www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 11: 561-568 (2011) DOI: 10.4194/1303-2712-v11_4_09

The Effect of Lead Bioaccumulation on Condition Indices of Zebra Mussel (*Dreissena polymorpha*) From Anzali Wetland-Caspian Sea

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Received 16 January 2011
Accepted 9 April 2011

Abstract

In this study the effect of lead on condition indices of zebra mussel (*Dreissena polymorpha*) together with the accumulation of lead in the soft tissue of mussels, were investigated. Studies showed no significant differences in condition indices (TCI and SCI) among the mussels in control and lead treatments. Mussels condition indices decreased during the experimental period, that it caused by function of time, not by metal accumulation. Besides the metal pollution, other environmental factors, such as food availability, could affect the mussel's condition. In our experiment we removed the effect of food by providing same food availability for mussels in control and lead treatments during the experiment. Therefore lead is the main effective factor on mussel's condition indices. Lead accumulation only observed in the soft tissue of mussels. Significant differences in lead accumulation in each treatment (based on time) and among treatments were observed. Our results suggest that the accumulation of lead in the soft tissue of mussels was affected by exposure time and lead concentration in the ambient water. In each treatment highest accumulation at longest exposure time (15 days) and lowest accumulation at shortest exposure time (5 days) were observed. Besides, among treatments, most accumulation of lead in the soft tissue of mussels at concentration of (455 $\mu g\ L^{-1}$) and lowest accumulation were observed at concentration of (2 $\mu g\ L^{-1}$).

Keywords: Condition index, *Dreissena polymorpha*, Bivalve Bioaccumulation, Lead contamination.

Anzali-Hazar Denizindeki Zebra Midyesine (*Dreissena polymorpha*) Kurşun Biyoakümülasyonunun Etkileri

Özet

Bu çalışmada zebra midyesinin (*Dreissena polymorpha*) yumuşak dokusunda kurşun birikiminin, midyenin kondüsyonuna etkileri araştırılmıştır. Çalışmalar, kondüsyon indislerinin (TCI ve SCI) kontrol grupları ve kurşun muamelesi görmüş midyeler arasında önemli bir fark olmadığını göstermiştir. Midyelerin kondüsyon indisleri deney süresince azalmıştır. Bunun nedeni de metal birikimi değil zaman fonksiyonudur. Metal kirliliğinin dışında, gıda temini gibi diğer çevresel faktörler de midyelerin kondüsyonunu etkileyebilir. Biz deneyimizde gıda temini faktörünü hem kontrol grubuna hem de muamele grubuna aynı gıdayı sağlayarak bertaraf ettik. Bundan dolayı, kurşun, midyenin kondüsyon indisine etki eden temel faktör olmuştur. Kurşun birikimi sadece midyelerin yumuşak dokusunda gözlemlenmiştir. Her muameledeki ve muameleler arasındaki zamana dayalı önemli farklar incelenmiştir. Sonuçlarımız midyelerin yumuşak dokularındaki kurşun birkiminin maruz kalma zamanından ve çevreleyen su ortamındaki kurşun konsantrasyonundan etkilendiğini göstermiştir. Her muamelede en uzun maruz kalma süresinde (15 gün) en yüksek birikme seviyesi ile en kısa maruz kalma süresinde (5 gün) en düşük birikme seviyesi incelenmiştir. Ayrıca muameleler arasında midyelerin yumuşak dokusunda biriken en yüksek kurşun seviyesi (455 $\mu g\ L^{-1}$) ile en düşük seviyesi (2 $\mu g\ L^{-1}$) incelenmiştir.

Anahtar Kelimeler: Kondüsyon indeksi, *Dreissena polymorph,*çift kabuklu yumuşakça, kurşun kontaminasyonu.

Introduction

Dreissena polymorpha is a sessile suspension-feeder, attached with byssal threads to firm substrate near to the water surface (Gundacker *et al*., 1998). *D. polymorpha* plays an important role in eutrophication control by grazing phytoplankton, bacteria and other inert detritic materials from water body (Noordhuis *et al*., 1992; Reeders *et al*., 1992). Filtration activity leads to high retention of water, particles and pollutants. Zebra mussel accumulates high amounts of potentially toxic metals and is therefore widely considered as a bio-monitoring organism (Neumann *et al*., 1992; Secor *et al*., 1993; Camusso *et al*., 1994).

Zebra mussels remove metals from the water column, incorporate metals in their tissues, and deposit metals on the bottom of the sediments in the form of pseudofeces (Klerks *et al.*, 1997). In aquatic ecosystem metals are present in physicochemical forms ranging from simple ions to colloids to metals sorbed on or incorporated in suspended particles. Dissolved metals are generally an important source of metals to suspension-feeding bivalves (Klerks *et al.*, 1997). Lead is one of the important metals in aquatic ecosystems which can be accumulated by zebra mussel in high amounts. *D. polymorpha* cannot regulate the concentration of lead in its body (Amiard *et al.*, 1987). Environmental lead contamination arises from various sources including mining, smelting, refining, manufacturing processes (battery plants), paints and pigments, also atmospheric emissions from motor vehicles, combustion of coal, recycling of batteries, incineration of municipal solid wastes and hazardous wastes (FDA, 1993). Lead particles emitted during the past use of leaded gasoline are also in the soil, especially near major highways (FDA, 1998). Because of ecological and physiological features (high filtration rate, high bioaccumulation capacities for metals) of the filter-feeding lamellibranch *D. Polymorpha*, it has been regularly used as an indicator of freshwater pollution, and particularly as a metal bioindicator (De Lafontaine *et al.*, 2000; Kraak *et al.*, 1991). Lead is found in several freshwater ecosystems such as, the south Caspian Sea basin wetlands. In this study the effects of lead on condition indices of zebra mussel with the its accumulation in the soft tissue of mussels from the Anzali wetland in the south Caspian Sea region are investigated in order to better understand the impact of this metal on the physiological integrity of mussels and food chain.

Materials and Methods

The mussels were sampled from relatively unpolluted area in Anzali wetland (Rahnama *et al.*, 2010). The mussels were picked up from the stems of aquatic plant (*Fragmites* sp). After collecting, mussels were carried to the laboratory and the acclimation period (14 days) was realized by plastic aquarium, containing 10 liters of filtered fresh water (0.45 µm) from the sample site. Mussels fed with the *Chlorella* spp (20,000 cells ml^{-1}) that cultivated used of Guillard cultivation environment (Guillard *et al.*, 1962) and the water was continuously aerated. After this period, mussels were sorted by length and only the ranges between 12 to 25 mm were taken. The average length did not differ between treatments. Exposure to lead consisted of four treatments of 2, 48, 93, 455 µg L^{-1} (they were relevant with natural lead levels in four sites of the Anzali wetland) that made up using stock solution of 1000 mg L^{-1} Pb $(NO_3)_2$ (Bleeker *et al.*, 1992). For doing the experiment 5 plastic aquariums were used. The volume of each treatment was 10 liters. Each treatment contained a group of 15 specimens. In this experiment also the control was tested. Lead was added to the aquaria at the start of the experiments. Mussels in each treatment, exposed to lead for 15 days. Two different indices were used to assess the effect of stress on the physiological integrity of the mussels. 15 mussels were placed in aquarium containing 10 L water without lead (0 µg L^{-1}) for 15 days. These mussels played control role for experiment. During the experimental period, mussels in control and four lead treatments fed with *Chlorella* spp (20,000 cells ml^{-1}). Five mussels collected from control and lead treatments every five days. Mussels were dissected and the shell length (up to 0.1 mm) and dry soft tissue weight (DW) and dry shell weight (SW) were determined on a Mettler H54 balance to the nearest 0.1 mg. These measurements were used to calculate two condition indices: Tissue Condition Index (TCI = tissue dry wt. /shell wt.) (Mersch *et al.*, 1996; Soto *et al.*, 2000) and the Shell Condition Index (SCI = tissue dry wt.*100/shell length) (Payne *et al.*, 1999).

During the experiment water temperature was kept constant at 25°C using a water heater, the hardness was 88.96 mgCaO L^{-1}. The pH and oxygen levels were (8.16 and 9.65 mg L^{-1} respectively). Also sampled mussels in every five days were analyzed for their lead content in soft tissue. Soft tissues of the mussels were removed from the shell, byssus threads were also removed. Soft tissue dry weight was determined with oven at 60°C for 24 h (Klerks *et al.,* 1997, Pessatti *et al.*, 2002). The soft tissues (Table 1) were digested in 5 mL concentrated HNO_3 with 2 ml distilled water added. After refluxing for 4 h at 120°C, 2 ml of H_2O_2 (30%, Fisher certified grade) was added and heating was continued for an additional 2 h. after cooling, the tissue digest was filtered using glass-fiber filters and brought up to volume (25 ml) using 0.4 M HNO_3. The samples were analyzed for lead by FAAS (furnace atomic adsorption spectrometer, Perkin Elmer 5100PC). The detection limit for Pb was 0.005 µg L^{-1}. Quality control of metal analysis was performed, using destruction blanks and reference

Table 1. The averages of mussel's soft tissues (gram dry weight) at every sampling time in four lead treatments. At every sampling time, 5 mussels were analyzed

Treatments (µg L^{-1})	Sampling Time (5 Days)	Sampling Time (10 Days)	Sampling Time (15 Days)
2	0.026	0.018	0.015
48	0.019	0.014	0.011
93	0.024	0.016	0.013
455	0.023	0.015	0.01

materials (IAEA shrimp MA-A-3/TM and NIST water SRM 1643c). The measured values were in good agreement with the certified values (<10% deviation).

The net accumulation rates of *D. polymorpha* for lead were determined as slope of the regression line between the metal concentration in the mussel's soft tissue and exposure time. Statistical software (SPSS ver.12) was used (Rahnama *et al.*, 2010). The one–way ANOVA was utilized to determine whether the differences among the lead concentrations in mussels soft tissues and condition indices in different treatments could be significant and least significance differences test (LSD) was used for comparing the significant differences among mean values of lead concentrations in mussels soft tissues and condition indices in different lead treatments.

Results

Figure 1 shows the averages of TCI of *D. polymorpha* exposed to control and four lead treatments (2, 48, 93, and 455 µg L^{-1}). As mentioned in the figure, in four lead treatments, the averages of TCI are in the same range and there were no significant differences between them. Also, when the condition indices of control mussels and treatments were compared with each other, no significant differences were observed (P>0.05). (Figure 2) shows the reduction of TCI during experimental period in four treatments (2, 48, 93 and 455 µg Pb L^{-1}). Within the individual treatment, however, the TCI changed in function of time with the lowest condition at the longest period of exposure. The averages of SCI of mussels in control and lead treatments are shown in (Figure 3). Like TCI, The averages of SCI of mussels in lead treatments are in the same range and there were insignificant differences between them and control (P>0.05). Within the individual treatment the SCI changed in function of time with the lowest condition at the longest period of exposure (Figure 4).

Absorbed metal in the tissue of mussels was also related to initial concentration in its ambient water. As shown in (Figure 5), the lead concentrations in the mussel's tissue plotted against the lead concentrations in the water, the assimilated concentration increases with the exposure time. In each treatment, lowest accumulation in the shortest exposure and highest accumulation in the longest exposure time occurred that showed significant differences (P<0.05) in lead

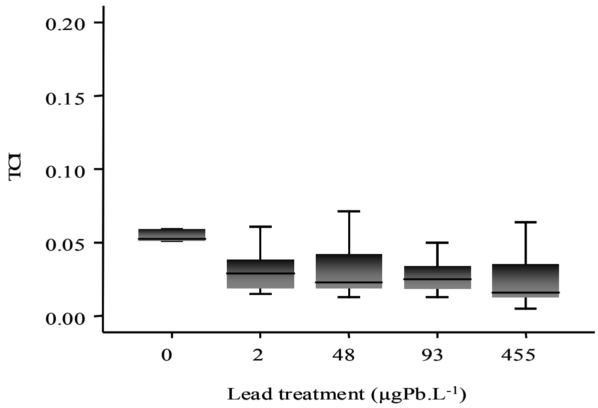

Figure 1. Mean values of TCI (±SD) in control mussels (0 µgPb.L^{-1}) and exposed mussels to four lead treatments (2, 48, 93 and 455 µgPb L^{-1}) during 15 days exposure.

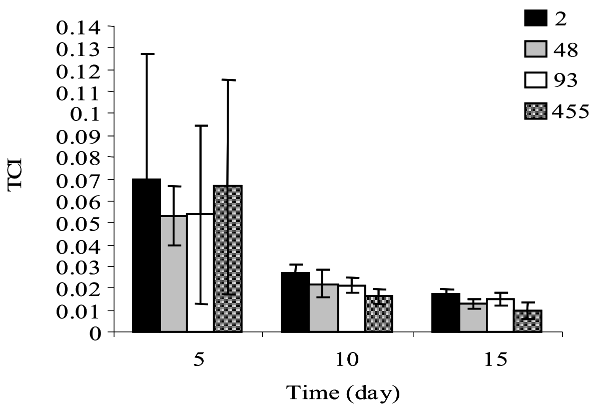

Figure 2. The evolution of reduction of TCI during experimental period in four treatments (2, 48, 93 and 455 µgPb L^{-1}).

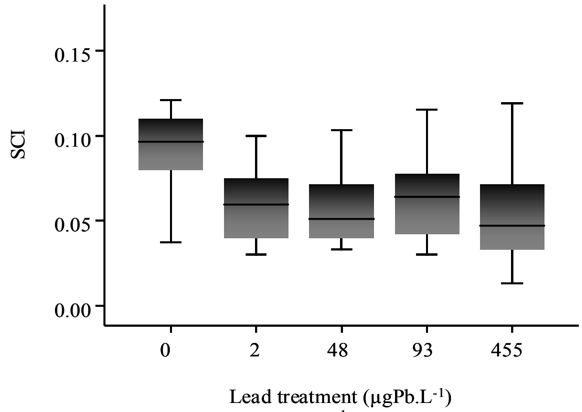

Figure 3. Mean values of SCI (±SD) in control mussels (0 µgPb L^{-1}) and exposed mussels to four lead treatments (2, 48, 93 and 455 µgPb L^{-1}) during 15 days exposure.

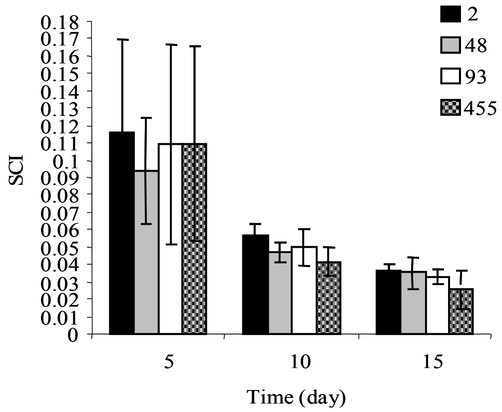

Figure 4. The evolution of reduction of SCI during experimental period in four treatments (2, 48, 93 and 455 µgPb L^{-1}).

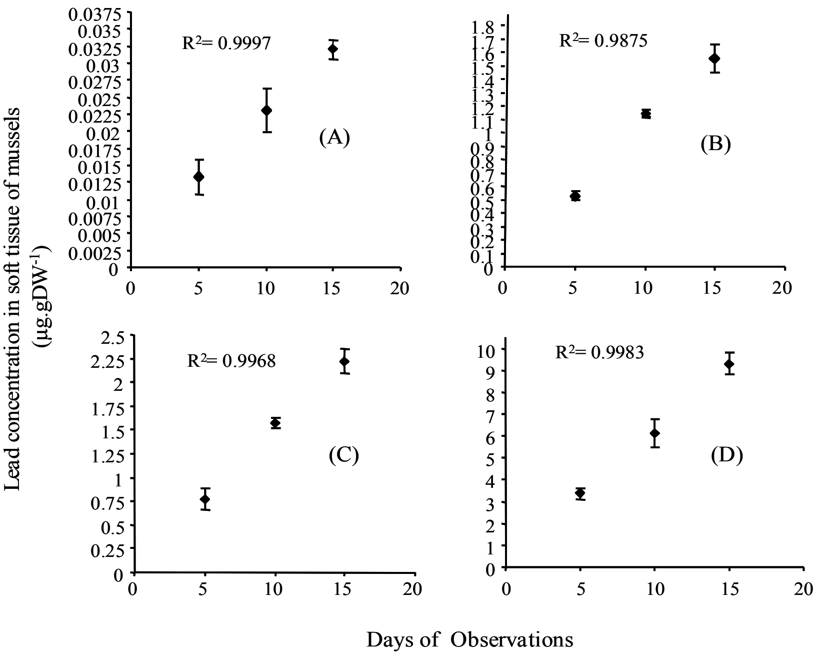

Figure 5. Lead concentration (±SD) in the soft tissue of the bivalve *D. polymorpha* after exposure to four lead treatments (A= 2 µgPb L^{-1}, B= 48 µgPb.L^{-1}, C= 93 µgPb L^{-1} and D= 455 µgPb L^{-1}).

accumulation in mussels soft tissue at sampling times (5, 10 and 15 days). The mean values of lead concentration in the soft tissue of mussels are shown in (Figure 6). When these values compared with each other, significant differences between some treatments observed (2 and 93, 455 µg L^{-1}, 455 and 2, 48, 93 µg L^{-1}) ($P<0.05$). Also, there were no significant differences between some treatments (2 and 48 µg L^{-1}, 48 and 93 µg L^{-1}) ($P>0.05$). Net accumulation rates of lead by mussels also varied between treatments. As it is showed in (Table 2), the least accumulation rate (0.0019 µgPb gDW^{-1} day^{-1}) of lead was measured at lowest concentration of 2 µg L^{-1}, while, the highest accumulation rate was observed in 455 µg L^{-1} (0.593 µgPb g DW^{-1} day^{-1}). These results still suggest that the lead specific accumulation (i.e. divided to its weight) to be dependent to ambient water concentration. It is possible that lead or another metals accumulation to be a non selective activity only related to respiration activity not measured in this study. Figure 7 shows the decrease in lead content of water in each treatment during the experiment. With consideration of results of four treatments as differences in initial concentration one can note that the reduction rates remained constant during 15 days of experiments. But the ability of reduction of metal by mussels decreased during the experimental period. It is clear that at the beginning of exposure maximum reduction rate was observed while after 15 days of exposure only a few part of lead was taken up from the water body. This suggests that mussels have a limit in their absorption activity. However, we must note that, some part of decrease in the exposure aquaria might also be due to absorption of lead to the faeces or aquarium walls.

Discussion

In this study the effect of lead accumulation on Condition indices of zebra mussel based on the tissue weight/shell weight (TCI) or tissue weight*100/shell length (SCI) investigated. These relationships are often used as measures for the well being of mussels (e.g. Mersch *et al*., 1996; Soto *et al*., 2000; Martel *et al*., 2003). Indices in control and treatments showed no significant differences. As showed in (Figures 1 and 3), the averages of condition indices of mussels in lead treatments are in the same range. When condition indices related to exposure times, decrease in these indices as function of time observed. Some studies have investigated the effect of pollution on the TCI or SCI (Mersch and Pihan, 1993; Kilgour *et al*., 1994; Smolders *et al*., 2002; Roméo *et al.,* 2003). Despite significant differences were found in accumulation for lead among the treatments, none of the condition indices were significantly different. The same was found by (Martel *et al*., 2003) who exposed the freshwater mussel *Elliptio complanata* along a river in Québec and did not find significant differences in the TCI. In contrast, (Mersch *et al*., 1996) found significant differences in TCI in transplanted zebra mussels along a pollution gradient. Also, (Smolders *et*

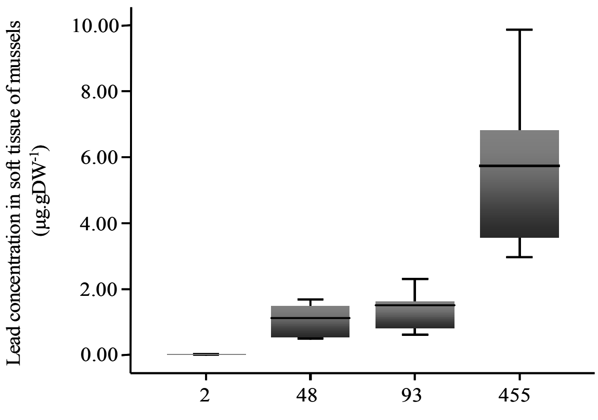

Figure 6. Mean values of Pb concentration (±SD) in the soft tissue of the bivalve *D. polymorpha* after 15 days exposure to four lead treatments.

Table 2. Net accumulation rates of lead of *D. polymorpha* exposed to four lead treatments

Lead Treatment (µgPb L^{-1})	Net Accumulation Rate ((µgPb.gDW^{-1}.day^{-1})
2	0.0019
48	0.102
93	0.144
455	0.593

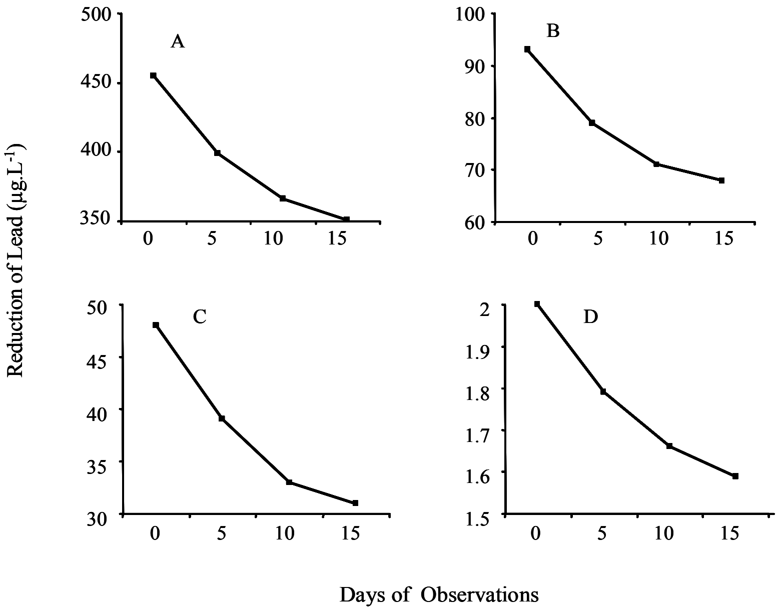

Figure 7. The evolution of reduction of lead during experimental period in four treatments (A= 455 µgPb L^{-1}, B= 93 µgPb L^{-1}, C= 48 µgPb L^{-1} and D= 2 µgPb L^{-1}). It is to be noted that, during the exposure time the reduction decreases compared to concentrations in ambient water body.

al., 2002) found significant differences in TCI values of zebra mussels exposed along a pollution gradient. Besides effects of pollution, the condition of zebra mussels affected by differences in food availability at the different sites (Smolders *et al*., 2002, Roméo *et al*., 2003). But, in our study, during the experiment, the food availability for mussels in control and lead treatments was same. Therefore, feeding had not any effect on differences in mussel's condition indices. Besides, mussels were continuously aerated and temperature was same for control and lead treatments. This indicates that in the absence of differences in food availability and other factors, the condition indices accurately reflect the effect of lead accumulation on zebra mussels.

Lead were taken up and accumulated by mussels. The lead concentrations that selected for this experiment were in agreement with the lead concentrations in four sites of Anzali wetland. The initial concentration of Pb used is in good agreement with the values usually found in bivalves of previous works (Mersch and Pihan, 1993; Kraak *et al*., 1994). The whole organism, lead bioaccumulation was concentration- and time- dependent as is typical for *D. polymorpha* and other bivalves exposed to lead in water.

Metals can be divided into two groups viz. the essential (e.g. Cu, Zn) and the non-essential metals (e.g. Cd, Pb). Essential metals are used in small amounts by animals as components of proteins and enzymes (Simkiss *et al*., 1982). Most animals can regulate the body concentration of these metals up to certain ambient water concentration above which accumulation of the metals starts and toxic effects may occur. Non-essential metals do not play any biological role and can not be regulated. So, zebra mussels are probably able to eliminate these metals of the ambient water in order to limit theirs body burden. These metals are therefore, potentially toxic at low concentration (Amiard *et al*., 1987). Results suggest that parts of dissolved concentration in the water are able to be accumulated in the soft tissue of mussels. This concentration as shown in (Figure 5) is related to initial concentration of lead components in the water body. Regressions in all four treatments indicate also more metal found in the water, more is accumulated in soft tissue. In whole four treatments the lead concentration in the water body decreased and a part of this pollutant was accumulated in the soft tissue of the mussels. Although, some part of decrease in the exposure aquaria might also be due to absorption of lead to the faeces or aquarium walls. With consideration to the results of four treatments as differences in initial concentration one can note that the reduction rates remained constant during 15 days of experiments. But the ability of reduction of lead by mussels decreased during the experimental period. It is clear that at the beginning of exposure maximum reduction rate was observed while after 15 days of exposure reduction rate decrease (compare to first 5 days).

Lead was accumulated at all lead concentrations in the water, indicating that this non-essential metal could not be regulated by *D. polymorpha*. This is in agreement with the results of acute experiments on lead (Bleeker *et al*., 1992) and holds for all bivalves (Amiard *et al*., 1987; Salanki *et al*., 1989), as well as for all other invertebrate taxa studied (Amiard *et al*., 1987; Borgmann *et al*., 1993). As presented above, two important factors are effective on lead accumulation in the soft tissue of zebra mussel: lead concentration in water and exposure time. In all lead treatments, highest lead accumulation at longest exposure period (15 days), and also lowest accumulation were found at shortest exposure period (5 days). Thus exposure time play a key role in the accumulation of metal in the body of mussels and this result is in accordance with previous works on other freshwater bivalves (Bleeker *et al*., 1992; Kraak *et al*., 1992, 1994, and 1999) Also Lecoer *et al*. (2004), and Marie *et al*. (2006) found the same pattern of bioaccumulation in the *D. polymorpha* and *Corbicula fluminea*. Other studies, on *Anodonta trapesialis* and lead and cadmium as metals (Tomazelii *et al*., 2003), show the same patterns.

Conclusion

In conclusion the results of our study showed that the condition indices TCI and SCI are useful tools for assessment of effects of metals on zebra mussels given that the variation in other environmental factors such as food availability are limited or are considered in the monitoring program. Further, in this study impact of concentration and exposure time on accumulation of lead as a water pollutant was examined. Both length and the concentration of lead considerably and significantly caused the enhancement of this metal intensity in soft tissue of mussels.

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