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COMPLEX EFFECT OF ROUNDUP AND HEATING ON BIVALVE MOLLUSK *UNIO TUMIDUS* UTILIZING *IN VIVO* AND *EX VIVO* APPROACHES

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Glyphosate, the modified amino acid and organophosphonate compound, N-(phosphonomethyl)glycine, is the most sprayed and most used herbicide in the world [1]. Whereas it acts as the specific inhibitor of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, intrinsic for the plant and bacteria, blocking the synthesis of aromatic compounds in them, it was originally marketed as absolutely non-toxic for the animals [4]. However, during the experience of its application, the understanding of its by-side effects on the non-targeted organisms were elucidated. In particular, it causes the loss of the immune defense of the animals and human due to the impact on the symbiotic microorganisms [1, 4]. Moreover, the commercial formulation of Glyphosate, namely Roundup, has strong chelating properties, which causes the immobilization of mineral nutrients by the organisms. Additionally, it was found to inhibit the cytochrome P450 enzymes. Plural effects of Glyphosate and its commercial formulations on the reproductive system, developmental abnormalities and carcinogenic effects in the non-targeted organisms were reported. However, the biochemical responses that serve for the early warning of ecotoxicity, are less known. In the realistic environment, the organisms are subjected to the complex exposures of different pollutants from non-pointed sources and deviations of temperature. The contribution of the heating is of particular danger for the ectothermic organisms. Therefore, the objective of this study was to evaluate the impact of complex exposure to Roundup and other confounding factors, namely pharmaceutics and heating, on the

bivalve mollusk *Unio tumidus*, with the utilizing of environmentally realistic conditions of exposure. The mussels, collected in the reference forestry area, were exposed to Roundup (33.8 μg L^{-1}), non-steroidal anti-inflammatory drug diclofenac (voltaren) (600 ng L^{-1}), or cardiac drug Ca-channel blocker nifedipine (700 ng L^{-1}) separately at the temperature 18° C and jointly at the temperatures 18° C and 25° C during 14 days. The utilized concentrations were correspondent to the levels indicated in the effluents of the municipal sewage treatment plants and/or freshwaters. In the *ex vivo* exposures, the samples of the digestive gland of the mollusks from the reference site were placed into experimental solutions of Roundup in Ringer's solution (pH 7.4) at the concentrations 13.3, 26.7, 66.8 and 133.6 μg L^{-1} during 2 h at 20° C followed by 15 h at ~2-4° C. The biochemical responses of stress and toxicity and metabolic characteristics were evaluated in the digestive gland. The metallothioneins from digestive gland were eluted chromatographically as thermostable proteins (only in the *in vivo* exposures) and assessed quantitative from their metal composition (Zn, Cu, Cd) and thiols (MT-SH). Lysosomal membrane stability was determined in the hemocytes (*in vivo*) or in gills (*ex vivo*).

It was shown the significant changes in the oxidative stress parameters under the exposures to Roundup, nifedipine and combine exposures, whereas the effect of diclofenac was lesser. The highest pro-oxidant shift was caused by the co-exposure to pollutants and heating with the depletion of the integral index of oxidative stress from 1 to about 0.1 relative unit. This down-regulation derived from the decrease of the total superoxide dismutase activity, the increase of the level of lipid peroxidation detected as the TBARS and the concentration of the protein carbonyls. Concentration of glutathione (GSH and GSSG) increased in most exposures, but redox index of GSH (GSH/GSSG ratio) decreased. The concentration of MT-SH increased in all exposures, particularly by Roundup and co-exposures (by 26-48%), detecting the stressful impacts. The level of metalated metallothionein dropped by Roundup (by 41%) indicating the loss of the metal-buffering ability of this protein that can lead to the disturbed distribution of these metals within the cells. The metal composition of metallothionein was also changed by the exposures with the increase

of the part of Cu and Cd.

Roundup and co-exposures provided the anaerobic shift and increased the redox state in the digestive gland, whereas nifedipine caused the opposite shift, particularly due to the increased level of pyruvate, while the level of lactate was rather stable. The reducing of the vitality (indicated as lysosomal membrane stability in hemocytes) was observed in all nifedipine-contained exposures (by ~27%) but was not caused by Roundup alone. The depletion of cholinesterase is the typical response to the organophosphorus compound. Indeed, this response was observed in the exposures, contained Roundup, except the co-exposure with heating. It is worth of mention, most of the indexes were not affected by the exposure to diclofenac. However, the level of cellular thiols was changed in this exposure. These results indicated that the impact of glyphosate is specific, particularly strong and evident both in the single and combine exposures. In general, co-exposure to selected substances caused mostly synergetic interaction at the 18^{o} C. However, most of the responses to combine exposure were distorted under the heating.

In the *ex vivo* exposure, the lowest concentrations of Roundup caused the most prominent changes of the indices: the decrease of metallothionein concentration (by ~ two times) and cholinesterase activity, whereas the level of GSH was decreased by the higher concentrations of Roundup. However, the levels of TBARS and protein carbonyls were not changed detecting the early stage of the injury in this acute experiment.

To summarizing, this study allows us to detect both earlier and long-term biological effects of Roundup in the low environmentally realistic concentrations. The co-exposures to the adverse effects could cause inconsistent and unpredictable consequences of the responses of stress and toxicity in the bivalve mollusks. The developing of the integrative indexes can be a prospective approach in the evaluation of the severity of the environmentally realistic impact of mixed chemical and thermal pollution of the surface waters

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ОСОБЛИВОСТІ ЛІПІДНОГО СКЛАДУ НИРОК КОРОПА ТА ЩУКИ ЗА ВПЛИВУ ПІДВИЩЕНИХ КОНЦЕНТРАЦІЙ ІОНІВ Fe^{3+}

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Ферум є одним з найбільш поширених елементів у земній корі, але через низьку міграційну здатність концентрація металу в природних водах дуже мала і його прийнято відносити до числа