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Evaluation of bivalve clearance rate (CR) as a physiological indicator of heavy metal toxicity in freshwater mussel, *Anodonta cygnea* (Linea, 1876)

F. Moëzzi[a,*], A. Javanshir[a], S. Eagderi[a], H. Poorbagher[a], M. Sallaki[b]
[a]*Department of Fisheries, Faculty of Natural Resources, University of Tehran, Iran.*
[b]*Department of Environmental Sciences, Faculty of Environment and Energy, Science and Research Branch, Islamic Azad University, Tehran, Iran.*

[*]Corresponding author; Department of Fisheries, Faculty of Natural Resources, University of Tehran, Iran. Tel: 0098-32223044

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This study was performed to evaluate suitability of clearance rate (CR) as a physiological indicator of zinc (essential element) and chromium (non-essential element) toxicity in *Anodonta cygnea* (Linaea, 1876). CR of bivalves was measured before and after 9 and 18 days exposure to 100 µg l^{-1} Zn and Cr during 120 min with 15 min intervals. 5, 25 and 125 µg l^{-1} concentrations of each metal were used for CR measurement. Irregular fluctuations were observed in pre-exposed CR of bivalves in all three concentrations of both metals during 2 hour period. There was significant difference (α=0/05; P < 0/025) between CR of Zn and Cr in lowest (5 µg l^{-1}) and highest (125 µg l^{-1}) exposure concentrations. Maximum CR for both metals was obtained in lower concentration. Also, calculated post-exposed CR for both metals in days 9 and 18 after exposure were significantly (α=0/05; P < 0/01) lower than control bivalves. Based on the results, clearance rate is a useful measure of heavy metal toxicity in *A. cygnea* as a model of freshwater bivalves.

1. Introduction

Nowadays, many of the water bodies are polluted by heavy metals. ‘Heavy metals’, generally is referred to a group of metals and metalloids, their pollution in aquatic environments can cause toxicity and ecological problems (Banfalvi, 2011). These metals are separated into two categories: essential and non-essential. Essential metals, such as Co, Cu, Fe, Mn, Mo, V, Sr, Se and Zn, have biological functions in organisms (Simkiss, 1981; Williams, 1981). Specific biological roles for non-essential heavy metals such as Cr, Hg, Pb and As were not reported yet (Banfalvi, 2011). These elements are one of the main pollutant groups in water bodies that low concentrations of them may have a high toxicity level on aquatic biota. Background levels of these elements in waters are within a range of ≤ 0.0001 to 0.02 mg l^{-1} (Alabaster, 1980).

Aquatic organisms are able to accumulate heavy metals in concentrations that are higher than their concentrations in the environment (Golovanova, 2008). Bivalves are a group of aquatic invertebrates that are widely used as bioindicator in environmental monitoring (Sanders et al., 1993; Livingstone et al., 2000). These filter-feeder molluscs are considered as sensitive indicators of chemical pollution (Fournier et al., 2001). The special features of bivalves, which considers them as important bioindicators in aquatic habitats, include: presence in coastal regions with low depth; immobility or sedentary situation; high abundance; ease of sampling; low to high resistance in exposure to different pollutants; accumulation of pollutants; and showing history of exposure (Philips, 1980; Green et al., 1985).

Detection and measuring physiological and biochemical responses of a bivalve can be used as an indicator of health status of larger population or community (Bayne et al., 1980). Clearance rate (CR) is a good physiological parameter in bivalves and its fluctuations shows the impacts of chemical pollution stress on the organism at the whole body level (Azarbad et al., 2010). This biological index reflects the performance of filtering surfaces, especially gills (Jørgensen, 1990). Investigation of filtration activity in mussels is a suitable approach in studying of biological parameters such as valve movements and water pumping pattern, as indices of short-term toxicity of pollutants (Kramer et al., 1989; Salanki and Balogh, 1989; Mouabad and Pihan, 1992). Numerous studies were taken on the effects of heavy metals on bivalve clearance rate, indicating the impossibility of this physiological index in exposure to these pollutants (Davenport, 1977; Manley, 1983; Viarengo, 1985; Sliverman et al., 1996; Anandaraj et al., 2002; Shi and Wang, 2004; Azarbad et al., 2010).

Among freshwater bivalves, mussels of the family Unionidae have been reported as good bioindicators (Philips, 1980; Green et al., 1985). *Anodonta cygnea*, which is also known as swan mussel, has belonged to this family and is found from the British and Irish Isles east to Siberia and south to northern Africa (MUSSELp, 2004). This species also is an endemic species of northern water bodies of Iran, connected to Caspian Sea (Parvaneh, 1990; Pourang, 1996). The aim of this study was to determine the effects of exposure to different concentrations of heavy metals, zinc (Zn) – essential element - and chromium (Cr) - nonessential element - on clearance rate of *A. cygnea*.

2. Materials and methods

Bivalve specimens have been collected from Semeskandeh region, Sari, Mazandaran Province, Iran (36°48′46′′N, 53°06′57′′E) and transferred to the laboratory. In order to acclimatization, bivalves were maintained for 14 days in fiberglass tanks with recirculating water at 16.3 ± 1.2 °C, pH 7.1 ± 0.3, dissolved oxygen 7.1 ± 0.2 mg l^{-1}. Dechlorinated tap water with aeration has been used in tanks during experiments.

CR of bivalves was calculated before exposure, and after 9 and 18 days exposure to 100 µg l^{-1} of metals. Before exposure CR was measured using 5, 25 and 125 µg l^{-1} of each metal. On days 9 and 18 after exposure, 25 and 5 µg l^{-1} concentrations of each metal were used for measuring CR, respectively. In each measurement time, 3 bivalve specimens with lengths in the range of 13 ± 0.3 cm were placed in a glass container with water volume of 5 l. Then, metal solution was added to water and sampling of water in 15 min intervals was taken. For preparation of metal solutions, $ZnSO_4$ and $K_2Cr_2O_7$ were used.

Water samples were maintained in 60 ml PET containers and kept at 4°C prior to analyses. For fixation of water samples, HNO_3 (pH < 2) was used. Element content of water samples was determined using Inductively Coupled Plasma Mass Spectrometer (VG PlasmaQuad 3- VG Elemental, Winsford, Cheshire, UK). All analyses were undertaken at least in triplicate on each sample and mean values were calculated. Bivalve specimens were dried to a constant weight at 90°C. Dry samples were kept in t = 500°C for 60 min and their AFDW were calculated.

CR of the bivalves was calculated by the following equation (Jørgensen, 1990):

$CR = [V (\ln CT_0 – \ln CT_n)] / [T×W]$

In that, CR: clearance rate (ml min^{-1} g^{-1} AFDW); V: volume of the container (ml); CT_o: metal concentration in t0 (µg l^{-1}); CT_n: metal concentration in T_n (µg l^{-1}); T: experimental period (min); W: ash free dry weight of bivalves (g AFDW).

The unpaired T-test was used to compare CR of bivalves in different concentrations and times. Differences were considered significant at $P < 0.05$.

3. Results

Figure 1 displays the trends of changes in pre-exposed CR values of bivalves in different concentrations of Zn and Cr. Irregular fluctuations in CR were observed in all three exposure concentrations. Minimum CR value (0.088 ± 0.007 ml min^{-1} g^{-1} AFDW) for Cr was seen in 125 µg l^{-1} and maximum CR values in 25 µgl^{-1} (3.445 ± 1.09 ml min^{-1} g^{-1} AFDW) and 5 µg l^{-1} (2.803 ± 0.672 ml min^{-1} g^{-1} AFDW) concentrations. CR of bivalves for Cr in 125 µg l^{-1} was significantly ($P < 0001$) higher than CR in 25 and 5 µg l^{-1}, but there was no significant difference between CR in 25 and 5 µg l^{-1} (figure 2). For Zn, there was an inverse relationship between concentration and CR value, so that minimum (0.369 ± 0.038 ml min^{-1} g^{-1} AFDW) and maximum (4.261 ± 0.458 ml min^{-1} g^{-1} AFDW) CR were obtained with highest and lowest concentrations, and significant differences ($P < 0.05$) were observed between CR of bivalves in different concentrations. In the other hand, significant differences were existed in CR values between Zn and Cr in 5 and 125 µg l^{-1} concentrations ($P_5 = 0.015$; $P_{125} = 0.025$), but there was no difference in 25 µg l^{-1} (Figure 2). Exposure to metals for 9 and 18 days caused to decrease in the CR of bivalves for both metals (Table 1). Post-exposed CR for both metals in day 9 in 25 µg l^{-1} was significantly ($P < 0.01$) lower than pre-exposed CR in that concentration. On day 18, post-exposed CR of both metals were significantly ($P < 0.02$) higher than CR on day 9, and significantly ($P < 0.013$) lower than pre-exposed CR in the same exposure concentrations. There was no difference in CR between Zn and Cr in days 9 and 18.

4. Discussion

The results of this study showed that type and concentration of metallic element can affect CR. For Zn as an essential element, pre-exposed CR was higher in lower concentration, shows that CR (and metal sorption) was related to the metal concentration. Although, maximum CR for Cr was observed in lowest concentration (lowest toxicity), but CR values in 25 and 125 µg l^{-1} concentrations were similar. Non-essential heavy metals have higher toxicity potential in lower concentrations than essential metals; therefore CR decreases in equal concentrations of essential and non-essential heavy metals, are higher for non-essential elements (Salanki and Balogh, 1989). It has been reported that CR was more affected by exposure to cadmium (non-essential element) than copper (essential metal) (Azarbad et al., 2010). Bivalves can regulate short-term biosorption of high concentrations of essential elements, but this situation is not seen for non-essential elements (Macrovecchio et al., 2007).

In our study increase in exposure time duration of both metals led to a higher decrease in CR, compared to pre-exposed CR of bivalves. This was maybe a result of tissue damages and impaired function of organs involved in filtration activity due to sorption and accumulation of metals during 9 and 18 days exposure period. Shi and Wang (2004) reported that the clearance rate of mussels in exposure to copper were significantly lower than the control group. In contrast, there was no difference in clearance rate of *Perna perna* after 8 days exposure to copper and control bivalves (Anandaraj et al., 2002). These conflicts may be due to differences in experimental conditions, species and metal concentrations that have been used. Decrease in CR also is likely due to gill damage and its performance abnormalities (Sliverman et al., 1996; Anandaraj et al., 2002) or valves closing response (Davenport, 1977; Manley, 1983). Also, the lack of significant difference between pre- and post-exposed CR in 25 and 5 µg l^{-1} may be the result of impaired specific absorption routes of metals.

5. Conclusion

Based on the results of this study it can be said that the type of heavy metal (essential and non-essential), its concentration and duration of the period of exposure to it in the aquatic environment can affect bivalve clearance rate and clearance rate can be used as a suitable physiological indicator of heavy metal toxicity in aquatic biota.

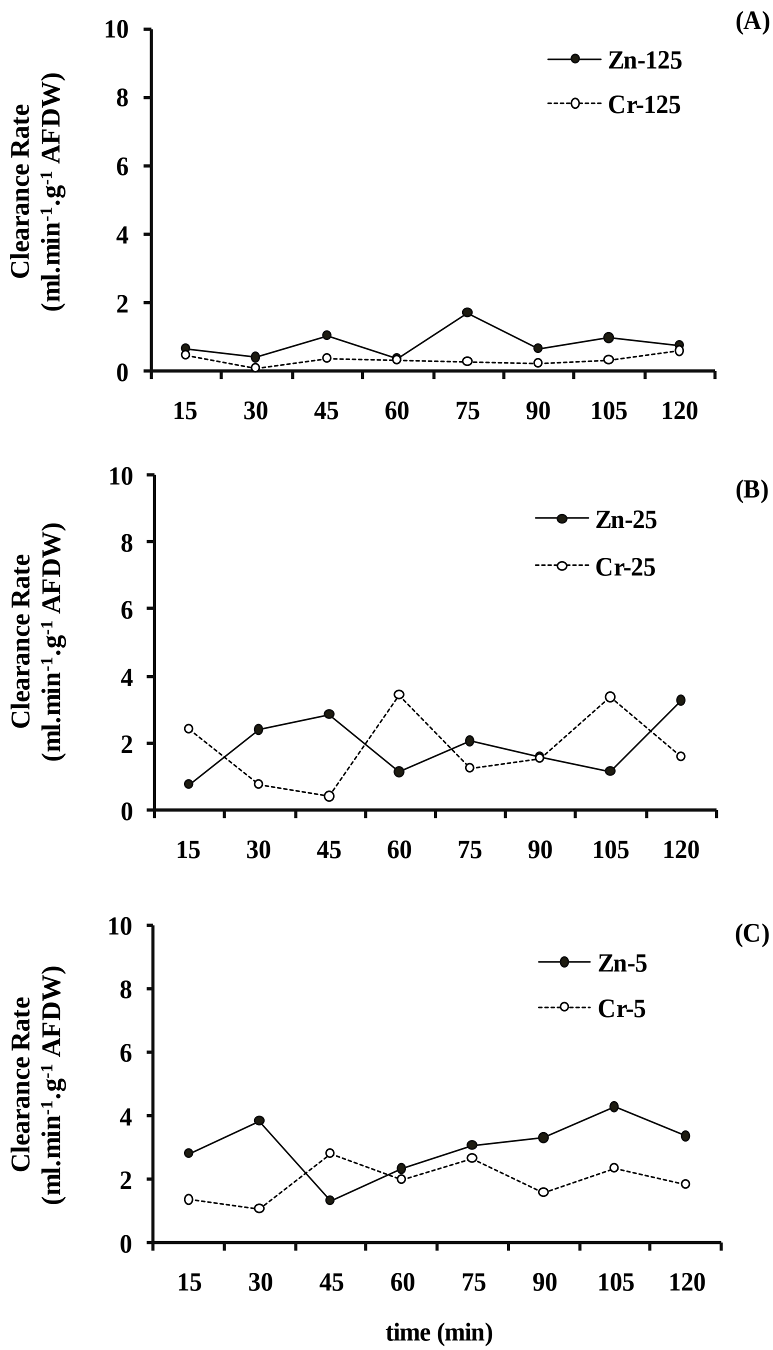

Fig. 1. Fluctuations of bivalves clearance rate (CR) during 2 hours period. A) 125 µg l^{-1} metal concentration; B) 25 µg l^{-1} metal concentration; C) 5 µg l^{-1} metal concentration.

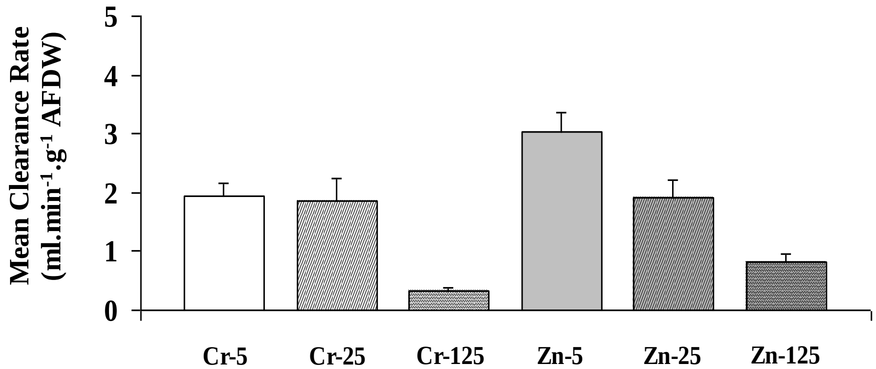

Fig. 2. Pre-exposed clearance rate (mean + SD) of bivalves in concentrations 5, 25 and 125 µg l^{-1} of Zn and Cr during 2 hours period.

Table 1
Clearance rate (CR) (mean ± SD) of bivalves. Post-exposure CR was calculated in days 9 and 18 after exposure in concentrations of 25 and 5 µgl^{-1} of Zn and Cr, respectively.

Metal	Concentration (µg l^{-1})	Pre-exposure CR (ml.min^{-1}.g^{-1} AFDW)	Post-exposure CR (ml.min^{-1}.g^{-1} AFDW)		P-value
			Day 9	Day 18	
Cr	25	1.844 ± 0.399	0.516 ± 0.058	---	P = 0.0087
	5	1.951 ± 0.217	---	1.022 ± 0.272	P = 0.0113
Zn	25	1.903 ± 0.314	0.534 ± 0.102	---	P = 0.0041
	5	3.028 ± 0.321	---	1.094 ± 0.282	P = 0.0096

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