

Biosorption and bioaccumulation of heavy metals by rock oyster *Saccostrea cucullata* in the Persian Gulf

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Abstract

Effective treatments of industrial waste streams and toxic spills containing heavy metals related with rapid removal of metal ions at high concentrations. Influences of clearance and absorption rates on two heavy metals (copper and cadmium) by *saccostrea cucullata* originated from mangrove ecosystem in the Northern Persian Gulf were investigated. Specimens were collected from sub-tidal band during neap tide; groups of 15 individual were placed in 1.5 L containers (2-liter volume), which were immersed in the water. After the acclimation time (i.e. the regulation of filtration activity caused by manipulation and any other sources), specific clearance and absorption performances were measured during alternation of stagnation and flow positions. Temperature, salinity and particle concentrations were maintained. The clearance rate is affected by the amount of cadmium (from 1.69 to 0.04 ml min/g/h AFDW) and copper (from 2.16 ml to 0.42 ml min/g/h AFDW). Although the clearance rate was significantly reduced, live oysters reduced the amount of cadmium and copper from 150 and 200 µg/l to 118.68 and 133.30 µg/l respectively. Oyster had the different clearance rates toward copper and cadmium when simultaneously introduced. The dead shells also had a good ability to reduce the metal condensation. According to the results it can be concluded that *Saccostrea cucullata* can be used as biofilters with good clearance ability.

Keywords: Persian Gulf, Retention rate, Mangrove, Bivalves, Heavy metal absorption

Introduction

There are two main sources of heavy metal contamination in aquatic ecosystems: natural processes or natural occurring deposits and anthropogenic activities. Anthropogenic activities have the highest importance (Francis 1994). The marine environment toxic metals are potentially accumulated in sediments and marine organisms, and subsequently transferred to man through the food chain.

Heavy metals are among dangerous parts of oil pollution. The large body of literature on the consequences of metal contamination of water attests to the fact that this is increasingly a worldwide problem growing in scale and degradation of global habitat (Vitousek et al. 1997). Metals can be divided in two groups: essential metals (e.g. copper, zinc, iron, etc.) and non essential toxic metals (e.g. cadmium, vanadium, nickel, etc.). Those metals are

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dissolved in the water or accumulated in the body of aquatic organisms (Amiard et al. 1987). Due to the rarity of some metals in the environment, they can be used as indicators of oil pollution. Metals are known to reduce the performance of bivalve molluscs (Kramer et al. 1989), because in the presence of a high concentration of heavy metals, bivalve molluscs keep their shells closed for a longer period of time (Doherty et al. 1987), producing fewer byssus threads (Martin et al. 1975) and reduce heart rates (Grace and Gainey 1987). Also some marine bivalves reduce their filtration rates (Watling 1981; Grace and Gainey 1987).

The Persian Gulf is a shallow basin with an average depth of 35–40 m and a total area of around 240 sq. km. It joins free international waters through the Strait of Hormuz (Banat et al. 1998). The turnover and flushing time have been estimated to be in the range of 3–5 yr indicating that pollutants are likely to reside in the Persian Gulf for a considerable time (Sheppard 1993). Due to shallow depth, limited circulation and high salinity and temperature Persian Gulf (Saeed et al. 1995), impacts of pollutants on the marine environment may be significant. Occurrence of several environmental disasters in the region during the recent years (including the biggest oil spill in the world in 1991) and increasing pollution, urges treatments that are efficient and cost-effective.

Bivalves have been extensively used for the assessment of the petroleum hydrocarbons and heavy metals (de Mora et al. 2005; Fowler et al. 1993) in the Gulf. Consideration of the ability of certain plants and animals to accumulate trace metals above ambient water concentration has led to the use of certain macrophytes (Jackson 1982; Fisher 1985) and mollusks (Bebiano et al. 1993; Walsh et al. 1995) as biofilter organisms. The purpose of this study was to examine the role of oyster filtration (alive oysters and dead shells) in mangrove habitat so as to reduce the pollutants in Persian Gulf. The Uptake and clearance rate of *saccostrea cucullata* was measured when it was exposed to heavy metals (cadmium and copper) in natural conditions in order to determine the capability of the oyster filtration rate as well as the impact of the heavy metals on the clearance rate.

Materials and methods

Study site

The in situ experiments were carried out in Queshm Island in the north of Hormuz strait (26°45'N 55°49'E) in the Persian Gulf in June 2009. Laboratory measurements of heavy metals were performed in the Department of Fisheries and Environmental Science, Faculty of Natural Resources, University of Tehran, Karaj, Iran.

Experimental set-up

Mesocosm was situated below the sea water surface in the selected coast of mangrove forest (based on easier availability, the existence of *saccostrea cucullata* communities and accessibility to basic data). First, an iron bench was installed on intertidal sediment near to subtidal zone during the low tide. The bench had two distinct levels (Figure 1), distanced by 1 m from each other.

Feeding reservoirs of 20-liter were placed on the upper level in order to allow the system flow by gravity. Recipients of 2-liter volume were placed below water surface at the lower level of the bench. The intermediate pipe between feeding reservoir and mesocosm recipient below the water was chosen from PVC tube. Flow taps were installed to control water flow rate and manipulate current-flow conditions. As it can be observed from Figure 1, water inlet was located at the bottom of recipient, while the water outlet was on the top.

Then, some of the alive oysters were washed, weighed and placed in the mesocosm (2-liter volume) provided with filtered water (MW cut-off: 50 μm). The second group of the experiment, which contains the dead shells, was also weighed and put in another mesocosm (2-liter volume). The water was kept and always air-saturated with oxygen. The beginning time of the experiment was started when bivalve siphons could be observed from the recipient walls (after adaptation to new condition).

At the beginning of the experiment, heavy metal solution (dissolved in distilled water), was added to the feeding tank situated 1.5 m higher than mesocosm. The initial concentrations for cadmium and copper were 150 $\mu\text{g/l}$ and 200 $\mu\text{g/l}$, respectively. CdCl_2 and CuSO_4 were used to prepare initial solutions. Each experiment involved two paralleled mesocosm for alive bivalves and dead shells. The absorption procedure was consisted of two minutes of flow followed by five minutes of stagnation. This stop-flow condition was repeated seven times, causing 50 minutes of experimental time for each metal. The sampling was done at the beginning of two minutes and at the end of five minutes.

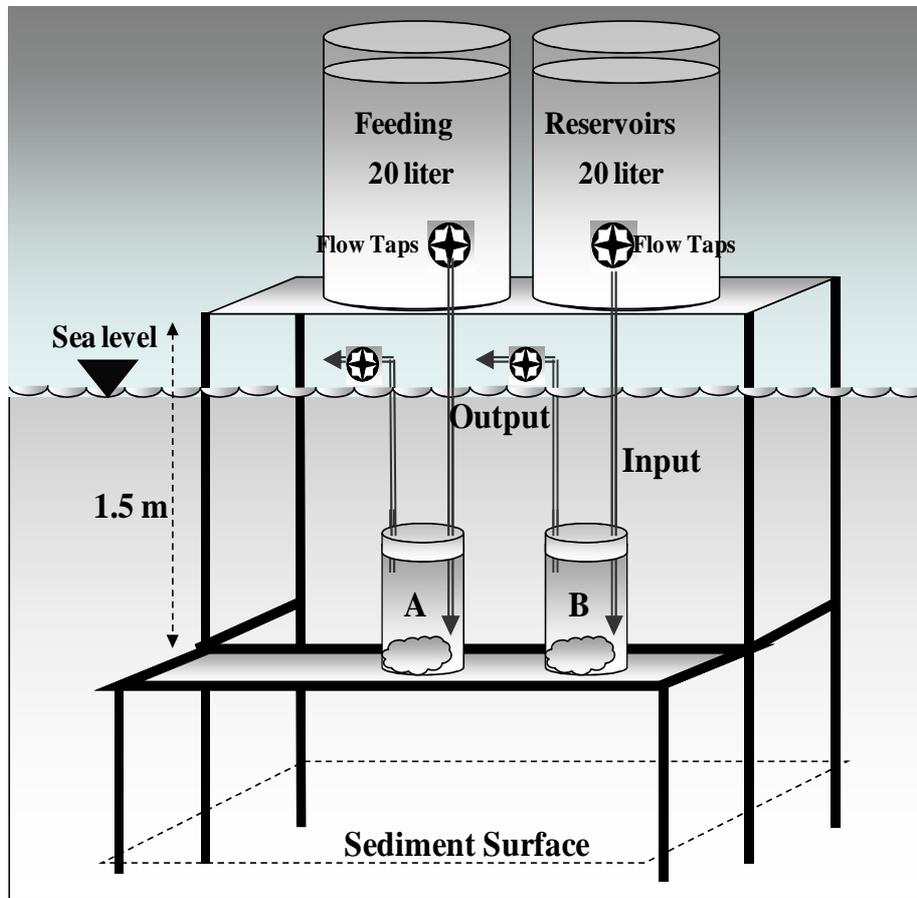


Fig. 1. In situ mesocosm settlement in subtidal zone, where parts are installed on a bench settled on the sediments. Contaminated sea water from reservoirs of 20 liters flowed into filtration chambers (A & B) of 2 liters, where each one contained alive or dead oysters Taps, installed in input and output flow pipes was installed to control water velocity in the system.

Clearance rate

After doing contamination experiments, enclosures were cleaned and filled again with filtered seawater (55 μm), containing a known concentration of the phytoplankton, *Dunaliella salina* ($\approx 10\,000$ cells/ml). This was considerably below the threshold value of 5 mg/l (Widdows et al. 1979) above which the organic material is deposited by bivalve molluscs as pseudofaeces (Foster-Smith 1975a, b). Water samples were taken after 30 minutes and the particle concentration was determined through visual counting under a microscope using a Neobar slide with five replicates. Clearance rate (defined as the volume water cleared of *Dunaliella* cells per time unit and biomass) was calculated by the following formula Jørgensen (1990):

$$\text{Clearance rate (CR: ml. min/g)} = V (\ln C_0 - C_1) / W.T$$

where:

C_0 = cell concentration at time T_0

C_1 = cell concentration at time T_1

W and T = AFDW biomass (g) and experimental period (min), respectively

V = volume of the recipient (ml)

Sample preparation

The water samples were prepared for analysis according to chelation-extraction method (Long and Martin, 1991). The samples were transferred to tightly-sealed polyethylene containers to avoid adsorption of metals from digested solution and kept at 4 °C prior to further analysis. Individuals were dried to a constant weight at 90°C and ashed at 538 °C for 5 hours to determine Ash-free dry weight (AFDW).

Analytical Method

Elements contents in samples were determined using Inductively Coupled Plasma Mass Spectrometer (VG PlasmaQuad 3 – VG Elemental, Winsford, Cheshire, UK). A known amount of an indium standard solution was added to HNO₃ solution as an internal standard. All analyses were undertaken at least in triplicate on each sample and the mean values were calculated.

Data analysis

The unpaired T-test was used to compare each group (alive and dead shell) at different time of experiment. Clearance rate data were log transformed to achieve homogeneous variances for statistical analysis (Bartlett's test, Sokal and Rohlf 1981). Differences were considered significant at $P < 0.05$.

Results

Clearance measurements

Preliminary results of clearance rates for copper and cadmium showed that oysters demonstrated different behaviors, when they were exposed to these metals, essential and non-essential, respectively. In case of cadmium, oyster cleared sea water as high as 2.39 ml/min in the beginning of exposure but this value decreased rapidly to 0.4 ml. Clearance rates remained constant after 49 minutes.

Clearance rate of live oysters exposed to cadmium at different exposure time were significantly different ($P < 0.05$). Average of clearance rate in live oysters was calculated to be 0.59 ml min/g AFDW and the least (at 35 minute) and maximum clearance (at 7 minute) in this group was 0.04 and 2.39 ml min/g AFDW respectively. With increasing exposure time with metal, clearance rate was reduced. The most clearance rate in treatment was measured at the beginning of experiment and this rate was decreased during Experimental period (Fig. 2). Clearance rate of dead shells exposed to cadmium with different exposure times was also different ($P < 0.05$). Average rate of clearance of the dead shells was 0.23 ml min/g DW and the lowest (at 14 and 35 minutes) and highest value (at me 28 minutes) in this group was 0.007 and 0.79 ml min /g DW, respectively.

In case of copper, clearance rate of alive oyster and dead shells exposed to this metal (200 µg/l) was significantly different between two groups ($P < 0.05$) (Fig. 2). Clearance rate of live oysters was different among tested times ($P < 0.05$). Average of clearance rate in the live oysters was 1.1 ml min /g AFDW and the minimum (at 28 min) and maximum values (during the 7 and 14 minutes) were 0.42 and 1.94 ml min /g AFDW respectively.

Ability of oyster in decreasing heavy metal concentration from sea water (reduction rate)

Figure 3 shows the removal of cadmium and copper from the sea water of about 150 and 200 ppb, by *saccostrea cucullata*, respectively. Reduction rate (the decrease in heavy metal concentration by alive or dead oyster in each experimental period, compared with initial concentration) of cadmium in two experimental groups was significantly different ($P < 0.05$).

For alive oyster, this value was different during the experiment. The average reductions in concentration of cadmium in alive oyster were 116.69 µg/l and the minimum (in 49 minutes) and maximum decreases (in 7 minutes) were 111.3 and 129.6 µg/l, respectively. The average reduction of cadmium concentration by shells, compared with initial concentration (150 µg/l), was calculated to be 138.73 µg/l (Fig. 2 A). Similarly, copper reduction rate in the two experimental groups was different ($P < 0.05$). Reduction rate of copper from water by live oysters was different at the experimental times ($P < 0.05$) and average reduction was 133.30 µg/l. The lowest values (in 7 minutes) and maximum reduction (in 42 minutes) were 126.9 and 145.7 respectively. Therefore, the absorption rate increased over time. It was noted that average decrease in copper concentrations by shells was 181.07 µg/l (Fig. 2 B). Shell clearance rate when exposed to copper was not significantly different with the tested time. Average rate of clearance in shells was 0.3 ml min/g DW.

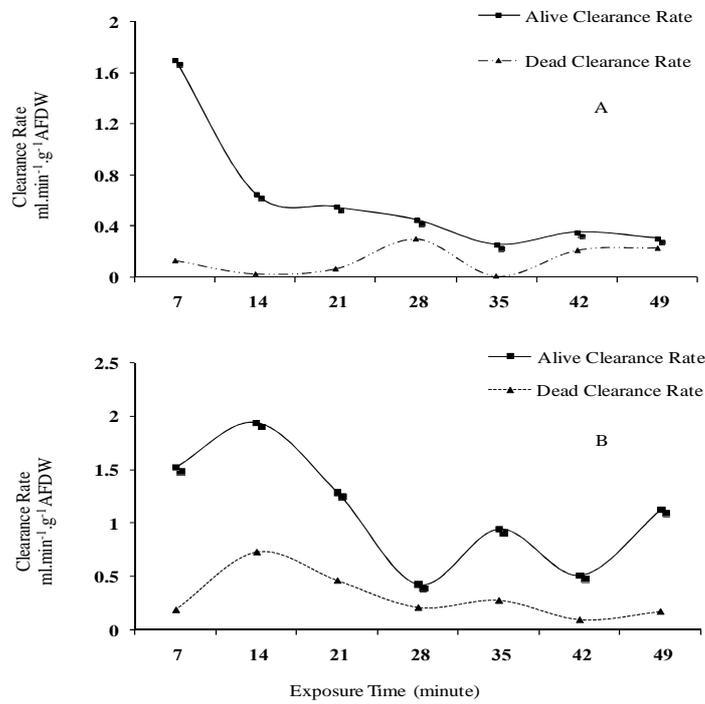


Fig. 2. Clearance rate of live oyster (treatment) and dead shells (control) exposed to cadmium (A) and copper (B)

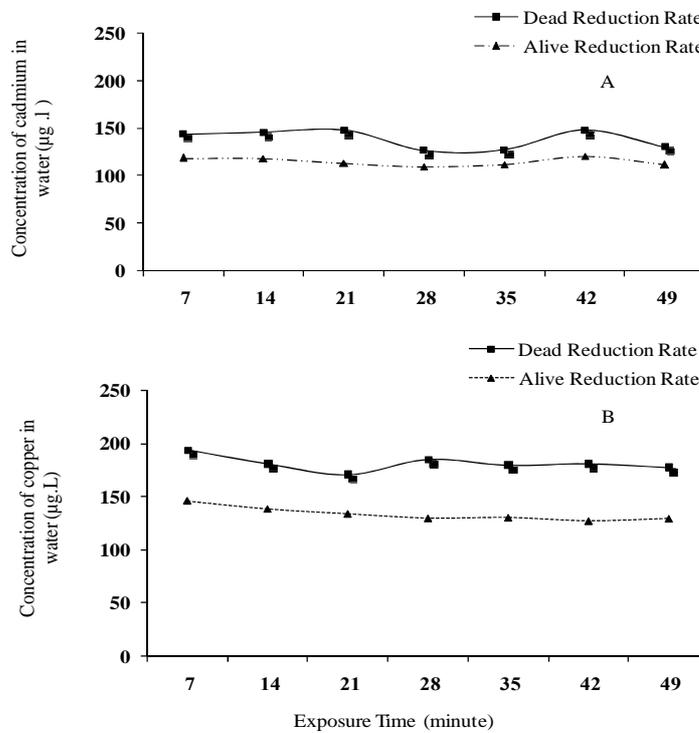


Fig. 3. Amount of cadmium (A) and copper (B) reduction by live oyster (treatment) and dead shells (control)

Table 1 shows the reduction of cadmium and copper from solution using live oyster and shells. After 49 minutes of continuous stirring, the average reduction of cadmium from solution compared with initial concentration in treatment and control reached 22.14% and 8.15% respectively. The total cadmium concentration in the water was reduced from 150 ppb to 116.69 and 138.73 ppb in treatment and control, respectively. The initial removal and the overall removal of cadmium from water decreased with increasing exposure times.

Table 1. Removal amounts of copper and cadmium in treatments (live oysters) compared to those of control (dead shells)

Time (min)	Percent of copper removed in control	Percent of copper removed in treatment	Percent of copper removed in control	Percent of copper removed in treatment
7	3.20	27.15	4.13	27.87
14	9.60	30.90	3.07	21.27
21	11.68	33.20	1.33	24.93
28	7.60	35.25	15.73	18.87
35	10.30	34.25	18.40	25.80
42	9.60	36.55	1.27	20.00
49	11.35	35.42	13.13	16.73

The average copper reduction in treatment and control of 33.35% and 9.05% respectively for copper removal from solution was achieved after 49 minutes of continuous stirring. The total copper concentration in solution was reduced from 200 ppb to 133.30 and 183.07 ppb in treatment and control, respectively. The initial removal and the overall removal of copper from water showed no decrease with increasing exposure times.

Discussion

The current work investigated the potential of rock oyster for rapid removal of high concentrations of metal ions and determines effect of heavy metals on clearance rate of oyster. The exposed individuals of *S.cucullata* to cadmium and copper showed a dramatic decrease in clearance rate.

This was more pronounced for cadmium, suggesting that this species is more sensitive to cadmium exposure than the copper. Reduced clearance rate could be the result of gill damages, since one of the recorded effects of sub lethal concentrations of cadmium is structural deformations of gills (Viarengo 1989). The reduction in clearance could also be the result of avoiding the cadmium and copper through partial valve closure, although this behavior was not observed in the present study. This behavior has been reported for the blue mussel, *Mytilus edulis*, when exposed to copper (Davenport 1977; Manley 1983).

Our results demonstrated that clearance rate was a sensitive parameter for determining the effects of metals on *S. cucullata*. In case of cadmium, clearance rate of live oyster at initial time of experiment was 2.39 ml min /g AFDW, while this value decreased to 0.5 ml min /g AFDW at the end of experiment. Clearance rate in live oysters exposed to copper at the first time (7 minutes) was 1.52 ml min /g AFDW and that obtained at the end (49 minutes) was 1.12 ml min /g AFDW. Thus, the oysters exposed to cadmium were more influenced than those exposed to copper. This was in agreement with results of other studies for marine bivalves, e.g. *Mytilus edulis* (Abel 1976; Watling 1981). Cadmium uptake by freshwater mussels has been shown to be highly dependent on concentration, duration of exposure (Das and Jana 1999), mussel length (Roseman et al. 1994), and detoxification mechanisms. In the experiments of clearance and uptake rate in both live oyster and dead shells exposed to cadmium and copper (Figure 2), re-dissolution of absorbed heavy metals was observed.

This was expected with the short exposure time. Effective treatments of industrial waste streams and toxic spills containing heavy metals depend on the rapid removal of high concentrations of metal ions (Demetra 2007). For dead shells, the first removal occurred, then re-dissolution, afterward removal again and so on. The re-dissolution could occur as a result of number of factors: a change in pH beyond a critical value, a change in temperature due to heating from the continuous operation of the stirrer, or a change in the crystal structure of the formed calcium/cadmium, copper compound following sorption (Elfwing 2002). It was concluded that in dead shells the

mechanism of the removal of heavy metal ions was rapid absorption of the metal and exchange of calcium ions of the shell with metal ions from the aqueous solution (Demetra 2007).

Maximum reduction rate of cadmium by live oyster was obtained in 7 minutes, although the highest clearance rate of live oyster was also found at this time. Similarly, the lowest clearance rate of live oysters was observed at the end of experiment (49 minutes) and at this time the minimum reduction of the metal concentration from water by *S. cucullata* was found (Table 1 and Figs. 1, 2). While this model was not found in the case of copper. The highest purification rate of live oyster was in the first time, while most of copper reduction took place at the end of experiment. This shows that at the beginning of the experiment oyster was able to control absorption of copper, but after being exposed to the metal, this ability was decreased (Table 1 and Figs. 1, 2). It was suggested that *S. cucullata* was capable of regulating the concentration of the essential metals Cu in the body, whereas Cd, which is a non-essential element, cannot be regulated. This has been found for several other organisms, including *Mytilus edulis* (Amiard et al. 1987; Rainbow and White 1989).

Rainbow and White (1989) showed that Zn regulation in the decapods, *Palaemon elegans*, is an active process; an increased rate of Zn uptake was matched by an increased Zn excretion (White and Rainbow 1982). When the effective concentrations for Cu and Cd, found in this study, are compared with quality criteria for metal concentrations in surface water (3 µg/l Cd and 0.2 µg/l) (Ministerie van Verkeer en Waterstaat 1988-1989), it appears that all effective concentrations were above these levels, suggesting that *S. cucullata* was sufficiently protected by these quality criteria.

This oyster lives in a constantly changing environment, rendering higher tolerance than species below the intertidal zone (Newell 1970). Since they are evolved in and adapted to such variable environments, they have a better ability to tolerate any toxic compound, and possibly every perturbation, than would morphologically similar organisms adapted to stable environments (Fisher 1977). Currently, the treatment of choice for reducing or removing dissolved heavy metals is chemical precipitation. Although this method is fairly inexpensive, it has disadvantages including slow reaction times, a need for pH control during treatment, subsequent pH readjustment, high residual metal solubilities at near-neutral pH, the need for adding polyelectrolytes or other chemicals for the coagulation and flocculation steps as well as reliance on numerous chemicals as coagulant aids to further promote rapid settling; high chemical costs and the concomitant disposal costs.

Use of shell and live oyster as an alternative to precipitation offers a benign process that could replace, or at least lessen, current reliance on the great quantities of generally corrosive chemicals that are essential with conventional precipitation treatment methods. According to our results, it can be concluded that *Saccostrea cucullata* (live and dead shells) can be used as biofilters with good clearance ability. The pollution pressure imposed by heavy metals is likely to increase even further in the area, due to the accelerating development throughout the coastal zone. A higher cadmium and copper load, e.g. released from anti-fouling paint used on most vessels in the gulf, will most certainly result from increasing shipping activities, but also from other sources, like industrial wastes, untreated sewage and leakage from sediments. As a conclusion, the use of *Saccostrea cucullata* for control of heavy metal pollution in the Persian Gulf can be a promising means.

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