UDC: 577.352.38:577.64

MOLECULAR RESPONSES OF BIVALVE MOLLUSKS TO COMPLEX EFFECT OF NANOPARTICLES OF ZnO AND HEATING DEPENDING ON THE HISTORY OF EXPOSURE *in situ*

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Nanooxide ZnO particles (n-ZnO) became one of the most common types of metal-based nanoparticles used in electronics and personal care products. Importantly, nZnO concentration in the aquatic environment in Europe has reached μg/L. The cooling ponds of the electrical power plants represent excellent model systems to study the effects of long-term acclimatization to anthropogenically modified environments on the ability of freshwater organisms to cope with multiple stressors and to determine the physiological and molecular mechanisms setting limits to the multistressor tolerance. Particularly, the fuel thermal power plants (TPPs) commonly release a mixture of pollutants (such as metals, radionuclides, aromatic and alkyl hydrocarbons and other hazardous compounds) in the discharge water and chronically elevate the temperature of the water in the vicinity of the discharge by as much as 5-8°C. Whereas vertebrate animals like fish or frog are able to release Zn from the nanoparticles [1], the bioavailability of Zn from nanoparticles in mollusks is questionable in general and unknown under the complex impact [4].

The aim of this study was to elucidate the ability of mollusks to utilize Zn from n-ZnO in the digestive gland. For that, we detected the binding of Zn with Zn-buffering proteins metallothioneins [3,5]. The effect of nanoparticles *per se* was evaluated from the integrity of lysosomal membranes and the activity of the ATP-dependent system of the efflux of xenobiotics from the cells (*p*-glycoproteins). Male

specimens of *Unio tumidus* from two cooling reservoirs (DPP and BPP) were exposed for 14 days to nZnO (3.1 μM), Zn^{2+} (3.1 μM) at 18ºC, elevated temperature (T, 25ºC), or nZnO at 25ºC (nZnO+T). Control groups were held at 18ºC.

It is important to mention that the differences between the populations of mussels were evident even after the depuration for 21 days in the tap water. All Zn-containing exposures resulted in the elevated concentrations of total and Zn-bound metallothionein (MT and Zn-MT) in the digestive gland demonstrating the bioavailability of Zn from nZnO. This cellular response was distinct from the response of the mussels from the reference pond studied previously [4]. The exposures to nZnO caused the decrease of *p*-glycoproteins activity in the mussels from both cooling ponds. Lysosomal membrane stability was a general stress biomarker that distinguished all Zn-exposed mussels (including those exposed to nZnO and Zn^{2+}) from the control groups. It was initially low in both DPP- and BPP-groups and was decreased in all Zn-related exposures but elevated or stable under the exposure to heating reflecting the adaptation of mussels to elevated temperature in both ponds. Generally, the lysosomal membrane stability was suppressed in nZnO- and Zn^{2+} -exposed mussels at 18°C and 25°C. Nanosized particles could cause mechanical damage of lysosome membranes, whereas dissolved metal can affect the lysosomal membrane indirectly, through multiple pathways including the metal-induced oxidative damage to the membrane. In our study, the intracellular Zn imbalance, caused by Zn depletion and/or trace metal release from oxidized MTs, might also contribute to the decrease in lysosomal membrane stability. The exposures to Zn and nZnO led to increase of ATP level and lipid stores (nZnO) in the digestive gland in DPP- and BPP-mussels. On the other hand, the heating caused the most prominent decline of the lipids (by 62.5% in BPP-group) and ATP levels. In combine exposures (nZno+T) the elevated temperature diminished several responses to nZnO [2]. Most common responses in all exposures were the decrease of pyruvate concentration and increase in the Lactate/Pyruvate ratio in digestive gland. The up-regulation of cathepsin D total and/or free activity demonstrates the activation of the processes of autophagy in the tissue. BPP-groups were distinguished by increased level of cadmium in the tissue reflecting the highest level of pollution.

The PCA analyses of the studied traits reflect population-specific differences in the biomarker response profiles to Zn^{2+}, nZnO and temperature exposures that were more dependent on the population than on the kind of exposure.

Hence, the mussels from the ponds of coal power plants can release Zn from the nanoparticles unlike the specimens from the pristine pond studied previously [4] and be affecting by the nanoparticles *per se.* However, their stress responses to additional heating could exceed the limits of adaptation.

This work has been granted by the Ministry of Education and Science of Ukraine (Projects ## 131B; M/70-2017, ## MV-1).

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