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Research Article

The effect of mercury ions on the metabolic activity of *Poecilia Reticulata* cells

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Abstract

Among anthropogenic factors polluting ecosystems and affecting living organisms, mercury, and its compounds are the most toxic. In this study, we investigated the effect of mercury ions on freshwater live-bearing fish *Poecilia reticulata*. The cytotoxicity of mercury was analyzed on *Poecilia reticulata* fry using the MTT test. Studies have shown that small concentrations of mercury can have a stimulating effect on the metabolic activity of fish cells, but at high concentrations, the mercury had a depressing effect.

Introduction

Intensive industrial and agricultural use of natural resources has caused a significant change in the direction and rate of migration, zones of removal, and accumulation of most chemical elements, including heavy metals in soils and water bodies, which has led to the deterioration of the environment and the health of the living population [1-4].

Pollution of natural waters is one of the global environmental problems of our time. Along with climatic changes in temperature, illumination, gas, and salt composition, pollution is almost the most important environmental factor to which aquatic organisms are exposed. The strength of this impact is determined by qualitative and quantitative features. The former is determined by the probability of toxicants entering the water, the latter by their concentration in the water, and the duration of the impact on biota.

Among anthropogenic factors that pollute ecosystems and affect living organisms, mercury, and its compounds are the most toxic [5-9]. Mercury contamination of natural waters, plants, and animals is currently characteristic of many regions of the planet. It is associated with the entry of a large amount of mercury into the biosphere as a result of both natural processes and anthropogenic activities. The danger is not only salvo discharges, but also moderate amounts of pollutants entering the waters, for example, flushing from the earth's surface with rain and meltwater [6]. Such substances are most often in concentrations that do not exceed the MPC, but, accumulating in bottom sediments, tissues of aquatic plants, and animals, pose a real threat of secondary pollution, when the concentration gradient comes from the deposited state into the aquatic environment [10]. The problem of mercury pollution is relevant even for aquatic ecosystems in the immediate vicinity of which there are no local sources of metal pollution [6,11,12].

Mercury is a typical complexing agent. Complexes formed as a result of the active interaction of ionic forms of mercury with organic ligands accumulate in bottom sediments, where their concentration can be ten times higher than in water [13].

In the case of ingestion of mercury compounds in the living organism, there are negative changes in various body systems, such as changes in the functional state of the central nervous system and neurotoxic effects [14].

The widespread use of mercury, as well as the pollution of the environment by it and its compounds, make the study of the impact of this metal extremely relevant.

Materials and methods

For breeding *Poecilia reticulata* (Peters, 1859), thermostatically controlled, aerated, illuminated aquariums with a capacity of 50 liters were used. The water temperature

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maintained in the aquariums was 27 °C, the average pH was 7.58 and the light regime was 10 hours. The average planting density was 1 male per 1-2 L of water, and 2-4 L per female. The diet of the fish was mixed and consisted of life (daphnia, gammarus) and dry (mixtures of flakes, pellets) feed.

Mercury solutions in concentrations of 1 and 10 MPC were investigated in the study. The MPC of mercury was taken as 0.0005 mg/L. Mercury (II) nitrate was used to prepare mercury solutions.

The cytotoxicity of mercury was analyzed on *Poecilia reticulata* juvenile fish of 3 weeks of age using the MTT assay according to the standard method. [15].

For the analysis, the fry from each experimental and control group was transferred into «Eppendorf»-type microtubes, and an MTT assay for metabolic activity was performed. For this purpose, water was removed from the microtubes, 100 µL of MTT solution was added, and incubated for 1 h at room temperature in the dark. At the end of incubation, the supernatant was carefully removed, and 200 µL of DMSO was added. After 10 min, the contents of the microtubes were homogenized using a pestle. After the dissolution of the formazan pellets, the supernatant from each test sample was transferred 200 µl to the wells of the plate and analyzed on a «StatFax 2100» immunoenzymatic analyzer («AwarenessTechnology», USA, VIS-model). The optical density was determined at a wavelength of 492 (operating) and 630 nm (noise).

The significance of the difference with the control was assessed by the Mann-Whitney test. The calculations were carried out using the software package Statistica for Windows, version 12.

Results of the study

The action of mercury compounds affects the activity of mitochondrial enzymes involved in energy production processes, which leads to a decrease in mitochondrial membrane potential, separation of components of the electron transport network, the accumulation of ROS in the cell, and oxidative stress [16-19].

The MTT assay is based on the ability of mitochondrial dehydrogenases to reduce MTT, integrally reflecting the number of reactive oxygen species, the ratio of living and dead cells, and the work of the antioxidant system. The subsequent photometric analysis allows us to compare the change in the optical density of the solution relative to the control with the change in the number of viable cells and evaluate the efficiency of the cytotoxic action of the analyzed compound. Cells with a low metabolic rate will recover MTT in small amounts, while rapidly dividing cells will show a high degree of recovery. Figure 1 shows the dependence of the cytotoxic action of mercury ions on fish cells obtained by the MTT assay.

1 MPC of mercury causes stimulation of the metabolic activity of Poecilia reticulate cells by 25.58% after 60 minutes of exposure and by 44.19% after 90 minutes of exposure (Figure 1).

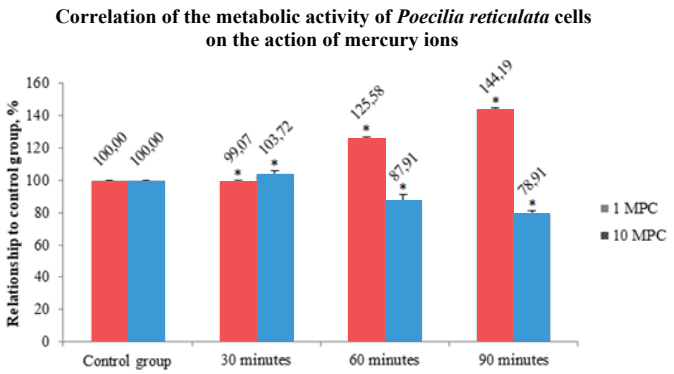

Figure 1: Correlation of the metabolic activity of *Poecilia reticulata* cells on the action of mercury ions. 1 MPC (0.0005 mg/L) and 10 MPC (0.005 mg/L) *$P < 0.05$ – Mann-Whitney test.

Exposure to mercury ions at a concentration of 10 MPC causes a decrease in metabolic activity by 12.09% at 60 minutes of exposure and by 21.09% at 90 minutes of exposure.

Discussion

Stimulation of metabolic activity of *Poecilia reticulate* cells upon exposure to 1 MPC of mercury is presumably caused by activation of detoxification systems represented by SH-containing compounds with high affinity for which mercury has, and effective work of the antioxidant system. It is impossible not to note the high resistance to peroxidation in fish [20-22].

The decrease in metabolic activity when exposed to 10 MPC of mercury can be explained, first of all, by the depletion of components of the antioxidant and detoxification systems, which causes increased generation of free radicals and reactive oxygen species, as well as "occluding" of ion channels and disorders of mitochondria.

Conclusion

Mercury pollution of natural waters is definitely an important problem that cannot be solved even if mercury is removed from industrial circulation. Mercury-containing waste has been causing significant damage everywhere for decades.

It has been found that low concentrations of mercury can have a stimulating effect on the fish *Poecilia reticulata*, which is presumably due to the effective functioning of the antioxidant system and, among other things, the metallothionein proteins at low concentrations of the pollutant.

High concentrations of the pollutant under study had a depressing effect, presumably caused by increased generation of free radicals and reactive oxygen species, as well as "clogging" of ion channels and disruption of mitochondria.

For a more detailed understanding of the effects of mercury compounds on various hydrobionts, it is worth considering a wider range of concentrations with shorter measurement intervals in the future, as well as investigating other mercury-containing compounds. Such studies will provide a holistic

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picture of the effects of mercury and its suppressive and stimulatory effects at various concentrations. This information can be used for the successful selection of test objects in biotesting.

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