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Effect of Polychlorinated Biphenyls on Biochemical Parameters of the Black Sea Bivalve Mollusk *Mytilus galloprovincialis* Lam.

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ABSTRACT

Polychlorinated biphenyls (PCBs) are known amongst the most dangerous toxicants entering the coastal marine waters from various polluting sources. Even the smallest PCBs doses are capable to change physiological and biochemical processes exerting toxic, mutagenic and carcinogenic effects. So, the aim of this study was to analyze the impact of PCBs at 1, 100, 1000 µg/L on oxidative stress parameters (level of oxidized proteins (neutral aldehydes and ketones, basic aldehydes and ketones) and lipid peroxidation), antioxidant enzyme activities (superoxide dismutase (SOD), catalase (CAT)) and cholinesterase (ChE) activity in the hepatopancreas of mussel *Mytilus galloprovincialis* during 5 days' of the toxicological experiment. Level of all forms of the oxidized proteins was found significantly increased at 100 µg/L and 1000 µg/L (+50-78% and +150-282%, respectively) compared to the control ($p<0.05$). Level of lipid peroxidation was considerably higher at 1, 100, 1000 µg/L (+59%, +134%, +269%, respectively) compared to the control ($p<0.05$). SOD activity significantly raised at 1, 100, 1000 µg/L (+63%, +200%, +118%, respectively) compared to the control ($p<0.05$), while CAT activity reduced at 1000 µg/L compared to the control, 1 µg/L and 100 µg/L (-29%, -66%, -40%, respectively) ($p<0.05$). ChE activity was found lower at 1, 100, 1000 µg/L (-60%, -93%, -30%, respectively) compared to the control ($p<0.05$). Possible mechanisms of *M. galloprovincialis* biochemical response to PCBs are discussed. Studied biochemical parameters can be suitable biomarkers for evaluating the toxicity of PCBs and *M. galloprovincialis* can be used as a bioindicator in the monitoring of marine ecosystems contaminated with these pollutants.

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INTRODUCTION

Ever-increasing anthropogenic load on marine coastal waters negatively affects the state of the aquatic communities. The main influencing factor is considered to be the chemical pollution. Various toxicants entering water and sediments adversely impact survival, growth, reproduction and the health of hydrobionts. Organochlorine compounds, including polychlorinated biphenyls (PCBs), are known the most dangerous anthropogenic pollutants inflowing from various sources into the coastal marine areas (Soldatov et al., 2005; Faria et al., 2009; Golovanova et al., 2011; Tao et al., 2013; Vidal-Linan et al., 2016; Kapranova et al., 2020; Malakhova et al., 2020).

For several decades PCBs have been extensively used all over the world as dielectrics, heat carriers, additives to adhesives, oils, lacquers, etc. Possessing high lipophilicity and resistance to physical and chemical factors, PCBs are actively and passively accumulated by different trophic levels hydrobionts, that explain their almost ubiquitous distribution. These

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compounds do not undergo metabolic degradation and are retained for a long time in tissues of marine organisms due to the specific features of their chemical structure (Soldatov et al., 2005; Malakhova et al., 2020). It has been found that PCBs, even in the smallest doses, cause changes in the physiological and the biochemical processes in the aquatic organisms, displaying toxic, mutagenic and carcinogenic effects (Soldatov et al., 2005; Vidal-Linan et al., 2016; Malakhova et al., 2020; Lomartire et al., 2021). Along with this, little attention has been paid so far to the study of PCBs effects on the physiological and the biochemical processes in hydrobionts. Changes in biochemical parameters reflect metabolism status and often indicate the development of a compensatory response to the pollutant effects. Biochemical parameters make it possible to identify the mechanisms of the toxic effects on the specific metabolism links, determine the main strategy and features of structural and functional changes in the body during adaptation to the adverse effects (Soldatov et al., 2005; Faria et al., 2009; Vidal-Linan et al., 2016; Sukharenko et al., 2017; Raisi, 2018; Malakhova et al., 2020).

When assessing pollutants' toxic effects on hydrobionts, the bivalve mollusk mussel *Mytilus galloprovincialis* is the most often used. Due to their survivability, the mussels exhibit high toxic resistance to various toxicants. Along with this, the threshold sensitivity of some physiological parameters in this mollusk is at the level of maximum permissible concentrations (MPC) for pollutants in water bodies. A wide range of prevalence and low mortality under abiotic changes make it easy to collect experimental material and keep mussels in the laboratory. Thus, the increased sensitivity of physiological functions to the toxicants allows to determine with high accuracy the suitability of the aquatic environment for the mollusks. Low concentrations of pollutants have practically no effect on the survivability of mussels, however, they can cause changes in the physiological and the biochemical processes (Soldatov et al., 2005; Fernandez et al., 2010; Attig et al., 2013; Franco et al., Vidal-Linan et al., 2015, 2016; Benali et al., 2017; Ozkan et al., 2017; Sukharenko et al., 2017; Lomartire et al., 2021).

Investigations of the biochemical responses in the mussels tissues under different PCBs concentrations are of significance to understand the mechanisms of the metabolism reorganization and the adaptive reactions occurring in hydrobionts when exposed to toxicants.

Thus, the aim of the research was to study the effect of PCBs on some biochemical parameters in the hepatopancreas of the Black Sea mussel *Mytilus galloprovincialis* in the toxicological experiment.

MATERIAL AND METHODS

Mussels were obtained in July 2020 from the collectors of the mussel-oyster farm in Karantinnaya Bay (Black Sea, Sevastopol) (Fig. 1). In this location the total concentration of PCBs did not exceed 3 ng/L; it corresponded to the average value of the Black Sea open areas (Kapranova et al., 2020). After sampling the mussels were immediately transported to the laboratory. The mollusks 50-60 mm of length were taken for the experiment and acclimated to the laboratory conditions during 7 days being kept in glass tanks with purified water in the ratio of five liters per one specimen with a constant aeration at the temperature 20-22 ° C. Water partially exchanged before the experiment daily. Mussels were not fed to avoid specific food exposure (Bakhmet et al., 2012).

Solution of PCBs mixture (Aroclor 1254, Supelco, USA) was added to glass tanks with filtrated marine water. Considering high resistance of mussels to chemical pollution, a wide range of PCBs concentrations was used in the experiment to detect the biochemical response. The concentration of PCBs affecting mussels was 1 μg/L (I experimental group), 100 μg/L (II experimental group) and 1000 μg/L (III experimental group). The control group was kept in water without toxic substances. Each treatment was replicated three times. Totally, the experiment lasted 5 days.

A biological analysis of mollusks was carried out at the end of the experiment. Since the

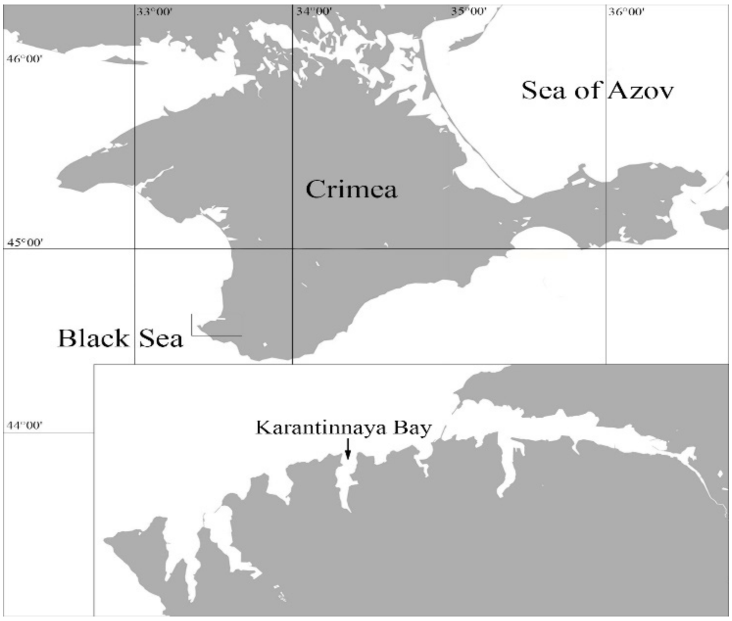

Fig. 1. Sampling area map

hepatopancreas of mussels is known to neutralize a wide range of toxic substances, it was used for the biochemical analysis. The hepatopancreas of 15 individuals from each group was washed several times by cold physiological solution, homogenized and centrifuged at 8000 g for 15 min at the temperature of 0–4˚C in a refrigerated centrifuge MPW-352 (MPW Med. Instruments, Poland). All biochemical parameters were determined in the supernatants.

The level of oxidized proteins (OP) was analyzed by using the method based on the reaction of interaction between the oxidized protein amino-acid residues and 2,4-dinitrophenylhydrazine with the formation of 2,4-dinitrophenylhydrazones (Dubinina et al., 1995). The optical density of the newly formed 2,4-dinitrophenylhydrazones was recorded at the following wavelengths (λ): 356 nm (neutral aldehydes), 370 nm (neutral ketones), 430 nm (basic aldehydes), and 530 nm (basic ketones).

The concentration of lipid peroxidation (LPO) secondary products - thiobarbituric acid reactive substances (TBARS) was determined by the reaction with thiobarbituric acid (λ = 352 nm) (Stalnaya and Garishvili, 1977).

Superoxide dismutase (SOD) activity was assayed in the nitroblue tetrasolium –phenazine methosulfate – NADH system (λ = 560 nm) (Nishikimi et al., 1972). Catalase (CAT) activity was measured based on the reaction of interaction between hydroperoxide and molybdite ammonium (λ = 410 nm) (Korolyuk et al., 1988).

Cholinesterase (ChE) activity was analyzed on the basis of hydrolysis of butyrylthiocholin to oil acid and tiocholine using standard assay kits OLVEX DIAGNOSTICUM (Russia) (λ = 405 nm).

All determinations were made on spectrophotometer SF-2000 (St. Petersburg, Russia). The biochemical parameters were calculated per mg protein. Total soluble protein concentration was quantified by biuret method using the standard assay kits OLVEX DIAGNOSTICUM (Russia).

The results were also processed statistically. Mean values +/- SEM (standard error of the mean) were established. The significance of the difference between the samples was evaluated by using Mann-Whitney U-test. The difference was found great at the significance level $p \leq 0.05$. The statistical analysis was carried out by using software programs Past 3 and Microsoft Office Excel 2016.

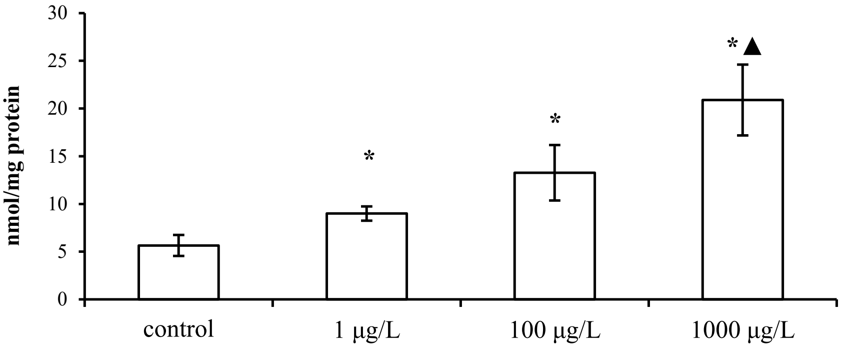

Fig. 2. Content of thiobarbituric acid reactive substances in the hepatopancreas of *Mytilus galloprovincialis* exposed to PCBs
* indicates statistically significant differences as compared to the control ($p<0.05$), ▲ - indicates statistically significant differences as compared to animals exposed to 1 µg/L PCB ($p<0.05$)

RESULTS AND DISCUSSION

The results obtained have shown that no animal mortality was registered during the experiment. It can indicate quite high toxic resistance of *M. galloprovincialis* to experimental PCBs concentrations. Along with this, it was stated that PCBs at 1, 100, 1000 µg/L caused remarkable changes in the biochemical parameters in the hepatopancreas of mussels. TBARS content was significantly higher at 1, 100, 1000 µg/L (+59%, +134%, +269%, respectively) compared to the control ($p<0.05$). Maximum values were registered at 1000 µg/L PCB (Fig. 2).

The level of all oxidized protein forms (D356, D370, D430, D530) dramatically increased at 100 µg/L and 1000 µg/L (+50-78% and +150-282%, respectively) compared to the control ($p<0.05$). The content of all oxidized protein forms at 1000 µg/L exceeded on 150-278% and 67-113% values at 1 µg/L and 100 µg/L, respectively ($p<0.05$) (Fig. 3).

SOD activity considerably raised at 1, 100, 1000 µg/L (+63%, +200%, +118%, respectively) compared to the control ($p<0.05$), while CAT activity reduced at 1000 µg/L as compared to the control (-29%), 1 µg/L (-66%) and 100 µg/L (-49%) ($p<0.05$) (Fig. 4).

ChE activity was lower at 1, 100, 1000 µg/L (-60%, -93%, -30%, respectively) compared to the control ($p<0.05$) (Fig. 5).

In the scientific literature it was shown that the technogenic pollutants under natural and experimental conditions cause changes in the biochemical parameters of hydrobionts (Soldatov et al., 2005; Faria et al., 2009; Fernandez et al., 2010; Bakhmet et al., 2012; Attig et al., 2013; Franco et al., 2016; Tao et al., 2013; Vidal-Linan et al., 2015, 2016; El-Khayat et al., 2015; Benali et al., 2017; Ozkan et al., 2017; Sukharenko et al., 2017; Malakhova et al., 2020). The mechanisms of toxic effects of many industrial organic xenobiotics are closely related to changes in the cell redox status. Violation in the cellular prooxidant-antioxidant balance and consequently the development of the oxidative stress is the main inducer of various pathologies - from molecular oxidative and genotypic damage to viability reduction and the organism death. The oxidative damage of biomolecules, primarily peroxidation of membrane lipids, oxidative modification of proteins and the DNA damage are the most significant reasons for developing cellular and functional pathologies, resulting in decrease of the organism viability. Therefore, the study and identification of molecular markers of cellular and functional disorders in aquatic organisms living under technogenic impacts are one of the major fields of the research in the hydrobionts biology (Fernandez et al., 2010; Franco et al., 2016; Ozkan et al., 2017; Sukharenko et al., 2017; Klimova et al., 2019).

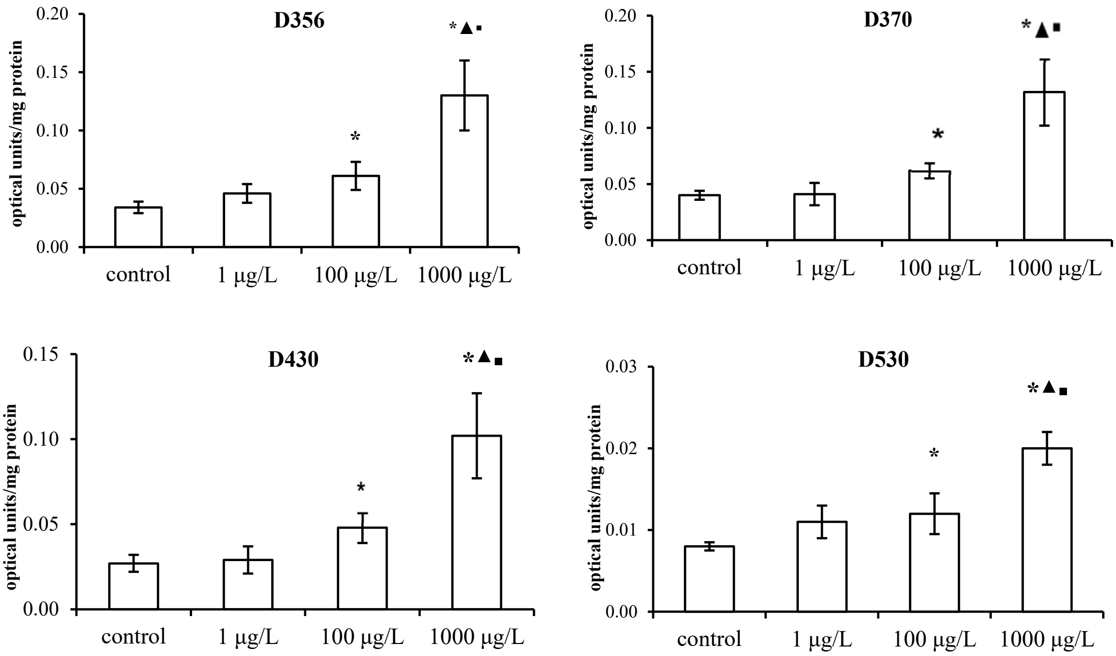

Fig. 3. Level of oxidized proteins in the hepatopancreas of *Mytilus galloprovincialis* exposed to PCBs, D356 - neutral aldehydes, D370 - neutral ketones, D430 - basic aldehydes, D530 - basic ketones
* indicates statistically significant differences as compared to the control (p<0.05), ▲ - indicates statistically significant differences as compared to animals exposed to 1 µg/L PCB (p<0.05), ▪ - indicates statistically significant differences as compared to animals exposed to 100 µg/L PCB (p<0.05)

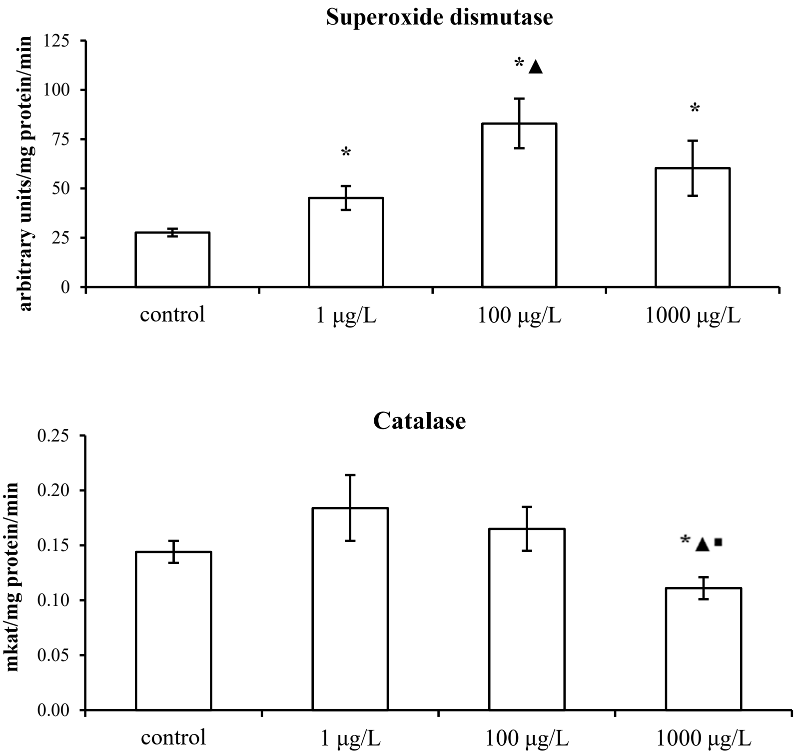

Fig. 4. Antioxidant enzyme activities in the hepatopancreas of *Mytilus galloprovincialis* exposed to PCBs
* indicates statistically significant differences as compared to the control (p<0.05), ▲ - indicates statistically significant differences as compared to animals exposed to 1 µg/L PCB (p<0.05), ▪ - indicates statistically significant differences as compared to animals exposed to 100 µg/L PCB (p<0.05)

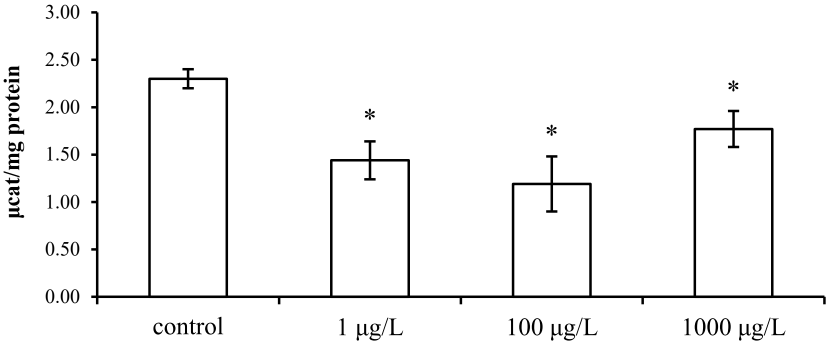

Fig. 5. Cholinesterase activity in the hepatopancreas of *Mytilus galloprovincialis* exposed to PCBs
* indicates statistically significant differences compared to the control ($p<0.05$)

Oxidative stress parameters are the indicators of the oxygen toxicity being suffered by an organism. Oxygen toxicity is caused by the reactive oxygen species (ROS) and oxygen free radicals, and is a result of either increased production of these oxidizing species, or a significant decrease in production of antioxidants. ROS may be produced endogenously, or as a result of the reduction of xenobiotic compounds, including PCBs. SOD and CAT are the antioxidant enzymes that form a part of a cellular defense system that inhibit and detoxify oxyradical formation, protecting cellular damage from the harmful effects of ROS (Kroon et al., 2017; Ozkan et al., 2017).

In our research, the increase in the oxidized proteins level at 100, 1000 µg/L and TBARS content at 1, 100, 1000 µg/L in the hepatopancreas of mussels indicates that PCBs initiate the development of the oxidative stress by intensifying lipid and protein peroxidation. Simultaneously, a significant increase in SOD activity in the hepatopancreas of mussels from all experimental groups is considered as an adaptive compensatory reaction to the toxicants (compensation stage) that demonstrates cellular response to raise in ROS production. However, a decrease in CAT activity in specimens from the III experimental group can be regarded as a consequence of the enzyme activity inhibition and/or a decrease in its buffer capacity resulted from the cumulative effect of PCBs indicating the oxidative stress as well.

The results of the experimental studies revealed that the main tissues accumulating PCBs in *M. galloprovincialis* are the hepatopancreas and the foot (Soldatov et al., 2005). In a chronic experiment it was shown that PCBs at 0.1, 0.3 and 0.9 µg/L concentrations inhibited the activity of glutathione-S-transferase (GST) and caused the induction of glutathione peroxidase activity in the gills of *M. galloprovincialis* (Vidal-Linan, 2016). CAT activity in the hepatopancreas of *M. galloprovincialis* was found significantly increased under the impact of oil polycyclic aromatic hydrocarbons (PAHs) (Lacroix et al., 2015). At the same time, there is the evidence that CAT activity in *Mytilus edulis* increases under PCBs exposition, but not under PAHs exposition (Damiens, et al. 2007). The reverse effect was observed in *Adamussium colbecki* bivalves when exposed to copper and mercury ions. It has been shown that a high dosage load of these metals causes a decrease in CAT activity in hydrobionts tissues (Orbea et al., 2002). On the basis of the experiment regarding the effects of the oil refining products, a significant increase in the level of the lipid peroxidation final products, SOD and CAT activities in tissues of *M. galloprovincialis* was found. The tissue differences in the indicators of the oxidative stress and the AO protection were shown. They demonstrated much higher sensitivity of hepatopancreas cells to the organic pollutants compared to gills (Sukharenko et al., 2017). An increase in SOD activity, the level of lipid peroxidation and a decrease in glutathione-peroxidase activity in hepatopancreas of mussel *Dreissena polymorpha* treated to 150 ng/L PCBs during 5 days were also demonstrated.

At that time, no changes in CAT activity were noted (Faria et al., 2009). The exposure of 0.005, 0.05 and 0.5 µg/L organochlorine pesticide endosulfan for 15 days increased the concentration of glutathione (GSH), MDA, glutathione-S-transferase activity and decreased SOD activity in gills and the hepatopancreas of *Ruditapes philippinarum*. Furthermore, the correlation analysis showed high positive correlation between endosulfan concentration and GSH content in the hepatopancreas only and GST activity, LPO, DNA damage in both tissues (Tao et al., 2013).

ChE plays an important role in the breakdown of acetylcholine. It is known as a behavioral marker for the organisms exposed to the environmental pollutants and is considered as a specific biomarker for organophosphate and carbamate insecticides. Recent studies have shown that other types of pollutants, such as heavy metals, surfactants, PAHs, PCBs and polybrominated diphenyl ethers (BDE-47) may also inhibit ChE activity (Banaee et al., 2013; Vidal-Linan et al., 2015, 2016; Raisi et al., 2018; Malakhova et al., 2020). In the present study a significant decrease in ChE activity in mussels from all experimental groups on the 5-th day of the exposure indicates enzyme inhibition with PCBs that can negatively affect the mollusks' behavior. A significant decrease in acetylcholinesterase activity in the freshwater snails *Galba truncatula* treated only with 0.1 and 0.2 mg/L of Pb and with carbamate pesticide Pirimicarb and Pb was demonstrated by Raisi and co-authors (Raisi et al., 2018). In other work neurotoxic effects of PCBs at 0.1, 0.3 and 0.9 µg/L were not found (Vidal-Linan et al., 2016).

Thus, it can be concluded that detected effects of the toxicants exposure may be considered as temporary and dependent on chemical properties and the concentration of toxicants, exposure time, taxonomic status of the test organism and the target organ/tissue (Soldatov et al., 2005; Faria et al., 2009; Fernandez et al., 2010; Bakhmet et al., 2012; Attig et al., 2013; Franco et al., 2016; Tao et al., 2013; Vidal-Linan et al., 2015, 2016; Benali et al., 2017; Ozkan et al., 2017; Sukharenko et al., 2017; Raisi et al., 2018). All these features must be taken into account when using biochemical parameters as the biomarkers in the environmental monitoring programs.

CONCLUSION

Hence, the results of the experiment have shown that no animal mortality was registered during the experiment. It can indicate quite high toxic resistance of *M. galloprovincialis* to experimental PCBs concentrations. Along with this, it was stated that PCBs at 1, 100, 1000 µg/L caused remarkable changes in biochemical parameters in the hepatopancreas of mussels. The level of oxidized proteins at 100 µg/L and 1000 µg/L as well as lipid peroxidation at 1, 100, 1000 µg/L was significantly higher compared to the control. SOD activity increased, while ChE activity decreased at 1, 100, 1000 µg/L and CAT activity reduced at 1000 µg/L compared to the control. The data obtained demonstrate the development of oxidative stress in the hepatopancreas of mollusks. It occurred due to shifting in the prooxidant-antioxidant balance towards intensification of protein (at 100, 1000 µg/L) and lipid (at 1, 100, 1000 µg/L) peroxidation as well as reduction in CAT activity (at 1000 µg/L). An increase in the SOD activity detected at all experimental concentrations can be considered as an adaptive compensatory reaction to the toxicants. The inhibition of ChE activity found at 1, 100, 1000 µg/L testified to PCBs neurotoxic effect. The level of oxidized proteins and lipid peroxidation, SOD, CAT and ChE activities can be suitable biomarkers to evaluate the toxicity of PCBs and *M. galloprovincialis* can be used as a bioindicator in the monitoring of marine ecosystems contaminated with these pollutants.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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