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Assessment of heavy metals bioconcentration factor (BCF) and genotoxicity response induced by metal mixture in *Salmo salar* tissues

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Abstract. The aim of this study was to evaluate metals bioconcentration factor (BCF) in gills, liver, kidneys and muscle in relation with genotoxicity effects of metal mixture in peripheral blood, kidneys, gills and liver erythrocytes of the Atlantic salmon (*Salmo salar*). Fish were exposed to maximum-permissible waterborne concentrations of Zn – 0.1, Cu – 0.01, Ni – 0.01, Cr – 0.01, Pb – 0.005 and Cd – 0.005 mg/L, respectively for 7 and 14 days. Genotoxicity was studied using the micronucleus test. In addition, erythrocyte nuclear abnormalities (ENAs) were analysed. Our study indicates that metal BCF in Atlantic salmon is tissue-dependent. Based on the BCF classification scale, the relatively low values of metals bioconcentration were assessed, except for Zn (gills) and Cu (liver) (359.6 and 594.0, respectively). Zn intensively concentrated in fish tissues, while Pb – least of all. Overall, metals were concentrated mostly in the liver, least – in the muscle. Significant differences among BCF values of Pb in gills and muscle and Cd in gills were measured between 7 and 14 d exposure groups. Treatment with metal mixture significantly increased micronucleus frequencies after 7 d of exposure in liver and peripheral blood erythrocytes. Significant genotoxicity response was not observed after 14 d treatment. The erythrocytic nuclei abnormalities determined in *S. salar* blood were nuclear bud on filament (NBf), nuclear bud (NB), blebbed (BL), kidney shaped, vacuolated (VacNuc), 8-shaped nuclei and fragmented-apoptotic (FA) erythrocytes. Significant elevation in total ENAs level was detected in kidneys and liver erythrocytes after 7 d treatment, while after 14 d – in gills and kidneys erythrocytes. No significant differences among analysed responses were measured between 7 and 14 d exposure groups, except total ENAs level in liver erythrocytes.

Keywords: *Salmo salar*, metal mixture, bioconcentration factor (BCF), genotoxicity, nuclear abnormalities, micronuclei.

Conference topic: Environmental protection.

Introduction

Metals are the chemical toxicants that can disturb environmental homogeneity due to their indeterminate persistence, non-degradation, affinity for bioaccumulation and complex interactions (Roy *et al.* 2011). Heavy metals (Zn, Cu, Ni, Cr, Pb, Cd) are assigned to priority hazardous substances (pollutants) in many countries (Directive 2008/105/EC; US EPA 2009). Bioconcentration and biomagnification processes are capable of leading to adverse effects of metals in fish, even at low exposure concentrations as metals integrate into important protein synthesis reactions and as a result perturb vital processes (Valavanidis *et al.* 2006). In the longer time, the pollutants present in the environment at very low levels may accumulate within the body of aquatic organisms by diverse mechanisms to the quantity that they exert noxious effects. Therefore, it is crucial to know the bioaccumulation potential of a pollutant (Palaniappan, Karthikeyan 2009).

Experimental measurement of bioconcentration factor (BCF) is used to assess the potential for a chemical to bioaccumulate (Parkerton *et al.* 2008). Bioconcentration is a situation in which the levels of a toxin in an organism exceed the levels of that toxin in the surrounding environment. This terminology is often used specifically in reference to aquatic environments and aquatic organisms. BCF is used to express bioconcentration levels in a numeric way. The BCF can be calculated as the ratio of a toxin concentration in an organism and the levels in the surrounding environment. The higher the ratio, the more intense the bioconcentration of toxins, in this case, metals in fish. Information on BCF is required for regulatory purposes within the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), which regulates chemicals in the European Union (EU) (Crookes, Brooke 2011).

Due to the ability to accumulate persistent pollutants, fish are excellent bioindicators revealing the relative health of aquatic ecosystems (Lasheen *et al.* 2012). Aquatic organisms can accumulate chemical compounds by two ways: directly from the environment (via skin or respiratory surface) and indirectly (by collecting and concentrating a chemical compound from food). Bioaccumulation (bioconcentration, biomagnification) is a dynamic process, by which chemicals accumulation and excretion occur at the same time (Ivanciuc *et al.* 2006). Due to specific biochemical,

physiological properties of the fish body tissues, diverse metal accumulation intensity may occur in different tissues. Therefore, it is important to identify and assess the potential for bioconcentration and to perform comparative analysis.

Aquatic organisms are typically exposed to mixtures of metals. Considerable amount of data show that certain metals affect accumulation of other metals in fish. Interactions among metals are related to their competitive uptake from the surrounding environment and different allocation in fish tissues. Interactions among metals may be different (additive, synergistic or antagonistic), therefore, the effects of their various mixtures on fish survival may also vary (Jezierska, Witeska 2001; Svecevičius *et al.* 2014).

Transition metals play an important role in oxidative reactions and are known for their potential to cause oxidative stress (Valavanidis *et al.* 2006). DNA is particularly susceptible to oxidative damage by reactive oxygen species (ROS). Micronucleus (MN) test together with erythrocyte nuclear abnormalities (ENAs) assay could be used as biomarkers of genotoxicity of a variety of genotoxic agents. As concluded by Luzhna *et al.* (2013), the role of metals in micronuclei formation and DNA damage arise from: binding to DNA and proteins, altered gene expression, mutations, altered cell cycle, chromosome non-disjunction, cytoskeleton dysfunction. Depending on the metal, clastogenic and aneugenic effects could lead to MN formation. Erythrocyte nuclear abnormalities could arise during the DNA replication process (Gomes *et al.* 2015).

Due to their susceptibility to water quality and commercial importance, Salmonids species were selected for experimental study (McCain 1998). The aim of this study was to evaluate metals bioconcentration factor (BCF) at steady-state in fish body tissues (gills, liver, kidneys and muscle) in relation with genotoxicity effects of metal mixture (Zn, Cu, Ni, Cr, Pb and Cd) in peripheral blood, kidneys, gills and liver erythrocytes of the Atlantic salmon (*Salmo salar*).

Material and methods

Experimental set-up

The experimental treatments were conducted on hatchery-reared one-year-old Atlantic salmon (*Salmo salar* Linnaeus, 1758) smolts, average total weight 42.1±4.43 g and average total length 167.4±7.17 mm (mean±SD, $N = 21$, respectively). The fish was obtained from Meškerinė fish hatchery (Švenčionys District, Lithuania) and kept for acclimation in holding tanks (1000-L volume) supplied with flow-through aerated deep-well water at least two weeks prior to testing (minimum water flow rate 1 L/g of their body mass per day). Fish were kept under a natural light cycle and fed commercial salmonids feed (ALLER PLATINUM) daily in the morning; the total amount was no less than 1% of their wet body mass per day. During the experiment, the fish were fed in the same manner. Fish were accepted as acclimated to a new medium when their behavior became normal and they fed well. Deep-well water was used as the dilution water.

Reagent grade metal salts («REACHIM» Company, Russia) were used as the toxicants. Stock solution was prepared by dissolving necessary amount of the salt in distilled water, the final concentration being recalculated according to the amount of metal ion.

The tests were conducted under semi-static rotating water-current conditions on 3 groups (2 treatments and one control, each group consisting of seven individuals) using polyethylene (PE) plastic tanks of 35-L total volume filled to a level of 30 L with continuously aerated dilution water. Test fish were exposed for the 7 and 14 days period to a six metal mixture at a concentration corresponding to Lithuanian inland water standards or Maximum-Permissible-Concentrations (MPC) for the receiving water-bodies (Directive 2008/105/EC) (Table 1). Test solutions and clean water were renewed every day, and test fish were transferred into freshly prepared solutions after they were fed.

Table 1. Metals and their test waterborne concentrations (mg/L) in test media

Metal	Source	Concentration	
		Maximum-Permissible-Concentration (MPC) (mg/L)	Measured (mean±SD)
Zn	$ZnSO_4 \cdot 7H_2O$	0.1	0.115±0.014
Cu	$CuSO_4 \cdot 5H_2O$	0.01	0.009±0.001
Ni	$NiSO_4 \cdot 7H_2O$	0.01	0.011±0.002
Cr	$K_2Cr_2O_7$	0.01	0.012±0.002
Pb	$Pb(NO_3)_2$	0.005	0.0045±0.0004
Cd	$Cd(CH_3COO)_2 \cdot 2H_2O$	0.005	0.0052±0.0003

Analytical procedures

The main physico-chemical parameters of the water (temperature, dissolved O_2, pH and conductivity) were measured routinely with a hand-held multi-meter (WTW Multi 340i/SET, Germany). Designed nominal metal concentrations in the tanks were checked during blank tests (without fish) ($N = 4$) with an atomic absorption spectrophotometer (SHIMADZU AA-6800, Japan) by graphite furnace technique using proprietary software. Each water sample was acidified with reagent-grade nitric acid (final concentration 0.5% v/v) and analysed in triplicate. Mean measured concentrations were within 5% – 20% of the target.

Metal bioaccumulation analysis

After the testing was completed, fish (of control and metal-exposed groups) were sacrificed. Fish were measured (total body length (L) and fork-length (l_C), mm) and weighed (total body weight (Q) and body weight without stomach weight (q), g). Later they were used in the removal of needed tissues: muscle without skin (~3 g), gills (whole organ), liver (whole organ) and kidneys (whole organ); organs were weighed to an accuracy of ± 0.001 g. Fish samples were hot air oven-dried at 85 ºC for 24 hours until reached constant weight, pre-digested tightly in a concentrated ultrapure HNO_3 (60%) and H_2O_2 (30%) (Lach-Ner, Chempur, respectively) at a ratio of 5:1 v/v for eight hours at a room temperature and then microwave-digested quickly (Jia *et al*. 2005). After that cooling solutions were filtered through a 0.45 µm glass filter and diluted with deionized water. Metal concentrations were measured by atomic absorption spectrophotometry on Varian Spectr AA 55 (USA) with a graphite furnace technique in accordance with standardized procedure ISO 15586:2003 final concentration being expressed as mg/kg of wet weight. Accuracy of analytical procedure was checked using certified reference material fish homogenate (IAEA–407). Recoveries were in acceptable range (within 10%) of the certified values.

Bioconcentration factors (BCF) estimations

Tissues with BCF greater than 1,000 are considered high, and less than 250 low, with those between classified as moderate (Landis *et al.* 2011).

BCF values in this study were calculated as reported by Gobas *et al.* (2009) where bioconcentration factor (BCF) is defined as the ratio of the steady-state metal ions concentrations in the fish vs the concentration in water:

$$BCF = \frac{C_{fish}\left(mg / kg\ wet\ fish\right)}{C_{water}\left(mg \diagup L\right)}. \quad (1)$$

Micronucleus (MN) and erythrocyte nuclear abnormalities (ENAs) analyses

Blood was immediately taken from the caudal vein. A drop of blood was directly smeared on microscopic slides and air-dried. After the sacrifice, small pieces of cephalic kidneys, liver and gills were dissected, softly dragged along clean slide and allowed to dry for 1-2 h (Baršienė *et al.* 2006). Dried smears were fixed in methanol for 10 min. and were stained with 10% Giemsa solution in phosphate buffer pH = 6.8 for 8 min. (Baršienė *et al.* 2004). A light microscope Olympus BX51 (Tokyo, Japan) was used to examine a total of 4,000 cells per sample. Final results were expressed as the mean value (‰) of sums of analysed individual lesions scored in 1000 erythrocytes per fish sampled from every study group. The formation of micronuclei (MN), nuclear buds (NB), nuclear buds on filament (NBf), 8-shaped nuclei, fragmented-apoptotic (FA), kidney-shaped, blebbed (BL), vacuolated (VacNuc) erythrocytes were identified using criteria described by Fenech *et al.* (2003) and Baršienė *et al.* (2014).

Statistical analysis

The statistical analysis was performed using STATISTICA 7.0 (StatSoft Inc., Tulsa, Oklahoma, USA) software package. Significance of differences between the non-exposed and treated groups were tested using one-way analysis of variance ANOVA followed by Bonferroni post hoc test. The results were expressed as mean ± standard error or standard deviation. The level of significance was established at $p < 0.05$.

Results and Discussion

Bioconcentration factor (BCF) assessment

BCF values were calculated in gills, liver, kidneys and muscle tissues after salmon exposure to metal mixture (composed of Zn, Cu, Ni, Cr, Pb and Cd) for 7 and 14 days. According to BCF classification scale, low BCF values of analysed metals were measured in fish tissues after 7 and 14 d exposure period (Fig. 1). However, average values of BCF for Zn [(in gills (356.8–359.6), in kidneys (293.6–294.0)] and Cu [in liver (566.7–594.0)] were recorded. As shown in Fig. 1, metal accumulation intensity in salmon tissues was similar after 7 and 14 d exposure period. Significant differences ($p < 0.05$) among BCF values of Pb in gills and muscle and Cd in gills were measured between 7 d and 14 d exposure groups. BCF shows the potential of particular metals to bioaccumulate in specific tissue. BCF values

of Zn, Ni, Cr and Cd in different tissues followed the same sequence: gills>kidneys>liver>muscle; while Cu – liver>kidneys>gills>muscle; Pb (after 7 d): muscle>kidneys>gills>liver, after 14 d – muscle>gills>kidneys>liver.

Generally, metal levels in fish body tissues usually follow the ranking: liver>gills>kidneys>muscle. The highest metal concentration was detected in the liver of salmon, the least – in muscle. Liver is an important target organ involved in metabolic and detoxification mechanisms (Liebel *et al.* 2013). Based on the data, metals exposure may cause an increase in metallothionein (MT) levels in animals, including fish (Hogstrand, Haux 1991). Reduced levels of proteins, lipids activities in the muscles were measured after fish exposure to metals. According to Allen-Gill and Martynov (1995), low levels of metals accumulated in muscles are due to slower synthesis of proteins in this tissue. BCF values revealed metals accumulation patterns in tissues of Atlantic salmon. Metals accumulation in the tissues showed the following sequences: gills: Zn>Cu>Cd>Cr>Ni>Pb; liver: Cu>Zn>Cd>Cr>Ni>Pb; kidneys: Zn>Cu>Cd>Cr>Ni>Pb and muscle: Cu>Zn>Pb>Cr>Ni>Cd. Zinc and Cu showed the highest levels of accumulation in the tissues. Essential metals (copper, zinc) are vital for the health of fish, involved in all aspects of biological function. However, an excess amount of such metals produces cellular and tissue damage, forming dangerous free radicals. Consequently, there is a fine balance between metal deficiency and surplus and it is crucial for organisms to maintain metal homeostasis via tight regulation by maintaining a balance between uptake and excretion (Bury *et al*. 2003). Bioaccumulation levels of Cd (in gills, liver and kidneys) and Pb (in muscle) take third place in the sequences. Cd and Pb have no known biological functions and are considered as non-essential toxic metals, which tend to accumulate in carnivorous fish tissues (Yousafzai *et al*. 2010). Pb and Cd disrupt calcium uptake in gills and may affect the metabolism of essential trace element by having an effect on normal tissue distribution of Zn and Cu (Komjarova, Blust 2009; Birceanu *et al.* 2008).

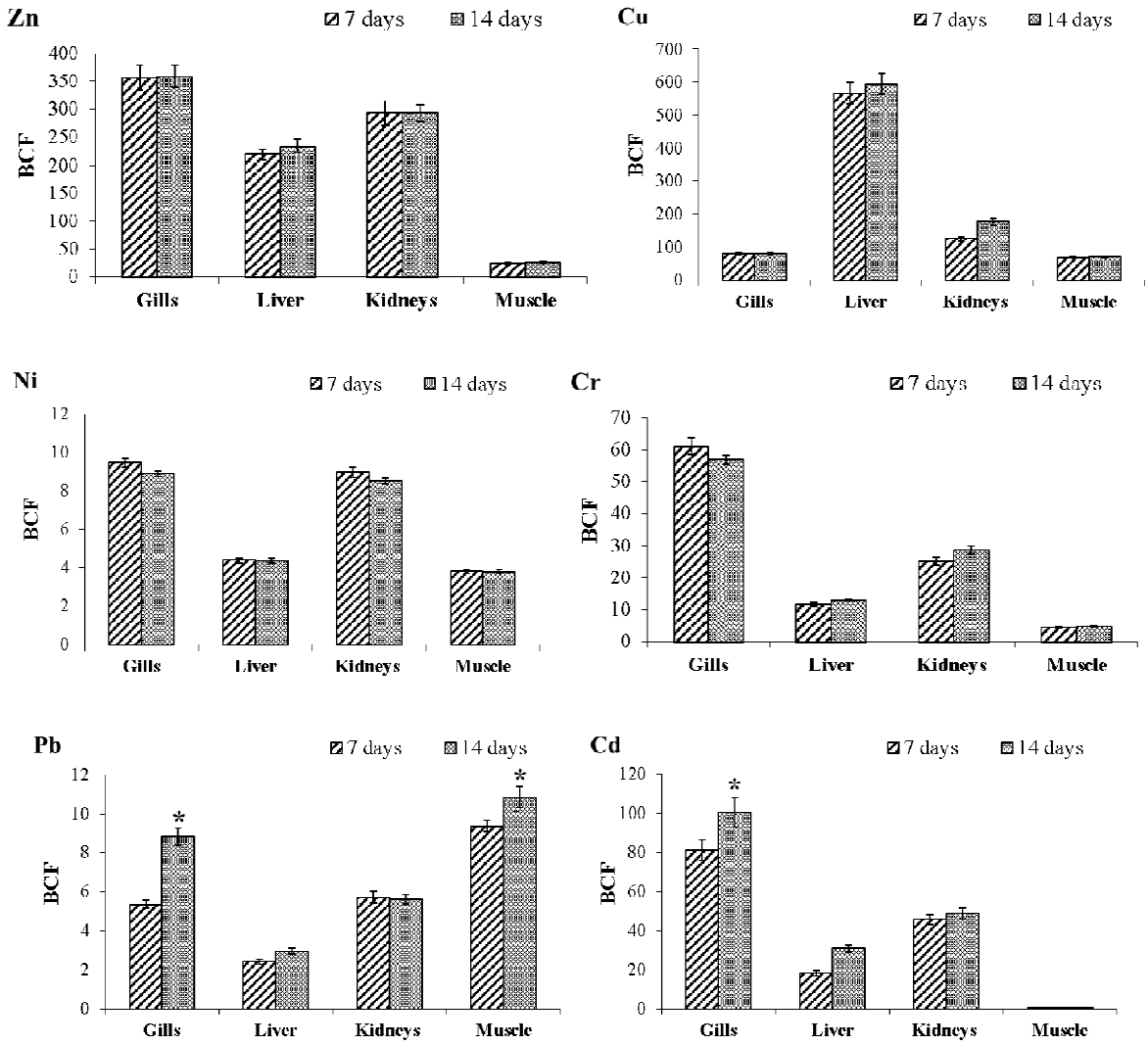

Fig. 1. Bioconcentration factor (BCF) in the selected organ tissues exposed to metal mixture for 7 and 14 d (mean ± SEM, $N = 7$). Asterisks (*) denote significant differences among exposure groups ($p < 0.05$)

According to Sauliutė and Svecevičius (2014) study result, salmon exposure to metal mixture (Zn, Cu, Ni, Cr, Pb, Cd) and single metal (Ni, Cr, Pb) at standing water-current conditions for 14 days showed different BCF values of

metals in fish tissues. BCF values of analysed metals were lower in comparison to this study. However, higher BCF level was measured for Zn in muscle (BCF = 283). Significantly higher BCF values of metals after fish exposure to metal mixture than to single metal were reported in that study. This could be influenced by synergistic interaction within metal mixture. Contrary to our result (during rotating water-current conditions), Sauliutė and Svecevičius (2014) study showed the highest BCF values in salmon muscle, the least in kidneys. The reasons why the Atlantic salmon accumulated the highest amounts of metals in the muscle are due to the experimental design and fish behavior (fish activity was low – lying on the bottom of the tank). Atlantic salmon is a very active rheophilous species which actively searches for food and performs distant and long-term anadromous and catadromous migrations. As confirmed by field studies, fish activity can promote the release of metals from the tissues (Svecevičius *et al.* 2014; Mohammadnabizadeh *et al*. 2014; Jezierska, Witeska 2001; Ray 1978). Therefore, in this study a new experimental system using rotating water-current condition was developed, which particularly reflect fish natural living conditions.

Micronucleus (MN) and erythrocyte nuclear abnormalities (ENAs) analyses

Fig. 2 shows MN frequencies in *S. salar* gills, kidneys, liver and peripheral blood erythrocytes. Significant increases occurred at 7 d of exposure in liver and blood erythrocytes. Significant genotoxicity response was not observed after 14 d treatment. The presence of micronuclei is an irreversible change and reflects genotoxic damage (Javed *et al*. 2016). Exposure to single metals such as Cd, Pb, Cr, Cu, Zn and Ni at high concentrations is known to induce MN in peripheral blood erythrocytes in different fish species (Gomes *et al*. 2015; Ahmed *et al*. 2013; Çavaş 2008; Bagdonas, Vosylienė 2006). There are experimental studies showing capacity of single metal (such as Cr) to induce MN even at low (environmentally relevant) concentrations (Zhu *et al*. 2004). Micronuclei induction after fish exposure to environmentally relevant metal mixture concentrations is scantily discussed. Significant MN induction was observed in blood after treatment with Cd, Cu, Pb, Zn and Cu, Zn metal mixtures using higher concentrations in several fish species (Harabawy, Mosleh 2014; Obiakor *et al*. 2010).

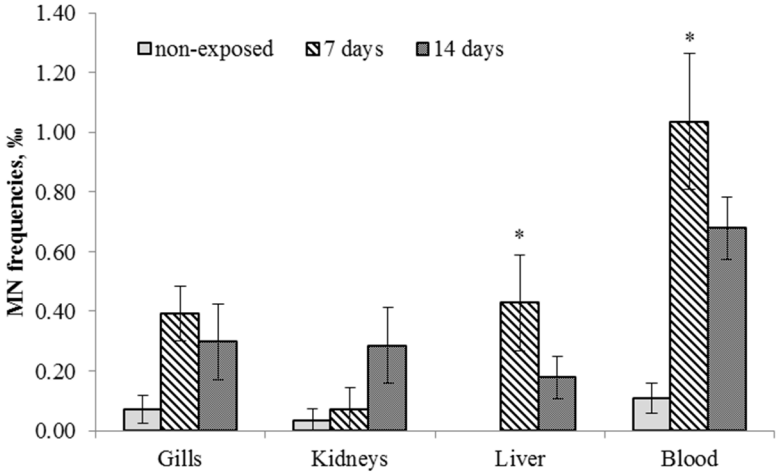

Fig. 2. Genotoxicity responses in *Salmo salar* gills, kidneys, liver and peripheral blood erythrocytes (mean ± SEM, $N = 21$). Asterisks (*) denote significant differences from control during exposure time ($p < 0.05$)

The erythrocytic nuclei abnormalities determined in *S. salar* blood were nuclear bud on filament (NBf), nuclear bud (NB), blebbed (BL), kidney-shaped, vacuolated (VacNuc), 8-shaped nuclei and fragmented-apoptotic (FA) erythrocytes. Levels of total ENAs are shown in Fig. 3. Significant ENAs induction was found in *S. salar* kidneys and liver erythrocytes after 7 days of exposure, while after 14 d of exposure – in gills and kidneys erythrocytes. No significant differences among analysed responses were measured between 7 d and 14 d exposure groups, with the exception of total ENAs level in liver erythrocytes. ENAs induction in different tissues erythrocytes is scantily discussed. Erythrocyte nuclear abnormalities are markers of genetic instability. It is suggested that ENAs could arise from the DNA replication process (Gomes *et al*. 2015). Induction of various ENAs such as lobed, blebbed, notched, bud, vacuolated and condensed nuclei after treatment with single Cd was reported (Gomes *et al*. 2015). Binucleated, kidney-shaped nuclei, blebbed nuclei, lobed nuclei, bilobed nuclei, notched nuclei, hook-shaped nuclei and vacuolated nuclei induction in Nile tilapia, *Oreochromis niloticus* was reported after treatment with Cd, Cu, Pb and Zn (1.25 mgL^{-1} of each) metal mixture (Harabawy, Mosleh 2014). As Gomes *et al.* (2015) study shows, MN induction exhibited the lowest frequency in most of Cd treatments, while the specific ENAs showed the highest frequencies in all treatments. As shown in Fig. 2, significant ENAs induction was noticed in *S. salar* gills and kidneys erythrocytes, while MN frequencies in these tissues were statistically insignificant during all exposure time. The results of this study suggest using MN test in combination with ENAs assay, which together bring better results considering genotoxicity evaluation after treatment with metal mixture at low exposure concentrations.

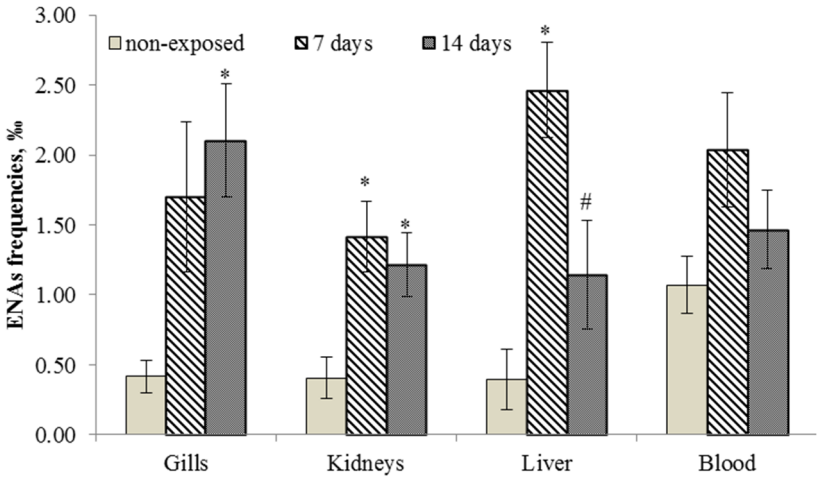

Fig. 3. Erythrocyte nuclear abnormalities level in *Salmo salar* gills, kidneys, liver and peripheral blood erythrocytes (mean ± SEM, $N = 21$). Asterisks (*) denote significant differences from control during exposure time, while # – differences among exposure groups ($p < 0.05$)

Conclusions

This study attempted to investigate the genotoxic potential and bioconcentration factor of complex metal mixture at environmentally relevant (maximum-permissible waterborne concentrations) concentrations, whose effects on aquatic organisms are still poorly investigated. The obtained results showed that used metal mixture caused genotoxic damage in *S. salar* erythrocytes. The results of this study suggest using MN test in combination with erythrocyte nuclear abnormalities assay, which together brings better results considering genotoxicity evaluation after metal treatment. Our study indicates that metal BCF in Atlantic salmon is tissue-dependent. However, measured BCF values of analysed metals were low in fish tissues after 7 and 14 d exposure period. The highest metal concentration was detected in the liver of salmon, the least – in muscle. Significant differences among BCF values of Pb (gills, muscle) and Cd (gills) were measured between 7 and 14 d exposure groups.

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Disclosure statement

The authors declare that have no conflict of interest.

References

Ahmed, M.; Kundu, G.; Al-Mamun, M.; Sarkar, S.; Akter, M.; Khan, M. 2013. Chromium (VI) induced acute toxicity and genotoxicity in freshwater stinging catfish, Heteropneustes fossilis, *Ecotoxicology and Environmental Safety* 92: 64–70. https://doi.org/10.1016/j.ecoenv.2013.02.008

Allen-Gill, S. M.; Martynov, V. G. 1995. Trace metals burdens in nine species of freshwater and anadromous fish from the Pechora River, Northern Russia, *Science of the Total Environment* 161: 653–659. https://doi.org/10.1016/0048-9697(95)93634-T

Bagdonas, E.; Vosylienė, M. Z. 2006. A study of toxicity and genotoxicity of copper, zinc and their mixture to rainbow trout (*Oncorhynchus mykiss*), *Biologija* 1: 8–13.

Baršienė, J.; Lazutka, J.; Syvokienė, J.; Dedonytė, V.; Rybakovas, A.; Bagdonas, E.; Bjornstad, A.; Andersen, OK. 2004. Analysis of micronuclei in blue mussels and fish from the Baltic and the North Seas, *Environmental Toxicology* 19(4): 365–371. https://doi.org/10.1002/tox.20031

Baršienė, J.; Dedonytė, V.; Rybakovas, A.; Andreikenaitė, L.; Andersen, OK. 2006. Investigation of micronuclei and other nuclear abnormalities in peripheral blood and kidney of marine fish treated with crude oil, *Aquatic Toxicology* 78S: S99–S104. https://doi.org/10.1016/j.aquatox.2006.02.022

Baršienė, J.; Butrimavičienė, L.; Grygiel, W.; Lang, T.; Michailovas, A.; Jackūnas, T. 2014. Environmental genotoxicity and cytotoxicity in flounder (*Platichthys flesus*), herring (*Clupea harengus*) and Atlantic cod (*Gadus morhua*) from chemical munitions dumping zones in the southern Baltic Sea, *Marine Environmental Research* 96: 56–67. https://doi.org/10.1016/j.marenvres.2013.08.012

Birceanu, O.; Chowdhury, M. J.; Gillis, P.; McGeer, J.; Wood, C. M.; Wilkie, M. P. 2008. Modes of metal toxicity, and impaired branchial ionoregulation in rainbow trout exposed to mixtures of Pb and Cd in soft water, *Aquatic Toxicology* 89: 222–231. https://doi.org/10.1016/j.aquatox.2008.07.007

Bury, N. R.; Walker, P. A.; Glover, C. N. 2003. Nutritive metal uptake in Teleost fish*, Journal of Experimental Biology* 206: 11–23. https://doi.org/10.1242/jeb.00068

Çavaş, T. 2008. In vivo genotoxicity of mercury chloride and lead acetate: micronucleus test on acridine orange stained fish cells, *Food and Chemical Toxicology* 46: 352–358. https://doi.org/10.1016/j.fct.2007.08.015

Crookes, M.; Brooke, D. 2011. *Introduction. In Estimation of fish bioconcentration factor (BCF) from depuration data*. Environment Agency. Environment Agency, Evidence. Horizon House, Deanery Road, Bristol, BS1 5AH. 11–12.

Directive 2008/105/EC of the European Parliament and of the Council of 496 16 December 2008 on environmental quality standards in the field of water 497 policy, amending and subsequently repealing Council Directives 82/176/EEC, 498 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 499 2000/60/EC of the European Parliament and of the Council. Official Journal of the European Communities L 500 348, 24/12/2008 p. 0084-0097.

Fenech, M.; Chang, W. P.; Kirsch-Volders, M.; Holland, N.; Bonassi, S.; Zeiger, E. 2003. Human MicronNucleus project. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures, *Mutation Research* 534: 65–75. https://doi.org/10.1016/S1383-5718(02)00249-8

Gomes, J. M. M.; Ribeiro H. J.; Procópio, M. S.; Alvarenga, B. M.; Castro, A. C. S.; Dutra, W. O., *et al.* 2015. What the erythrocytic nuclear alteration frequencies could tell us about genotoxicity and macrophage iron storage?, *PLoS ONE* 10(11): e0143029. https://doi.org/10.1371/journal.pone.0143029

Gobas, F. A. P. C.; Wolf, D.; Burkhard, W.; Verbruggen, L. P.; Plotzke, K., *et al.* 2009. Revisiting bioaccumulation criteria for POPs and PBT assessments, *Integrated Environmental Assessment and Management* 5: 624–637. https://doi.org/10.1897/IEAM_2008-089.1

Harabawy, A. S. A.; Mosleh, Y. Y. I. 2014. The role of vitamins A, C, E and selenium as antioxidants against genotoxicity and cytotoxicity of cadmium, copper, lead and zinc on erythrocytes of Nile tilapia, *Oreochromis niloticus, Ecotoxicology and Environmental Safety* 104: 28–35. https://doi.org/10.1016/j.ecoenv.2014.02.015

Hogstrand, C.; Haux, C. 1991. Mini review. Binding and detoxification of trace metals in lower invertebrates with references to metallothionin, *Comparative Biochemistry and Physiology* 100: 383–390.

ISO 15586:2003. Water quality – determination of trace elements using atomic absorption spectrometry with graphite furnace. Geneva, International Organization for Standardization.

Ivanciuc, T.; Ivanciuc, O.; Douglas, K. D. 2006. Modeling the bioconcentration factors and bioaccumulation factors of polychlorinated biphenyls with posetic quantitative superstructure/activity relationships (QSSAR), *Molecular Diversity* 10: 133–145. https://doi.org/10.1007/s11030-005-9003-3

Javed, M.; Ahmad, I.; Ahmad, A.; Usmani, N.; Ahmad, M. 2016. Studies on the alterations in haematological indices, micronuclei induction and pathological marker enzyme activities in *Channa punctatus* (spotted snakehead) perciformes, channidae exposed to thermal power plant effluent, *Springerplus* 5(1): 761. https://doi.org/10.1186/s40064-016-2478-9

Jezierska, B.; Witeska, M. 2001. *Metal toxicity to fish*. Siedlce, Poland: Wydawnictwo AP.

Jia, H.; Ren, H.; Satoh, S.; Endo, H.; Hayashi, T. 2005. Comparison of pretreatment condition of cadmium in fish sample and diet by microwave digestion method for ICP-AES, *Journal of the Tokyo University Marine Science and Technology* 1: 41–46.

Komjarova, I.; Blust, R. 2009. Multimetal Interactions between Cd, Cu, Ni, Pb, and Zn Uptake from Water in the Zebrafish *Danio rerio*, *Environmental Science & Technology* 43(19): 7225–7229. https://doi.org/10.1021/es900587r

Landis, W.; Sofield, R.; Yu, M. H.; Landis, W. G.; Sofield, R. M. 2011. *Introduction to environmental toxicology: molecular substructures to ecological landscapes.* 4th ed. Boca Raton, Florida: CRC Press. ISBN 9781439804100.

Lasheen, M. R.; Abdel-Gawad, F. Kh.; Alaneny, A. A.; Abd El bary, H. M. H. 2012. Fish as bio indicators in aquatic environmental pollution assessment: a case study in Abu-Rawash area, Egypt, *World Applied Sciences Journal* 19(2): 265–275.

Liebel, S.; Tomokake, M. E. M.; Oliveira Ribeiro, C. A. 2013. Fish histopathology as biomarker to evaluate water quality, *Ecotoxicology and Environmental Contamination* 8(2): 9–15. https://doi.org/10.5132/eec.2013.02.002

Luzhna, L.; Kathiria, P.; Kovalchuk, O. 2013. Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond, *Frontiers in Genetics* 4: 131. https://doi.org/10.3389/fgene.2013.00131

McCain, B. B. 1998. Uptake of aromatic chlorinated hydrocarbons by juvenile chinook salmon (*Oncorhynchus tshawytscha*) in an urban estuary, *Archives of Environmental Contamination and Toxicology* 19: 10–16. https://doi.org/10.1007/BF01059807

Mohammadnabizadeh, S.; Pourkhabbaz, A.; Afshari, R. 2014. Analysis and determination of trace metals (Nickel, Cadmium, Chromium, and Lead) in tissues of *Pampus argenteus* and *Platycephalus indicus* in the Hara Reserve, Iran, *Hindawi Publishing Corporation Journal of Toxicology*. https://doi.org/10.1155/2014/576496

Obiakor, M. O.; Okonkwo, J. C.; Ezeonyejiaku, C. D.; Ezenwelu, C. O. 2010. Genotoxicology: single and joint action of copper and zinc to *Synodontis clarias* and *Tilapia nilotica, Journal of Applied Sciences and Environmental Management* 14(3): 59–64. https://doi.org/10.4314/jasem.v14i3.61468

Palaniappan, P. L. R. M.; Karthikeyan, S. 2009. Bioaccumulation and depuration of chromium in the selected organs and whole body tissues of freshwater fish *Cirrhinus mrigala* individually and in binary solutions with nickel, *Journal of Environmental Sciences* 21: 229–236. https://doi.org/10.1016/S1001-0742(08)62256-1

Parkerton, T. F.; Arnot, J. A.; Weisbrod, A. V.; Russom, C.; Hoke, R. A; Woodburn, K.; Traas, T.; Bonnell, M.; Burkhard, L. P.; Lampi, M. A. 2008. Guidance for evaluating in vivo fish bioaccumulation data, *Integrated Environmental Assessment and Management* 4(2): 139–155. https://doi.org/10.1897/IEAM_2007-057.1

Ray, S. 1978. Bioaccumulation of lead in Atlantic salmon (*Salmo salar*), *Bulletin of Environmental Contamination and Toxicology* 19: 631–636. https://doi.org/10.1007/BF01685850

Roy, U. S.; Chattopadhyay, B.; Datta, S.; Mukhopadhyay, S. K. 2011. Metallothionein as a biomarker to assess the effects of pollution on Indian major carp species from wastewater-fed fishponds of East Calcutta wetlands (a Ramsar site), *Environmental Research, Engineering and Management* 4(58): 10–17.

Sauliutė, G.; Svecevičius, G. 2014. Sunkiųjų metalų biokoncentracijos potencialo atlantinės lašišos (*Salmo salar* L.) kūno audiniuose įvertinimas veikiant modeliniu mišiniu ir atskirais metalais, *17-osios Lietuvos jaunųjų mokslininkų konferencijos „Mokslas – Lietuvos ateitis“* straipsnių rinkinys, Vilnius, Technika. 228 p. 142–148.

Svecevičius, G.; Sauliutė, G.; Idzelis, R. L.; Grigelevičiūtė, J. 2014. Accumulation of heavy metals in different body tissues of Atlantic Salmon, *Salmo salar* L., exposed to a model mixture (Cu, Zn, Ni, Cr, Pb, Cd) and singly to nickel, chromium, and lead, *Bulletin of Environmental Contamination and Toxicology* 92: 440–445. https://doi.org/10.1007/s00128-014-1237-2

US EPA. 2009. National recommended water quality criteria. Office of Water, Office of Science and Technology (4304T) [online], [cited 10 October 2016]. Available from Internet: http://water.epa.gov/scitech/swguidance/standards/criteria/current/

Valavanidis, A.; Vlahogianni, T.; Dassenakis, M.; Scoullos, M. 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants, *Ecotoxicology and Environmental Safety* 64: 178–189. https://doi.org/10.1016/j.ecoenv.2005.03.013

Yousafzai, A.; M.; Chivers, D. P.; Khan, A. R.; Ahmad, I.; Siraj, M. 2010. Comparison of heavy metals burden in two freshwater fishes *Wallago attu* and *Labeo dyocheilus* with regard to their feeding habits in natural ecosystem, *Pakistan journal of zoology* 42(5): 537–544.

Zhu, Y.; Wang, J.; Bai, Y.; Zhang, R. 2004. Cadmium, chromium, and copper induce polychromatocyte micronuclei in carp (*Cyprinus carpio* L.), *Bulletin of Environmental Contamination and Toxicology* 72(1): 78–86. https://doi.org/10.1007/s00128-003-0243-6