# RESPONSE OF OXIDATIVE STRESS AND NEUROTOXICITY BIOMARKER IN RAINBOW TROUT (*Oncorhynchus mykiss*) AFTER EXPOSURE TO SIX-METALS MIXTURES

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Heavy metals toxicity to aquatic organisms includes their ability to induce oxidative stress due to an imbalance between the production of reactive oxygen species and the activity of antioxidant defence system. Catalase (CAT) is one of the most important enzymes in the antioxidant defence system, which protects organisms from oxidative stress. Acetylcholinesterase (AChE) is a key enzyme in the nervous system and its activity is a widely used as biomarker of neurotoxicity in various organisms after different metals exposure [1, 2, 3]. Adverse effects of metals on the structure or function of the organism 's central or peripheral nervous system is described [4, 5], but it is still lacking information on the toxicity effects of metals mixtures.

The aim of this study was to assess two different biochemical parameters – liver CAT and brain AChE activity of rainbow trout (*Oncorhynchus mykiss*) after exposure for 7 and 14-days with: 1) six metals (Zn – 0.1, Cu – 0.01, Ni – 0.034, Cr – 0.01, Pb – 0.014 and Cd – 0.0015 mg/L) mixture (metals in a mixture were at maximum permissible concentrations (MPC) (2013/39/EB; 2008/105/EB)) and 2) the same six metals mixtures, with reduced concentration of Cu and Cr ions for 10 times, respectively.

The experimental treatment was conducted on one-year-old O. *mykiss*, acclimated fish were exposed for 7 and 14-days with six metals mixtures. After exposure rainbow trouts were dissected, brain and liver tissues for biochemical analysis were collected. Later in the laboratory the brain and liver tissues were homogenized and centrifuged for 20 min. at 15 000× g (4 °C). The supernatants were used immediately and were frozen at −80 ◦C for CAT and AChE assays, respectively. Total protein concentrations were determined according to the Bradford method [6] using bovine albumin as the standard. CAT activity was determined according to the method of Aebi [7] with minor modifications. CAT activity was calculated as μmol $H_2O_2$ decomposed/min/mg protein. AChE was determined using a modified Ellman method [8]. AChE activity was expressed in nmol of hydrolysed acetylthiocholine iodide/min/mg protein.

Statistically significant increase of catalase and acetylcholine esterase activity (26 % and 29 % respectively) was observed in rainbow trout (*Oncorhynchus mykiss*) after 7-days exposure to six-metals mixture at MPC. However, after 14-days treatment, neither CAT nor AChE did not show significant response to this mixture exposure. Six metals mixtures, where concentrations of Cu or Cr were reduced 10 times, inhibited liver catalase activity in the rainbow trout after 7 days exposure (20% for Cu and 22% for Cr, respectively). After 14-days treatment CAT did not showed significant response. The levels of AChE activity after 7 and 14 days of exposure, with reduced Cu or Cr ions concentrations in a mixture, did not vary significantly in comparison to control groups.

The results of present work suggest, that exposure for 7 and 14-days with six metals (Zn, Cu, Ni, Cr, Pb and Cd, metals are at MPC) mixture and with mixtures where Cu or Cr ions were reduced for 10 times, induce oxidative stress and neurotoxicity as metal's toxicity response in *O. mykiss* tissues. It's known that fish utilize enzymatic defence and cells are protected by an interacting antioxidant enzyme against oxidative stress [2, 3, 5]. In addition, the results provide evidence that enzymic biomarkers of oxidative stress and neurotoxicity can be sensitive indicators of aquatic pollution.

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