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Exposure to polybrominated diphenyl ethers impairs the health condition of Zebra Mussel, *Dreissena polymorpha* (Pallas, 1771)

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Abstract. The present pilot 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 Mussels (*Dreissena polymorpha* Pallas, 1771). Thus, we calculated different condition factors based on the mussel's weight and length to determine the possible adverse effects of acute and subchronic exposure to PBDEs under laboratory conditions.

Key words: Zebra Mussel, pollution, PBDEs.

Introduction

Polybrominated diphenyl ethers are a class of persistent organic pollutants known as additive synthetic brominated flame retardants, widely used in household and commercial items to meet flammability standards of consumer electronics, textiles, and furnishings (Portet-Koltato *et al.* 2021; Jiang *et al.* 2023). Diosis *et al.* (2016) explain that the group consists of 209 congeners. PBDEs are highly lipophilic, have low water solubility, and tend to accumulate in high-fat tissues (Li *et al.* 2022; *Yang et al.* 2023). According to Giandomenico *et al.* (2013), they are now included in the EU Priority Pollutants List (Decision 2455/2001/CE) because of their environmental persistence and toxic effects on wildlife and humans.

In the marine environment, mussels, such as *Mytilus* sp., were proven to be excellent bioindicators for monitoring various organic pollutants, including PBDEs decades ago (Goldberg 1975). Zebra Mussel is considered the freshwater equivalent species, although it is also highly invasive (Yancheva *et al.* 2022). In this regard, Zebra Mussel is distributed broadly in Bulgaria, including the Danube River and the Black Sea coastal lakes and rivers.

Various condition factors are applied to determine pollution's effects, which are simple mathematical calculations. For example, the condition factor, proposed by Fulton (1902), which represents the relationship between the weight and length of fish, indicates the organisms' health status - their growth, nutrition, and energy reserves. According to several studies, the deteriorated state of health affected by pathogenic infections or various chemical agents reflects on the normal biology of aquatic organisms, including bivalves, and changes the condition factors' values.

Therefore, the present pilot study aimed to calculate different condition factors based on the mussels' weight and length and determine the possible adverse effects of PBDEs' acute and subchronic exposure under laboratory conditions.

Material and Methods

About 100 Zebra Mussels were hand-collected, transported, and placed on the same day 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. Acute (96 hours) and subchronic (30 days) tests were carried out. The mussels (n=30 in each water tank) were exposed to two different test concentrations of a mixture of PBDEs congeners (PBDE 28, PBDE 47, PBDE 99, PBDE 100, PBDE 153, PBDE 154) based on the maximum allowable concentrations (MAC) for water and biota according to the EU and national legislation – 0.14 µg/L and 0.0085 µg/kg, and one tank with clean tap water was used for control. The mussel dissection was performed according to the bioaccumulated pollutant methodology described in the EMERGE protocol of Rosseland *et al.* (2003), which we adapted slightly for mussels.

Each mussel (n=5 from experimental concentration) was weighed with an analytical balance (Kern, Germany) (g) and measured with a caliper (cm) to calculate different condition factors as follows: CI 1 - soft tissue weight/total weight; CI 2 - soft tissue weight/shell weight; CI 3 (state index) - soft tissue weight/shell length; CI 4 (shell component index) - shell weight/shell weight + soft tissue weight and CI 5 (condition factor) - soft tissue weight/shell lenght^3

The data from the experiment are presented as average for all treated individuals. Past 3.03 (Hammer *et al.*, 2001) and GraphPad Prism 7 for Windows (USA) were used to evaluate the obtained 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 groups by the Kruskal-Wallis test, followed by the Mann-Whitney test (medians comparison).

Results and Discussion

The results on the calculated condition factors of Zebra Mussels after the 96th and 30 days' exposure to both experimental concentrations of PBDEs are presented in Table 1.

Table 1. Average results on the values of different condition factors in Zebra Mussels treated with maximum allowable concentrations (MAC) of PBDEs in water and biota after 96 hours and 30 days of exposure.

Duration and Test concentrations	CI	CI 2	CI 3	CI 4	CI 5
96 hour MAC - water	0.51	0.002	0.35	0.0005	0.007
96 hour MAC - biota	0.50	0.001	0.33	0.00045	0.006
96 hour - Control	0.50	0.0025	0.35	0.0005	0.008
30 day MAC - water	0.49	0.0015	0.33	0.0004	0.005
30 day MAC - biota	0.47	0.0009	0.31	0.0003	0.0045
30 day - Control	0.51	0.0025	0.35	0.0006	0.007

No statistically significant difference ($P > 0.05$) was proven in the values of the examined factors of the treated mussels and those of the control after 96 hours' exposure to PBDEs. We think four days is a short period to detect changes in the values of such parameters. Summarizing the results obtained for the computed factors on day 30, we can say that with the increase in the exposure period, the values of the calculated factors decrease. However, no significant differences were proven again between the control and treated mussels ($P > 0.05$). Furthermore, we consider that one month is also not sufficient to register significant changes in the values of condition factors, which in turn provide information on the overall physiological state of mussels. On the other hand, our results are in close agreement with other reports and confirm our previous studies with heavy metals and various organic pollutants (Stoyanova *et al.*, 2020; Wiatt *et al.*, 2014; Yancheva *et al.*, 2018, 2019).

There is evidence that aquatic organisms in a polluted environment use their energy reserves and include them in detoxification mechanisms, reducing their growth and development. According to Widdows (1985), such condition factors are easy and fast to use and reflect changes in the nutrient state of mussels, such as stored energy reserves and metabolic responses to environmental stress.

Conclusions

In summary, the condition factors' values of the treated with PBDEs Zebra Mussels were overall lower than those of the control ones. The study shows the adverse effects of an emerging environmental pollutant and the adverse effects of the permitted by law concentrations in water and biota.

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