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Full Length Research Paper

Influence of calcium presence on the absorption of cadmium by the rock oyster *Saccostrea cucullata* from Persian Gulf (Ostreidae; Bivalvia) in laboratory conditions

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Bioaccumulation of Cadmium as a function of its concentration and exposure time in *Saccostrea cucullata* (Ostreidae; Bivalvia) has been studied in filtered sea water and in laboratory. Results showed that the accumulation of Cd by gills was greater than other organs such as visceral mass. At 150 µg /l saturation as a function of time appeared in uptake of Cadmium by gills, all binding sites for Cd may be occupied; Cadmium may then be redistributed to the other organs. Also total Calcium concentration in the gills of control animals Ca was decreased by Ca 20% By Verapamil. Comparisons (t -tests) between animals pre-exposed and not pre-exposed (after 48 h and after 85 h) were significant at $p < 0.05$. The fact that verapamil decreased total calcium concentration in gills of *S. cucullata* may imply that calcium channels are present in this organ. The only significant effect was that of Cadmium concentration in the medium ($p < 0.05$) on Calcium content of gills. ANOVA, performed on Calcium concentrations after 85 h of Cd exposure, showed a significant effect of Cadmium on Calcium concentration in gills ($p < 0.05$). Results concerning gills after 48 h Cd exposure were demonstrated a decrease in Calcium concentration was observed at 150 µg Cd/l (referred to -VP) compared to controls (t-test significant at $p < 0.01$). No significant effect was noted when animals were pre-exposed with Verapamil (referred to as +VP).

Key words: Bioaccumulation, cadmium, verapamil, *Saccostrea cucullata*, metal uptake**.**

INTRODUCTION

There are relatively few experimental studies of metal transport from the dissolved phase in marine invertebrates. Carpene and George (1981) reported that uptake of dissolved Cd proceeds by diffusive processes without energy expenditure in isolated gills of mussels (*Mytilus edulis*). Trace metals can also be taken up through various channels, including Ca channels (Simkiss, 1996). Several metals, including Cd, Pb, Mn and Zn, have been shown to be transported through Ca channels in vertebrates (e.g., fish) (Anderson, 1979; Spry and Wood, 1989). In aquatic invertebrates, there are few studies on the involvement of Ca channels in transporting metals (Roesijadi and Unger, 1993). Mechanisms by which metals are transported into aquatic invertebrates' tissues have been proposed (Simkiss and Taylor, 1989). In the carrier-mediated facilitated transport model, dissolved metals are first decomposed by a membrane protein ligand and then further by an intracellular protein ligand which may have a higher binding stability constant for the metals than the membrane protein (Simkiss and Taylor, 1989, 1995). Metals are then trapped by the intracellular ligand, resulting in a lower intracellular free metal amounts and thus a continuous removal of extracellular metal (Williams, 1981). Binding with protein ligands may greatly affect the rate at which a metal is transported across biological membranes. Metals that tend to associate with protein ligands (e.g., Ag, Zn, Cd) have the highest dissolved uptake rate constants in

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marine mussels, whereas metals that tend to bind with oxygen (e.g., Co, Cr, Am) generally have lower uptake rates (Wang et al., 1996, 1997). This trend has also been observed in other marine organisms, including polychaetes (Bryan, 1984) and copepods (Wang and Fisher, 1998). Metal uptake in aquatic invertebrates is generally inversely related to salinity (Wright, 1995). In mussels, lowering the salinity from 33 to 15 ppt, results in an increase in trace element influx by 1.5 - 2 times (Wang et al., 1996). The mechanisms underlying this salinity effect are not yet fully understood (Wright, 1995; Wang et al., 1996).

Physiological changes and physico-chemistry (e.g., metal speciation) can both affect metal uptake as the salinity is decreased. Among several possible explanations, variations in Ca concentration may partially account for metal uptake patterns at different salinities, where greater metal uptake at low salinity could be due to decreased competition with Ca (Wright, 1977). In addition, Bjerregaard and Depledge (1994) documented a significant interaction of Ca and Cd in the intertidal periwinkle *Littorina littorea* and the crab *Carcinus maenas*, possibly due to a change in the general permeability of body surface associated with varying Ca concentrations. However, they found no evidence of Ca and Cd interaction in the mussel *M. edulis*. The objective of this study was to test the effects of Ca concentration and different Ca channel and metabolic blockers on Cadmium as a trace element, uptake in a marine bivalve, the rock oyster, *Saccostrea cucullata*. This species has not yet been widely employed as biomonitors of in other coastal and estuarine contamination. *S. cucullata* (Rock oyster; Ostreidae, Bivalvia), a common bivalve mollusks in the coastal waters of northern coast lines of the Persian Gulf and the sea of Oman, has been studied for trace metal concentrations. In this paper, the uptake of Cadmium was experimentally studied in different organs of *S. cucullata* since metals such as Cadmium, Copper and Mercury may alter Calcium homeostasis (Allemand et al., 1989; Viarengo et al., 1988; Gnassia-Barelli et al., 1995; Tran et al., 2003), Calcium uptake was also studied in the animals treated with Cadmium.

Cadmium and Calcium have very similar atomic radii (Jacobson and Turner, 1980; Ya et al., 2004), thus Cadmium may be taken up through the Calcium channels (Hinkle et al., 1987; Banaoui et al., 2004). We measured trace element influx in this bivalve, *in situ* by exposing animals to two different concentrations of Ca channel and metabolic blockers. Among the trace elements studied before, Cadmium was selected because that tends to bind with protein. The aim of this work is to suggest the effect of variation in free calcium amount available to calcium channel leading Cadmium to rock oyster tissues.

MATERIALS AND METHODS

S. cucullata was collected at depths between 0 and 2 m on the coastal waters of northern coast lines of the Mangrove forests in northern Persian Gulf. Animals were then transported to the laboratory and placed in plastic tanks filled with natural, aerated seawater (S = 36°/°°, T = 21°C ± 1°C) under a light-dark regime (12 h: 12 h). After 3 days of acclimation, Cadmium uptake experiments were carried out at two Cadmium concentrations (20 and 150 µg/L). Oysters were exposed to heavy metal for 48 and 85 h. The uptake of Cadmium was also studied on animals previously treated with Verapamil (at 100 µM, treatment 1 h before adding Cadmium), an organic inhibitor of Calcium channels. During exposure, the bivalves were not fed, the seawater was renewed after 48 h and the same treatment was applied (1 h with Verapamil in the case of pre-exposure followed by addition of Cd).

Control animals were held in the same manner. Consequently, those pre-exposed with Verapamil and those not pre-exposed gills, visceral mass and the remainder of the soft tissues (that is, mantle, muscles and gonads were dissected out. They were then, carefully rinsed with a buffer (10 mM Tris (hydroxymethyl aminomethane) - HCl and 1 M glycine; pH = 7.8). Rinsing organs with this Solution removed surface-bound Ca. In all cases, three experimental samples consisting of ten specimens each were taken from both the control and exposed animals. Experiments were carried out in triplicate. Samples were dried to constant weight (50°C). Digestion of tissue samples was performed in a microwave oven (CEM-MDS81D) as follows. First, samples were placed in high-pressure vessels and concentrated nitric acid (65%) (Merck Suprapur) was added. The digestion procedure then followed 4 steps: microwaving at 240 W for 4 min; microwaving again at 180 W for 4 min, cooling for 10 min and finally at 220 W for 5 min. Metal determinations were carried out by flame spectrometry for total Calcium and (for control samples) atomic by flame or furnace absorption spectrophotometry (GBC 904 AA equipped with Deuterium background correction GF 3000) for Cadmium. Material used. reference (Lobster was Standard Hepato-pancreas Reference Material for Trace Metals, TORT 2 provided by the National Research Council of Canada) was analyzed; the results compared well with certified values (26.3 ± 0.5 µg Cd/g dry weight, n = 5, versus the certified values of 26.7 ± 0.6 µg Cd/g). The level of detection was 0.05 µg Cd/g dry weight) for flameless analyses of Cadmium. Data were tested for homogeneity of variance and for normal distribution. The following statistical treatment of the results consisted of two–way analysis of variance (ANOVA), which allowed the evaluation of Cd (Ca concentration and Verapamil pre-exposure on metal and Cd) concentration of samples. Pair wise comparisons were made to determine which values differed significant overall ANOVA was found.

RESULTS AND DISCUSSION

Figure 1 represents the accumulation of Cadmium in *S. cucullata* in specific organs as a function of exposure time (48 and 85 h) and of Cd concentration (20 µg/l and 150 µg Cd/l). Cadmium uptake increased as a function of Cd concentration in the medium and exposure time.

In all cases, gills accumulated Cadmium to a much greater extent than the other organs. Accumulation of Cadmium in gills suggested being mainly in function of initial concentration more than exposure time duration. In low concentration accumulation in gills was lower than high concentration (40 versus 65 µg in 20 and 150 µg as initial concentrations respectively). A decreasing range of accumulation was noted as follows: gills > visceral mass = remainder. As experiments were performed in natural sea water (without any supplement of food), the accumu-

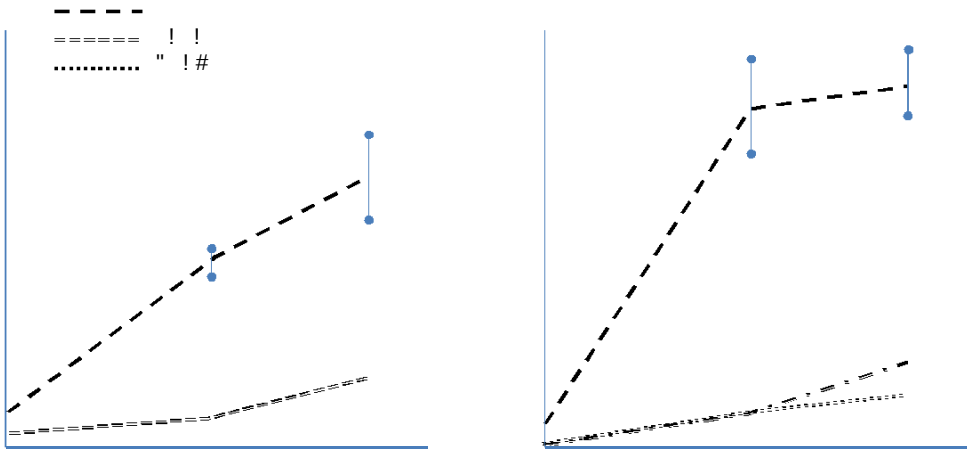

Figure 1. Accumulation of Cadmium in *S. cucullata* as a function of exposure time and of Cd concentration (20 and 150 µg Cd/l) in the medium (s.d. shown).

lation occurred by a direct mechanism of uptake where gills play a leading part. Regoli et al. (1991) and Liaoa et al. (2005) reported a similar preferential metal (Cd, Cu and Mn) accumulation in gills of the wedge shell, *Donacilla cornea* and *Corbicula fluminea* treated with these metals. At 150 µg/l saturation as a function of time appeared in uptake of Cadmium by gills, all binding sites for Cd may be occupied; Cadmium may then be redistri-buted to the other organs. Previous work (Sidoumou 1991; Sidoumou et al., 1992) suggested that Cadmium uptake by *Donacilla rugosus* linearly increased as a function of Cd concentration in the medium (from 10 µg Cd/l to 500 µg Cd/l). According to Viarengo (1989), this phenomenon indicates that a diffusion process is involved in Cadmium uptake. In a previous work, Sidoumou (1991) reported that *M. edulis* Cd levels remained unchanged following a detoxication period of 8 days, suggesting that Cd was bound firmly and seques-tered in the animal by metallothioneins. Comparisons (t-tests) between animals pre-exposed and not pre-exposed (after 48 h, Figure 2 and after 85 h, Figure 3) were signifi-cant at $p < 0.05$, which suggest that total Calcium concentration in the gills of control animals was decreased by Ca 20% by Verapamil.

It must be noted that the calcium channel blocker verapamil (VP) did not cause apparent toxicity to *S. cucullata*. Verapamil is known to block a class of calcium channels (Guerrero and Martin 1984). The fact that verapamil decreased total Calcium concentration in gills of *S. cucullata* may imply that calcium channels are present in this organ. ANOVA was performed on results obtained in the case of *S. cucullata* after 48 h of Cd ex-

exposure on Calcium concentrations in the specific organs of *S. cucullata*. The only significant effect was that of Cadmium concentration in the medium ($p < 0.05$) on Calcium content of gills. ANOVA, performed on Calcium concentrations after 85 h of Cd exposure, showed a significant effect of Cadmium on Calcium concentration in gills ($p < 0.05$). Results concerning gills after 48 h Cd exposure are shown in Figure 2 (left part). A decrease in Calcium concentration was observed at 150 µg Cd/l (referred to -VP) compared to controls (t-test significant at $p < 0.01$). No significant effect was noted when animals were pre-exposed with Verapamil (referred to as +VP).

Figure 3 shows the Calcium specific concentration after 85 hr of Cd-exposure. A significant increase in Calcium concentration was found in gills of animals exposed to 20 µg Cd/l for 85 h (11.05 ± 3.5 µg/g compared to controls 3.96 ± 0.51 µg/g, significance of t-test shown in Figure 3). The same increase was found for visceral mass (t-test significant at $p < 0.01$) and for the remainder of the tissues ($p < 0.05$). The increase in Calcium concentration in animals exposed to 20 µg Cd/l was not found when they were pre-exposed with Verapamil (Figure 3). Exposure of animals with 150 µg Cd/l (pre-exposed or not with VP) did not change Calcium concentration compared to controls in specific organs. ANOVA performed on Cad-mium concentrations found after 48 and 85 h of exposure in specific organs of *S. cucullata* demonstrated that the effect of Cadmium concentration in the medium on Cadmium concentrations was significant at $p < 0.0001$. ANOVA showed that Verapamil had an effect on Cadmium concentration in the gills ($p < 0.05$) after 48 h, whereas after 85 h this effect was significant in the visce-

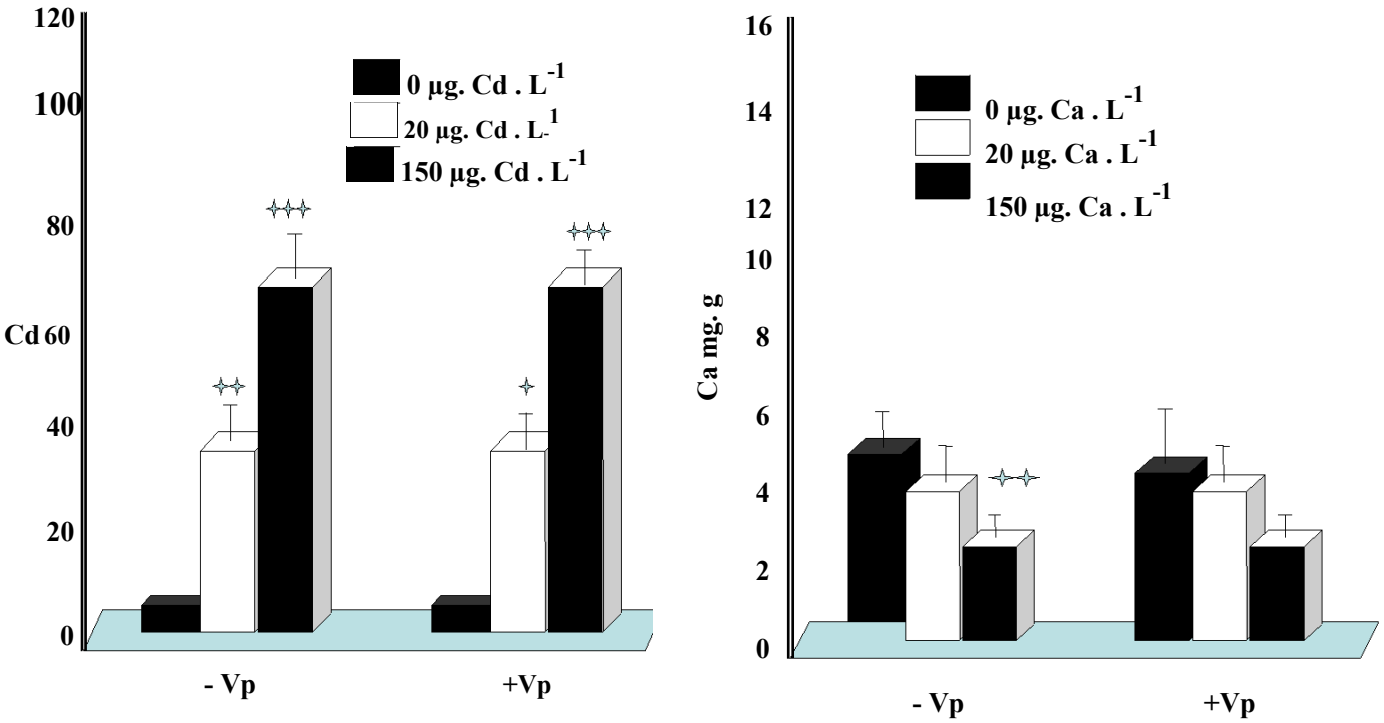

Figure 2. Ca and Cd concentrations mean values ± 1 rugosus after 48 h of Cd in gills treatment (20 and 150 µg Cd/l). The pre-exposure with the absence of pre-exposure Verapamil is shown by +Vp; t-tests were performed between Cadmium-treated by -Vp. **p < 0.0l; controls (*p < 0.05; animals and appropriate ***p < 0.00l).

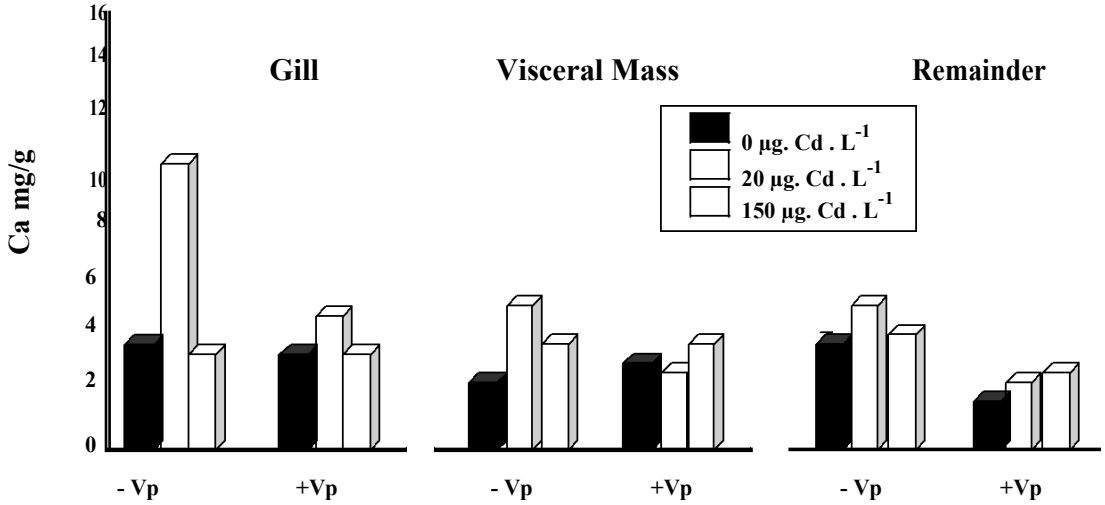

Figure 3. Ca concentrations (mean values ± 1 s.d.) in *S. cucullata* after 85 h of Cd of specific organs. The pre-exposure with treatment (20 and 150 µg Cd/L). The absence of pre-exposure Verapamil is shown by +VP, t-tests were performed between Cadmium-treated by -Vp. **p<0.0l; animals and appropriate controls (*p<0.05; ***p<0.00l).

ral mass (p < 0.05) and remainder (p < 0.001).

Results concerning Cadmium concentration in gills, after 48 h of Cd exposure, are shown in Figure 2 (right part) Cadmium in gills decreased when animals were pre-exposed -Vp/+Vp significant at p < 0.001 at to Verapamil (t-test 20 µg Cd/l; n.s. at 150 µg Cd/l). Cadmium concentrations in the different organs after 85 h of Cd exposure are shown in Table 1.

After 85 h of exposure (Table 1), Verapamil generally decreased Cadmium concentration of treated animals (20

Table 1. Cadmium concentrations in specific organs of *S. cucullata* after 85 h of treatment.

µg Cd/L Added to the medium	Gill µg Cd/g	Visceral mass µg Cd/g	Remainder µg Cd/g
0	6.2±1.8	1.6±1.1	0.3±0.1
0+Vp	5.2±2.0	8.2±0.8	0.6±0.3
20	52.1±9.3	8.2±0.6	11.3±0.7
20+Vp	37.3±2.7***	5.5±0.8*	7.6±0.4**
150	72.1±6.8	13.3±1.4	14.7±0.9
150+Vp	74.2±10.3n.s	10.7±0.5*	11.3±0.6**

and 150 µg Cd/l) in gills, visceral mass and the remainder (significance of t-tests -VP/+VP shown in Table 1). Nevertheless, Verapamil had no effect on Cadmium uptake by gills at 150 µg Cd/l. As mentioned above, gills had already reached saturation at this exposure time and this Cadmium concentration, when animals were treated only by Cd. Results presented in this paper demonstrate that Cadmium had an effect upon Calcium homeostasis (either by decreasing Calcium concentration or by increasing this concentration) in specific organs, particularly gills, of *S. cucullata*, Cadmium, introduced into the medium at 150 µg Cd/l for 48 h, significantly decreased total Calcium concentration in gills, whereas at 20 µg Cd/l exposure and for a longer time 85 hr, Calcium concentration increased in gills, visceral mass and the remainder *S. cucullata*. Alteration of Calcium homeostasis by Cadmium seemed, therefore, to depend on Cadmium concentration and on exposure time. Sauer and Watabe (1988) reported that Cadmium inhibited by Calcium uptake by *Fundulus heteroclitus* gills and the authors suggested that Cd may have affected Ca uptake by competing with Ca^{2+} ions for the same binding sites. On the contrary, increased Calcium uptake was observed in the gills of the mussel *Mytilus galloprovincialis* treated with copper (Viarengo et al., 1988) and in all the organs of the clam *Ruditapes decussates* (Gnassia- Barelli et al., 1995) treated with copper. *Donacilla cornea* exposed to Cadmium or copper showed an increase in Calcium concentration in whole soft parts and in the digestive gland (Regoli et al., 1991; Gregory et al., 2002). According to Viarengo (1989), heavy metals in invertebrates may alter the plasma membrane Ca-extruding systems. This would result in a decreased capacity for pumping Ca^{2+} out of the cell and therefore, in an enhanced concentration of Ca in the cytoplasm in an attempt to restore low physiological concentration of free cytosolic Ca^{2+}. *S. cucullata* appeared to counter- act the toxic Cadmium effect on Calcium homeostasis by restoring the initial Calcium concentration.

The overall results of this work may suggest that, when concentration of dissolved calcium changed, which may in the other hand related to water temperature, the absorption of heavy metals (cadmium in this work) may be changed. This kind of result synthesis may suggest that in cold waters (and/or seasons), the absorption of some heavy metals such as cadmium may be greater than warm ones.

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