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BIOCHEMICAL RESPONSES OF THE *DREISSENA POLYMORPHA* FROM MUNICIPAL POND TO CAFFEINE, MICROPLASTICS, AND HEATING IN SINGLE AND COMBINED EXPOSURES

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Background. Pharmaceuticals have become the aquatic pollutants of growing concern. Caffeine is one of the most widely distributed in the surface waters among them. However, the environmentally relevant models of its effect, which include combined exposures with probable confounding factors, are unknown. Microplastics are a suspected vector that influences caffeine bioavailability. The temperature dependence of response, considering the increase of temperature in surface waters, can also be anticipated. The aim of this study was to analyze the input of caffeine, microplastics and elevated temperature into their combined effect on the zebra mussel *Dreissena polymorpha* (Pallas, 1771).

Materials and Methods. Molluscs were exposed to caffeine (Caf, 20.0 μg·L^{-1}), microplastics (MP, 1 mg·L^{-1}, 2 μm in size), or elevated temperature (T, 25 °C) in the single and combined (Mix- and MixT-) exposures for 14 days. The concentrations of metallothioneins, metallothioneins-bound zinc, total Zn and Cu concentration in the tissue, total glutathione level, antioxidant (superoxide dismutase) and metabolic (citrate synthase) enzymes activities, acid phosphatase activity as the lysosomal functionality marker were determined.

Results and Discussion. The decrease in Zn/Cu concentrations ratio in the soft tissues shared the common response in all exposures, reflecting the metal imbalance as the most sensitive marker. The MP-group was distinguished by the decrease in the levels of total Zn and extra lysosomal acid phosphatase activity, proving injury of Zn

transportation and Zn-related enzyme activities. All other exposures (T-, Mix-, MixT-) caused citrate synthase and superoxide dismutase activation. Caf-related groups demonstrated the elevation of the levels of phosphatase lysosomal membrane-linked latency, metallothionein total protein and its apo-form. However, glutathione level was stable in all exposures.

Conclusion. These data revealed the adverse effect of MP and shared beneficial effects in the exposures that involved caffeine, which can be explained by the antioxidant activity of this substance. Exposure to elevated temperature partially alleviated the effect of caffeine in the mixture. Thus, the results indicate the importance of multi-stress exposures modeling, which allows the evaluation of environmentally realistic responces of an organism to xenobiotics.

Keywords: zebra mussel, pharmaceuticals, zinc, copper, acid phosphatase, oxidative stress

INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is an alkaloid that is naturally present in more than 80 plant species and is known as one of the most consumed psychoactive drugs because it acts as an adenosine antagonist and affects psychomotor functions and sleep-wake regulation (Cruz *et al.*, 2016). Due to high water solubility (13.0 $g \cdot L^{-1}$), relatively high stability and slow pace of degradation under different environmental conditions, caffeine has been well-accepted as one of the most ubiquitous pharmaceutically active compounds in the natural environment (Lia *et al.*, 2020). Commonly, concentrations of caffeine in surface waters typically lay below 1 $\mu g \cdot L^{-1}$, with a maximum concentration in Europe of 59.5 $\mu g \cdot L^{-1}$ (Li *et al.*, 2020; Wilkinson *et al.*, 2022). A few known studies provide sometimes contradictive results on the effects of caffeine on aquatic habitants (Aguirre-Martinez *et al.*, 2013; Cruz *et al.*, 2016). In the higher vertebrates, the antioxidant effect of caffeine and its major metabolites, 1-methylxanthine and methyluric acid (Barcelos *et al.*, 2014) and the inhibition of the activity of NF-kB, which plays a key role in regulating the immune response to infection (Aguirre-Martinez *et al.*, 2013), were indicated. However, the studies of aquatic invertebrates indicate the damage to DNA and lysosome integrity, increased lipid peroxidation and biotransformation activity in crab *Carcinus maenas* (Aguirre-Martinez *et al.*, 2013), and an increase in the antioxidant and biotransformation enzymes activity in clams (Cruz *et al.*, 2016). Lysosomal injury was the most frequent manifestation in these studies (Aguirre-Martinez *et al.*, 2013; Cruz *et al.*, 2016). Consequently, these studies need more substantial clarification of the molecular targets of caffeine and suitable bioindicators, taking into accout environmentally relevant concentrations and combined effects.

Plastics of all sizes have become the main form of surface water litter. Microplastics (MP, particles less than 5 mm) are reported to account for 92.4 % of marine plastic debris (Li *et al.*, 2019; Martinho *et al.*, 2022). The primary sources of MP are freshwaters (Lasee *et al.*, 2017; Scherer *et al.*, 2020). One of the most well-studied effect of MP on aquatic species is the oxidative stress response (Alomar *et al.*, 2017; Magni *et al.*, 2018; Sewwandi *et al.*, 2022). However, the MP potential ecotoxicity cannot be restricted by its effect *per se*. MP can adsorb hydrophobic contaminants from water, increase their environmental sustainability and is a suspected vector that influences their bioavailability

(Li *et al.*, 2019; Martinho *et al.*, 2022). Particularly, due to the intensive usage of MP and caffeine, they are expected to co-occur in wastewater (Alfaro-Núñez *et al.*, 2021), but research on vector transportation of caffeine associated with MP is still in its infancy (Sewwandi *et al.*, 2022). Zebra mussel *Dreissena polymorpha* (Pallas, 1771) is of exceptional interest as the prospective bioindicator of MP due to its extensive filter-feeding activity (Li *et al.*, 2019).

Water temperature is an important environmental factor for ectotherm aquatic organisms that affects their physiological state (de Souza *et al.*, 2013). Seasonal temperature extremes in shallow freshwater habitats are ordinary phenomena, and are assumed to increase due to global climate change (Intergovernmental Panel for Global Climate Change (IPCC), 2013). In recent years, the understanding of the cumulative impacts of anthropogenic activities was evolved in a complex scenario of multiple stressors (De Marchi *et al.*, 2022).

Therefore, to reflect the environmental multi-stress reality, the aim of this study was to analyze the input of caffeine, microplastics and elevated temperature into their combined effect on the *D. polymorpha*. We tried to adjust the environmentally realistic conditions of sub-chronic exposure according to the concentrations and size (for MP) of the substances utilizing the mussels from the municipal pond that are chronically subjected to the complex micropollutants effect. Using *D. polymorpha* as a well-approved model organism (Farkas *et al.*, 2017), we determined Zn and Cu accumulation in the tissues and the level of low-molecular weight metal chaperones and redox-active thiols glutathione (GSH) and metallothioneins (MTs). The general cell response and cytotoxicity were assessed by determining the antioxidant activity (superoxide dismutase, SOD), metabolic status (citrate synthase, CS), and acid phosphatase (total AcP (AcPt) and lysosomal membrane-linked latency of phosphatases (L)) activities. This spectrum of methods was expected to select the valuable biomarkers for the specific and multi-stress exposures.

MATERIALS AND METHODS

Methodology used is given in detail in Supplementary information (SI 1)

Chemicals. All reagents were of the Reagent grade or higher (S1 Appendix). They were obtained from Sigma-Aldrich (USA) or the Synbias (Ukraine).

Detailed information on exposures and assay characteristics is given in the Supplement available via the Mendeley Data by the following link:

http://dx.doi.org/10.17632/mh4fhd5tw3.1

Study area and experimental groups. The specimens of zebra mussels, *Dreissena polymorpha* (Pallas, 1771) (2.5–3 cm in length and 1.83–2.67 g weight in average) were sampled from a site in the upstream portion of the Seret River (the tributary of the Dnister, West of Ukraine) at the pond of the city of Ternopil (49° 33′ 12.6576″ N and 25° 35′ 41.1612″ E) in August, 2021. About 600 specimens were transported to the laboratory in 25 L cages with aerated native water and then acclimated to the laboratory conditions for up to seven days in 80 L tanks filled with aerated, dechlorinated, and softened tap water. Molluscs were fed 500 mg of Tropical SuperVit Basic containing beta-1.3/1.6-glucan twice a week.

After the acclimation period, mollusks were distributed randomly to six groups. We treated mollusks with microplastics (MP-group, 1 mg·L^{-1}, pore size 2 μm), caffeine of

pharmaceutical quality (Caf-group, 20.0 μg·L^{-1}), their combination (Mix-group), elevated temperature (T-group, 25 °C) or a combination of microplastics, caffeine and elevated temperature (MixT-group) for 14 days. Untreated mollusks were also examined after the same length of time of being in the laboratory tanks. Two replicates per group were used (50 specimens per 25 L tank). The mortality during the exposure was: 13.67 % of the average number of individuals, in particular, the highest mortality was observed under exposure to T (26 %), MixT (21.5 %), and Mix (15.5 %), and to a lesser degree – in exposures to Caf (11.5 %) and MP (7.5 %).

The concentration of MP and its size corresponded to the typical MP characteristics in freshwater (Lasee *et al.*, 2017; Scherer *et al.*, 2020), and the time of exposure correlated with the period of the greatest accumulation of microplastics in bivalve mollusc tissues exposed to 1 mg·L^{-1} (Martyniuk, 2022). Caffeine concentration was selected based on the data reported for freshwaters that can reach 20 μg·L^{-1} of caffeine taking into consideration the data from the least and the most polluted sites of Europe (0.05 μg·L^{-1}-59.5 μg·L^{-1}) (Li *et al.*, 2020; Wilkinson *et al.*, 2022). Water was changed, and freshly prepared chemicals were replenished every two days.

After exposures, molluscs were examined for sex and the presence of parasites under a light microscope, and only parasite-free male molluscs were used for the investigation. The samples were frozen (-40 °C) until analyses. For all biochemical traits, except the study of thermostable protein extract, soft tissue samples were prepared individually from eight molluscs. To analyze thermostable protein extract, the pool of tissue (350 mg) from five specimens in each group was utilized. The concentration of the proteins was determined according to O. H. Lowry *et al*. (1951), using bovine serum albumin as the protein standard.

Assays for the low weight thiols. Metallothionein protein (MT-SH) concentration was determined by the method of A. Viarengo *et al.* (1997) using tissue homogenate in 20 mM *Tris*-sucrose buffer, pH 8.6 (1:3 w/v) with 0.5 mM PMSF, 0.01 % ß-mercaptoethanol, 6 μM leupeptine as it is described in V. V. Khoma *et al.* (2020). Low-weight (approximately 7 kDa) fractions with high absorbance at 254 nm and a high D_{254}/D_{280} density ratio were identified as putative MTs-containing peak and pooled for Zn determination.

Total glutathione (GSH: reduced plus oxidized) concentration was quantified using the glutathione reductase recycling assay (Griffith, 1980). The rate of 5-thionitrobenzoic acid formation was monitored spectrophotometrically at 412 nm. Standards were prepared from reduced glutathione, and concentrations were expressed as μmol per g wet weight.

The concentration *of Zn* was measured in the soft tissue (Znt) and the pooled MT-containing eluate (Zn-MT) utilizing the reaction of the complexation of Zn(II) with 2-(5-bromo-2-pyridylazo)-5-[N-propyl-N-(3-sulfopropyl) amino]phenol disodium salt dihydrate (5-Br-PAPS) (Wang *et al.*, 2018); after the digestion of samples with HNO_3, it was evaluated by the absorbance of the metal-5-Br-PAPS complex at 560 nm, expressed as μg/g fresh weight (FW).

Cu assay in the soft tissues was accomplished according to R. E. Peterson and M. E. Boiler (1955) by the spectrophotometric assay utilizing cuprizone (Copper Test. 1.14767 – Merck Millipore). The samples of tissue were pretreated to release Cu and convert Cu(I) to Cu(II). The pH of the samples was adjusted to 8.0. Calculation was made utilizing the molar extinction coefficient of 16000 M^{-1} cm^{-1} at 600 nm (Marczenko & Balcerzak, 2000).

Biomarkers of oxidative stress and metabolic activity. Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured according to the non-enzymatic assay based on aerobic reduction of nitro-blue tetrazolium (NBT) in the presence of phenazine methosulphate (PMS) and NADH (Fried, 1975). The reduction of NBT was measured at 560 nm and expressed as SOD units per mg of soluble protein (one unit of SOD is defined as the amount of enzyme that causes 50 % inhibition of NBT reduction).

Citrate synthase (CS, EC 4.1.3.7) activity was assayed according to E. E. Flynn *et al.* (2015) in the supernatant of 10 % w/v homohenate of soft tissue in the buffer solution. Absorbance was monitored at 412 nm for 3 min. CS enzyme activity was expressed in nmol/min/mg soluble protein.

The activity of acid phosphatase (AcP, EC3.1.3.2) was estimated after S. Lin and D. J. Steichen (1994). The supernatant of 10 % homogenate in cold 0.25 M sucrose solution buffered with citric acid/0.1 M K_2HPO_4 (pH 8.0) was used for determination. The reaction mixture for the determination of free AcP (AcPf) contained supernatant, 5 mM *p*-nitrophenylphosphate in 50 mM Na-acetate buffer (pH = 5.0), 0.25 M sucrose, and 1 mM EDTA. For the estimation of total acid phosphatase (AcPt), Triton X-100 (0.125 mg/mL) was added to the mixture. The absorbance was spectrophotometrically recorded at 410 nm. Latency, L, which is the percentage of total enzymes that are bound in lysosomes (latent), was calculated by the formula L = (AcPt – AcPf)/AcPt·100 %. The activity of AcP was quantified using the molar extinction coefficient of *p*-nitrophenol ($16000 \cdot M^{-1}$ cm^{-1} in the presence of EDTA) (Zhang *et al.*, 1992) and expressed in pmol/min/mg protein.

Statistical analysis. Results were expressed as mean ± SD. For all traits, except Zn-MT (poolled homogenates), sample size was eight from eight individuals, and for Zn-MT, the sample size was five from the two replicates in each group. Data were tested for normality and homogeneity of variance using the Shapiro–Wilk test and Levene's tests, respectively. Whenever possible, data were normalized by Box-Cox common transforming method. For the data that were not normally distributed, non-parametric tests (Kruskall–Wallis ANOVA and Mann–Whitney U-test) were performed. The IBM SPSS Statistics version 24 software for Windows was used for calculations.

RESULTS

The MT-SH concentration increased in the groups exposed to caffeine and, particularly, mixtures. In the Mix-group it was 1.83 times higher in comparison with C-group (**Fig. 1*A***). On the contrary, determining the GSH level, we did not observe a significant difference between control and exposed groups (**Fig. 1*B***). MTs-containing proteins were eluted by gel-exclusion chromatography as the thermostable proteins with the molecular mass (about 7 kDa) and typical maximum absorbance at 245–250 nm with a high D_{254}/D_{280} density ratio (**Fig. S2*A*, S2*B***). The content of MT-Zn decreased under exposures to Caf and Mix and was correspondent to control in the other groups (**Fig. 1*C***).

The determining of the total metal concentration in the tissues indicated the decreased level of Zn under exposure to MP, whereas it increased under exposure to Mix (**Fig. 1*D***). The concentration of Cu in the tissues increased in all exposures, and the highest meanings were in groups, affected by heating (T and MixT-groups, by 2.9 and 3.0 times higher correspondingly against control) (**Fig. 1*E***). Tissue Zn to Cu ratio decreased in all groups from 4.28 in control to 1.25 in the T-group (**Fig. 1*F***).

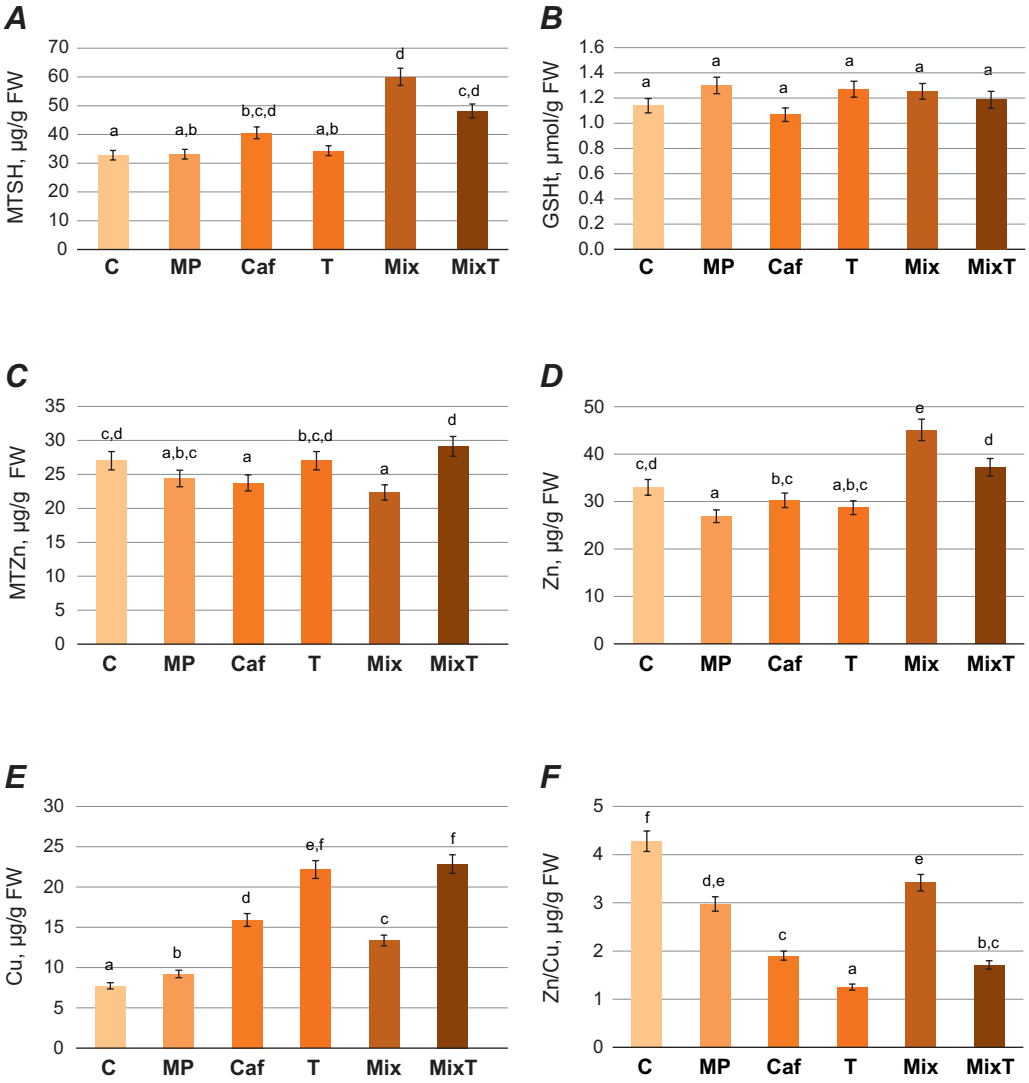

Fig. 1. Low weight thiols and the concentration of metals in the soft tissues of *D. polymorpha* under the influence of microplastics (MP), caffeine (Caf), their mixture (Mix), an elevated temperature (T) and a mixture of microplastics and caffeine at an elevated temperature (MixT): ***A*** – MT-SH; ***B*** – GSH total; ***C*** – concentration of zinc in the metallothioneins; ***D*** – zinc total levels; ***E*** – concentration of copper; ***F*** – zinc/copper ratio, presented as mean ± SD (n = 8). Here and in **Fig. 2**, all groups were compared with each other, if the letters above the bars are the same, it indicates that the values do not differ significantly (P >0.05)

The CS activity enhanced compared to control in all exposures, except MP (by 1.34–1.74 times) (**Fig. 2*A***), whereas the activity of the antioxidant enzyme SOD elevated after the exposures to Mix, T and MixT (**Fig. 2*B***). The AcPt decreased in the MP-group and significantly elevated by 1.40 times in exposure to the Mix (**Fig. 2*C***), whereas in all caffeine-containing exposures, the part of membrane-bound acid phosphatase (L) increased (by 3.42 and 3.56 for Mix and MixT-exposures correspondly) (**Fig. 2*D***).

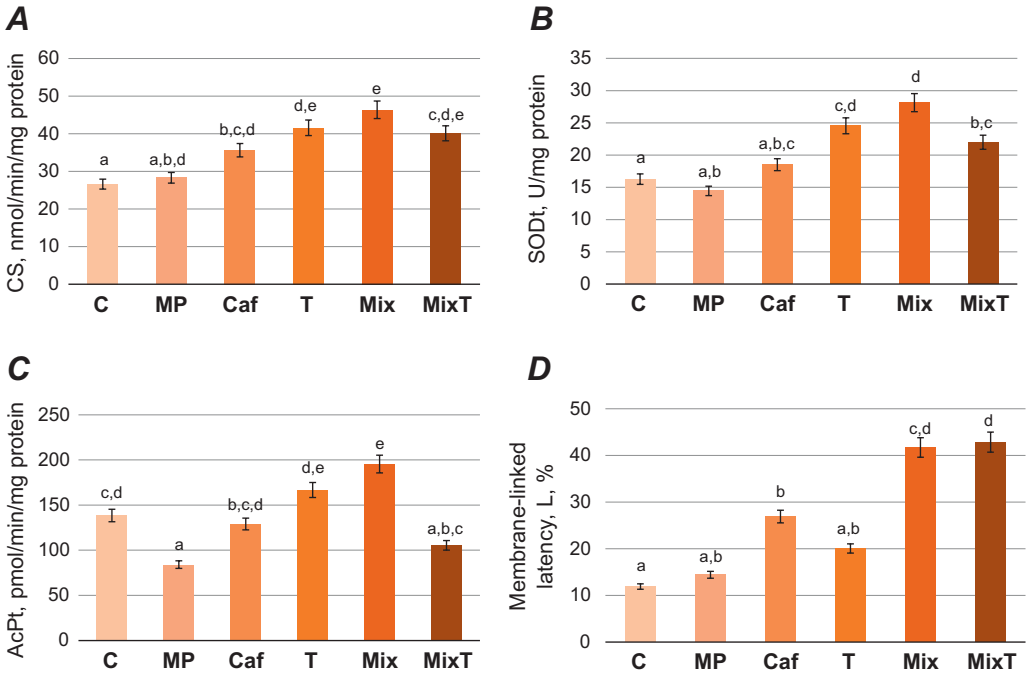

Fig. 2. Markers of oxidative stress and metabolic activities in the soft tissue of *D. polymorpha* under exposures to microplastics (MP), caffeine (Caf), their mixture (Mix), an elevated temperature (T) and a mixture of microplastics and caffeine at an elevated temperature (MixT): ***A*** – citrate synthase activity (CS); ***B*** – total SOD activity; ***C*** – total acid phosphatase activity (AcPt); ***D*** – lysosomal membrane-linked latency of phosphatases (L), presented as mean ± SD (N = 8)

DISCUSSION

In this study, we aimed to identify the consequences of the multi- stress effect of common pharmaceuticals, personal care products, and abiotic factors on the zebra mussel. Generally, it was expected that the molluscs adapted to the moderately polluted city pond will be resistant to this loading (Farkas *et al.*, 2017). However, the data indicated significant changes in all exposures and indexes, except GSH. In all exposures, the shared manifestation was a decrease in the Zn/Cu ratio. Although the concentrations of essential metals, such as Zn and Cu are frequently determined in the tissues of molluscs, their ratio is rarely utilized for the assessment of their health status (Khoma *et al.*, 2021). However, in the higher vertebrates, the perturbation of the Zn/Cu ratio in the blood serum and other fluids is an approved sign of pathology, particularly inflammation and aging (Schneider *et al.*, 2020; Matskiv *et al.*, 2021; Kazi Tani *et al.*, 2021). As it is evident from our results, the disturbance of Zn/Cu ratio can be a useful index in the bivalve molluscs too.

In the present study, this imbalance was most evident in the T- and MixT-groups indicating the thermal sensitivity of the metal bioavailability. The consequences of this imbalance can be the distortion of Zn signaling functions and the promotion of prooxidant processes, namely, the oxidation of low-weight thiolome (Atrián-Blasco *et al.*, 2017). However, no other common regularities were found in these exposures. Therefore, the utilization of this sensitive index for bioindication needs substantial investigation.

The particular effect was revealed for the MP, whereas all other exposures demonstrated plural similarities in the responses. To recognise which mode of effect was adverse or nonadverse, we, first of all, relied on the AcP responses. The AcP in invertebrates is considered as a biomarker for lysosomal membrane damage (Gaikwad, 2010). In the present study, MP caused a decrease in the AcPt activity in accordance with the decreased Zn total concentration in the tissues and without changes in the rate of membrane-related activity. This response was indicative only for MP. In all other exposures, these indexes were either unchanged compared to control or increased. Since the number of lysosomes is correlated with the AcP activity (Prinz *et al.*, 2020), the particular decrease in this activity without changes in the part of its latency testifies he reducing of lysosomal biogenesis. The concentrations of lysosomes within the cell can change according to their biogenesis activity, and, in turn, Zn plays a crucial role in this processes (Kim *et al.*, 2022). Therefore, the particular disturbance of Zn transport through the cell membrane by MP (Wang *et al.*, 2022) in the single exposure can be the reason for the decreased AcP activity.

The data concerning the effect of MP on the AcP are consistent with the research by M. Revel *el al.* (2020), where the AcP activity of coelomocytes in *H. diversicolor* decreased after exposure to MP for 10 days. Several reports confirm that the decrease of the AcP activity in aquatic species can be caused by different contaminants. For example, AcP activity decreased in the algae *Pseudokirchneriella subcapitata* under exposures to metals and linear alkylbenzene sulphonate (Jonsson *et al.*, 2009), in the blood clam *Anadara granosa* under extreme heating (32–35 °C) (Patel & Patel, 1985), and was more vulnerable than cathepsin activity in cuttlefish (*Sepia officinalis*) eggs exposed to Cd and Cu (Lacoue-Labarthe *et al.*, 2010). A reduced AcP activity may contribute to a decrease in the resistance of organisms to a variety of other threats, such as bacterial infections, other pollutants, or abiotic environmental factors (Revel *el al.*, 2020). Importantly, in this study we have identified AcP as the prospective biomarker of MP impact, whereas the studies that involved a set of stress markers did not give a uniquocal evidence of such toxicity for bivalve molluscs (Hamm & Lenz, 2021; Martyniuk *et al.*, 2022a,b, 2023).

Contrary to the MP effect, Mix caused AcPt activation. Study of Y. B. Gaikwad *et al.* (2010) suggests that AcPt activity increased in the silkworm *Bombyx mori* at a low level of oxidative stress. Moreover, the membrane-bound activity (L), which is a more accurate measure of stress, increased in all caffeine-related exposures, especially combinations, indicating a powerful activation of the functional activity of lysosomes (Lin & Steichen, 1994).

Caffeine is suspected to affect the oxidative stress balance in different species including molluscs (Aguirre-Martinez *et al.*, 2013; Cruz *et al.*, 2016), particularly as a scavenger of $OH^{\bullet}$ radical (Schlater *et al.*, 2022). An important evidence of antioxidant activity of caffeine was the increase of MTs total concentration in all caffeine-related groups. It attested their involvement in the response to stress. Moreover, a decrease in the metalation of metallothioneins can increase the antioxidant activity of these thiols (Buico *et al.*, 2008). On the other hand, the release of Zn from the thiolate clusters assists in the transfer of Zn to other cellular targets, which is particularly important in the circumstances of the distorted Zn/Cu balance (Saad *et al.*, 2016). Hence, the exposure to caffeine caused beneficial changes in the studied markers. According to the study by D. Cruz *et al.* (2016), GSH content in the clams (*Ruditapes philippinarum*) was decreased with the increasing level of caffeine in exposure, and the lowest values were observed in specimens exposed to the highest concentration (18.0 $mg \cdot L^{-1}$). It seems that

in the present study, the zebra mussels provided the more successful involving of thiols in the antioxidant response to metallothionein participation. Additionally, the activation of CS indicates mobilizing of energy resources in all exposures, except MP. Caffeine is a popular ergogenic substance for humans, that has been shown to increase the metabolic rate, mitochondrial content and biogenesis as well as oxidative and total metabolism through binding of phosphodiesterase that results in an increased cyclic adenosine monophosphate (Vaughan *et al.*, 2012; Giráldez-Costas *et al.*, 2023). Particullarly, the activation of CS was shown in the cell cultures exposed to caffeine ods (McConell *et al.*, 2010; Schlater *et al.*, 2022). The increased temperature had the similar to combined exposures effect on the SOD and CS, indicating the suitability of the explored model with elevated temperature to the adaptive limits of mussels. These manifestations were confirmed in other studies of molluscs (Rahman & Rahman, 2021; Martyniuk *et al.*, 2022a). Similar activation of CS under higher temperature was observed in the horse mussel *Modiolus modiolus* (Lesser & Kruse, 2004). However, under the combined exposure in the MixT-group, some of the beneficial responses were less evident than in the Mix-group, and the distortion of metal ratio was the highest, indicating the distorting effect of heating on the metal metabolism. The positive relationship between temperature and an increased metal uptake was demonstrated in various aquatic species (Serra *et al.*, 1999; Serafim *et al.*, 2002; Baykan *et al.*, 2007) confirming our results. Similar alleviation of the effects of mixtures on molluscs under heating was also shown in other studies (Khoma *et al.*, 2021; Martyniuk *et al.*, 2022a,b).

Generally, the applied exposures demonstrated the adverse impact of MP on the bivalve mollusk and the ability of caffeine to alleviate this impact in combined exposures. As it is evident from the Discussion, the combination of caffeine and MP caused the cumulative effect on the mussels, that was clear from the signs of an increased lysosomal and mitochondrial biogenesis, and also antioxidant activities with the involvement of cellular low weight thiols metallothioneins. The findings of M. Sewwandi *et al.* (2022) state that in mixtures microplastics may potentially serve as a vector for caffeine, allowing it to pass into cells in larger quantities and, correspondingly, to increase its own effects. Indeed, in the combined exposures the highest magnitudes of the increase of the Zn total and metallothioneein-related thiols concentration, AcPt, CS and SOD activities were found. Moreover, the traces of caffeine effect were more evident in the combined exposures.

CONCLUSION

The findings from our study highlight the importance of assessing the multiple stressors effects to understand the environmentally relevant consequences of water pollution. The data revealed an adverse effect of microplastics and shared beneficial effects in the exposures that contained caffeine, partially alleviated by the heating. The responses were best distinguished by the assessment of acid phosphatase and metallothioneins. The future prospects of this study are related to the elucidation of the validity of Zn/Cu ratio as the sensitive biomarker of the health status in bivalve mollusks.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal studies: This article does not contain any studies with vertebrate animal subjects performed by any of the authors.

AUTHOR CONTRIBUTIONS

Investigation, [M.T.]; visualization, [M.T.]; validation, [M.T.]; software, [M.T.]; writing original draft, [M.T.], conceptualization, [S.O.]; data curation, [S.O.]; writing – writing – review & editing, [S.O.]; project administration [S.O.].

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БІОХІМІЧНІ ВІДПОВІДІ ОРГАНІЗМУ *DREISSENA POLYMORPHA* З МІСЬКОГО СТАВУ НА КОФЕЇН, МІКРОПЛАСТИК І НАГРІВАННЯ ЗА ПООДИНОКОГО ТА КОМБІНОВАНОГО ВПЛИВІВ

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Вступ. Фармацевтичні препарати стали одними з найзначущіших забруднювачів водного середовища і спричиняють дедалі більше занепокоєння. Проте зовсім невелика кількість досліджень стосується їх комбінованому впливу на водних мешканців у екологічно значущих моделях. Крім того, вивчення векторного транспортування кофеїну мікропластиком і, зважаючи на підвищення температури у поверхневих водах, температурної залежності цього процесу, все ще перебуває у зародковому стані. Метою дослідження було проаналізувати вклад кофеїну, мікропластику та підвищеної температури у їхньому комбінованому впливі на прісноводного двостулкового молюска *Dreissena polymorpha* (Pallas, 1771).

Матеріали та методи. Молюски піддавали дії кофеїну (Caf, 20,0 мкг/л), мікропластику (MP, 1 мг/л, розміром 2 мкм) і підвищеної температури (T, 25 °C) за одиничного та комбінованого (Mix, MixT) впливів протягом 14 днів. Було проаналізовано концентрацію загального білка металотіонеїну (MT-SH), MT-зв'язаного цинку (MT-Zn), концентрацію загального Zn і Cu у тканинах, рівень загального глутатіону (GSH), індукцію антиоксиданта (супероксиддисмутаза, SOD), метаболічний статус (активність цитратсинтази (CS)), активність загальної кислої фосфатази (AcPt) та лізосомальну мембранно-зв'язану латентність фосфатази (L).

Результати і їхнє обговорення. Спільною реакцією під час усіх експозицій було зниження співвідношення Zn/Cu у м'яких тканинах, що відображає дисбаланс металів як найчутливіший маркер впливу. За впливу МР спостерігали зниження рівнів Zn і загальної кислої фосфатази, що свідчить про пошкодження лізосом. Усі інші експозиції (Т, Міх, МіхТ) спричиняли активацію цитратсинтази та супероксиддисмутази. Усі групи, які піддавалися дії кофеїну (Caf, Міх, МіхТ), продемонстрували підвищення лізосомальної мембранно-зв'язаної латентності фосфатази і концентрації загального білка металотіонеїну та його неметальованої частини. Рівень глутатіону був стабільним за усіх впливів.

Висновки. Отримані результати виявили несприятливий вплив мікропластику, тоді як за впливу на організм кофеїну спостерігали загальний позитивний ефект, частково нівельований впливом температури. Це вказує на важливість вивчення комбінованої дії стресорів, що дасть змогу оцінити реальні реакції організму на ксенобіотики зовнішнього середовища.

Ключові слова: тригранка річкова, лікарські препарати, цинк, купрум, кисла фосфатаза, окислювальний стрес

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