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Original article

The study of zinc bioaccumulation in internal organs of swan mussel, *Anodonta cygnea* (Linea, 1876)

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A B S T R A C T

Heavy metals as important pollutants in aquatic ecosystems can cause adverse biological effects on live organisms of these environments. This research was conducted to study the bioaccumulation of zinc (Zn) in internal organs (mantle, gill, hepatopancreas and foot) of the freshwater bivalve, *Anodonta cygnea* during 27 days in vitro exposure period. Results showed that this species has a high potency of metal accumulation. There was no significant difference between basal Zn levels in studied organs. Maximum accumulation rate were recorded in gill and hepatopancreas and their Zn content was significantly (α=0.05; $P < 0.02$) higher than mantle and foot. The minimum Zn accumulation occurred in mantle. Zn levels in gill, hepatopancreas and foot were increased during exposure period, but a decreasing trend was observed in Zn level of mantle between days 18 and 27. The results of this study indicated that accumulation potential of different organs and their sensitivity and impressibility are different and gill and hepatopancreas are appropriate organs to study heavy metal bioaccumulation and their pollution in freshwaters.

1. Introduction

Heavy metals are considered as an important group of main pollutants of aquatic environments and their concentrations above tolerable physiological levels in cells can cause toxic effects (Banfalvi, 2011). These elements are divided into two groups: essential (with biological structural and functional roles); and non-essential elements (with no defined biological role) (Simkiss, 1981; Williams, 1981; Banfalvi, 2011). Occurrence of both groups in concentrations higher than critical levels of them in water bodies may have dangerous effects on aquatic biota (Forstner and Wittman, 1983; Rainbow, 2002). Zinc (Zn) is one of the essential heavy metals that play important roles as a compartment of more than 200 metalloenzyme and other metabolic complexes, as well as DNA and biological structures such as membranes and ribosome (Leonard and Greber, 1989). In recent years, this element is reported as an important pollutant in freshwaters (Buhl and Hamilton, 1990; WHO, 2001). The major anthropogenic sources of this element are processing of metal ores, electroplating, domestic and industrial effluents, combustion of fossil fuels and agricultural soil erosion (Liobet *et al*., 1988; Buhl and Hamilton, 1990).

Among different aquatic organisms, bivalves are usefull bioindicators for investigating chemical pollution in water bodies (Fournier *et al*., 2001). These organisms are in direct contact with polluted compartments of water and sediments, and can accumulate high levels of heavy metals in their soft tissues (Golovanova and Frolova, 2005). Bivalve species which are belonged to Unionidae family have high bioaccumulation potency of wide range of chemical pollutants such as heavy metals (Cairns and Pratt, 1995). The swan mussel, *Anodonta cygnea* (Linea, 1876) is one of the unionid mussels, which is an endemic species of northern waters of Iran and also are found from the British and Irish Isles to Siberia and south to northern Africa (Pourang *et al*., 2010).

This study was performed to investigate Zn accumulation in internal organs of *A. cygnea* includes mantle, gill, hepatopancreas and foot during 27 days exposure to Zn. The results of this study can provides clear views and insights about the potential of this species for introducing as good bioindicator for heavy metal (and specially Zn) pollution monitoring in aquatic environments.

2. Materials and methods

2.1. Bivalve collection and experimental condition

30 specimens of *Anodonta cygnea* were collected in October 2011 from the Tajan River estuary, Mazandaran province, Iran (36°48′46′′N, 53°6′57′′E). After transmission 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 and bivalve specimens were not fed.

2.2. Exposure plan

According to previous studies (Naimo, 1995; Anandraj *et al*., 2002; Rainbow, 2002; Azarbad *et al*., 2010) and also the natural concentrations of Zn (WHO, 2001), bivalves were exposed to 125 µg l^{-1} of Zn for 27 days, with 3 replicates. Metal solution was prepared using zinc sulphate salt ($ZnSO_4$, Merck). Before exposure, bivalves were sampled for determining the basal levels of Zn. Exposed bivalves were sampled in days 3, 6, 9, 18 and 27 after exposure beginning in order to obtaining tissue samples of studied organs.

2.3. Measuring metal content

Tissue samples were placed in oven for 48 h at 75°C. Then, dry masses were weighted and again were placed in oven for 1 h at 500°C to obtaining ash free dry weight (AFDW) (Jørgensen, 1990). Digestion of dry tissue samples of studied organs was performed according to Fukunaga and Anderson (2011). Metal content in samples were determined using Inductively Coupled Plasma Mass Spectrometer (VG Plasma Quad 3- VG Elemental, Winsford, Cheshire, UK).

2.4. Statistical analysis

Statistical analyses were performed using SPSS version 17.0. All results are presented as means + S.D. Significant differences between groups and in different times were determined using unpaired T-test. Level of significance was determined as $P < 0.05$.

3. Results

Basal levels of Zn in bivalves before exposure showed no significant difference between target organs (mantle: 5.834 ± 0.983; gill: 6.793 ± 1.273; hepatopancreas: 7.501 ± 1.938; foot: 6.978 ± 1.958 µg g^{-1} AFDW). At the end of the study period, maximum Zn levels were belonged to hepatopancreas (25.418 ± 1.832 µg g^{-1} AFDW) and gill (23.71 ± 3.112 µg g^{-1} AFDW), and minimum level was related to mantle (8.109 ± 0.531 µg g^{-1} AFDW).

The trend of changes in Zn contents (mean + SD) in mantle, gill, hepatopancreas and foot during exposure period are showed in figure 1. During 27 days period, metal content in gill, hepatopancreas and foot were increased significantly (α = 0/05; *P < 0/02*). Zn content in mantle has no significant change until 9th day, but after it, there was a significant (α = 0/05; *P < 0/001*) increase in day 18, and then a significant (α = 0/05; *P < 0/015*) decrease was occurred in day 27 (figure 1). Increasing trend of metal accumulation in gill was occurred in shorter period than other organs, so that Zn level in gill in 6th day was significantly (α = 0/05; *P < 0/05*) higher than earlier times, as well as than Zn level in other organs.

Figure 2 shows accumulated amounts of Zn in three 9 days intervals. Generally, maximum accumulated Zn amounts were observed in the first 9 days, where highest values were related to gill and then hepatopancreas. Accumulated amounts of Zn in gill in second and third 9 days were significantly (α = 0/05; *P < 0/0001*) decreased. In hepatopancreas, metal accumulated amount during first and second 9 days was almost constant, but during third 9 days was significantly (α = 0/05; *P < 0/015*) decreased. Highest Zn accumulation in foot was occurred during second 9 days. In mantle, there was a significant (α = 0/05; *P < 0/035*) decrease in accumulated amount of Zn, where in third 9 days accumulated value was negative and this indicates that in this time Zn releasing from mantle tissues into the water was occurred.

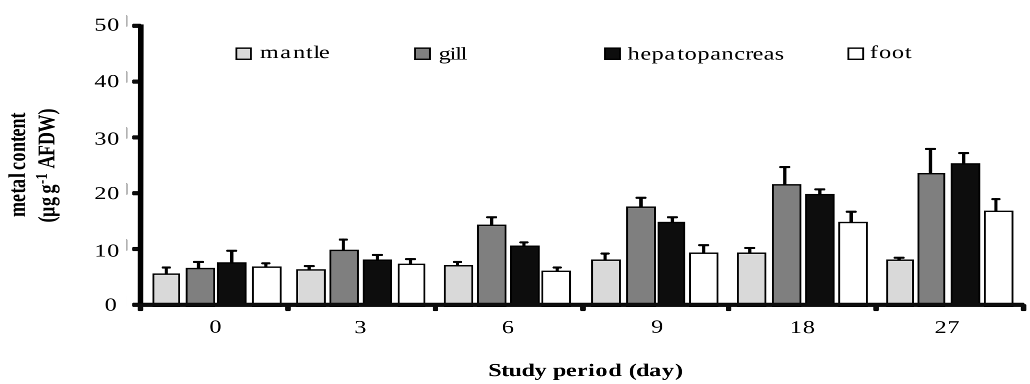

Fig. 1. Zinc content of mantle, gill, hepatopancreas and foot during 27 days exposure period. Error bars represent standard deviation.

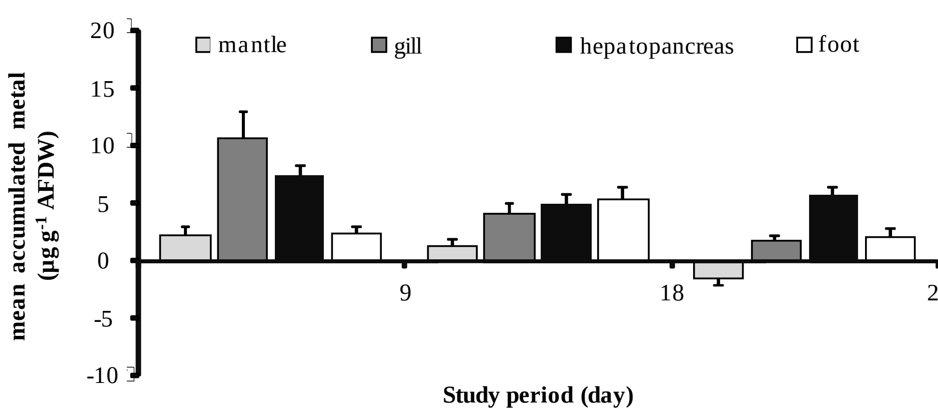

Fig. 2. Accumulated Zn level in mantle, gill, hepatopancreas and foot in three 9 days intervals. Error bars represent standard deviation.

4. Discussion

In recent years, bivalve molluscs have widely been used as bioindicators of chemical pollution in aquatic environments (Phillips, 1990; Garcia-Rico *et al*., 2001; Hung *et al*., 2001). Investigation of heavy metals bioaccumulation trends in exposure to them has been offered as an efficient tool for monitoring heavy metal impacts (Rainbow, 2002; Zorita *et al*., 2006; Golovanova, 2008). Results of the present study showed that *A. cygnea* can accumulate remarkable amounts of Zn in its internal organs (mantle, gill, hepatopancreas and foot) in exposure to 125 µg l^{-1} concentration of Zn during 27 days.

Bioaccumulation of Zn in studied organs showed important differences in metal accumulated level and time pattern of accumulation between organs. Our results showed that highest accumulation was occurred in hepatopancreas and gill. Similar results have been reported in *Mytilus edulis* (Viarengo *et* al., 1981; Al-Subiai *et* al., 2011) and *Chlamys varia* (Metian *et al*., 2009). In present study, minimum Zn accumulation was belonged to mantle, so that in third 9 days not only no accumulation in mantle was occurred, but also Zn releasing from mantle into the aqueous environ was occurred and metal level of mantle was decreased during this period. This situation reflects the greater sensitivity of mantle to Zn accumulation and possibly was occurred due to tissue damages resulted from metal accumulation in this organ. In the study of Metian *et al*., (2009) that was conducted on *Chlamys varia*, it has been reported that with increase in exposure time, accumulated level of Zn in mantle compared with other organs was negligible. In contrast, Stewart (1990) reported that Cd accumulation in mantle was higher than hepatopancreas. The results of these studies are not consistent with our results about metal accumulation in mantle. Pourang *et al*., (2010) reported that accumulated amounts of Zn as an essential element were almost equal in gill and foot in *A. cygnea*, but accumulation of cadmium and lead as nonessential metals in gill were higher than foot. In our study, Zn accumulation in foot was lower than in gill and hepatopancreas. Observed differences in bioaccumulation trend of Zn as an essential element between different organs may be due to different metal regulation systems that includes metal sorption, storage, detoxification and excretion processes (Phillips and Rainbow, 1989). Also, conflicts between our results and prior studies may be were due to differences in experimental conditions or bivalve species that has been used. Overall, considering our results and the results of other studies it can be said that metal accumulation potential in different organs of bivalves is dependent on the type of metallic element and bivalve species.

5. Conclusion

The results of our study showed that swan mussel, *Anodonta cygnea*, is a good species for using in Zn pollution monitoring studies in water bodies. Among studied organs, mantle had minimum accumulation and higher sensitivity in exposure to Zn and highest accumulations were belonged to gill and hepatopancreas and foot had an intermediate situation about metal accumulation. Generally, we can say that gill and hepatopancreas are more appropriate organs in order to investigation occurred metal levels in waters.

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