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Genotoxicological Assessment of Waters from Kazanka River (Tatarstan, Russia)

Maxim V. Trushin and Irina A. Arkharova

Kazan Federal University (Institute of Fundamental Medicine and Biology), Kazan, Russia

Abstract: The waters of Kazanka according to *Allium*-test were characterized by a pronounced mitotoxic activity. A survey of cytogenetic analysis had the highest mutagenic potential of water at some stations. The most common genetic defect was the formation of micronuclei. *Allium*-test showed a high sensitivity and can be recommended as a tool for assessing the risk of mutagenic treatment of other water bodies of Kazan and Tatarstan.

Key words: Genotoxicity · Waters · Genetic aberrations · Kazanka · Tatarstan · Russia

INTRODUCTION

Water is necessary for life of all living organisms, as well as for human activities. To maintain human health and biodiversity in different ecosystems, it is require to monitor water quality [1]. The main water pollutants of Tatarstan are the products of human activities: oil products, agricultural fertilizers, insecticides and herbicides, as well as other organic and inorganic compounds. The literature notes that the above-mentioned pollutants can have a negative impact on the qualitative and quantitative composition of phytoplankton and zooplankton, as well as other aquatic organisms [2]. In addition, some researchers have shown that higher aquatic vegetation is able to recover some biological balance in polluted ecosystems through both direct disposal of pollutants and by stimulating the reproduction of some invertebrate organisms involved in the process of autopurification of water [3]. However, the long-term effects of water pollution are reflected in changes in the genetic apparatus of aquatic organisms still remain outside the field of research. In the absence of the data, to determine the genetic risk of Kazanka river waters seems very relevant. The aim of this work was to study the mutagenic potential of the waters of Kazanka. The following tasks were stated: to identify possible mitotoxic activity of samples of water from the river Kazanka in its lower reaches (in the city of Kazan); to identify the most contaminated areas of study, as well as to detect the most common genetic defects; to conclude on the appropriateness of *Allium*-test to analyze the mutagenic potential of surface waters.

MATERIALS AND METHODS

The study was conducted on water samples collected in August-September 2011 along the left bank of the river Kazanka. Water (1.5 liter in volume) was collected at 11 stations, whose coordinates are given below. Water immediately after sampling was used for sprouting onions.

Method of Accounting for Genetic Disorders in Meristem Cells of *Allium cepa*: The mutagenic activity of water in the cells was evaluated meristem roots *Allium cepa* [4]. The most common violations detected by this method are chromosomal bridges and fragments that result from chromosomal damage such as deletions and translocations, In the experimental apparatus bulbs germinated on fresh samples of water for 5 days. In the control variant distilled water was used. The experiment was in five replicates. The tips of the roots were fixed in a fixative of Clark (3 parts 96% ethanol and 1 part of glacial acetic acid) for 48 hours and stored in 75 % ethanol. Colouring roots was held 2% acetocarmine dye.

For the analysis of the mutagenic effect of water, samples were prepared from root meristem. Painted roots were placed in a drop of 45 % acetic acid and covered with

Corresponding Author: Dr. Maxim V. Trushin, Department of Genetics, Kazan Federal University, Institute of Fundamental Medicine and Biology, 420008 Kazan, Russia.

Fig. 1: Station 1. Coordinates: Latitude: 55° 49'19. 25 "N (55.822015) Longitude: 49° 10'15. 51" E (49.170975)

Fig. 2: Station 2. Coordinates: Latitude: 55° 48'40. 63 "N (55.811287) Longitude: 49° 9'51.41" E (49.164281)

Fig. 3: Station 3. Coordinates: Latitude: 55° 48'25.32 "N (55.807033) Longitude: 49° 9'46.47" E (49.162907)

Fig. 4: Station 4. Coordinates: Latitude: 55° 48'20.45 "N (55.80568) Longitude: 49° 8'50.23" E (49.147286)

Fig. 5: Station 5. Coordinates: Latitude: 55° 48'7.22 "N (55.802006) Longitude: 49° 8'8.83" E (49.135785)

Fig. 6: Station 6. Coordinates: Latitude: 55° 48'13.83 "N (55.803843) Longitude: 49° 7'5.17" E (49.118104)

Fig. 7: Station 7. Coordinates: Latitude: 55° 48'9.31 "N (55.802586) Longitude: 49° 6'22.53" E (49.106259)

Fig. 8: Station 8. Coordinates: Latitude: 55° 48'5.13 "N (55.801425) Longitude: 49° 6'4.28" E (49.101189)

Fig. 9: Station 9. Coordinates: Latitude: 55° 47'51.9 "N (55.797751) Longitude: 49° 5'34" E (49.092778)

Fig. 10: Station 10. Coordinates: Latitude: 55° 47'36.93 "N (55.793593) Longitude: 49° 5'29.3" E (49.091471)

Fig. 11: Station 11. Latitude: 55° 47'29.68 "N (55.791577) Longitude: 49° 5'41" E (49.094723)

coverslip. Further spine was crushed to obtain a monolayer of cells. Preparations were analyzed under a microscope Carl Zeiss AxioLab A1 at magnifications of 10, 40, 100. For each sample, we viewed the cells with well-dyed monolayer with intact cell walls. We took into account the total number of dividing cells at the stage of analysis, telophase and the number of cells with chromosome aberrations. Then we calculated the frequency of chromosome aberrations and micronuclei.

The mitotic index was calculated using the formula: MI = (R + M + A + T) / N where (S + M + A + T) - the sum of the cells in the stage of prophase, metaphase, ana and telophase and N - the total number of analyzed cells (Prokhorov, 1991).

RESULTS AND DISCUSSION

We studied 10 samples of water taken from different points (stations) of Kazanka River in its lower reaches. The total number of analyzed cells was 34348. The results of this work are presented in Table 1.

Examples of some of the genetic defects found in the cells of the test object and caused by exposure to water samples from the river Kazanka are presented in Figure 12.

Figure 13 presents the data for the calculation of frequency of genetic defects, depending on the total number of the cells.

As seen from the results of analysis of the data presented in Figure 13, the stations 1, 7 and 8 were the most contaminated. Perhaps the water in these stations experienced the greatest human pressure.

Thus, our analysis revealed a pronounced mitotoxic activity of water samples from the river Kazanka. In addition, we identified a number of genetic disorders related to chromosomal aberrations (bridges, C-metaphase, chromosomal sticking) and alterations of nucleus (micronuclei containing aborted genetic material). Sampling was carried out in August and September, so it

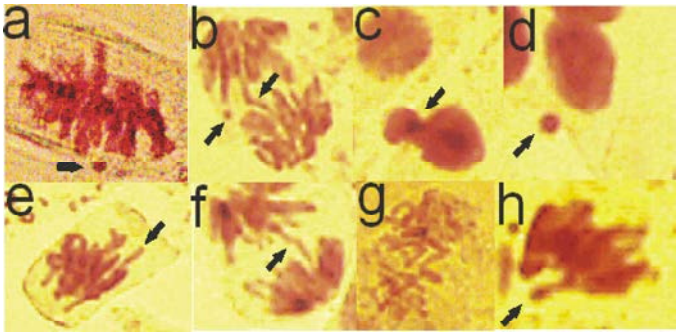

Fig. 12: Examples of some genetic defects in *Allium cepa* cells. a - the micronucleus (station 1), b - micronucleus and chromosomal bridge (station 1), c - lobulated nucleus (station 1), r - a large micronucleus (station 1), e - loss of chromosomes (station 4), f - chromosomal bridge (station 4), g - C-metaphase (station 6) h - sticking chromosomes (station 7)

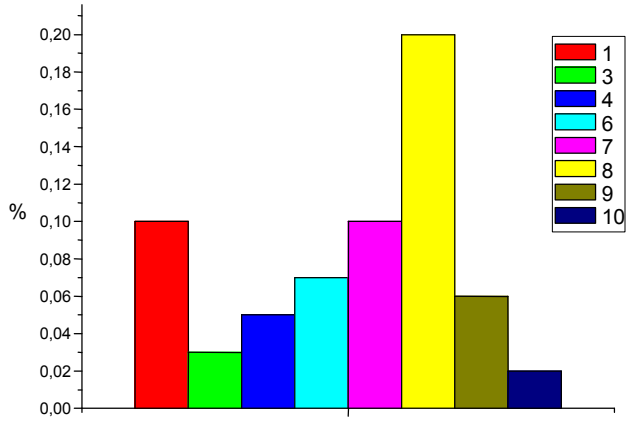

Fig. 13: Frequency of genetic defects. The Y-axis - % of cells with genetic defects, the X-axis - the experimental variants (station numbers correspond to different colors in the diagram)

Table 1: The values of the mitotic index and genetic disorders identified in the *Allium*-test in the study of the waters of Kazanka

Station	Total number of analyzed cells	Mitotic index (%)	Genetic disorders
Control	2933	8.3	Not detected
1	4609	3.65	Micronuclei (small and large) (4), bridge, lobulated nucleus
2	3148	1.19	Not detected
3	3025	0.89	Micronucleus
4	3476	2.7	Bridge, chromosome loss
5	3856	0	Not detected
6	4043	2.47	Bridge, Ñ-metaphase, micronucleus
7	1540	1.85	Micronucleus, chromosomal sticking
8	423	4.85	Micronucleus
9	1573	1.78	Micronucleus
10	3413	6.54	Micronucleus
11	2309	1.74	Not detected

should be expected that the seasonal characteristics of autopurification of aquatic ecosystems have played a role. Typically, the water has a much higher degree of contamination of the spring and early summer, when the autopurification capacity of the ecosystem (due to the action of aquatic plants) are the least pronounced.

CONCLUSIONS

The waters of Kazanka according to *Allium*-test were characterized by a pronounced mitotoxic activity. A survey of cytogenetic analysis had the highest mutagenic potential of water at stations 1, 7 and 8. The most common

genetic defect was the formation of micronuclei. *Allium*-test showed a high sensitivity and can be recommended as a tool for assessing the risk of mutagenic treatment of other water bodies of Kazan and Tatarstan.

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