UDC: 502/504:57(477.81) 577.47: 504.054

ACCUMULATION OF MICROPLASTIC AND ITS IMPACT ON THE RESPONSES TO PHARMACEUTICAL IN THE BIVALVE MOLLUSC *UNIO TUMIDUS*

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Microplastic (MP) is one of the most persistent pollutants in the surface waters. MP refers to plastic particles smaller than 5 mm that are formed after large plastic wastes enter the aquatic environmental and are destroyed by environmental exposure, physical destruction and biodegradation [1]. Importantly, 70–80% of the total number of MPs found in the marine environment enter the environment from freshwater rivers, however, the freshwater pollution by MP is studied lesser [2]. MP enter primary freshwater ecosystems through untreated sewage and agricultural and storm water discharges flows. Importantly, sorption of some novel pollutants, such as pharmaceuticals on these particles with a large total surface area, for which treatment facilities are also ineffective, is especially dangerous. Bivalves as filters have highly developed processes of intracellular internalization of MP [3,4]. However, they are able to excrete a significant amount of MP *in vivo* in the form of pseudofaeces, therefore the utilizing of bivalves in the bioindication of MP is discussed.

The aim of this study was to elucidate the ability of freshwater bivalve mollusk to accumulate microplastic from the environment and assess its own toxicity and its impact on the responses to other chemical contaminants. To our knowledge, the information concerning the MP level in the freshwaters and in molluscs in Ukraine is lacking generally.

For this study, the specimens of bivalve mollusks *Unio tumidus* were collected from two populations: in pristine (Pr) and contaminated

(Ct) sites. The specimens were depurated during 7 days and exposed to microplastic PET particles (M, 1 mg L^{-1}, obtained from the powder which was sieved with a standard mesh of size < 0.5 mm), ibuprofen of pharmaceutical quality (IBU, PJSC SIC Borshchahivskiy CPP, M01A E01, 0.8 μg L^{-1}), or with their combination (Mix) for 14 days. Untreated mussels from both sites (PrC and CtC) were also examined after the same time of being in the laboratory tanks. The specimens were collected and water was replaced every 2 days with the renewing of MP content. The duration of exposure was 14 days. The molluscs from Pr-area exposed to MP, were examined each two days for the accumulation of MP. All exposed groups were examined after 14 days of exposure to determine the biomarkers of toxicity. Additionally, the molluscs from the contaminated site were analyzed in the day of sampling to determine the concentration of microplastic in the organism. The amount of MP in the water was calculated after filtering it and coloring the existing particles. To estimate the number of particles in each individual tissue sample, we used an alkali-based potassium hydroxide/hydrogen peroxide digestion method that provide the digestion of any residual bioorganic material. The amount of MP in water was calculated after filtering, staining and counting the number of particles. The filters with MP particles obtained from the tissues and the samples of water were stained with Nile red (9-diethylamino-5H-genso [alpha] phenoxazin-5-one) and examined under optical microscope with additional UV (395 - 405 nm) illumination. The specimens were collected and water was replaced every 2 days with the renewing of MP content. Among the biomarkers, the enzymes of apoptosis, metallothionein functionality and redox state (NADH/NAD; GSH/GSSG levels) in the tissues of digestive gland were analyzed.

During the exposure, the number of MP in the tissues of molluscs and in the water was changed simultaneously with the opposite regularity. The number of particles in the tissues was negligible at the 0 day and increased sharply until the 2-th day. Generally, during the 14-th days exposure, it was indicated a *bell-shaped* response curve for MP accumulation with maximum correspondent to 15.62 items/g FW at 10-th day. The maximum accumulation of MP relating the length of molluscs was also detected

at 8-th-10-th days comparing to the start of exposure. However, it remained elevated at the 14-th day. In the experimental tank, the number of particles was changed in the limits of 590 -790 items L^{-1}, decreasing from the initial level of 2-th day by 1.3 times at the 10-th day of exposure but returned to the level of 2-th day till the 14-th day. Correspondingly, the most prominent accumulation of MP from water was detected at the 10-th day of exposure. The dynamic of the accumulation of MP in the soft tissues (in relation to total in tissues) and in the water had significant negative correlation (r = -0,799, $P < 0.05$).

The MP concentration in the soft tissues of field specimens from polluted area was higher than in the depurated molluscs, and the level of MP in the river water was about 103 items L^{-1}. When we compared the ability of molluscs to accumulate the MP from the water (number of items per g tissues/number of paticles per L of water), its higher effectivity was shown for the field group (~75%). In the short experimental exposure of the molluscs from the reference site, this function was also rather high (~55% after 10 days of exposure). Moreover, the depurated molluscs from the reference area were not cleaned totally from the MP and indicated the presence of these particles in the body at the 0 day of exposure despite their almost total absent in the water.

The comparison of two control and exposed groups after 14 days of exposure indicated that the PrC and CtC groups showed remarkable differences, with lower levels of metallothionein protein (MTSH), NADH and NAD^+, but higher levels of GSH, GSSG, caspase-3 and cathepsin D (CTD) in the CtC group. These data indicate a chronic stress impact in the Ct population and could be particularly related to chronic pollution by MP. Under exposures, we found an almost common strategy in both populations for NAD^+/NADH and MTSH suppression and CTD induction. Additionally, IBU did not change GSH in both populations. However, the typical response to IBU – the suppression of caspase-3 – was indicated only in PrIBU- and PrMix-mollusks. CTD efflux increased dramatically only in PrMP- and PrMix- groups. According to discriminant analysis, exposed Pr-groups were highly differentiated from control, whereas Ct-groups had common localization demonstrating high resistance to environmental stress.

These data indicate the high accumulative ability of *U. tumidus* and attract the attention to the utilizing of this species in the biomonitoring of pollution by MP and for the depuration of surface waters. Collectively, this study suggested that the accumulation of MP *per se* in molluscs did not cause particular toxicity during the sub-chronic exposure. However, it substantially distort the particular effects of ibuprofen in the specimens from the reference site that are not well adapted to the chronic pollution.

This work has been granted to Oksana Stoliar by the Ministry of Education and Science of Ukraine to (Projects No M-70/2021 and No M-13/2022 under the French-Ukrainian Cooperation Program) and the Avard of visiting professor of the University of Messina (Italy).

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