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EVALUATION OF CELLULAR STRESS RESPONSE IN BIVALVE MOLLUSK *U. TUMIDUS* EXPOSED TO MIXTURE OF WATERBORNE PHARMACEUTICALS AND GLYPHOSATE-BASED HERBICIDE

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The common use of different xenobiotics, such as pharmaceuticals, personal care products, pesticides, metals and their combination with heating waves has increased the outpouring of emerging contaminants into wastewater. It is well-known that pharmaceuticals constitute a significant class of aquatic contaminants and can seriously threaten the health of aquatic organism [3]. In addition, eliminating these chemicals can pose a challenge due to their typically low concentrations, their poor degradation, higher consumption rates and the inability to be removed by the conventional sewage treatment plants. Analysis of different pharmaceutical categories in the influent revealed that non-steroidal anti-inflammatory drugs (NSAIDs) were the predominant category. Among them, diclofenac (Dcf) is listed as emerging pollutants in surface water in Europe according to the recent Directive 2020/1161 EU and also considered as one of the most frequently reported contaminants of emerging concern in Ukraine [5]. However, there has been limited research on the combined effects of Dcf along with other year-round use organic compounds, for instance, pharmaceuticals or/and pesticides that may leads to the creation of a novel potential contaminant.

Bivalves, a diverse group of mollusks, have recently gained

significant importance in various biological and ecotoxicological research fields because of their unique life history traits: filter feeding capacity, limited mobility and their sedentary lifestyle, etc. It makes them good candidates as promising model organisms for biomonitoring environmental health hazards in aquatic ecosystems, especially in the monitoring of pharmaceuticals to observe the changes in the concentration and distribution of these compounds [4]. Furthermore, environmental conditions, such as temperature, can impact both the chemical and biological characteristics of pharmaceuticals, including their metabolism and toxicity [2]. Therefore, our research is focused on the evaluation of cellular stress response in freshwater bivalve mollusk exposed to mixture of waterborne pharmaceuticals and glyphosate-based herbicide.

Thus, in our study we used model xenobiotics to elucidate the characteristics of the cellular stress response in concentration close to environmentally realistic conditions of exposure and were correspondent to the levels indicated in the effluents of the municipal sewage treatment plants. With this aim we treated the bivalve mollusk *Unio tumidus* (Philipsson, 1788) with the mixture containing of diclofenac (Dcf, Diclofenac-Darnitsa, 2 nM), a dihydropyridine calcium channel blocker nifedipine (Nf, Nifedipinum Retard-Darnitsa, 2 nM) and herbicide Roundup with active ingredient glyphosate (Rn, formulation Roundup MAX, Monsanto, USA, 33.8 μg L^{-1},corresponding to 80 nM of glyphosate) separately and in a mixture at 18°C (Mix) and 25°C (MixT) during 14 days.

The Integrated Biomarker Index (IBR) was calculated according to the indices of antioxidant defense system (superoxide dismutase (SOD) and catalase (CAT) activities, the level of reduced/oxidized glutathione (GSH/GSSG), the content of lipid peroxidation (TBARS) and protein carbonyls (PC)), biomarkers of toxicity (lysosomal membrane stability (NRR), caspase-3 (Cas-3) and cathepsine D (lysosomal, CtDL, and its efflux, CtDe) activities, metallothionein (MTSH) level, metals content and their ration Zn/Cu [1]: IBR = (SOD*TBARS + TBARS*PC + PC*GSH + GSH*GSSG + GSSG*MTSH + MTSH*NRR + NRR*CtDl + CtDl*CtDe + CtDe*Cas3+Cas3*Zn/Cu + Zn/Cu*SOD)/2.

The obtained data show that the response to the mixture at 18°C (Mix) is more similar to the action of the individual pharmaceuticals

(Dcf and Nf), while the general responses to the effect of the mixture at 25°C corresponds to the Rn impact, mainly due to the ratio of Zn/Cu in the MixT-group and the MT reaction in Rn-group. However, both parameters are related to the metabolism of essential metals zinc and copper in the tissue.

According to the results of group identification using Canonical Discriminant Analysis, it is observed that discriminant variables of Rn-, Mix- and MixT-groups are well separated from the set of C-, Dcf- and Nf-groups. In addition, the last three groups are also separated within the used parameter set by their centroids located along the Function 2 axis (-5.240 (C), -3.744 (Dcf) and -2.782 (Nf)). Therefore, according to the sum of the determined indices, the action of Dcf and Nf has the least effect on the cellular response of the mollusk, while the Rn- and MixT-exposed groups, especially at 25ºC, are clearly localized at a considerable distance from the others. This distribution demonstrates the predominant role of the effect of 80 nM glyphosate in the mixture and the presence of a cumulative effect, especially for MixT-group at 25ºC, which enhances the effect of individual components.

In conclusion, the complex impact of pharmaceuticals/herbicide mixture and heating caused some specific effects that are more severe and opposite to those indicated in all other individual exposures.

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List of references

1. Beliaeff B., Burgeot T. Integrated biomarker response: a useful tool for ecological risk assessment. *Environmental Toxicology and Chemistry.* 2022. Vol. 21(6), P. 1316–1322.
2. Fabbri E., Franzellitti S. Human pharmaceuticals in the marine environment: Focus on exposure and biological effects in animal species. *Environmental Toxicology and Chemistry*. 2016. Vol. 35(4), P. 799-812.
3. Hejna M., Kapuścińska D., Aksmann A. Pharmaceuticals in the aquatic environment: a review on eco-toxicology and the remediation potential of algae. *International Journal of Environmental Research and Public Health*. 2022. Vol. 19(13), P. 7717.

4. Khan B., Ho K.T., Burgess R.M. Application of biomarker tools using bivalve models toward the development of adverse outcome pathways for contaminants of emerging concern. *Environmental Toxicology and Chemistry*. 2020. Vol. 39(8), P. 1472–1484.
5. Vystavna Y., Frkova Z., Celle-Jeanton H., Diadin D., Huneau F., Steinmann M., Crini N., Loup C. Priority substances and emerging pollutants in urban rivers in Ukraine: Occurrence, fluxes and loading to transboundary European Union watersheds. *Science of the Total Environment.* 2018. Vol. 637–638, P. 1358–1362.

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THE INFLUENCE OF GERMINATION ACTIVATION ON THE CHANGE OF THE PROOXIDANT-ANTIOXIDANT BALANCE IN THE TISSUES OF LILIOPSIDA

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Relevance of research. The research of changes in the state of the components of the pro-oxidant-antioxidant system (PAS) that initiate the process of seed germination opens the prospect of the possibility of regulating and correcting this stage of plant ontogenesis, increasing the germination and friendliness of crops, is especially relevant and economically justified in the conditions of intensification of crop production [1-3].

Aim of the research: to identify changes in the value of indicators of the state of the prooxidant-antioxidant system (PAS) in the tissues of monocotyledonous plants at rest and the initiation of its germination processes..

Methodology. Quantitative determination of indicators of the state of PAS was performed on tissue samples of seeds of the following plants: *Panicum miliaceum L., Oryza sativa L., Avena sativa L., Zea mays L., Hordeum vulgare L., Triticum durum Desf.* The concentration of superoxide anion radical ($\bullet O_2^-$), TBA-active