Evaluation of the cytogenotoxic activity of mercury by analyzing chromosomal aberrations in wheat root meristem cells

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Abstract. The work evaluates the cytogenotoxic effect of mercury by performing a primary screening on a plant object. The authors determined the qualitative and quantitative characteristics of a cytogenotoxic effect depending on the exposure time and the dose of mercury nitrate (Hg(NO₃)₂) in anaphase and telophase of mitosis on wheat apical meristem cells of the Memory of Aziev variety. In the process of primary screening to assess the cytogenotoxic effect of the test substance, the nature and diversity of chromosomal abnormalities in the wheat meristem cells of the presented variety were studied. The frequency of occurrence of various types of chromosomal aberrations was analyzed. The complex shows the effect of mercury salts on viability indices and chromosomal instability of wheat. The cumulative pathology curves for different exposures of (Hg(NO₃)₂) are presented. A significant increase in the number of pathologies was shown in accordance with the increase in mercury concentration in anaphase of mitosis. A similar pattern was observed in the change in the total number of chromosome aberrations in accordance with the increase in the soaking time of seed material. The results of the study of the cytogenotoxic activity of mercury make it possible to assess the toxicity of mercury (Hg(NO₃)₂) for plant objects and recommend the object used in the experiment as an adequate model of test systems for studying xenobiotics.

1. Introduction
Contamination with heavy metals, and mercury in particular, is a fairly strong stress factor of the environment [1,2]. Understanding how living organisms react to it, allows us to assess the risk of its impact [3, 4]. The most dangerous is the effect at the subcellular level, manifested in changes in the genotype as a result of chromosomal mutations. Such changes subsequently change the gene pool of populations [5]. Despite the prevalence of mercury in the environment, there are only a few data in the literature about its direct or indirect mutagenic effect. In the works of a number of authors, mercury is ranked as a number of chemical mutagens [6, 7, 8]. This paper investigates the effect of mercury on toxicity and induction of pathological mitoses in the cells of the root meristem of the plant organism using the example of the Aziev memory wheat variety.

2. Materials and methods of research
Studies were conducted on wheat grown in an environmentally friendly environment, without the use of fertilizers. This test object was chosen because of its widespread use, as well as its sensitivity at all levels of the organization to environmental pollution [9].
The exposure to mercury nitrate was carried out in three concentrations; the control plants were treated with distilled water. The study of cells (at least 1000) in ana- and telophase was carried out after 24, 48 and 72 hours after application. The following types of cellular mitosis pathology of the wheat apical meristem were taken into account: fragmentation, single and multiple chromosome bridges, combined pathology, as well as lag at divergence to the poles. The results of the study were processed using student's criterion, correlation analysis and the Kruskal-Wallis H-test.

3. Results and discussion
In anaphase, the greatest number of pathologies at concentrations of maximum permissible concentration (MPC) and MPC100 was recorded at an exposure of 72 hours, and at a concentration of MAC of 10, at an exposure of 48 hours. At the same time, when exposed for a short time, mainly pathologies were subjected to fragments, and during long-term, all other pathologies under study.

The number of pathologies detected after exposure to mercury nitrate is shown in figure 1 a, b. The number of pathologies during daily exposure increased from 13.45 ± 1.1 to 17.05 ± 1.3 (p <0.05-0.001). When exposed for 48 hours, the number of chromosomal aberrations increased from 13.4 ± 0.9 to 16.05 ± 1.2; at 72 hours of exposure, from 17.3 ± 1.3 to 18.1 ± 1.3, respectively.

![Figure 1](image_url). Pathology in anaphase.
With an exposure of 24 hours in concentrations of MPC (7.15 ± 0.7) and MPC10 (6.25 ± 0.4), the number of fragments in anaphase exceeded the control values (5.4 ± 0.6) by 1.3 and 1.1 times, respectively. A similar trend was observed in relation to other pathologies. With regard to single bridges: at MPC10, the indicator was 1.5 times higher than the control variant; sets of bridges with MPC100 - 1.3 times (r = 0.65, p≤0.05); combined pathology with MPC100 - 1.1 times (r = 0.67, p≤0.05). Exposure 48 hours exceeded the anaphase values compared with the control are fixed in the following variants: single bridges at MPC 10 exceeded control values 1.4 times, combined pathology at concentration MPC10 - 1.6 times, and at MPC100 - 1, 3 times. At an exposure of 72 hours, the fragments exceeded the control values at MPC and MPC100 by 1.0 and 1.2 times, respectively. Single bridges at MPC100 exceeded the control by 1.1 times, the set of bridges - 1.0 times, combined pathology - for all exposure periods: MPC - 1.1 times, MPC10 - 1.4 times, MPC100 - 1, 2 times.

The graph of the cumulative curves of the spectrum of pathologies is constructed to denote the space of changes in pathologies. Accumulated frequencies are determined by sequential summation of frequency intervals. In accordance with the schedule, changes in anaphase occur in all pathologies studied.

A positive connection of the combined pathologies in anaphase with single bridges (r = 0.54, p ≤ 0.05) and a set of bridges (r = 0.59, p ≤ 0.05) were also found; negative fragments with single bridges (r = –0.37, p ≤ 0.05) and a set of bridges (r = –0.82, p ≤ 0.05).

In telophase, the greatest number of pathologies at all concentrations was observed at 48 hours exposure, and the predominant type of pathology was lagging chromosomes (Figure 2 a-c). When exposed to 24 hours, a decrease in the number of pathologies was observed with an increase in the concentration of mercury nitrate from 18.05 ± 1.0 in the concentration of maximum concentration limits to 15.35 ± 1.4 at MPC100. At a concentration of MPC 10, a decrease in the total number of pathologies to 13 ± 0.9 was recorded.
At the telophase stage, there is a decrease in the number of chromosomal aberrations with an increase in mercury concentration, as well as a term for soaking the seeds. For example, when exposure to seeds with mercury nitrate for 24 hours, the number of pathologies was $65.5 \pm 1.2$, with an increase in the exposure period $- 64 \pm 1.3$.

The pathologies in telophase in the order of their increase are placed in the following order: the set of bridges ($5.4 \pm 0.3$), combined pathology ($6.4 \pm 0.3$), single bridges ($8.8 \pm 0.4$), fragments ($15.1 \pm 0.6$) and lagging chromosomes ($29.8 \pm 0.8$).

The graph of the cumulative curves of the spectrum of pathologies in telophase turned out to be similar to that in anaphase.

In some cases, there was an increase in the percentage of aberrant cells after exposure. For example, when processed for 24 hours at concentrations of MPC and MPC100, the number of sets of
bridges exceeded control values by 1.2 and 1.9 times, respectively. Combined pathology with MPC100 exceeded control by 1.3 times.

When treating with mercury nitrate within 48 hours, excess values were noted in the following cases. Single bridges: MPC - 1.4 times, MPC10 - 1.7 times, MPC100 - 2.2 times. Many bridges: MPC - 2.5 times, MPC10 - 1.4 times, MPC100 - 2.3 times. Combined pathology with a concentration of MPC exceeded control by 1.7 times, MPC10 - by 1.8 times, and with MPC100 - by 2.6 times.

When exposed for three days, the fragments exceeded the control values at MPC by 1.2 times, for MPC 10 - by 1.1 times and for MPC 100 - by 1.1 times. Single bridges are exceeded by 1.5 times at a concentration equal to MPC.

4. Conclusion
The mutational effect of mercury on plant organisms was revealed, which is manifested in chromosome changes in the phase of mitosis in the apical meristem. A comparative analysis of the characteristics of the cytogenetic effect of mercury revealed a greater number of chromosomal aberrations in anaphase compared with telophase. The mutagenic effect of mercury in anaphase increases with increasing concentration and exposure time. Such a pattern was not found in telophase. This phenomenon is explained, from our point of view, by the so-called “saturation effect”. And it can also be caused by the activation of the protective mechanisms of the plant organism.

In anaphase, pathological changes mainly affect fragments. Apparently, this is due to disturbances in the discrepancy of chromatids in mitosis. Combined pathology is least affected.

In telophase under the action of mercury, the most significant decrease in lagging chromosomes is observed. This phenomenon is associated with a disorder in the kinetochore area when chromosomes diverge towards the poles. Changes in other chromosomal aberrations with respect to control plants in telophase have no statistically significant value.

In general, the conducted study allows to conclude about the average level of mutation under the action of mercury nitric acid cells of the root meristem of the Aziev memory wheat variety.

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