Heavy Metal Effects on the Lysosomal Membrane Stability and Respiratory Rate in Chinese Pond Mussel (Anodonta woodiana) under Ex situ Exposure

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Introduction & Aim

The Chinese pond mussel (*Anodonta woodiana*) is an unionid mussel species that has recently been reported as being invasive worldwide. As a filter feeder, it is known to accumulate heavy metals, making the species useful for biomonitoring. However, the effects on some physiological functions of this species, after acute exposure to heavy metals, is still poorly studied.

The current study aimed to investigate the lysosomal membrane stability in haemocytes of Chinese pond mussel (Anodonta woodiana) by applying the neutral red retention assay (NRRA), as well as changes in the respiratory rate under acute metal exposure.

Materials & Methods

The mussels were treated with different concentrations of Ni and Pb in laboratory conditions for 72 h. These metals are considered as priority substances according to **Directive 2013/39/EU** of the European parliament and the Council. The metal concentrations were prepared as 75, 50 and 25% of the maximum permissible levels set by law.

Lysosomal membrane stability was measured according to the method of Lowe & Pipe (1994) и Lowe et al. (1995).

Respiratory rate was measured at beginning of the experiment, 24th hour and the end of the experiment (72nd hour). At the given time the mussels were transferred in 6 I tank with water from their original tank (one mussel per tank). Oxygen levels were measured, using oximeter "Oxi 315i/SET". The tanks were then covered with plastic foil (water level must be to the edge of the tank) so the oxygen transfer from the air can be eliminated. The tanks were left for one hour, when the oxygen level was measured again. We calculated the respiratory rate by determining the difference in the dissolved oxygen levels before and after the passed hour, following **Tsekov (1989)**.



Fig. 1. LMS stages in mussel haemocytes: (α) intact haemocytes (β) enlarged lysosomes (γ) more enlarged lysosomes and NR loss from the lysosomes to the cytosol (δ) total dye loss from the lysosomes to the cytosol. (Patetsini et al. /Aquatic Toxicology 126 (2013) 338–345)

Results and Conclusions

After the 72nd h of exposure to Ni and Pb the lysosomes retained the dye between 30 to 60 minutes in the mussels exposed to the higher metal concentrations. We registered a negative, statistically significant correlation between the metal concentrations and the average time the lysosomes retained the dye after the 72nd hour. The respiratory rate also increased in a dose-dependent manner, i.e the mussels reacted by increasing their respiratory rate with the increase of the metal concentrations.

At the beginning of the experiment (0 hour) there is low, statistically insignificant, negative correlation between the heavy metals concentration and the respiratory rate index (Pb - S=-0.2, p>0,05; Ni - S=-0.35, p>0,05). After 24 h of exposure we recorded very strong, positive, statistically significant correlation between the concentration of both metals and the index of respiratory rate (S=0.97, p<0,05). At the end of the experiment, after the 72nd hour this correlation had changed significantly and was very low, positive and statistically insignificant for both metals (S=0.2, p>0,05). In most cases the respiratory rate increases with the increase of the pollutant concentration and level of toxicity (Kumar et al., 2012). It seems in our case, the mussels react by increasing their respiratory rate with the increase of the metal concentration after the exposure to Ni and Pb (24 hours). After 72 hit seems that the initial stress, caused by the pollutants is on some level overcome, since there is no such correlation between the respiratory rate and the heavy metal concentrations, similar to the beginning of the experiment.



We can conclude that the acute metal exposure, including all metal concentrations below the allowable concentrations, lead to destabilization of the lysosomal membrane stability and changes in the respiratory rate of the Chinese pond mussel, thus altered physiological functions. Fig. 2. Neutral red retention time in mussels exposed to different Ni and Pb concentrations, representing 75, 50 and 25% of the Bulgarian maximum permissible levels.

We suggest that further research in this particular area need to be carried out in order to better understand the negative effect of heavy metals on this mollusc species.

<u>Acknowledgements</u>: This paper is supported by the NPD - Plovdiv University "Paisii Hilendarski" under Grant No NI15-BF-003 "Integrated biological approaches for monitoring priority substances in water".