Biomarkers responses in *Salmo salar* exposed to multicomponent metal mixtures

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Abstract
Pollution of freshwater from industrial, domestic and agriculture sources is one of the major factors responsible for the decline of Atlantic salmon (*Salmo salar*) populations in Europe. Biomarkers responses (bioaccumulation, glucose content, cytogenetic and behavioral endpoints) elicited by the most common metal contaminants at Maximum-Permissible-Concentrations (MPC: Zn – 0.1, Cu – 0.01, Ni – 0.01, Cr – 0.01, Pb – 0.005 and Cd – 0.005 mg/L) accepted for the inland waters in EU was assessed in fish after 4 h, 1, 2, 4, 7, 14 or 28 days of exposure. Experimental studies were performed using whole mixture approach and by reducing environmentally realistic concentration of single metal in the mixture by 10-times (represents background exposure in the aquatic environment). Bioaccumulation of metals was assessed in gills, liver, kidneys and muscle tissues. In the most of the investigated tissues, steady-state metals concentrations were reached within 14 days. All metals have attained steady-state in muscle. Metal mixture at MPC and 10-fold reduction of single metal in the mixtures significantly affected cytogenetic, behavioral endpoints, blood glucose content and bioaccumulation of several metals in analysed tissues of fish. Therefore, discharges of metals at MPC into the aquatic environment can lead to health problems of juvenile *S. salar.*
Keywords: bioaccumulation, steady-state, genotoxicity, cytotoxicity, behavioral alterations.

1. Introduction

Metals are the chemical toxicants that can disturb environmental homogeneity due to their toxicity, persistence, bioaccumulation, and interactions properties (Ali et al., 2019). Due to the ability to accumulate chemical compounds, fish are important bioindicators disclosing the relative health of aquatic ecosystems (Lasheen et al., 2012). Moreover, *S. salar* is an ecologically and economically important fish species in the Europe. Fish are usually exposed to multicomponent metal mixtures (Zhu et al., 2004). The large amount of data show that particular metals affect the accumulation of other metals in fish. Interactions (additive, synergistic or antagonistic) among metals are related to their competitive uptake patterns from the surrounding environment and their different distribution in fish tissues (Jezierska, Witeska, 2001).

The objectives of this study were: (1) to assess bioaccumulation of metals in different *S. salar* tissues after exposure to multicomponent metal mixtures at MPC, (2) to measure the time necessary to attain the steady-state metals concentrations in tissues, (3) to assess cytogenetic damage, (4) to determine behavioral alterations, (5) and to measure glucose content in peripheral blood of fish.

2. Materials and Methods

2.1. Study animals

The research was conducted on hatchery-reared one-year-old Atlantic salmon (*Salmo salar* Linnaeus, 1758) smolts, average total weight 45.8 ± 6.3 g and average total length 171.8 ± 8.0 mm (mean ± SD, N = 205). The fish kept for acclimation in holding tanks (1000-L volume) supplied with flow-through aerated deep-well water at least two weeks prior to testing. Fish were kept under a natural light cycle and fed commercial salmonids feed daily in the morning. During the experiment, both water and diet was of the same type. Fish were accepted as acclimated to a new medium when fish started to swim freely in all directions and they feed well (Stankevičiūtė et al., 2017, 2018).

2.2. General laboratory procedures

The experiments were conducted under semi-static rotating water-current conditions in polyethylene tanks of 35-L with continuously aerated deep-well water. Test solutions and clean water were renewed every day. Experimental design, including types of performed treatments, biomarkers analyses (bioaccumulation, glucose content, cytogenetic and behavioral endpoints), number of replicates, exposure duration, number of fish used and statistical data analyses in this research are described in detail by Stankevičiūtė et al. (2017, 2018).

3. Results

3.1. Bioaccumulation and the steady-state metals concentrations in tissues

The obtained data showed that metal bioaccumulation in *S. salar* was time-related and tissue specific. The time necessary to reach steady-state was metal specific and varied between tissues. It should be mentioned that all metals have reached steady-state in muscle, but at different exposure period. To summarize, steady-state for metals were reached within 14 days in most of the explored tissues. In tissues, quantitatively maximum levels were found for essential (Zn, Cu) while the minimum levels for nonessential (Pb, Cd) metals. Overall, metals were accumulated mostly in gills, liver and kidneys, at least – in muscle.

The results of this study demonstrated that reduction (↓) of even low (MPC) metal concentration in mixture had a significant effect on metals distribution in tissues.

3.2. Erythrocytic nuclear abnormalities (ENAs)

Considering frequencies of single genotoxicity endpoints, such as micronucleus (MN), nuclear bud (NB) and nuclear bud on filament (NBf), the highest frequencies were detected after Cr↓ treatment in most cases, fallowing by MIX and Cu↓ treatments. The frequencies of cytotoxicity endpoints, showed the highest induction after Cr↓, Cu↓ or Pb↓ treatment. Moreover, significant elevation in total ENAs in peripheral blood was measured after Cd↓ treatment. It is important to emphasize, that mixture with reduced concentration of Cr caused the highest induction of genotoxicity and cytotoxicity endpoints frequencies. While, metal bioaccumulation data shows that treatment with reduced Cr resulted in a decreased accumulation of nonessential metals such as Cd or Pb in tissues.

3.3. Behavioral alterations and blood glucose level

The obtained data demonstrates changes in locomotor activity patterns of the *S. salar* exposed to multicomponent metal mixtures. Fish exposed to MIX and Cu↓ showed a presence of the different peak over time in average velocity endpoint, while no peak in response of fish exposed to Cr↓ was observed. Only MIX treatment was found to be a significant compared to control for both endpoints. The meaningful changes in swimming activity of *S. salar* to mixtures were observed after 10 min of exposure. Moreover, this behavioral study showed the rapidness of the behavioral response of fish to metal mixtures treatment indicating the sensitivity of *S. salar* to water contamination.

Blood glucose levels were observed to increase significantly after 120 min treatment with MIX, Cu↓ and Cr↓ mixtures in comparison to control. However, the level of blood glucose in fish did not differ between mixtures investigated.

4. Conclusions

In summary, this study showed induction of geno- and cytotoxicity response after metal mixture exposure at MPC and established the time (14 days) necessary to attain the steady-state concentrations of metals in tissues of *S. salar.* This study shows that multicomponent metal mixtures induce changes in *S. salar* behavior and blood glucose level. The most severe genotoxicity and cytotoxicity endpoints were detected after treatments with MIX, Cr↓ (10 times reduced Cr^{6+} concentration) and Cu↓ (10 times reduced Cu^{2+} concentration) mixtures. Consequently, this research disclosed the meaningful influence of background exposure on chosen toxicological endpoints.

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