Impact of water hardness on cadmium absorption by four freshwater mollusks *Physa fontinalis*, *Anodonta cygnea*, *Corbicula fluminea* and *Dreissena polymorpha* from south Caspian Sea region

Arash Javanshir 1*, Maryam Shapoori 2 and Fateh Moëzzi 1

1 Department of Fisheries and Environmental Sciences, Faculty of Natural Resources, University of Tehran, Karaj, PO Box 4314, Iran. 2 Savadkooh Branch, Islamic Azad University, POBox 155, Mazandaran Province, Iran. *e-mail: arashjavanshir@hotmail.com

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Abstract
The modalities of cadmium biosorption were studied in four freshwater mollusk species from south Caspian Sea region. Cadmium absorption was measured under hard and soft water conditions as well as under original Tajan River water conditions. Special attention was paid to inter-specific variations of cadmium absorption. Under laboratory conditions the estimated bioaccumulation efficiency was 1350-20,000 for the whole soft body, calculated on a dry mass basis. Concentrations were highest in *Dreissena polymorpha*, followed by those in *Corbicula fluminea, Physa fontinalis* and *Anodonta cygnea*.

Key words: Cadmium, biosorption, bioaccumulation, freshwater mollusk, freshwater pulmonate snail, freshwater bivalves.

Introduction

It has already been recognized that bioaccumulation of cadmium differs in different aquatic species and is influenced by water chemistry conditions 1-3. Especially, freshwater organisms are known to be more sensitive to heavy metal contamination than marine animals of soft water environments 4,5. Cadmium absorption rates and bioaccumulation may be regarded as indicators of the toxicity and were therefore studied in more detail in the following. In general, bioaccumulation of chemicals is the result of uptake efficiencies. It is known, that the speciation of trace metals occurring in the investigated system influences the uptake by aquatic organisms 6. Cadmium concentrations of bivalves are affected by cadmium concentrations in the ambient water and suspended material, such as phytoplankton which is taken up by the bivalves. During the filter feeding process, suspended particles (detritus, zooplankton, and phytoplankton) are retained. Experimental results of uptake rates are known to vary considerably according to the conditions chosen. However, biotic and abiotic factors influencing cadmium retention and toxicity have often been neglected. For this reason, it is laborious to compare available data and assessment of potential endangering by aquatic organisms is very difficult. It was mentioned 6, that most studies with heavy metals were performed with test waters with CaCO₃ concentrations clearly above 5 mg/l. These results cannot be directly applied to low alkalinity conditions. In particular, there is a lack of data dealing with low cadmium concentration levels in comparative studies under natural and artificial water conditions. Investigations with only one species do not allow generalizing the results for a whole group, such as the mollusks. We therefore studied four species, the freshwater pulmonate snail *Physa fontinalis* and the freshwater bivalves *Dreissena polymorpha*, *Anodonta cygnea* and *Corbicula fluminea*. Physa and *Dreissa* live on hard substrates like bank reiforcements. The bivalve *Anodonta cygnea* and *Corbicula fluminea* were found in sediments with different grain compositions. All the species used in this study have been reported to be suitable for biomonitoring heavy metal bioconcentration 7-10. In a previous study, we have presented a scheme of the water-mussel system with some of the crucial factors affecting bioaccumulation in mollusks 11. In the present study i) cadmium uptake capacities of four mollusk species were described; ii) the influence of experimental conditions was studied and different results of investigated species were compared and iii) the suitability of tested species as biomonitoring organisms was also examined.

Materials and Methods

Specimens of *Anodonta cygnea*, *Corbicula fluminea* and *Dreissena polymorpha* were obtained from the Tajan estuary (Tajan river, Mazandaran Province, North of Iran), which is characterized with fluctuated water input which changes during seasons (Fig. 1). There is inflow of agricultural waste water of an important catchment (up to 4000 km²) to this river. The river mouth joining to the sea may be dry during dry months (August-September). Climate is temperate with lack of severe winters. The salinity of the southern Caspian Sea fluctuates between 8.5 and 12.5‰. This river has 500 × 10⁶ m³ annual input to the Caspian Sea. These conditions make the Tajan River mouth a suitable habitat for four species studied in this work. With *Anodonta cygnea* we studied the cadmium kinetics under three experimental conditions. In the first experiment, animals were exposed to artificially cadmium enriched Tajan river water (median Ca 70.5 and Mg 10 mg l⁻¹). In a second experiment we used in laboratory prepared water, one set with high concentrations of Ca⁺ (63-69
mg l$^{-1}$ and Mg$^{++}$ (7.9-10.1 mg l$^{-1}$) similar to Tajan river water conditions, second one with low concentrations of Ca$^{++}$ (2.8-6.1 mg l$^{-1}$) and Mg$^{++}$ (1.3-3 mg l$^{-1}$). In experiments with *Corbicula fluminea*, *Dreissena polymorpha* and *Physa fontinalis* we only used Tajan river water and laboratory prepared hard water.

The exposure time of 12 weeks remained constant for all of treatments. These three laboratory prepared test waters were all enriched with 15 µg l$^{-1}$ (= 0.133 µmol l$^{-1}$) cadmium. The experiments were kept at 15±0.3°C and pH 6.1-7.8. During the exposure period the animals were not fed. The dissected organs from *Anodonta cygnea* and the whole soft parts from the other species were stored at -30°C refrigerator. For analyses, tissues were defrosted and cut into small pieces. Aliquots were dried for 24 h at 60°C. The colorless digest was filtered and filled up with deionized water to the final volume. Cadmium concentrations were analyzed by furnace-AAS, equipped with Zeeman compensation. To assess the cadmium absorption potential, the following equation which describes the processes of cadmium accumulation under the present experimental conditions in mollusk species was used.

Cadmium concentration = a $[1-0.5 (\text{week}/h)]$

a = saturation concentration, Cd uptake potential (Cd µg g$^{-1}$); h = half saturation time (week).

To determine if there were significant differences in the estimated Cd concentrations and the absorption rates between the species and the different treatments, we used Student’s t-test for separate variances. Differences were considered significant at the 0.05 probability level after correction of P-values. Single P-values were multiplied with the number of comparisons in this study to reduce accidental significance of any statistical test. The statistical procedures were performed using Statistica 5.2 software for PC.

**Results**

Cadmium saturation concentration potential could be estimated from the increase of cadmium concentrations during the exposure period. Cadmium absorption histograms were almost equal for animals from Tajan river water and hard water experiments. Mostly, uptake of cadmium was faster in the soft water experiment, except in the muscle tissue.

As can be seen from the Fig. 2, high cadmium concentrations were found in the hepato-pancreas, gills and heart of *Anodonta cygnea* in each experiment. In contrast to the Tajan and hard water experiments, the concentration in the mantle tissue was evidently high in the soft water experiment. The midgut gland has turned out to be negligible for overall bioaccumulation figures. We found the lowest cadmium saturation concentrations in the foot and gut/gonad complex as well as in the muscle tissue. In all treatments we determined short half saturation times for the gills, the first absorber tissue.

The cadmium saturation concentrations in the soft water experiment differ from the other experiments in their high values. We found significant differences in midgut gland, foot and mantle between hard and soft water experiments and in mantle tissue between laboratory prepared soft water and Tajan river water. There were no significant differences between Tajan water and hard water experiments in any of the investigated tissues (Table 1). Hard water was prepared to reflect important ions of Tajan river water, especially calcium, magnesium and chloride.

There were no clear differences between cadmium concentrations in the two experiments with Tajan river water and hard water in the whole soft parts of tested mollusk species (Fig. 3).
Like the separated tissues of Anodonta cygnea, no significant differences of cadmium saturation concentrations in whole soft parts of mollusks were found between Tajan river water and artificial hard water. However, we partly found significant interspecific variations of cadmium saturation concentration after correction of p-values (Table 2). The figures suggest a different uptake capacity under identical conditions. Dreissena polymorpha showed high cadmium saturation levels, whereas Physa fontinalis showed relatively low absorption levels in both experiments.

**Discussion**

Incorporation of pollutants may enhance their persistence in the ecosystem and directly affect the biological community. For this reason, understanding bioaccumulation processes is important. However, certain tissues are more efficient accumulators than others (Fig. 2). It is in these tissues, where harmful effects will probably occur first. Cadmium concentrations in each tissue separated from Anodonta cygnea and the soft bodies from the remaining mollusks strongly increased during the first four weeks (Figs 2 and 3). The course of cadmium absorption under these experimental conditions appears to be a rapid process and indicate equilibrium between absorption and elimination. However, the saturation level was reached mostly during the 4th and 10th week of the exposure period and probably corresponds to the initial stages of dominant cadmium binding by low molecular weight protein. Based on the final plateau level, hepato-pancreas tissue revealed a high cadmium absorption capacity under all conditions. Concentration of cadmium in the gills varied to a great extent between individual tests. It is probable, that glochidia or parasites present in the fills are influencing cadmium concentrations in this tissue. Cadmium concentrations in glochidia or parasites generally were neglected. When Anodonta cygnea comprises glochidia in the present study, nearly 5% of the total amount was measured in glochidia,

![Graph showing cadmium saturation concentrations for whole soft parts from Anodonta cygnea, Physa fontinalis, Corbicula fluminea and Dreissena polymorpha. Bars indicate standard error of the mean.](image)

**Figure 3.** Cadmium saturation concentrations for whole soft parts from Anodonta cygnea, Physa fontinalis, Corbicula fluminea and Dreissena polymorpha. Bars indicate standard error of the mean.

**Table 2.** Significance of differences of cadmium saturation concentrations comparing the studied mollusk species in two laboratory experiments.

<table>
<thead>
<tr>
<th>Comparison: species</th>
<th>T-Value</th>
<th>P value corrected (p × 36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tajan river water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dreissena × Physa</td>
<td>3.97</td>
<td>0.016</td>
</tr>
<tr>
<td>Dreissena × Corbicula</td>
<td>2.61</td>
<td>0.508</td>
</tr>
<tr>
<td>Physa × Corbicula</td>
<td>4.15</td>
<td>0.009</td>
</tr>
<tr>
<td>Laboratory prepared hard water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dreissena × Physa</td>
<td>1.91</td>
<td>2.384</td>
</tr>
<tr>
<td>Dreissena × Corbicula</td>
<td>1.59</td>
<td>4.444</td>
</tr>
<tr>
<td>Physa × Corbicula</td>
<td>1.96</td>
<td>2.145</td>
</tr>
</tbody>
</table>
indicating that bivalves excrete a considerable part of their cadmium burden by this way. In all experiments, the heart has turned out to be an effective accumulator, the mantle, however, was so only under soft water conditions. The high cadmium concentration in the heart is probably based by the close contact to the haemolymph.

Inorganic ions compete for transport pathways in living organisms. Presumably essential elements influence the absorption of nonessential heavy metals 12,13. Experimental studies in fishes suggested that low aqueous concentrations of calcium probably enhanced the bioavailability and toxicity of metal cation, because the permeability of membranes was inversely related to aqueous calcium concentration. Calcium ions apparently compete with other metal cations for binding sites on the gill surface, decreasing the direct uptake of cationic metals 13-15. It was suggested that the relation of metals in some fish tissues could be influenced by aqueous and dietary calcium. Anodonta cygnea is able to obtain calcium from water as well as from the diet 16. It was described 17 that water hardness influences the absorption rate and total residues of cadmium. It was concluded 18,19,20 that cadmium storage takes place faster under lacking calcium conditions. It is conceivable that as a consequence of different calcium concentrations in the tissues, cadmium absorption capacity and distribution mechanisms are affected. Cadmium accumulation increased until free binding places are engaged. An elevated calcium level in the haemolymph may reduce the transport of cadmium from the gills to secondary tissues. These figures diminish the assessment of cadmium absorption by the gills. Comparing cadmium concentrations in freshwater mussels from over twenty lakes in relation to local water chemistry, it was found the strongest correlation between cadmium concentration in mussels and the water pH 20,21. Cadmium exists largely as the free divalent ion along a pH range 4-7 and is usually not complexed by dissolved organic material. The pH influences both the chemistry of metal and macromolecules of surface structures. Modifications of membrane permeability causes an alteration in metal diffusion. Additionally, changes in membrane potential modify the transport of polar metal species 4,22,23. A general regulation of physiological processes in aquatic organisms is likely. Calcium, total inorganic carbon and pH are interrelated and so it was not possible to determine the importance of individual parameters. Changes in water hardness affected toxicity of cadmium to Daphnia pulex 24.

The figures of the different experiments in the present study support the presumption that the toxic Cd²⁺ competes with the essential Ca²⁺ for binding sites (or Ca²⁺ reduces membrane permeability) in some tissues (Table 2). The influence of Mg seems to be negligible. The results of the laboratory experiments indicate that changes in cationic ions especially Ca²⁺ affected cadmium uptake more than the unknown disturbing factors of Tajan river water. No relationship was found between cadmium saturation concentration and body size of the different mollusks.

Differences of physico-chemical parameters, between laboratory and natural habitat influences the biology of tested organisms as well. In contrast to natural habitat, in our laboratory experiments there was only one route of uptake (via aquatic phase). In other studies, it was found that cadmium concentrations increased during the first weeks and may demonstrate biphasic accumulation patterns 25,27. After this initial time no remarkable rise was found 26,28. Different responses to cadmium contamination in biased organisms were described in comparison to individuals from unpolluted areas. Furthermore, an explanation for the fast achievement of the saturation state may be a decreasing filtration activity in mussels. The valve closure response of Corbicula fluminea is dependent on the exposure concentrations of dissolved Cd and Zn 29. Also it was described that heavy metals influenced the filtration activity of Anodonta cygnea 7,30. It was also observed that mussels reduce filtration activity if no food is available 31. The concentration of Cd 9 µg l⁻¹ reduced the filtration rate of Dreissena polymorpha 31. In such a case, changes in ventilation rate and valve closure can affect the cadmium absorption rate, and cause decrease in cadmium accumulation.

Estimated levels of cadmium bioaccumulation were specific for the different species and the different experimental designs. High cadmium absorption capacities in the laboratory experiments show Dreissena polymorpha has a deciding feature for bioindicators. However, in the past years decreasing individual densities of Dreissena polymorpha were found in the Tajan river, due to increased competition, predation and pollution. For this reason it is necessary to look for other indicator species. A number of studies have proved the application of Corbicula species as biomonitor 4,28,30,34. Our study confirms that Corbicula fluminea, a mass invader in the Tajan river, processes important features of a biomonitoring organism.

Only few data are available on the effect of cadmium exposure on complex ecosystems. The results showed that it is important to pay attention on chemical conditions for assessing toxicity and the absorption potential of cadmium in freshwater animals. It is conceivable that community structure is affected by selective toxicity. There were no visible direct toxic effects during exposure period, but possible changes in ventilation rate, and moreover a supply of metal binding proteins 31. If in this stress situation harsh circumstances, like oxygen deficiency or an accidental release of organic pollutants, occur additionally to metal contamination, the community structure might eventually become affected 35,36.

References

26 Javanshir, A. 2001. Influence of
23 Azarbad, H., Javanshir, A., Mirvaghefi, A., Danekar, A. and Shapoori,
22 Javanshir, A., Shapoori, M., Azarbad, H., Mirvaghefi, A. and Danekar,
21 Gerhardt, A. 1993. Review of impact of heavy metals on stream
20 Ciocan, C. M. and Rotchell, J. M. 2004. Cadmium induction of
19 Bebianno, M. J. and Serafim, M. A. 2003. Variation of metal and
18 Andreev, G., Simeonov, V. and Stoikov, S. 1994. Occurrence and
16 Wicklund, A. and Runn, P. 1988. Calcium effects on cadmium uptake,
14 Wicklund, A. and Runn, P. 1988. Calcium effects on cadmium uptake,
12 Streit, B. and Winter, S. 1993. Protein binding lead and cadmium in
7 Andreev, G., Simeonov, V. and Stoikov, S. 1994. Occurrence and
6 Javanshir, A., Shapoori, M., Azarbad, H., Mirvaghefi, A. and Danekar,
5 Javanshir, A., Shapoori, M., Azarbad, H., Mirvaghefi, A. and Danekar,
4 Aspholm, O. O. and Hylland, K. 1998. Metallothionein in green sea
2 Tedengren, M. 2004. Physiological and proteomic responses in