

HAEMOTOLOGICAL AND BIOCHEMICAL INDICES IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AFTER 4, 7- AND 14-DAYS EXPOSURE WITH METALS MIXTURE

Eglė Stasiūnaitė^{2*}, Brigita Čapukoitienė¹, Reda Eglinskaitė¹, Milda Stankevičiūtė¹, Tomas Makaras¹, Laura Butrimavičienė¹

¹ Nature Research Centre, Institute of Ecology, Akademijos Str. 2, LT-08412 Vilnius, Lithuania

² Vilnius University, Life Sciences Center, Institute of Biosciences, Saulėtekio av. 7, LT-10257 Vilnius, Lithuania
egle.stasiunaite96@gmail.com

Chemical agents can able to induce changes in the various haematological components [1]. Estimation of haematology and biochemical parameters (determination of glucose concentration in blood) is used as an index of fish health status in a number of fish species to detect physiological changes following variety of stressors [2, 3, 4]. Furthermore, non-destructive haematological technique and changes in the proportion of blood parameters may be indicative and sensitive biomarker of the biological effects of metals in fish [5].

The aim of this study was to analyse the changes in haematological and biochemical parameters in Rainbow trout's (*Oncorhynchus mykiss*) peripheral blood after exposure with metals (Zn – 0.1, Cu – 0.01, Ni – 0.034, Cr – 0.01, Pb – 0.014 and Cd – 0.0015 mg/L (2013/39/EB; 2008/105/EB)) mixture at various time points (4, 7 and 14 days). Haematological biomarkers were assessed as differentiation of leukocytes and the relative abundance of thrombocytes and different types of leukocytes in fish blood and the measurement of blood haematocrit levels. Biochemical indices – assessed concentration of glucose in *O. mykiss* blood.

O. mykiss peripheral blood samples were taken from fish caudal vein with syringes (rinsed with 3.8% sodium citrate solution). The differential count of leukocytes and thrombocytes was performed by microscopy at a magnification of ×1,000 (Olympus BX51, Japan) in Giemsa-stained peripheral blood samples. Differentiation of leukocytes and the relative abundance of analysed cell types was determined by counting a total of 300 leukocytes on each slide [4]. Haematocrit levels were determined directly by microhematocrit centrifugation. Concentrations of glucose in fish blood were determined by using the automatic Glucose Analyser (EKSAN-Gm, ANALITA, Joint-Stock Company Ltd, LITHUANIA). The minimum detection limit of the blood glucose method is from 2 to 30 mmol/L and the error for repeated measurements (precision) is ≤5%. Minimal blood sample volume per measurement is 50 µL.

Statistically significant induction of thrombocytes was estimated in all exposure groups of treated *O. mykiss*. The number of thrombocytes in fish from 7-days group was 5 times higher, while after 14 days treatment there was found only 4 times higher induction in comparison to control. In all exposure groups there was observed two times higher elevation of neutrophils number. Moreover, induction in neutrophils (neutrophilia) and monocytes after 7 days exposure differed significantly in comparison to control. The levels of haematocrit and concentrations of glucose did not vary significantly between exposure groups and in comparison, to control.

Based on the presented results it can be concluded that chemical stress (exposure with six metals mixture) induces both thrombocytosis and neutrophilia in fish. The measuring of haematological parameters in fish is useful biomarker and was performed in wide range of fish species after exposure to different trace metals [5, 6, 7]. The understanding of toxicant uptake, behaviour and responses in fish may, therefore, have a high ecological relevance and pollution risk prediction.

-
- [1] J.C. Bloom, J.T. Brandt, Toxic responses of the blood. In: Casarett, L.J., Klaassen, C.D. (Eds.), Casarett and Doull's, Toxicology: The Basic Science of Poisons, 7th ed. McGraw-Hill Medical, New York, 455–484 (2008).
- [2] F. Ejraei, M. Ghiasi, H. Khara, Evaluation of hematological and plasma indices in grass carp, *Ctenopharyngodon idella*, with reference to age, sex, and hormonal treatment – Arch. Pol. Fish, 23, 163-170 (2015).
- [3] P. Satheeshkumar & G. Ananthan & D. Senthil Kumar & L. Jagadeesan, Haematology and biochemical parameters of different feeding behaviour of teleost fishes from Vellar estuary, India, Comp Clin Pathol DOI 10.1007/s00580-011-1259-7, (2011).
- [4] F. Fazio, V. Ferrantelli, G. Piccione, C. Saoca, M. Levanti, M. Mucciardi, Biochemical and hematological parameters in European sea bass. (*Dicentrarchus labrax* Linnaeus, 1758) and Gilthead sea bream (*Sparus aurata* Linnaeus, 1758) in relation to temperature, Vet. arhiv 88, 397-411, (2018).
- [5] R. Maheswaran, A. Devapaul, S. Muralidharan, B. Velmurugan, S. Ignacimuthu, Haematological studies of fresh water fish, *Clarias batrachus* (L.) exposed to mercuric chloride, International Journal of Integrative Biology IJIB, Vol. 2, No. 1, 49 (2008).
- [6] S. F. Alwan, A. A. Hadi, A. E. Shokr* Garyounis, Alterations in Hematological Parameters of Fresh Water Fish, *Tilapia zillii*, Exposed to Aluminum, University Press Journal of Science and Its Applications Vol. 3, No. 1, 12-19, April (2009).
- [7] Sheeba Ali Siddiqui, C. M. Noorjahan, Toxicity of copper nanoparticle on haematology and biochemistry of fish, *tilapia mossambica*. International research journal of pharmacy, Int. Res. J. Pharm, 2018, 9 (10).