contents were quantified in the whole first tissues removed from control and treated specimens (n=4 for each group). Then, the second tissues removed were used to malondialdehyde (MDA) and reduced glutathione (GSH) measurement in control and treated-cadmium groups (n=4 for each group).

Protein and lipid analysis

Protein and lipid of each tissue of control and treated clams were extracted [22] and quantitative evaluation was done according respectively to [23] and [24]. Content of each metabolite was expressed as µg of metabolite per mg of fresh tissue.

Malondialdehyde analysis

MDA determination was carried out in the gills, mantle, adductor muscles and digestive gland using the colorimetric method [25] which is based on the reaction of thiobarbituric acid with MDA. Malondialdehyde levels were estimated at 532 nm. The concentration of lipid peroxidation in organs is expressed as µg of MDA per mg of proteins.

Glutathione analysis

Content of GSH in the tissues (gills, mantle, adductor muscles and digestive gland) was quantified according to the colorimetric method [26]. Glutathione levels were estimated at 412 nm and expressed as µM of glutathione per mg of proteins.

Statistical analysis

Differences between control and treated-cadmium groups were evaluated by an analysis of variance (one-way ANOVA) with significance level of 0.001 using the MINITAB software. Normality and homogeneity of variances were verified and a parametric one-way analysis (ANOVA) was performed on data.

All results are expressed as mean ± standard error.

RESULTS

Protein and lipid contents

Protein and lipid contents analysed in four tissues of *R. decussates* control and Cd-treated are presented in Tab.1 and Tab.2 respectively. Cadmium exposure at 100µg.l^{-1} did not result from changes in protein concentration of all tissues studied, while at 200µg.l^{-1}, a significant increase of the protein content ($p < 0.05$) was observed.

Table 1: Protein contents (µg/mg of tissue) in mantle, adductor muscles, gills and digestive glands of *R. decussatus* control and Cd-treated (m ± s, n = 4). For each tissue, means followed by same letter are not significantly different (p <0.05).

Tissues	Control	Cd 100µg.l^{-1}	Cd 200µg.l^{-1}
Mantle	2.450 ± 0.386a	3.619 ± 1.104ab	5.796 ± 2.164b
adductor muscles	2.586 ± 0.518a	5.145 ± 2.55ab	6.119 ± 2.624b
Gills	2.012 ± 0.202a	3.866 ± 1.141ab	4.620 ± 0.420b
Digestive gland	2.362 ± 0.522a	3.631 ± 2.246a	3.824 ± 1.543a

Lipid concentrations was significantly higher in clams treated with the two doses with a dose-dependent manner ($p<0.05$), in all tissues studied except the mantle where there is an increase only with 200 µg of Cd.l^{-1}.

Table 2: Lipid contents (µg/mg of tissue) in mantle, adductor muscles, gills and digestive glands of *R. decussatus* control and Cd-treated (m ± s, n = 4). For each tissue, means followed by same letter are not significantly different (p <0.05).

Tissues	Control	Cd 100µg.l^{-1}	Cd 200µg.l^{-1}
Mantle	0.320 ± 0.139a	0.320 ± 0.039a	0.553 ± 0.305b
adductor muscles	0.123 ± 0.046a	0.665 ± 0.292b	0.707 ± 0.086b
Gills	0.162 ± 0.061a	0.201 ± 0.093b	0.267 ± 0.091bc
Digestive gland	0.097 ± 0.008a	0.273 ± 0.074b	0.254 ± 0.078b

Effect of Cd on MDA concentration

Evaluation of MDA concentrations in tissues of *R. decussates* after a 21 days Cd exposure is presented on Fig.1.

Cadmium treatment causes a significant increase in MDA concentrations in all tissues with a concentration-dependent manner, reflecting lipid peroxidation. Moreover, the strongest increase was observed in mantle (+1100%) followed by adductor muscle (+850%) and finally gills and digestive gland (+600% and +640% respectively).

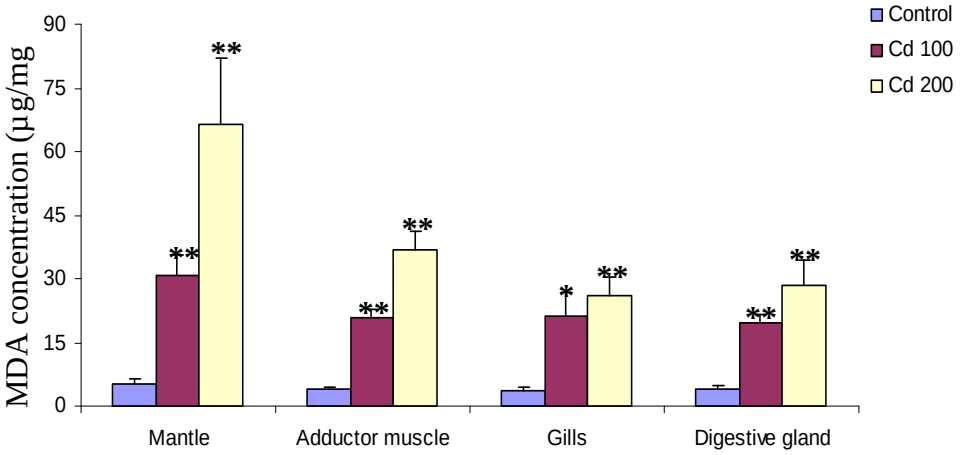

Figure 1: Effect of cadmium exposure (100 and 200 µg/l) on MDA concentration (µg/mg of protein) in mantle, adductor muscle, gills and digestive gland of *R. decussatus* (m ± s, n = 4). For each tissue, different from control ** : P<0.01 ; *** : P<0.001).

Effect of Cd on GSH concentration

The determination of GSH in tissues of control and Cd-treated *R. decussatus* is shown in Fig.2. Treatment with Cd (with 200µg.l^{-1} dose) results in a drastic decrease of GSH concentration, explained by enzyme system activation (including glutathione-S-transferase). The largest decrease was observed in mantle (-98%), followed by gills (-93%), digestive gland (-92%) and adductor muscle (-72%). No significant effect was observed with the 100µg.l^{-1} dose except in digestive gland.

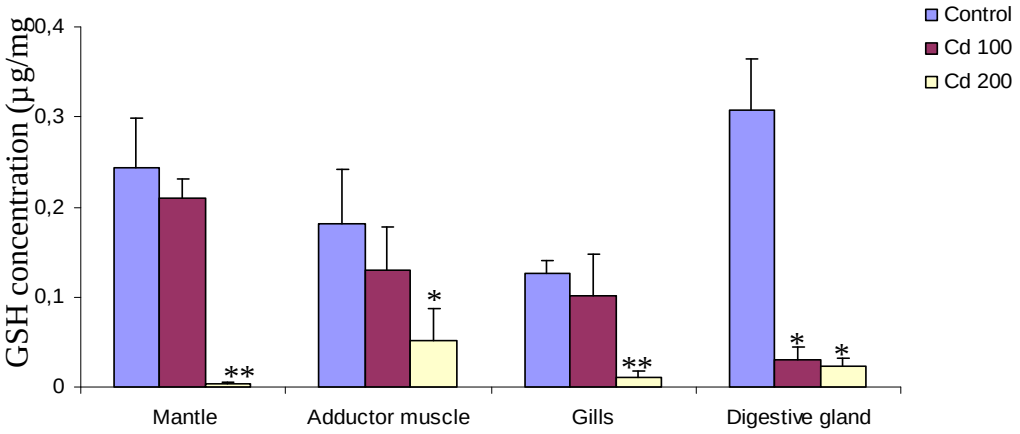

Figure 2: Effect of cadmium exposure (100 and 200 µg/l) on GSH concentration (µg/mg of protein) in mantle, adductor muscle, gills and digestive gland of *R. decussatus* (m ± s, n = 4). For each tissue, different from control *: P<0.05; **: P<0.01; ***: P<0.001).

DISCUSSION

Industrial discharges and waste disposal in urban estuaries and coastal regions are the main sources of water pollution [2]. The clam *Ruditapes decussatus* strongly accumulates hydrocarbons, metals (copper, iron, mercury, manganese, cadmium, nickel, zinc, lead) organophosphorus compounds (PCBs), pesticides and herbicides [27, 28].

The Gulf of Annaba is the most important tourist attraction and economic installed on the east coast of Algeria. Its fisheries resources are threatened by pollution-related economic activity booming. In this context and within the coastal biomonitoring, we evaluated the effects of cadmium exposure in laboratory on the responsiveness of the clam by the measurement of two biomarkers (MDA and GSH) in various tissues and to assess sensitivity of each tissue.

Cadmium has no essential function in physiological processes for pelagic organisms as a biological non-essential heavy metal and is toxic to many aquatic organisms including zooplankton even at micrograms per litter level, which can be accumulated by aquatic organisms and affect their survivals [29].

Marine bivalve such as molluscs and mussels are appropriate sentinel species [30] for most of the biomarkers studies except for the induction of the cytochrome P-450 system.

In this work, the determination of protein and lipid content was conducted in four organs of the clam. The mantle contains organic materials and minerals [31]. The gill is the main tissue in contact with the outside environment. The high level of filtration and transport of suspended particles make this organ a preferred site for the concentration of microorganisms and bioaccumulation of toxic substances [32]. The adductor muscle contraction provides their closing valves and the digestive gland is a privileged contact with contaminants in the marine environment [33].

Our results showed that the rate of protein increased only at 200 $\mu g.l^{-1}$ Cd exposure. These results were according to those of [34]. Some studies also showed a significant increase of total protein as a result of chemical stress in various biological models (protists ciliates, rabbits) [35, 36]. Concerning the assessment of lipid content after Cd-treatment (100 and 200 $\mu g.l^{-1}$), an increase of lipid in the tissues studied, except mantle, was observed. This increase can be explained by the impact of cadmium on the physiology of the clam. A study on a bivalve mollusc *Donax trunculus* showed an increased concentration of lipids [37, 38]. Moreover, there is a correlation between lipid content and levels of contaminants in molluscs [39].

The concentrations of malondialdehyde (MDA), a break down product of the oxidative degradation of cell membrane lipids, increased along the metal gradient. Increased levels of MDA following Cd exposure have been reported in other species of bivalves [33, 40, 41].

Our results indicate a significant increase of MDA levels in all organs studied after cadmium exposure. Moreover, the mantle and adductor muscles appear more sensitive to cadmium than the gills and digestive gland witch are yet in direct contact with the pollutant. This could be due to the different physiological roles of these organs. The gills and digestive gland have been noticed to be a storage organ for a short time, whereas absorption has led to an accumulation of toxic metals for a longer time in mantle and, to a lesser degree, in adductor muscles [42]. An increase in lipid peroxidation was also reported in *Ruditapes decussatus* [43]. Similarly, exposure of bivalves to cadmium affects the activation of antioxidant enzymes and increased lipid

peroxidation [34], and to cadmium, copper and mercury stimulates lipid peroxidation in the mussel *Mytilus galloprovincialis* [44].

Glutathione plays a central role in the process of intracellular defence and exists in two forms, oxidized GSSG and reduced GSH. GSH deficiency exposes the cell to a risk of oxidative damage [45], through its ability to bind to heavy metal ions [46]. The glutathione-enzymes include glutathione peroxidase (GSH-Px) and glutathione S-transferase (GST) involved in the detoxification reaction intermediates and oxygen radicals [47]. Our results showed, overall, a drastic decrease of GSH content for the highest dose compared with controls in all organs studied. The mantle, gills and digestive gland showed the largest declines. Several studies confirm the results and help to better explain the relationship between the decrease in GSH and the level of contamination. This has been observed in mussels, *Crassostrea virginica* [48] and in the bivalve, *Unio limidus* exposed to copper [40]. Decreased GSH and increased MDA were also reported in *Perna viridis* exposed to cadmium [34] and *Mytilus galloprovincialis* exposed to copper [49].

In conclusion, the results obtained in this study confirm that the clams are rightly regarded as sentinel species of coastal pollution. In addition, metabolic organs as mantle appear more sensitive to pollution than gills or digestive gland. Thus, evaluation of biomarkers in the mantle of clams is more suitable for monitoring metal pollution.

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