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Mutagenic activity of water samples from Kaskelen River (Kazakhstan) on plant test systems

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Abstract. Rivers are pivotal in the life processes of all living organisms, including humans. The engagement of rivers in daily human economic endeavours has precipitated and continues to precipitate disturbances in the ecological equilibrium, long-established by the forces of evolution. Consequently, providing clean, high-quality water is paramount for sustaining all life forms. This investigation aimed to examine the mutagenic potential of water samples from the Kaskelen River, which is subjected to anthropogenic influences, employing plant-based test subjects. The water's mutagenic properties were assessed through an assay for chromosomal aberrations (utilizing the metaphase approach) on *Hordeum vulgare* L. The results demonstrated a statistically significant decline in the mitotic index under all experimental conditions compared to the negative control ($p<0.05$), indicating the cytotoxic effects of the water samples under study. Further analysis of the water's mutagenic activity elucidated its capacity to induce structural mutations within the root meristem cells of barley, manifesting a significant uptick in the frequency of chromosomal aberrations relative to the negative control ($p<0.05$). The extensive array of observed chromosomal rearrangements underscores the existence of mutagenic agents in the river's water, possessing a broad spectrum of effects.

1 Introduction

Water, an invaluable resource, fulfils the essential needs of all forms of life and supports a wide array of human economic activities and everyday tasks. Nevertheless, its critical importance is increasingly overshadowed by the growing menace of contamination, characterized by the influx of diverse chemical and physical pollutants into aquatic ecosystems [1, 2]. Academic research highlights that many rivers, lakes, springs, and other surface water bodies, especially in densely populated areas, are extensively contaminated [3, 4]. The irrigation of agricultural land with river water has been identified as a cause for the presence of various chemical substances in plants, which exhibit toxic and genotoxic activities [5, 6]. A significant proportion of Kazakhstan rivers are continuously affected by pollution from heavy metals and organic substances [7-9]. The intensification of industrialization, urbanization, agricultural development, the growth of domestic tourism and

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the unregulated exploitation of natural resources lead to a deterioration in water quality and a change in the characteristics and properties of various ecosystems, including aquatic ecosystems [10]. As a result, continuous monitoring is required through environmental supervision of natural surface waters, including genetic monitoring using cytogenetic tests. In the context of the Almaty region and its urban vicinities, research into the mutagenic and genotoxic properties of natural surface waters has unveiled a troubling situation: numerous water bodies demonstrate mutagenic activity, thus posing a genetic threat to both local wildlife and human populations [9].

According to the Environmental State Bulletin of Kazakhstan for 2023, only seven out of 134 surveyed surface water bodies are classified as class 1 (indicating the highest quality), while 17 rivers are classified as more significant than class 5, rendering their water unfit for any usage according to the "Unified Water Quality Classification System in Water Bodies" [11]. The Kaskelen River, which flows through the Almaty region and is classified as a class 3 water body polluted with total phosphorus, has a length of approximately 170 km. It originates from the northern slope of the Trans-Ile Alatau ridge and flows into the Kapchagai Reservoir. The Kaskelen town, located along the river, practices maral farming upstream; is home to various species of fish such as bream, pike-perch, catfish, crucian carp, and carp; and uses the river for irrigation of local farms and for supplying water to the residential population, with a quail farm situated downstream. These activities undoubtedly subject the river to anthropogenic pollution. This study uses plant test subjects to assess the mutagenic effect of water from the Kaskelen River exposed to considerable anthropogenic pressure.

2 Materials and Methods

The research methodology employed in this study involved the systematic collection of water samples from three strategically chosen locales along the Kaskelen River, spanning two consecutive years in January 2022 and 2023. These locations were precisely identified as Point No. 1 – located in the upper echelons of Kaskelen city (43°10'03.8"N, 76°36'58.4"E); Point No. 2 – positioned within the central sector of the city (43°11'52.1"N, 76°37'40.9"E); and Point No. 3 – situated in the city's lower vicinity (43°13'34.9"N, 76°38'41.3"E). Fig. 1 offers a visual delineation of these water sampling sites along the course of the Kaskelen River, with the distance between Points No. 1 and No. 3 approximating 12 km.

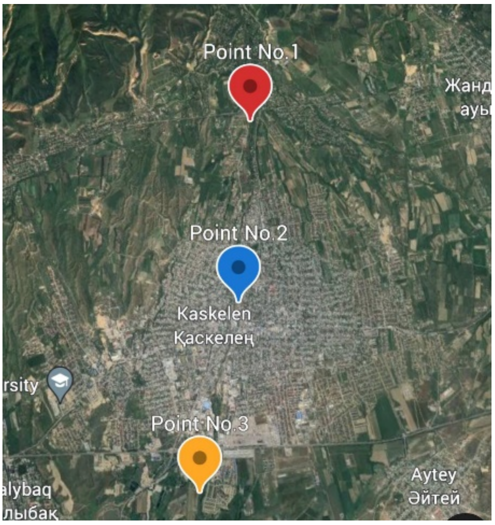

Fig. 1. Geographic Distribution of Water Sampling Sites on the Kaskelen River.

Following their acquisition, the water samples were filtrated and treated with nitric acid (HNO_3) to curtail bacterial proliferation following the guidelines prescribed by the Kazakhstan State Standard (GOST 31861-79 2012, 2013) [12]. Subsequently, the samples were stored at a temperature of -16°C to ensure their preservation.

The assessment of mutagenic activity within the water was facilitated through the chromosome aberration count test, utilising the metaphase method, on the barley [13] variety Baisheshek cultivated across Kazakhstan. The baseline mutation rate in seeds germinated in distilled water was designated as the negative control, whereas the mutagenesis induced by Methyl methanesulfonate (MMS) was employed as the positive control [15].

The seed germination process in the water samples under examination was conducted within Petri dishes, maintained at controlled temperature regulation at 25±1°C. After developing the primary roots to a length of 0.5 cm, these were transferred to filter paper saturated with a 0.01% colchicine solution. The roots were then fixed in an ethanol-acetic acid mixture (1:1 ratio) for 24 hours and stored in 70% ethanol. The preparation of cytological specimens and their subsequent staining with fuchsin-sulfuric acid were performed in line with established protocols [16]. These prepared cytological specimens were frozen at -85°C on a freezing table FS-06 before undergoing alcohol processing to produce permanent slides. The examination of metaphase plates was carried out using an Olympus BX 43F optical microscope with at least 400 metaphase plates.

Statistical examination was conducted using StatPlus software, employing the Student's t-test to identify statistically significant differences. Significance was determined at a confidence probability level of 0.95 ($p<0.05$).

3 Results and Discussion

The cytotoxic potential of river water was meticulously assessed by examining proliferative activities within the meristematic cells located in the root meristem of barley germinated in the water samples under study. Fig. 2 presents the results concerning the mitotic index (MI) within the cells of the apical region of the primary roots of barley post-exposure to the water samples.

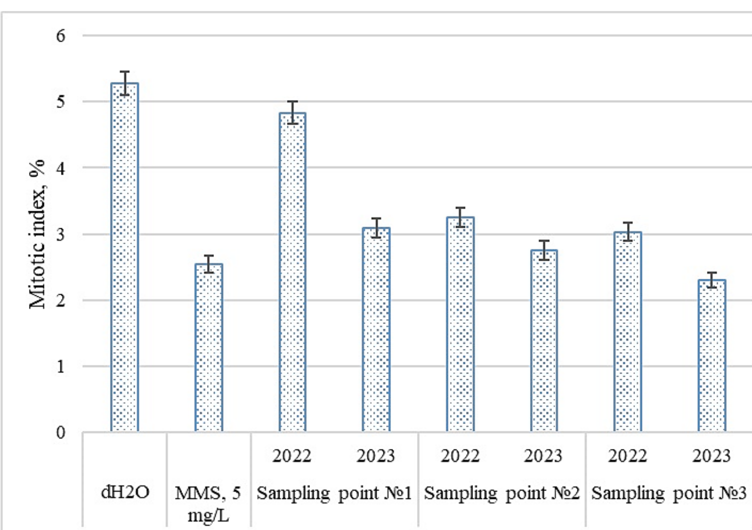

Fig. 2. Proliferative activity levels in the root apical meristem of barley germinated on water samples from the Kaskelen River

This analysis unveiled that the MI across all experimental conditions exhibited a notable decrease against the negative control. Due to the water samples' influence, a statistically significant diminution in proliferative activity was observed in 2022 at points No. 2 and No. 3 ($p<0.05$), and a similar trend was noted across all conditions in 2023. Upon exposure to a cytotoxic factor, the reduction in the MI denotes the factor's cytotoxic impact. In contrast, an increase in the MI suggests a mitosis-stimulating effect. The uniform decrease in the MI across all experimental configurations, relative to the negative control, accentuates the cytotoxic nature of the Kaskelen River water. This heightened cytotoxicity, particularly as the river meanders through urban locales, is likely attributable to the presence of ecotoxic substances within the river, adversely affecting the proliferative potential of cellular populations.

Environmental genotoxic agents can elicit a broad spectrum of alterations in the genetic material of organisms. The augmentation of anthropogenic stressors upon the habitats of numerous species, including humans, correlates with a heightened incidence of various pathologies and an elevated genetic burden, potentially culminating in population declines or even extinction [16]. Consequently, genetic monitoring is paramount to detect entities with genotoxic and mutagenic capabilities within the environment.

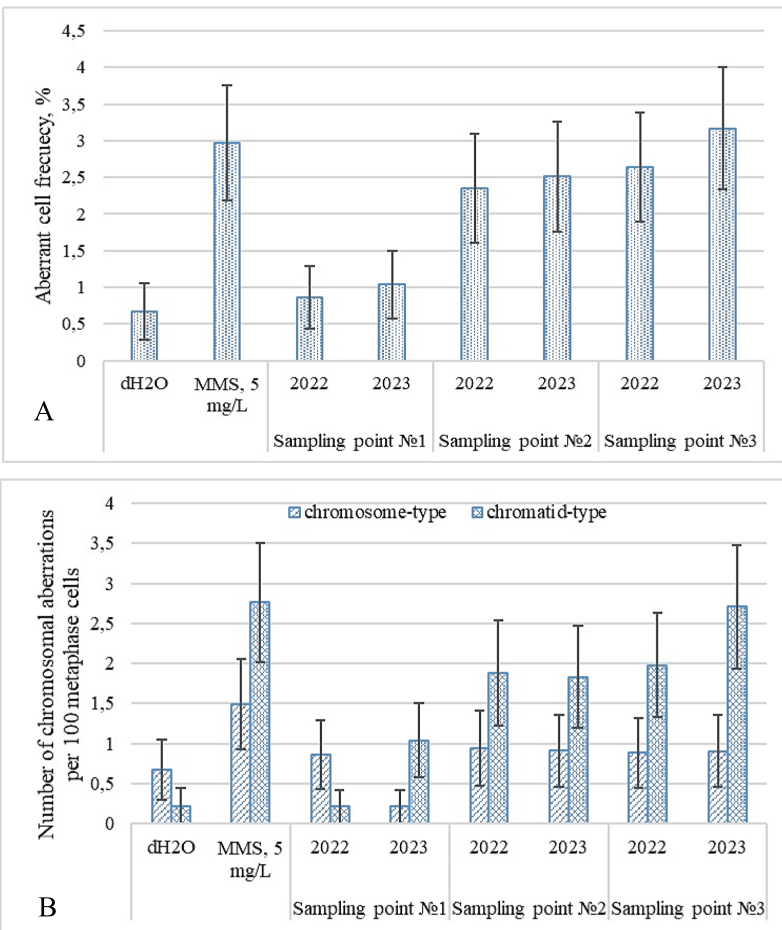

Fig. 3. Frequency (A) and spectrum of chromosomal aberrations (B) in barley seeds prompted by water samples from the Kaskelen River

Fig. 3 illustrates the cytogenetic analysis performed on barley seeds germinating on water samples from various sampling sites along the Kaskelen River. The introduction of MMS significantly augmented the occurrence of aberrant cells and aberrations per 100 metaphases by 4.4 and 4.8 times, respectively ($p < 0.01$), against the negative control. This examination of the water's mutagenic properties, collected during winter 2022, demonstrated its ability to induce structural mutations within the barley root apical meristem cells.

Exploring the genotoxic potential of water from the Kaskelen River uncovered significant variation across different sampling sites. Water from site No. 1 was associated with a modest 1.2-fold escalation in chromosomal aberrations compared to the negative control, although this increase did not attain statistical significance. In contrast, water samples from points No. 2 and No. 3 exhibited a statistically significant enhancement in both the prevalence of aberrant cells and the frequency of chromosomal aberrations per 100 metaphase plates ($p<0.05$). Furthermore, these samples also showed a statistically significant rise in the occurrence of chromatid-type aberrations ($p<0.05$), signalling a marked mutagenic impact of the water, comparable to the effects observed with the positive control (Methyl Methanesulfonate).

This discernible pattern was consistent in the analyses of water samples collected from the same points during winter 2023. While water from point No. 1 showed an increase in mutagenicity indicators against the negative control, this observed increase did not reach statistical significance. However, the samples from points No. 2 and No. 3, collected in 2023, disclosed a marked increase in the occurrence of aberrant cells, with increments of 3.8 ($p<0.05$) and 4.7 ($p<0.01$) times, respectively. In a similar vein, the frequency of chromosomal aberrations per 100 metaphase cells also registered significant elevations of 3.1 ($p<0.05$) and 4.1 ($p<0.01$) times, respectively. When juxtaposed with the control group, the incidence of chromatid-type abnormalities was notably higher. The spectrum of structural chromosomal rearrangements observed was comprehensive, encompassing both chromosomal and chromatid-type abnormalities, as depicted on Fig. 4, with a predominant incidence of the latter.

A comparative analysis of the mutagenic activity in the river water collected during 2022 and 2023 revealed no statistically significant variances between corresponding points. Nonetheless, a marked decrease in the mitotic index was noted across all sampling sites in 2023, signifying a considerable change.

Rivers play an integral role within the biosphere, supporting the existence and flourishing of many life forms, including humans. They are repositories of more diverse hydrobionts than other aquatic ecosystems, acting as essential channels for ecological sustainability and biodiversity.

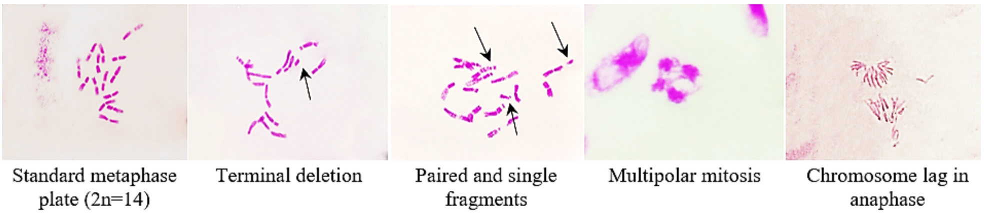

Fig. 4. Chromosomal aberrations within the root meristem of barley induced by water from Kaskelen River, magnified 1000 times.

The employment of rivers in human economic endeavors has led to ongoing disruptions in the ecological balances established through millennia of evolution. Consequently, maintaining water purity and quality is paramount for the well-being of all organisms. Against this backdrop, the continuous monitoring of natural water quality, especially those sources designated for human consumption, is imperative. The anthropogenic impacts on river ecosystems resulting from industrialization, urbanization, manufacturing and disposal of plastic products, and the reckless use of water resources present formidable challenges. These challenges exacerbate the deterioration of aquatic environments and contribute to shifts in the environmental and ecological equilibrium [17].

The marked increase in chromosomal aberrations observed in the cells of the barley apical meristem, germinated in water from the Kaskelen River, emphasizes the mutagenic impact of this river's water. The wide array of chromosomal rearrangements identified signifies the existence of mutagenic agents in the river's water, with a comprehensive range of actions. Additionally, the noted reduction in proliferative activity within the root meristem cell signifies an escalation in the cytotoxic effects exerted by the river's water. This underscores the urgent requirement for advanced conservation strategies and rigorous environmental oversight to mitigate the adverse effects and ensure ecological and public health protection.

4 Conclusion

In this study, we sought to explore the cytotoxic and mutagenic characteristics of water from the Kaskelen River, an essential source for the population of the corresponding city and surrounding areas, utilizing plant-based subjects in a chromosome aberration assay. The outcomes of this research shed light on the inherent cytotoxic and mutagenic properties present in the water from the Kaskelen River. The demonstration of cytotoxicity was confirmed by a statistically significant reduction in the mitotic index observed within the cell population of the barley root apical meristem germinated in water samples collected from various points along the river's course. Simultaneously, mutagenic activity was evidenced by a statistically valid rise in structural aberrations in the barley seeds.

Considering the critical importance of the Kaskelen River as a primary water resource, these revelations are of substantial consequence for the community dwelling near the river. Detecting cytotoxic and mutagenic activities in the river's water underscores the necessity for immediate and thorough research efforts, coupled with implementing corrective actions to mitigate potential impacts on the biodiversity of aquatic organisms and public health infrastructure. Extending the research scope to include animal objects is essential, as it will provide deeper insights into the ecological and health ramifications of the detected mutagenic activities. By expanding the investigative purview in such a way, a more holistic comprehension of the broader ecological effects can be attained, markedly aiding in formulating efficacious strategies to protect both environmental and human health.

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