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Polybrominated diphenyl ethers' exposure causes changes in the physiology of zebra mussel, Dreissena polymorpha (Pallas, 1771)

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Abstract. The present laboratory study (96 hours and 30 days) aimed to provide for the first time the possible adverse effects of different concentrations of PBDEs congers (PBDE 28, PBDE 47, PBDE 99, PBDE 100, PBDE 153, PBDE 154) based on Water Framework Directive 2000/60/EC (WFD) in zebra mussel (*Dreissena polymorpha* Pallas, 1771). Therefore, we analysed the gill histochemical structure by applying the Periodic acid–Schiff staining method to assess the exposed individuals' physiological status and linked them to the tested PBDEs concentrations in the contaminated water.

Key words: mussels, pollution, organic contaminants, PBDEs, biomarkers.

Introduction

Persistent organic pollutants (POPs) are well-known compounds that are toxic to humans and wildlife because they are lipophilic, have low degradation rates, biomagnify through the food chain, and can travel long distances far from the releasing use source (Ahrend et al., 2023). POPs of most significant concern fall into several categories, including polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), perfluorinated chemicals (PFCs), and polybrominated diphenyl ethers (PBDEs) (Medina, 2024). According to Yang et al. (2023), PBDEs are a class of typical POPs with 209 congeners. Three types of commercial PBDE mixtures, namely penta-, octa, and deca-BDE, have been ubiquitously used as flame retardants in various industrial products, such as plastic products, electronics, building materials, and textiles (Portet-Koltalo et al., 2021). However, the widespread usage of PBDE-containing products, combined with their long-distance migration, high lipophilic ability, and accumulation in fat tissues, has resulted in global environmental problems (Abbasi et al., 2019). For instance, among the PBDE congeners, tetrabromodiphenyl ether (PBDE 47) is the dominant congener found in the environment, and numerous studies have already indica-

ted that PBDEs are endocrine-disrupting chemicals in both, animal and human bodies (Al-Harbi et al., 2021; Li et al., 2024).

Mussels are widely used as bioindicators and probably the most commonly used organisms in field or laboratory pollution experiments and for aquatic monitoring along with fish because they are filter-feeders and sessile, they have a wide geographical distribution and availability throughout the year and due to their efficiency in accumulating POPs, and tolerance to various environmental conditions (Yancheva et al., 2018a; Gecheva et al., 2020; Georgieva et al., 2022). The freshwater zebra mussel is suggested as an equivalent of *Mytilus* spp., which is notably used in large-scale programs, such as the Mussel Watch (Binelli et al., 2015; Beyer et al., 2017). This type of monitoring was first applied in a marine environment using blue mussels (Goldberg, 1975), but later in freshwaters with zebra mussels (Secor et al., 1993; Faria et al., 2010; Lepom et al., 2012; Louis et al., 2020; Hani et al., 2021; Baratange et al., 2023). However, the zebra mussel is a wide-spread invasive bivalve species commonly found in freshwaters of the northern hemisphere and identified by the IUCN as one of the 100 of the World's worst invasive alien species, but it has also been used actively as a model organism for freshwater toxicology since the late 1970s (Stoyanova et al., 2020; Yancheva et al., 2016, 2017a,b, 2018b, 2019a, 2020; 2021, 2022). In this regard, the zebra mussel is also widespread in most European freshwaters, including Bulgaria. In Bulgaria, this species was reported initially in the Danube River, the lowest reaches of its tributaries, some Black Sea lagoons, and the Black Sea coastal lakes and rivers (Valkanov, 1957; Russev et al., 1994; Angelov, 2000; Hubenov, 2005), including the Veleka River.

This study aimed to evaluate the zebra mussels' health status after acute (96 hours) and subchronic (30 days) exposure to different PBDEs concentrations under laboratory conditions. To achieve this, we applied the histochemical biomarker approach.

Materials and Methods

Experimental setup

The experimental setup was conducted by national and international guidelines of the European Parliament and the Council on protecting animals used for scientific purposes (Directive 2010/63/EU).

Mussel collection

About 100 zebra mussels were hand-collected and transported on the same day in polyethylene containers, thoroughly cleaned with distilled water, and then filled with water from the water source. The mussels were placed in a 50 L aquarium, pre-filled with dechlorinated water, and equipped with oxygen pumps in the vivarium of the Faculty of Biology at the University of Plovdiv to acclimatize for two weeks.

PBDEs' exposure

Acute (96 h) and subchronic (30 days) test was carried out. The zebra mussels (n=30 in each water tank) were exposed to two different test concentrations of a PBDEs mixture (PBDE 28, PBDE 47, PBDE 99, PBDE 100, PBDE 153, and PBDE 154) based on the maximum allowable concentrations according to the EU and national legislation – 0.14 μg/L and 0.0085 μg/kg. A water tank was used for control with PBDEs-free tap water. The water physicochemical parameters including pH (ISO 10523:2008), temperature (BDS: 17.1.4.01:1977), electrical conductivity (μS/cm, ISO 7888:1985), and dissolved oxygen (mg/L, ISO 17289:2014) were read three times per day using a multi-device (WTW, Germany).

Histochemical assessment

Mussel dissection was performed according to the methodology for bioaccumulated pollutants described in the EMERGE protocol of Rosseland et al. (2003), which we adapted for mussels. Each mussel was weighed with an analytical balance (KERN, Germany) (g) and measured with a caliper (cm) to calculate different condition factors further. The dissected-out gills were placed in sterile polyethylene plastic bags and deep-frozen.

Frozen gill tissue samples were fixed on pre-cooled aluminum chucks for cryostat sectioning. Deep-frozen multiple zebra mussel's gill sections (5 μm) were prepared using a cryotome (Leica CM 1520, Wetzlar, Germa-

ny) at a chamber temperature of -25°C. The sections were stored after that at − 80°C for further staining procedures following a standard PAS methodology for polysaccharides according to McManus (1948) and Culing (1974) with slight modifications as previously described in our studies (Georgieva et al., 2013; Yancheva et al., 2019b; Kovacheva et al., 2022). The samples were fixed in absolute alcohol, rinsed quickly with distilled water, hydrolyzed with periodic acid solution, rinsed with tap and distilled water, stained with Schiff's reagent at room temperature, rinsed with warm tap water (35°C minimum), then rinsed quickly with distilled water, counterstained with hemalum solution, colored in blue in flowing tap water, dehydrated by ascending alcohol series (50, 70, 80, 90 and 100%), cleared with clearing agent, dried at room temperature and capped with Canada balsam. Five slides per treated specimen (n=15 from each tank) were prepared.

The gill histochemical changes, including the controls, were evaluated blindly and appraised individually and semi-quantitatively using the grading system of Mishra & Mohanty (2008) adopted for this study. A positive PAS reaction was presented in pink to violet staining, and the cell nuclei were colored in blue, respectively. Each grade represented specific histochemical characteristics and was categorized as follows: (0) – negative reaction of the histochemical staining; (1) – very weak positive reaction of the histochemical staining; (2) – weak positive reaction of the histochemical staining; (3) – moderate positive reaction of the histochemical staining; (4) – strong positive reaction of the histochemical staining.

Statistical analysis

The results from the conducted experiment were presented as mean±SD for all evaluated individuals per experimental concentration. Past 3.03 (Hammer et al., 2001) and GraphPad Prism 7 for Windows (USA) were used to assess the data statistically. The normality of data was tested by the Shapiro-Wilk test. The homogeneity of variances was tested with the Levene's test. The results were also analyzed for the significance of differences among the control and the treated mussel groups by the Kruskal-Wallis test, followed by the Mann-Whitney test (medians comparison). The levels of statistical significance were set at $p < 0.05$.

Results and Discussion

The physicochemical parameters of the water contaminated with PBDEs stayed relatively constant throughout the experiment. No significant changes between the control and the tested aquaria ($p > 0.05$) were observed; hence, they will not be further discussed as we did not link the changes in the glycogen amount with their values, but rather with the PBDEs' toxicity.

The degree of intensity of the applied histochemical PAS reaction in the zebra mussel gills after the acute and subchronic exposure to both experimental PBDEs concentrations is presented in Figures 1 and 2. Overall, the results showed a decrease in the amount of glycogen compared to the control group. There were no detectable differences in the amount of glycogen between the two experimental PBDEs concentrations administered after the 96 hours' exposure. Such were not found on day 30, either. The only difference was found in the glycogen amount when comparing the results between the 96 hours' and 30 days' exposure, but they were not statistically significant ($p > 0.05$). We consider that this data could indicate that, in our case, the PBDE toxicity depends on the time of action of the toxicant, but not on the administered dose, which, from our perspective, requires further research.

Our results are in line with Ansaldo et al. (2006), that the glycogen levels are negatively correlated with the level of chemical stress, and with Hamed et al. (2023), who found that after seven days of exposure to several concentrations of ZnO nanoparticles, the glycogen levels exhibited a significant reduction in the gill tissues. As explained by Arrighetti et al. (2019), the gills of bivalves play an essential role in the feeding process, and they are also the primary organ for the entry of pollutants. In addition, glycogen is the molecule that functions as secondary long-term energy storage in animal and fungi cells, it is found in the

form of granules in the cytosol and plays a vital role in the glucose cycle (Brenner et al., 2014). Therefore, glycogen is the primary energy reserve in bivalves. According to Brenner et al. (2014), glycogen forms an energy reserve that can be quickly mobilized to meet a sudden need for glucose. When living in a stressful environment affected by various anthropogenic pollution, the glycogen levels tend to decrease to supply different energetic requirements due to elevated metabolism in the exposed organisms (Hyötyläinen et al., 2002). We agree that changes in the amount of glycogen in the cells indicate changes in the carbohydrate metabolism of the organism under the adverse effect of various pollutants and that the reduced glycogen storage represents a non-specific reaction of the zebra mussels against the PBDE exposure in our case. We also express the same opinion of Au (2004) that such histochemical biomarkers are unspecific and easy to determine, and their advantage is that they could be directly related to the health status of the organism. Also, as an early warning tool for assessing environmental risk, histochemical methods can be used in combination with biomarkers at other organizational levels, such as different enzyme activities (cellular level), condition factors, and shell analysis (organismal level), etc. (Brooks et al., 2015; Rementeria et al., 2017; Bebianno et al., 2018).

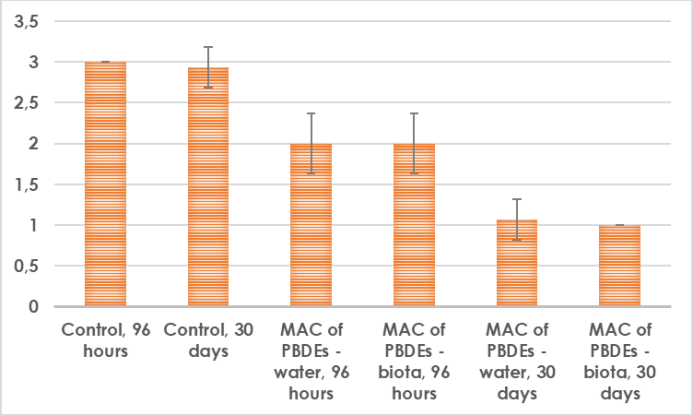

Fig. 1. Glycogen levels (PAS-reaction) decrease in the gills of zebra mussels (n=15 individuals per treatment) after exposure to two different concentrations of PBDEs (maximum allowable concentrations, MAC in water and biota) after 96 hours and 30 days of exposure (average±standard deviation).

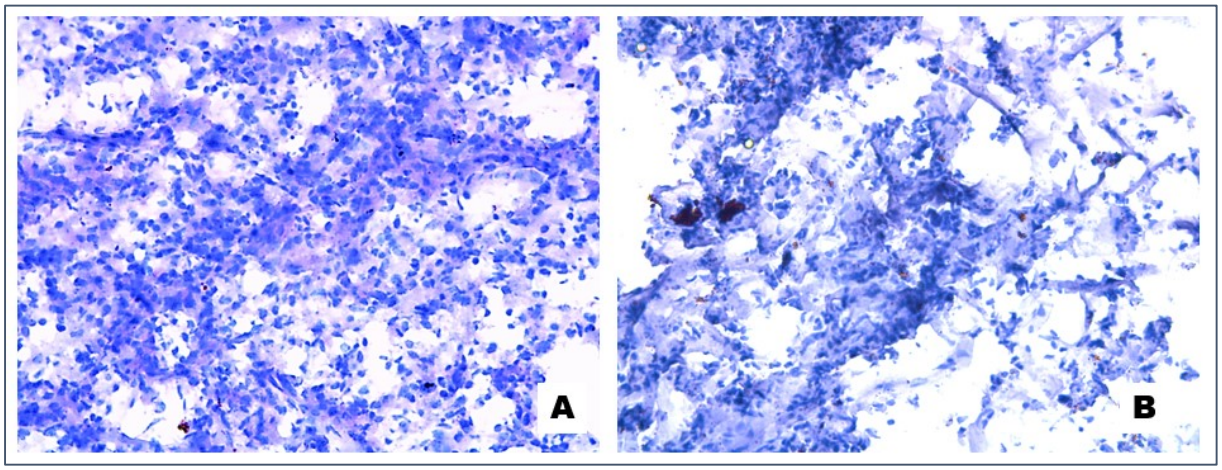

Fig. 2. Glycogen levels (PAS-reaction) decrease in outer lamella and middle mantle of the gills of zebra mussels after 96 hours' exposure to PBDEs (maximum allowable concentrations, MAC in biota): A) control and B) treated individuals, x 400.

Conclusions

In summary, we can conclude that the changes in the intracellular glycogen levels and storage capacities correlate with the duration of chemical stress. We also suggest that the PAS reaction can be used as a general tissue biomarker of nutritional status and health, and the effects of exposure of freshwater mussels to PBDEs, along with other biological tools in terms of the multi-biomarker approach.

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