Genotoxicity Assessment of Bottom Sediments of the Chumysh River Using the Allium-Test

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Abstract. We performed a bioassay of aqueous extracts from the bottom sediments of the Chumysh River near the settlement of Talmenka (the Altai Territory of Russia) using the Allium test. The presence of toxic components that caused a decrease in the mitotic activity of onion root meristem cells was revealed. The frequency of pathological mitoses, significantly exceeding the background value (on average, 16.3 times), indicates the presence of factors with total mutagenic activity in the samples. We found violations of chromosome divergences (emissions beyond the spindle, lagging, running off and premature chromosome divergence), abnormalities of the mitotic apparatus (multipolar, asymmetric, monocentric mitosis, polyploidy), bridges (single and multiple). The prevalence of spindle pathologies suggests chemical contamination of the bottom sediments. Stress factors present in bottom sediments activate adaptive mechanisms of the test-object cells, aimed at maintaining their viability and reliability of the transmission of genetic information to the next cellular generations. Such adaptation mechanisms include the formation of micronuclei and cell polyploidization.

1. Introduction

Anthropogenic impact on the hydrosphere leads to a widespread increase in toxic pollution of water systems. The problem of maintaining the cleanliness of inland waters is one of the main among modern global environmental challenges [1]. A consequence of the accumulation of various chemical substances in water is the migration and transformation of toxicants, their transfer along trophic chains, and the synergistic and antagonistic effects in multicomponent systems. Chemical elements that are not mutagenic, when interacting in an aqueous medium, can form compounds with a genotoxic effect. Reservoirs in the Altai Territory of Russia are no exception to the general trend of a worsening ecological state and are under significant anthropogenic pressure. Most of the water bodies in our Region belong to the “very polluted” and “dirty” categories according to the water quality class. According to annually published official reports on the state of the environment, the most characteristic substances polluting the surface waters of the Altai Territory are petroleum products, volatile phenols, nitrogen compounds, phosphates, and iron. Most of these substances are xenobiotics and can have toxic, carcinogenic, teratogenic or allergenic effects on living systems. A large role in the functioning of the aquatic ecosystem is assigned to bottom sediments, which on the one hand are the habitat of benthic organisms and a food source for many aquatic organisms, and on the other hand, a place for the deposition of pollutants. Moreover, the degree of accumulation of toxicants, especially organic ones, can be so high that they are able to completely suppress the self-cleaning process in the bottom layer of water. In this regard, the study of the potential impact on biological objects of substances accumulated in bottom sediments is relevant [2].

Plant test systems are currently widely used as indicators of the genotoxicity of various environmental factors [3]. Biotesting with onion (Allium cepa L.) has shown its high efficiency for assessing the toxic and mutagenic activity of a large number of compounds [4]. This object allows one to register various types of chromosomal mutations induced both by direct mutagens that directly damage DNA and by mutagens that acquire genetic activity in the living body during metabolism [5]. The performance of the Allium-test correlates well with the results obtained at other objects, including mammalian cells [6]. This system is also widely used to evaluate the biological effects of water pollution.
The purpose of this work is to evaluate the total mutagenic activity of bottom sediments of the Chumysh River (Russia, the Altai Territory) using the Allium-test.

2. Materials and methods

2.1. Sampling site
Samples of bottom sediments (BS) of the Chumysh River were taken during high water (end of April) and a decreased potential for self-cleaning of the reservoir (first decade of October) (Galakhov, 2003). The Chumysh River is one of the largest tributaries of the Upper Ob. It flows mainly along the Biysk-Chumysh Upland and in mainstream and downstream it crosses a number of settlements in the Altai Territory of Russia, including the industrial city of Zarinsk and the settlement of Talmenka. We examined 4 sites located in the vicinity of Talmenka (Latitude: 53° 49′05″ N, Longitude: 83° 34′03″ E) and selected 24 samples (12 in each season, 3 repetitions for each site): BS-1 - the right bank, 1 km above the settlement; BS-2 - the left bank, 1 km above the settlement; BS-3 - the right bank, 1 km below the settlement; BS-4 - the left bank, 1 km below the settlement. Samples were taken near the coast from the upper layer up to 20 cm deep, wiped through a polyethylene network with a mesh size of 0.9 cm, and stored at 4–5 °C in a refrigerator.

2.2. Sample preparation
To prepare aqueous extracts of bottom sediments, each sample was mixed with tap water in a volume ratio of 1 : 4, shaken for 2 hours, then precipitated for 1 hour. The supernatant was drained and centrifuged for 10 min at a speed of 4000 rpm.

2.3. Preparing a test-object
The potential biological effect of aqueous extracts was evaluated using the Allium-test. Before testing, the outer scales and the lower plate were removed without damaging the primary roots. In order to synchronize cell divisions, standard bulbs were soaked in tap water and kept for 24 hours in the dark at a temperature of + 4 °C, then the temperature was raised to + 22 °C. When the roots reached a length of 2-3 mm, the bulbs were transferred into glasses containing extracts of bottom sediments, and cultivated for several days at a temperature of + 24-25 °C. The control was a sample with filtered tap water of medium hardness. In addition, a variant was studied in which the bulbs were germinated in glasses with distilled water (sample 5). Roots 10-12 mm long were fixed by the Carnoy method in a mixture of 96 % ethanol and acetic acid (3 : 1). The material was stored in 70% ethanol at a temperature of + 4 °C. A cytological analysis of mitosis in the root meristem cells was performed using an AXIO ZEISS Imager.Z1 microscope with magnification of 10×15×90 on temporal preparations after staining with acetoorcsein [8].

2.4. The analyzed parameters
The total number of cells on the preparation, the number of dividing cells, the number and type of pathological mitoses were taken into account. The volume of analysis was at least 2000 pcs. per sample. The cytotoxicity of bottom sediments was evaluated by the mitotic index (MI, %). To assess the genotoxicity of the samples, the proportion of pathological mitoses among the total number of dividing cells (PM, %), as well as the types and frequencies of various abnormalities of cell division, were determined. Mutagenic activity of bottom sediments was assessed by the total mutagenic activity (TMA), the level of which was determined by the indicator "Severity of Mutagenic Activity" (SMA). SMA was calculated as the difference in the excess (%) of the frequency of visible mutations in Allium cepa caused by exposure to aqueous extracts over the control level (Table 1).

| Parameter | Lack of TMA | Weak TMA | Medium TMA | Strong TMA |
|-----------|-------------|----------|------------|------------|
| The excess frequency of CA over the control level (difference) | No significant differences | Significant differences of less than 10% | Significant differences of 10-25% | Significant differences of more than 25% |

Table 1. Assessment of the level of total mutagenic activity (TMA) when using the method of counting chromosomal aberrations (CA) in the meta- and ana- telophase of mitosis in Allium cepa L.
2.5. Statistical analysis
The significance of differences in the results was evaluated using Student's t-test at p ≤ 0.05. Statistical data processing was performed using the Microsoft Excel 2010 application software package.

3. Results and discussion
Cytogenetic methods are considered as the most sensitive for the effective assessment of the mutagenic and toxic effects of adverse environmental factors on the environment. Analysis of mitotic cycle disorders makes it possible to identify early changes in the body's cytogenetic system in the absence of phenotypic manifestations and make adequate predictions of the further state of the system in changing environmental conditions. It is known that a number of mutagens have synergistic and cumulative effects on the cellular apparatus, which makes it possible to evaluate their complex effect even with a small level of contamination.

One of the main characteristics of the mitotic regime of a living organism is the mitotic activity of cells of meristematic tissues, the indicator of which is the mitotic index (MI).

Figure 1 shows the proliferative activity of the cells of the apical meristem of the test-object.

![Figure 1](image)

**Figure 1.** Mitotic activity of cells of the root meristem of onion, induced by aqueous extracts of bottom sediments of the Chumysh River (The Altai Territory, Russia).

The water extracts of the bottom sediments of the Chumysh River, regardless of the sampling period, had a depressing effect on cell division during the formation of the onion root system. The average MI value was 5.67% (BS-1 – BS-4), which was 1.7 times lower than the control indicator (9.66%, p ≤ 0.05). The most significant effect was observed in samples taken in the first decade of October, when the mitotic activity index reached only 50.52% relative to the control value (p ≤ 0.01). Bottom sediments of the right-bank section of the river (BS-1, BS-3) caused a significant decrease (p ≤ 0.05) of the mitotic index of the biotestor cells in comparison with the BS of the left bank (BS-2, BS-4) by 21.3 and 23.3% during high water (April) and autumn low-water (October), respectively. Statistically significant differences in the action of samples collected above and below the settlement have not been established.

Thus, the results obtained indicate the presence of toxic components in the bottom sediments that cause depression of the mitotic activity of the apical meristem of the onion roots. Our earlier study of water samples from the Chumysh River revealed a similar heterogeneity in the temporal and spatial distribution of characteristics within the same riverbed. However, the samples taken in April during the high water, unlike the samples of the autumn period, had a mitosis-stimulating effect on proliferating tissues, which is typical for weak stressful influences of pollutants or their synergistic effect [9]. Stimulation of cell divisions in the tissues of the test-object cultivated on aqueous extracts of bottom sediments was not observed, which indicates higher concentrations of negative components or differences in their composition. River water and bottom sediments of the same reservoir have different environmental effects on the tested objects. This is probably due to the fact that there are substances in the water that are in transit from upstream sections of the river. As a result, short-term acute pollution is possible, which in the future does not have a noticeable negative effect on the ecosystem of the reservoir. The accumulation of pollutants and their transformation over a long period take place in the bottom sediments, which leads to a more noticeable toxic effect on the biota. This thesis can be confirmed by the above noted fact of a significantly stronger inhibitory effect of water extracts of bottom sediments, selected in the autumn, during the period of a decreased potential for self-purification of the reservoir, on cell proliferation.
It is of interest to discuss the results of germination of a test-object immersed in distilled water (sample 5). The average MI value was 12.25%, which significantly exceeded the control (p ≤ 0.05). The mitosis-stimulating effect of distilled water as a medium for the cultivation of onion was similar in effect to the experimental samples of the spring period [9], which indicates its toxic effect on a living object. Therefore, the use of distilled water as a control during biotesting various media or substances is undesirable.

Figure 2 presents the results of a cytogenetic study of the level of pathological mitoses in the cells of the apical meristem of the onion roots, which allows us to evaluate the total mutagenic activity of the components of the bottom sediments of the Chumysh River.

![Figure 2. The frequency of pathological mitoses induced by water extracts of bottom sediments of the Chumysh River in the apical meristem of onion roots, %.

The background value of various disorders of cell division was 0.86%. Pathologies induced by water extracts of bottom sediments exceeded the control by an average of 16.3 times. Most often, abnormal cells were found in the roots of onions grown on samples taken in October (15.34%). A rather high level of cytogenetic disturbances is an indicator of the genotoxic effect of BS on the plant organism. According to the SMA indicator, the level of total mutagenic activity of water extracts from the bottom sediments of the Chumysh River is classified as “high”. In contrast to the general level of mitotic activity of the root meristem of the test-object, the spatial differences in the frequency of pathological mitoses depending on the place of sampling are statistically insignificant. The increased level of various cell division abnormalities in the root meristem of onion (3.58%) cultivated in distilled water (sample 5) confirms the conclusion that it is incorrectly to use distilled water to estimate the background values of the frequency of spontaneous disturbances in mitotic cycles of plant test-systems.

A comparative analysis of the frequencies of the main types of mitosis disturbances also did not reveal significant differences between the samples taken in different areas of the riverbed during the same season. In this regard, the results of both the autumn and spring periods of the study of the samples are combined into groups depending on the type of pathology. These are violations of chromosome divergences (emissions beyond the spindle, lagging, running off and premature chromosome divergence), abnormalities of the mitotic apparatus (multipolar, asymmetric, monocentric mitosis, polyploidy), bridges (single and multiple). Other anomalies, the frequency of which was small, are grouped into the “other aberrations” group, which included fragmentation, agglutination, and pulverization of chromosomes (Figure 3). It should be noted that the spectrum of mitotic disturbances in this experiment did not differ from the aberrations noted earlier in the study of water samples from the Chumysh River [9].
Among the revealed abnormalities, various types of uneven chromosome divergence dominated both in the control and experimental samples (64.6–88.6%). Lagging of chromosomes in metakinesis and anaphase occurs, as a rule, when the kinetochore is damaged. Such chromosomes “drift” in the cytoplasm without making movement to the equatorial plate, and at the telophase stage they are forced into one of the daughter nuclei or subsequently eliminated. The second largest group turned out to include various anomalies of the mitotic apparatus. Their frequencies ranged from 3.6 (control) to 22.9% (samples taken in autumn). Such violations of cell division, as a rule, cause a delay in mitosis at the metaphase stage. The studied samples induced asymmetric, mono- and multipolar mitoses. Such anomalies are most often associated with the disorganization of centrioles and the spindle of division, which indicates the presence of mitotic poisons in the aqueous extracts that affect the polymerization-depolymerization of the cytoplasmic globular tubulin protein, which is part of microtubules. The described pathologies result in multinuclei, aneuploidy, and polyploidy. Chromosomal aberrations in the form of anaphase bridges were detected in 2.68.9% of dividing cells. In addition, cells with centric and acentric fragments, chromosome and chromatid rings were recorded. Fragmentation of chromosomes is a sign of destruction of their structure associated with the lysis of DNA molecules by enzymes and serves as an indicator of genome instability. Chromosome fragmentation is known to result from exposure to ionizing radiation or chemical mutagens. Most fragments lack centromeres and kinetochores involved in the movement of chromosomes. Therefore, acentric fragments remained motionless and lagged during metakinesis and anaphase. In rare cases, pulverization of the entire chromosome complex into fragments was observed. During mass fragmentation of chromosomes, the fragments randomly scattered across the cytoplasm and also did not participate in the general movement of the chromosomes. The presence of bridges indicates that the studied samples contain substances that can cause adhesion of the telomeres or breaks in DNA, leading to non-reciprocal translocations. In asymmetric exchange, as a result of connecting fragments with centromeres, a dicentric forms (a chromosome with two centromeres), which leads to the appearance of bridges when the chromosomes diverge in anaphase.

The result of fragmentation or lagging of individual chromosomes around which the nuclear membrane forms in the telophase is the appearance of micronuclei. Newly formed micronuclei are either preserved throughout the entire cell cycle, or undergo pycnosis, are destroyed and removed from the cell. All investigated samples of the bottom sediments of the Chumysh River induced micronuclei. These structures revealed at the telophase stage were small, well-shaped rounded formations of nuclear material located in the cytoplasm of the cell at some distance from the main nucleus. In the meristematic tissue of the test object, cells with one micronucleus, less often with two or three, predominated. By the size of micronuclei, one can judge the changes that occurred in the chromosome set. Thus, the formation
of large micronuclei is associated with genomic disorders. Small micronuclei are due to violations in the structure of chromosomes. A comparative analysis of cell diversity with this type of abnormality showed the predominant appearance of large micronuclei, which is probably due to the spectrum of pathological mitoses. As indicated above, various disorders of the mitotic apparatus and chromosome divergences dominated among other pathologies (Figure 3).

According to some researchers, the formation of micronuclei is a consequence of the manifestation of the adaptive mechanism of the population of the tester's meristem cells under the influence of aggressive environmental factors. The biological meaning of this process is to isolate chromosomes or their fragments damaged by mutagens, which prevents their further participation in cell division and the transfer of genetic information to the next generation of cells. Since micronuclei, as a rule, are eliminated when passing checkpoints of the cell cycle, their isolation is aimed at maintaining cell viability. This can be considered as self-defense of the genetic apparatus from subsequent abnormal divisions, in some cases leading to the death of this cell. In our experiment, it was found that samples inducing a high frequency of mitotic disorders caused the formation of the maximum number of cells with micronuclei. So, in the material selected in the autumn period, where the proportion of pathologies was highest, 5.4% of cells with micronuclei were found. The frequency of induction of such structures in the root meristem of onion cultivated on spring samples was significantly lower and amounted to 3.2%. The use of distilled water for germination of the test-object also caused the isolation of a certain number of micronuclei (0.41%) in daughter cells during mitotic division, while they were absent in the control (tap water). The fact of the absence of micronuclei in the cells of control samples and the intensification of the process with an increase in the frequency of pathologies associated with disorientation and violations of chromosome divergences, in our opinion, confirms the hypothesis of the launch of adaptation mechanisms at the cellular level.

As noted above, cytogenetic testing of the BS of the Chumysh River revealed the presence of a certain number of polyploid cells among the pool of various types of pathological mitoses, mainly when evaluating samples taken in October. In the control in the meristematic tissue of onion, the phenomenon of polyploidy was not observed. It is known that the ploidy of the genome is also controlled by a system of checkpoints [10]. In response to a change in ploidy, this system blocks further cell progression in the cell cycle. So, when studying mitosis in Drosophila larvae of the f13 line (mutant in the gene of the cell cycle), an increase in the nucleus ploidy was observed in individual cells after monopolar divergences of chromatids in anaphase, resulting in tetraploid cells. However, further blocking of the division of tetraploid cells and their complete absence in the metaphase occurred [11]. Thus, in our experiment, the appearance of polyploid cells in the tissues of the test-object at the maximum mutational pressure of the incubation medium (aqueous extracts of bottom sediments) is most likely associated with the induction of adaptation mechanisms at the cellular level. It can be assumed that the cell cycle control system will block further division of such cells, preventing the preservation of the genomic mutation. In addition, studies on Drosophila showed that hyperploid cells (2n + 1), unlike tetraploid cells, overcame checkpoints, retaining the ability to divide, and were subsequently detected in metaphase [11]. Other cases of overcoming of “checkpoints” of the cell cycle by mutant aneuploid cells are known. This phenomenon is called “adaptation of control points” [12]. It is possible that activation of micronucleus formation processes in the onion meristem under the influence of substances present in the studied extracts is associated with an increase in the proportion of hyperploid cells. In turn, they arise as a result of pathologies of the spindle and chromosome kinetochore anomalies. The unhindered passage by such cells of the third control (M-reconciliation) point of the cell cycle triggers the mechanism of isolation of "extra" chromosomes and their fragments in the form of isolated micronuclei.

Thus, depression of mitotic activity, the induction of a large number of pathological mitoses and a wide range of aberrations in the dividing cells of the test-object indicate a multicomponent composition of aqueous extracts of the bottom sediments of the Chumysh River, which are capable of exhibiting toxic and mutagenic effects. It is believed that the nature of cell division abnormalities may reflect the nature of the factor present in the environment. For example, the effect of radiation leads to an increase in the frequency of chromosomal aberrations in the cell (deletions, translocations), and the action of chemical mutagens most often causes gene mutations or damage to the mitotic spindle [1-2]. The prevalence of division spindle pathologies in our study, leading to disorientation of chromosomes in the metaphase and multipolar and asymmetric mitosis in the ana-telophase, suggests that the bottom sediments of the Chumysh River are chemically contaminated in the vicinity of Talmenka. It is
interesting to note that a higher level of pathological mitosis induced by autumn samples (15.34%) is also due to an increase in the spectrum of anomalies in the proportion of mitotic apparatus pathologies (22.9%), which confirms the chemical nature of mutagenic factors.

4. Conclusion
As a result of the biotesting of water extracts from the bottom sediments of the Chumysh River, the presence of toxic components was revealed that caused the depression of the mitotic activity of the root meristem of the onion. The frequency of pathological mitoses, significantly exceeding the background value, indicates the presence in the samples of factors with total mutagenic activity (TMA). According to the indicator “Severity of Mutagenic Activity”, the TMA level of experimental samples is classified as “high”. The prevalence of spindle pathologies suggests that there is chemical contamination of the bottom sediments of the Chumysh River in the vicinity of Talmenka. Stress factors present in the bottom sediments activate the adaptive mechanisms of the cells of the test-object, aimed at maintaining their viability and reliability of the transmission of genetic information to the next cellular generations. Such adaptation mechanisms include the formation of micronuclei and cell polyploidization.

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