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Toxic and mutagenic activities of surface water from the Chumysh River

L.P. Khlebova[1], N.N. Chernysheva[2], A.P. Kraynov[1]

[1]Altai State University, Barnaul, Russia
[2]Altai State Agricultural University, Barnaul, Russia
E-mail: hlebova61@mail.ru; nnchernisheva@mail.ru; tema_rid@mail.ru

The Allium test system is worldwide used for biological monitoring of environmental pollutants. We have studied toxic and mutagenic activity of water from the Chumysh River, which is one of the largest tributaries of the Upper Ob. Water samples were taken near industrial settlement Talmenka (Altai Territory of Russia) during the spring flood and low autumn water level in 2015. Root tips of *Allium cepa* L. was used as a biological model. There were examined 30 samples. Purified tap water of medium hardness served as a control sample. After the synchronizing the cell divisions, the onion bulbs with roots reaching a length of 2-3 mm were transferred to glass cups containing the selected samples of River water and cultured for several days at a temperature of +24 ± 1°C. After exposure, root tips were rinsed in distilled water and fixed in a cold mixture of ethanol and acetic acid (3:1). Fixed samples were used after 12-24 h or transferred to 70% alcohol and stored in refrigerator at a temperature of +4°C until required. The fixed materials were hydrolyzed in 1N HCl at 60°C for 5-8 min and squashed in aceto-orcein. Prepared slides were viewed under the microscope at a magnification of ×90. The mitotic index (MI), the phase indices, the frequency of abnormal mitosis, and chromosomal aberrations were determined by the examination of 500 cells per a replicate (100 cells per slide). We established that the decreased or increased levels of mitotic activity and the frequencies of pathological mitoses (up to 7.9%, $P < 0.05$) in onion root tips revealed the presence of mitotoxic and genotoxic agents in the Chumysh River water. We found that the most number of chromosomal abnormalities occurs at the stages of meta- or anaphase. The main abnormalities are chromosome laggings in meta- and anaphase, chromosome bridges, chromosome fragments and micronuclei. Their number increased in 5.0-10.8 times compared with the control value. It has been discovered the temporal and spatial distribution of compounds with different toxicity and genotoxicity within a stream. The greatest level of mitotic depression and the highest frequency of chromosomal mutations were observed in the tissues of the onion root tips germinated on the samples collected in low autumn water. The mechanisms of plant adaptation to unfavorable environmental factors are discussed.
Keywords: cytogenetic monitoring, genotoxicity, mitotic activity, mitotic index, chromosome aberrations, Allium test, root tip cells, surface water, the Chumysh River.

Introduction

Environmental health is one of the most acute global problems around the world. Various ecosystems, including water ones, are subjected to the combined effect of the negative factors of different nature. The assessments of the environment, as well as the forecast of the hazard from complex pollution are based on the results of biological monitoring using the responses of living organisms. Biomonitoring is a regular investigation of behavioral, functional, and morphological changes in organisms and their populations under the action of chemical, physical, and ecological factors. Genotoxicity is the most important area of biomonitoring because it can cause carcinoma of somatic cells and harmful mutations of ova and sperm cells, affecting the health of the next generation (Kotelevtsev et al., 2009; Shevtsova & Gudkov, 2009; Zaitseva et al., 2013; Geras'kin et al., 2016). Genotoxicity tests can be classified according to the information they provide. There are chromosomal aberration tests, including a cytogenetic analysis of structural disturbances of chromosomes in metaphase and anaphase of mitosis, and a micronucleus test; recombination assays, based on registration of sister chromatid exchanges in eukaryotic systems, as well as reciprocal mitotic crossing-over and mitotic gene conversion in yeast; detection for recessive (on Drosophila) and dominant (on mice) lethal; the micro array techniques, which allow detecting changes in the gene expression; gene mutation analyses on microorganisms or cultured animal cells (Seth et al., 2008; Kotelevtsev et al., 2009; Kang et al., 2013; Alimba & Bakare, 2016; Hemachandra & Pathiratne, 2016).

Higher plants are recognized as excellent genetic models to detect environmental mutagens, and are, therefore, frequently used in monitoring studies (Kovalchuk et al., 2001; Shevtsova & Gudkov, 2009; Geras'kin et al., 2011a; Goncharova & Lyashenko, 2011; Sadowska et al., 2011; Khlebova & Ereschenko, 2012; Barberrio, 2013; Kalaev & Popova, 2014; Shevtsova et al., 2014; Alexeyeva et al., 2016; Geras'kin et al., 2016; Khlebova & Bychkova, 2016; Medvedeva & Bolsunovsky, 2016; Prysedskyj, 2016). Among plant test systems, *Allium cepa* L. is characterized by high informative properties on the cellular and genetic levels. This test-object allows recording various types of chromosomal mutations induced both by mutagens directly damaging DNA and promutagenes acquiring genetic activity in the living organism in the process of metabolism (Fiskesio, 1985; Seth et al., 2008; Leme & Marin-Morales, 2009; Yildiz et al., 2009; Geras'kin et al., 2011b; Barberrio, 2013; Trushin et al., 2013; Firbas & Amon, 2014; Dutta & Ahmad, 2016; Khlebova et al., 2016; Silveira et al., 2016; Cabuga et al., 2017; Duarte et al., 2017). It has been established that the results obtained by using *A. cepa* are highly correlated with those carried out on mammalian cells, including human ones (Ghosh et al., 2011).

Water objects in the Altai Territory (Russia) are not an exception to the general trend of a deteriorating ecological situation. They also experience a significant anthropogenic load. Most of the reservoirs of the region belong to the categories of "very contaminated" as well as "dirty" waters. According to the annual published reports on the state of the environment, the most frequently detectable substances polluting the surface waters in the region are petroleum products, volatile phenols, nitrogen compounds, phosphates, and total iron (http://altaipriroda.ru/). Most of these substances belong to the group of xenobiotics and may have toxic, carcinogenic, teratogenic or allergenic effects on living systems.

The aim of our work is to assess the cyto- and genotoxic effects of surface water from the Chumysh River on living organisms by the Allium-test.

Materials and Methods

The Chumysh River is one of the largest tributaries of the Upper Ob. The River originates on the Salair Ridge in the Kemerovo Territory (Russia), flows mainly along the Biysk-Chumysh Upland and crosses some settlements in the Altai Territory, including Zarinsk city and industrial settlement Talmenka. It belongs to the eastern European type of Rivers, characterized by high spring water level, low summer and winter water levels, and high runoff in autumn. The share of snow-fed is more than half the flow rate per year, and the maximum flood is in April (Galakhov et al., 2003). We investigated the Chumysh River water near Talmenka automobile bridge. The water samples were collected in 2015 during the periods of high spring water in the end of April (April 28) and low autumn water (low potential for self-cleaning capacity) in the first ten days of October (October 10): sample 1 – right bank, 1 m from the shore, surface; sample 2 – right bank, 1 m from the shore, depth 1 m; sample 3 – the middle of the stream, the surface; sample 4 – left bank, 4 m from the shore, surface; sample 5 – left bank, 4 m from the shore, depth 1 m. Samples were taken in triplicates from each sampling point aseptically into plastic containers and kept in an ice box. Thus, 30 samples of River water were examined.

For cytogenetic analysis, a bulb onion was used as a test object (Barberrio et al., 2011). Approximately equal sized healthy onion bulbs, weighing 30-35 g each were selected for the experiment. To synchronize cell divisions, 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 ± 1°C. When the roots reached a length of 2-3 mm, the bulbs were transferred to glass cups containing the selected samples of River water and cultured for several days at a temperature of +24 ± 1°C. As a control, we used filtered tap water of medium hardness. After the exposition, the rootlets were fixed in aceto-alcohol according to Carnoy's method (1:3). The material was stored in 70% ethyl alcohol at a temperature of +4°C. Before the analysis the root tips were hydrolyzed in 1N HCl at 60°C for 5-8 minutes after which they were washed in distilled water. Aceto-orcein squash technique was used in preparing the root tip for cytological examination. Each preparation considered the total number of cells, several dividing cells that are in one or another stage of mitosis, a number and a type of pathological mitoses. The mitotic index (MI), the phase indices (P, M, T, A) and chromosomal aberrations were determined by the examination of 500 cells per a replicate (100 cells per slide). Mitotic index was estimated as a number of dividing cells to the total number of the cells counted. It was expressed in percentage. The mitotic phase indices were estimated as several cells in each mitotic phase to several dividing cells expressed in percentage. Similarly, the percentage abnormal cells were calculated as a number of aberrant cells to a number of dividing cells. Cytological analysis of the root meristem cells was carried out under the AXIO ZEISS Imager. Z1 microscope at a magnification of 10×15×90. Microphotographs of dividing cells were made using the AxioCamMRc5 digital camera.

Data are presented as mean ± standard error (SE). The reliability of differences in the results obtained was assessed using the Student's t-test. Statistical processing of data was carried out using the application package Microsoft Excel 2010.

Results and Discussion

The change in mitotic activity of meristematic tissues can be considered an integral indicator of the negative impact of factors on the plant. The indicator of the level of mitotic activity is the mitotic index, which reflects the fraction of dividing cells. The purpose of the mitotic index is to measure cellular proliferation. It can state the normal course of mitosis, the inhibition of cell divisions or, conversely, the intensification of cell mitotic activity. Based on the results obtained, a conclusion is made about the mitosis-modifying action of the studied factor. Thus, the mitotic index can be used to quantify differences in cell division when an environmental parameter is changed.

The results of the impact of surface water on the *A. cepa* root meristem are presented in Table. The testing water samples affected different depending both on a place of sampling and on the sampling time. The samples collected in April during high water had a stimulating effect on the mitotic activity of the onion root meristem, significant exceeding the control value. The mean level was 14.15 ± 0.18%, varying from 12.98 ± 0.18 to 15.14 ± 0.13%. Minimal MI was observed in sample 3. It was collected from the middle of the stream.

Table 1 The mitotic regime in the root meristem cells of *Allium cepa* L.

Sample number	MI, %	Mitotic phase index, %			
		prophase	metaphase	anaphase	telophase
		April 28, 2015			
Control	**11.72±0.14**	**68.92±0.41**	**9.17±0.48**	**10.83±0.24**	**12.08±0.24**
Sample 1	14.51±0.09*	57.78±1.28*	18.23±0.74*	13.33±0.64*	10.66±1.11*
Sample 2	15.14±0.13*	56.27±0.57*	18.91±0.99*	15.01±2.57*	9.81±1.56*
Sample 3	12.98±0.18*	65.78±3.60*	9.92±0.32	11.97±2.32*	12.33±2.46
Sample 4	13.39±0.16*	62.14±1.83*	9.98±0.73	12.48±1.60*	15.40±3.53*
Sample 5	14.71±0.11*	61.29±4.03*	8.11±0.52	12.70±0.52*	17.90±3.00*
Mean ± SE	**14.15±0.18***	**60.64±3.91***	**10.63±0.88***	**13.10±0.73***	**13.22±0.55***
		October 10, 2015			
Control	**12.64±0.09**	**81.50±0.79**	**10.23±0.23**	**6.70±0.39**	**1.57±0.46**
Sample 1	4.27±0.14*	92.86±0.69*	5.95±0.69*	1.19±0.00*	0.00±0.00*
Sample 2	5.18±0.09*	93.11±0.86*	5.17±0.99*	1.72±0.49*	0.00±0.00*
Sample 3	9.72±0.08*	86.74±1.06*	7.65±0.61*	3.57±0.29*	2.04±0.29*
Sample 4	5.99±0.04*	94.02±0.85*	4.27±0.85*	0.00±0.00*	1.71±0.49
Sample 5	5.64±0.09*	92.23±1.48*	5.83±1.12*	0.97±0.00*	0.97±0.56
Mean ± SE	**6.16±0.09***	**91.79±0.99***	**5.77±0.85***	**1.49±0.16***	**0.94± 0.45***

Note: * indicates significant differences compared with the control at $P < 0.05$.

The samples from the right bank of the River, taken both from the surface and from the depth of 1 m, revealed a more stimulating effect. For example, the effect of sample 2 on the mitotic activity of cells was 29% higher than the control value. It is known that stimulation of mitotic activity in proliferating tissues is manifested, as a rule, under weak stress effects of pollutants or their synergistic effect, when the values of each one individual do not exceed the maximum permissible concentration (Khlebova & Ereschenko, 2014). Similar results were obtained in the earlier studies of the region when genotoxicity of the Ob River water near Barnaul city (the Altai Territory) was investigated (Larikova, 2012). The reaction of the test object, the bulbs of which were germinated on the samples taken in October, turned out to be diametrically opposite to the above described events. The average level of mitotic activity decreased almost two times in comparison with the control value (Table 1). The minimal inhibition of cell division was observed in the sample from the middle of the River (9.72 ± 0.08%). The samples from the left side (samples 4 and 5) showed a less inhibitory effect on the frequency of cell proliferation compared to the samples from the right bank, as evidenced by higher values of MI. The depressive nature of the mitotic activity of the cells points to the presence of substances with mitotoxic action in water of the autumn period.

Mitotic phase indices allow one to judge the relative duration of individual stages of cell division. The distribution of cells in phases of mitosis also differed depending on the time of selection of the material. Meristematic cells of onion rootlets germinated on the samples which were collected during high spring water revealed delays at later stages, beginning with metaphase, while the prophase duration was significantly lower than control. Cell delay in metaphase may indicate violations in the division spindle formation, and at the stages of anaphase-telophase does the violation in the cell wall formation (Butorina & Kalaev, 2000). A study of autumn samples showed that against the background of a decrease in the mitotic index of the test object, the proportion of prophase cells increased, which is probably due to damages of the supra-molecular structure of chromosomes, which prevent the transition to next stages of mitosis. The delay of cells at the prophase stage can also be caused by the activation of the checkpoint system, which is controlling the integrity of the genetic material, leading to the induction of repair processes as a response to emerging genome damage (Kalaev & Popova, 2014).

Along with the phenomenon of non-uniform passage by cells of separate stages of the division, the increase of pathological mitoses in the tested samples of River water was observed. The average level of meristematic cells with various disturbances was 4.24 ± 0.42 and 5.63 ± 0.48% during the high spring and low autumn periods, respective, exceeding the control value by 5.0 and 10.8 times (Fig. 1). This fact allows us to state the presence in the River water of some factors with potential mutagenic activity for the period of sampling. The level of spontaneous mutation of the test object was low, not exceeding 1%.

The samples from the middle of the stream (sample 3) had a much less negative effect on the cell division, while the samples from the right side (samples 1 and 2) induced a maximum of pathologies. The greatest number of aberrations was observed in River water during the autumn period, which indicates a low water self-purification. We found that the maximum number of chromosomal abnormalities occurs at the stages of meta- or anaphase.

An analysis of a wide range of pathologies revealed that the main disturbances are chromosome laggings in meta- and anaphase, chromosome bridges, chromosome fragments and micronuclei (Fig. 2).

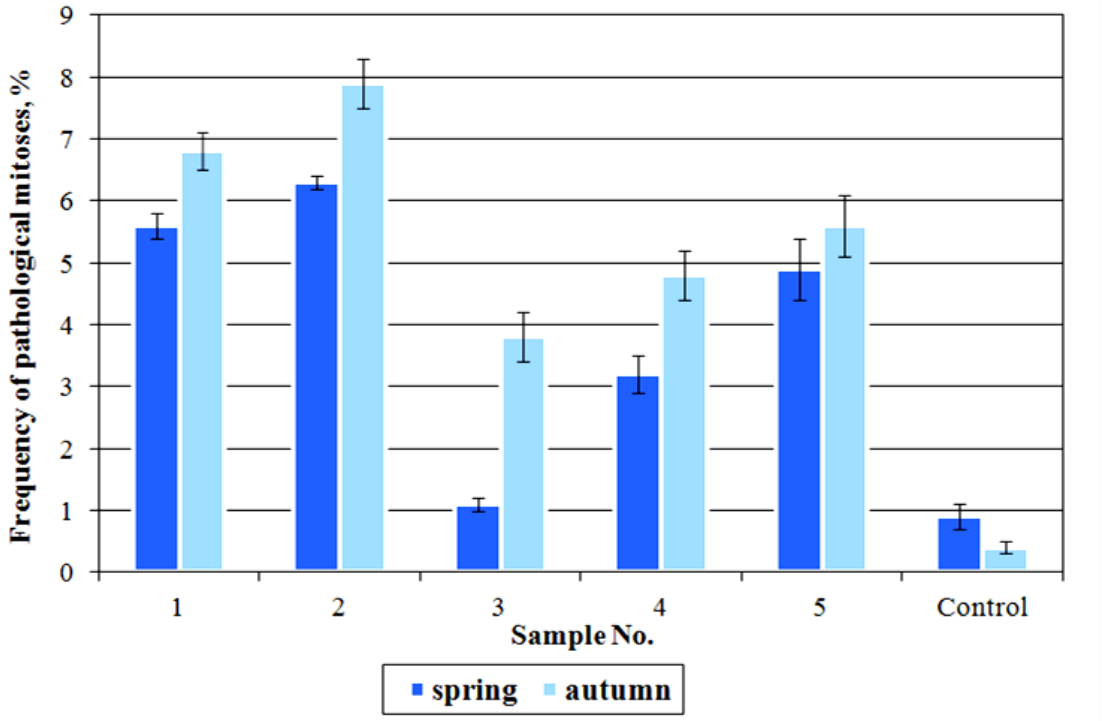

Figure 1. The frequency of pathological mitoses induced by the samples of the Chumysh River water in the onion root meristem, %

The presence of chromosome bridges indicates that the samples contain substances capable of causing clumping of telomeric chromosomal regions or ruptures in DNA leading to non-recurrent translocations. When asymmetric exchange occurs, a dicentric is formed (a chromosome with two centromeres) as a result of the connection of fragments containing centromeres. This leads to the appearance of bridges when the chromosomes divide in the anaphase (Fig. 2a). In some cells, along with the bridges, chromosome fragments, laggings and emissions were observed, which can be considered indicators of "fresh" chromosomal rearrangement (Kalaev & Popova, 2014). Fragmentation of chromosomes is a sign of the destruction of their structure, associated with the lysis of DNA molecules by enzymes and serves as an indicator of the genome instability. It is known that in some cases dicentric chromosomes pass through mitosis as a result of the "rupture-fusion-bridge" cycle and persist for several cell generations. Fragments in this case are not included in the forming daughter nuclei and are lysed by enzymes or remain in the cell cytoplasm in the form of micronuclei (Butorina & Kalaev, 2000). The chromosome laggings (Fig. 2b) occur with violations both in the chromosome itself and in the achromatin spindle.

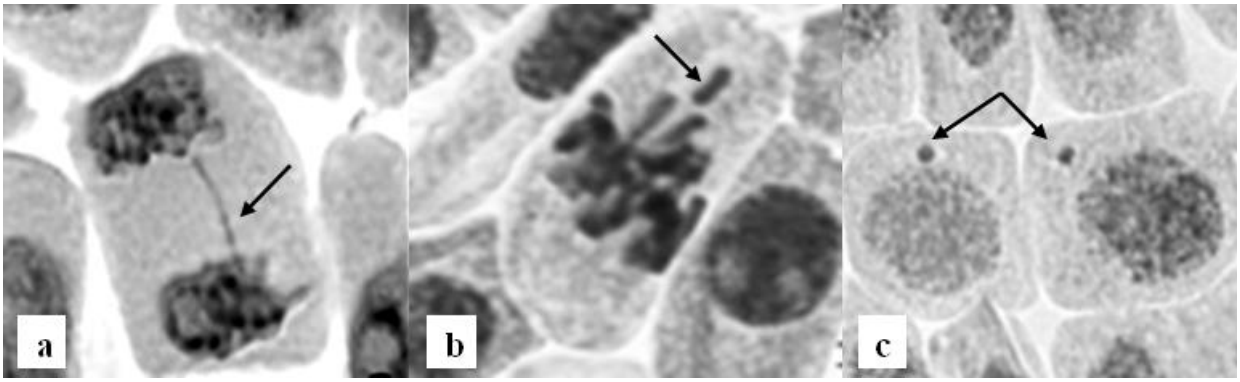

Figure 2. Pathologies of mitosis in the onion root meristem cells induced by the samples of the Chumysh River water:
a – chromosome bridge; b – chromosome lagging; c – micronuclei

Micronuclei detected at the telophase stage were small, well-formed rounded formations of nuclear material located in the cytoplasm of the cell at a certain distance from the main nucleus (Fig. 2c). As mentioned above, they are the result of the lag of the chromosomes or their fragments in the previous stages, which are not included in the daughter nuclei and subsequently, as a rule, they eliminate, which leads to aneuploid mutations. Cells with micronuclei were noted predominantly in samples with a high frequency of disturbances.

According to the data of Altai Center for Hydrometeorology and Environmental Monitoring, in the Chumysh River water for several years, the content of petroleum products and volatile phenols many times exceeded the Maximum permissible

concentration, MPC (http://altaipriroda.ru/). It is probably one of the causes of high frequency of chromosomal abnormalities during cell division of the model object. Along with these substances, some other compounds not exceeding the permissible threshold are present in the River water, but they are dangerous from the point of view of the effect on the genetic apparatus. Also, the genotoxic effect can also be caused by promutagenes acquiring genetic activity during metabolic reactions in the living body. The presence in the River water of the factors modifying the mitosis in onion root meristem poses a danger to the health of the population using it as a recreation and for economic needs.

Conclusions

Thus, the level of mitotic activity in the onion root meristem, as well as the frequency of chromosomal aberrations, state the presence in the Chumysh River water substances with mitomodifying and genotoxic activities. The main abnormalities are chromosome laggings in meta- and anaphase, chromosome bridges, chromosome fragments and micronuclei. It has been established the temporal and spatial distribution of compounds of different genotoxicity within a stream. The maximum level of mitotic depression and the highest frequency of chromosomal mutations were observed in the tissues of the onion root tips germinated on the samples of the autumn period. Also, the diverse nature of the mitotic activity in the onion meristems under the influence of the components of River water in the high spring and low autumn periods were discovered. This may indicate a variety of mechanisms for adapting plant organisms to changing environmental conditions. In one case, this is an increase in the rate of cell division compensating for damage by the number of new formed cells, in another case, activation of repair systems aimed at correcting existing disorders takes place.

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