Neuro-, geno- and cytotoxicity responses in mussels *Anodonta cygnea* after six metals mixture treatment

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Abstract
Concerning toxicity, bioaccumulation and persistence in the aquatic environment, six metals such as Zn, Cu, Ni, Cr, Pb and Cd are attributed to priority hazardous substances in many countries throughout the world (Water Framework Directive 2008/105/EC; US EPA 2009). In this study, time-dependent and tissue-specific induction in geno- and cytotoxicity was determined in haemocytes and gills cells of *Anodonta cygnea* after treatment with six metals (Zn-0.1, Cu-0.01, Cr-0.01, Ni-0.01, Pb-0.005 and Cd-0.005 mg/L, at Maximum Permissible Concentrations (MPC), accepted for the inland waters in EU) mixture at various time points (1, 2, 4, 7, 14 and 28 days). The highest genotoxicity levels in gill cells and haemocytes were determined after 4 days exposure. After 2 days treatment in gill cells of exposed mussels, there was found the highest and statistically significant induction of cytotoxicity level. Neurotoxicity studies have shown, that prepared metals mixture has ability to inhibit AChE activity in mussel's hemolymph after 4 days and 28 days exposure. Time-depended metals accumulation in *A. cygnea* gills and statistically significant relations between Cu, Cd, genotoxicity (in gill cells) and cytotoxicity (in gill cells) demonstrates bioavailability of used trace metals for the bioindicator and time-related DNA damage.
Keywords: trace metals, genotoxicity, cytotoxicity, *Anodonta cygnea*, AChE

1. Introduction

Metal pollution from anthropogenic origin in the world's water ecosystems is still continually rising up. In order to achieve good ecological and chemical status in transitional and coastal waters, Water Framework Directive (WFD, 2000/60/EC) has defined a list of priority pollutants, which are deemed to have detrimental effects on the environment, with a requirement for substantial monitoring of these pollutants. Usually in water environment, many of the metals exist in various mixtures. Due to the interactions between chemicals, even low metal concentrations in the mixture (at MPC) are able to induce DNA damage in aquatic organisms (Stankevičiūtė et al. 2017). Genotoxic and cytotoxic effects caused by trace metals, leading to changes in biological parameters and indices, may affect health at individual and at population levels in time. Bivalve mussels are considered as reliable-sentinel species for the bioindication of aquatic pollution. Freshwater mussel *Anodonta cygnea* (Unionidae) can be found in rivers and lagoons all over the Europe and the Northern America (Lopes-Lima et al. 2017). In some countries, this species is listed as near threatened or threatened owing to severe declines in abundance of distribution (Lopes Lima et al. 2017).

Acetylcholinesterase enzyme (AChE) is an essential enzyme in the prevention of acetylcholine accumulation and the termination of the nerve impulse transmission frequently used in marine and freshwater pollution monitoring as biomarker of exposure to neurotoxic compounds (Benali et al. 2015).

In order to assess geno- and cytotoxic effects of six metals (at MPC) mixture treatment at various exposure time points, micronucleus and nuclear abnormalities' test has been applied in gills and hemolymph cells of fresh water mussels *Anodonta cygnea*. Neurotoxicity of metal mixture was determined in *A. cygnea* by measuring AChE in hemolymph of exposed mussels.

2. Materials and Methods

Adults of *Anodonta cygnea* at approximately the same size were collected at the bank of River Neris (Lithuania) in Verkiai Regional Park (54° 44′ 47.3″ N and 25° 17′ 39.4″ E). Before the experiment mussels were kept 2 weeks for acclimation. The experiment was conducted under semi-static rotating water current conditions. During the experiment, both water and diet were of the same type. Test mussels were exposed for the 1-, 2-, 4-, 7-, 14-, and 28-day period to a six-metal (Zn, Cu, Ni, Cr, Pb and Cd) mixture (at MPC). After treatments, mussels were dissected, and their tissues (gills and hemolymph) collected for further (neuro-, geno-, cytotoxicity and metal bioaccumulation) analyses.
The acetylcholinesterase activity was determined in hemolymph samples using the method of Ellman et al. (1961) modified for microplates. Preparation of test solutions, samples for geno- and cytotoxicity assessment and criteria for geno- and cytotoxicity endpoints identification are presented in Butrimavičienė with co-authors (2019) publication. For metal determination in mussels' tissue samples, a procedure was adapted from Sen and co-authors (2011).

3. Results and Discussion

After 4-day exposure, the highest and statistically significant levels of genotoxicity in gills (40.80‰) and hemolymph (34.76‰) tissues were assessed. Moreover, after 4 days of exposure in hemolymph of investigated mussels, there were measured a significant decrease in AChE activity (12.37±1.462 nmol ACTC min-1 mg), when compared to the control (18.19±1.58 nmol ACTC min-1 mg; p=0.048) and pre-exposure (17.81±1.977 nmol ACTC min-1 mg; p=0.038) groups. After 2 days treatment in gills' cells of exposed mussels, there was found the highest (38.9‰) and statistically significant induction of cytoxicity level. In gills of exposed mussels time-dependent increase in accumulated metal amount was found. The highest and statistically significant accumulation of most of trace metals has been determined in gills of *A. cygnea* after 28 days treatment. According to the obtained geno- and cytotoxicity data, it is suggested that gills are more sensitive tissue in comparison to investigated hemolymph. Genotoxic properties of trace metals are related evidently to the accumulation of DNA damaging free radicals, clastogenic process, or simultaneously to clastogenic and aneugenic action in aquatic organisms (Nepomuceno et al. 1997). Induction of DNA damage in mussels after exposure to trace metals was described previously (Falfushynska et al. 2013; Nugroho et al. 2015; Sohail et al. 2017; Khan et al. 2018). Inhibition in AChE activity in various mussels and in their different tissues after exposure to trace metals was presented in experimental and *in situ* studies (Bainy et al. 2006; Vidal-Liñán et al. 2014). Decrease in AChE activity observed initially in the present study might be a response to metal mixture toxicity.

The study results suggested that due to discharges of metals at MPC into the aquatic environment, the occurrence of threats to *A. cygnea* health is a likely scenario.

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