# Assessment of mutagenic potentials of water from the Kapshagai reservoir (Republic of Kazakhstan) utilising barley as a test organism

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**Abstract**. The intensification of human-induced pressures on the environment leads to significant disturbances in the integrity and stability of ecosystems. The presence of pollutants can mutate genetic material, thereby heightening the rate of genetic mutations within organisms due to environmental exposure. The accumulation of xenobiotics in essential habitats like soil and water underscores the necessity for continuous genetic monitoring of surface waters in daily contact with humans. This study analyses the mutagenic effects of water from the Kapshagai Reservoir and the Ile River at its confluence with the reservoir, a site of significant economic activity. Utilising cytogenetic analysis to examine chromosomal aberrations in *Hordeum vulgare* L., the study investigated the mutagenic and cytotoxic impacts of water samples gathered during the spring and summer of 2023. The results indicate that water from the Kapshagai Reservoir displayed mutagenic and cytotoxic activities, causing structural mutations in barley seeds at a rate markedly exceeding spontaneous mutation levels ($p<0.01$). Additionally, the appearance of polyploid cells, which were not present in the control group, reduced the proliferative activity of the barley root meristem cells. These findings underscore the detrimental effects of the examined water samples on genetic stability.

## 1 Introduction

The growing global population and increasing consumption have intensified environmental harm, mainly driven by increased pollutants from industrial, agricultural, and household sources. These pollutants often contain toxic compounds from increased reliance on chemical fertilisers, pesticides, synthetic building materials, pharmaceuticals, and more. Such anthropogenic activities destabilise natural ecosystems, imposing a significant anthropogenic load on the environment. This results in the pervasive pollution of all environmental components with an array of xenobiotics, whose diversity and quantity rise annually. All environmental contaminants potentially contribute to the genetic burden on the gene pool of living organisms' populations [1-3].

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Consequently, genetic monitoring is imperative to assess the impact of a complex mix of environmental pollutants on organisms' genetic material. This issue is of particular concern to healthcare professionals [4, 5]. Agents with genotoxic activity are known to induce harmful mutations, potentially leading to hereditary disorders and cancer [6]. Given the considerable pollution of natural surface waters near populated areas, investigating the mutagenicity of these waters becomes crucial [7].

The Ile River, a critical water source for Kazakhstan's third-largest endorheic lake, Lake Balkhash, originates in the People's Republic of China. The region continuously develops hydraulic structures to support irrigated agriculture and energy production [8]. The Kapshagai Reservoir, situated along the middle course of the Ile River, stands as Kazakhstan's second-largest artificial water body. This reservoir serves multiple purposes, including water regulation for various economic sectors and acting as a regional tourist attraction. The economic activities in populated areas like the village of Bakanas and the city of Konaev adversely affect the ecosystems of the Ile-Balkhash basin. Even at minimal concentrations, the synergistic effect of these chemicals can be detrimental to organisms, including their genetic material [9]. Thus, examining the mutagenicity of water used by the population in diverse activities is pertinent. This study aimed to assess the mutagenic and cytotoxic activities of water from the Kapshagai Reservoir and the Ile River at its confluence with the reservoir, employing the chromosomal aberration count test on the model organism barley.

## 2 Materials and Methods

Water samples for this study were collected from five distinct locations: four within the Kapshagai Reservoir (labelled as Nos. 1-4) and one from the Ile River at its confluence with the reservoir (No. 5), as depicted on Fig. 1.


**Fig. 1.** Map of Water Sampling Locations. Sampling point' coordinates: 1 – Kapshagai Reservoir, 43°53'29"N, 77°09'06"E; 2 – Kapshagai Reservoir, 43°53'22"N 77°12'55"E; 3 – Kapshagai Reservoir, 43°56'31"N, 77°19'04"E; 4 – Kapshagai Reservoir, 43°56'13"N, 77°22'50"E; 5 – Ile River, 43°50'46"N, 78°12'03"E.

Sampling took place during the spring-summer period of 2023. Each sample was collected into 5-litre plastic bottles and stored at -16°C. Collecting, filtering, and storing the water samples was conducted following the Kazakhstan State Standard (GOST 31861-2012, 2013 [10]).

Cytogenetic analysis was performed on the barley (*Hordeum vulgare* L.) variety Baisheshek, acclimatised to Kazakhstan. The assessment of mutagenic activity utilised the chromosomal aberration counting test (metaphase method). The baseline for the negative control was established by the natural mutation rate in barley seeds germinated in distilled water. Conversely, the positive control was determined by the mutagenesis level induced by methyl methanesulfonate (MMS, $C_2H_6O_3S$) at a concentration of 5.0 mg/l.

Seeds were soaked in each water sample for four hours and germinated in Petri dishes containing distilled water under a steady temperature of 25±1°C in a thermostat. After 24 hours, seeds germinating with primary root lengths of approximately 0.5 cm were transferred to filter paper soaked in a 0.01% colchicine aqueous solution to facilitate the accumulation of metaphase plates. Cytogenetic slides were prepared from the apical meristem cells of barley roots using a conventional protocol with fuchsin-sulfurous acid staining [11]. These cytological slides were preserved at -85°C on a freezing table (FS-06, China) to produce permanent slides.

The statistical analysis of the collected data was undertaken utilising StatPlus and WinPepi software. For each variant, the mean values of the indicators alongside the standard errors of these means were computed. The Student's t-test was applied to ascertain the significance of disparities between the mean values across different variants. Distinctions were deemed statistically significant at a confidence probability of 0.95 ($p<0.05$), ensuring a rigorous assessment of the data's reliability and the conclusions' validity.

## 3 Results and Discussion

A cytogenetic analysis was conducted on the root germ meristem of barley exposed to water samples from the Kapshagai reservoir and the Ile River to evaluate their mutagenic potential (see Figure 2). In the control experiment, where barley seeds were immersed in distilled water, the natural mutation rate was 1.37%. In contrast, seeds treated with Methyl Methanesulfonate (MMS) as a positive control exhibited a statistically valid rise in the aberrant cells to 5.45% ($p<0.001$). This treatment also led to a significant rise in the incidence of structural chromosomal disturbances, with the aberrations per 100 observed metaphases increasing to 6.91 due to aberrant cells harbouring more than one structural mutation ($p<0.001$). Water samples from all collection points, including Kapshagai (No. 1-No. 4) and the Ile River (No. 5), prompted chromosomal aberrations at a frequency that significantly surpassed the spontaneous mutation level. Specifically, water from Kapshagai collection points No. 1 to No. 4 rise aberrant cells' frequency by 2.6 to 2.8 times against the distilled water ($p<0.05$). The chromosomal aberrations per 100 observed metaphases in these samples were significantly higher, by 2.9 to 3.4 times, than that noted in the control ($p<0.01$). Similarly, water from the Ile River induced structural mutations in barley seeds at a high frequency, markedly exceeding that of the negative control. Exposed to this water, there was a 3.1-fold increase in the prevalence of aberrant cells ($p<0.01$) and a 3.7-fold escalation in the rate of chromosomal aberrations per 100 observed metaphases ($p<0.001$) relative to distilled water. A comparative analysis of the frequency and range of structural chromosome aberrations induced by the water samples from Kapshagai Nos. 1-4 and Ile River No. 5 with those from the positive control revealed no statistically significant differences, underscoring the considerable mutagenic activity of the tested water samples akin to that of recognised mutagens. No significant variations were detected in the mutagenic potency across the sampling points.

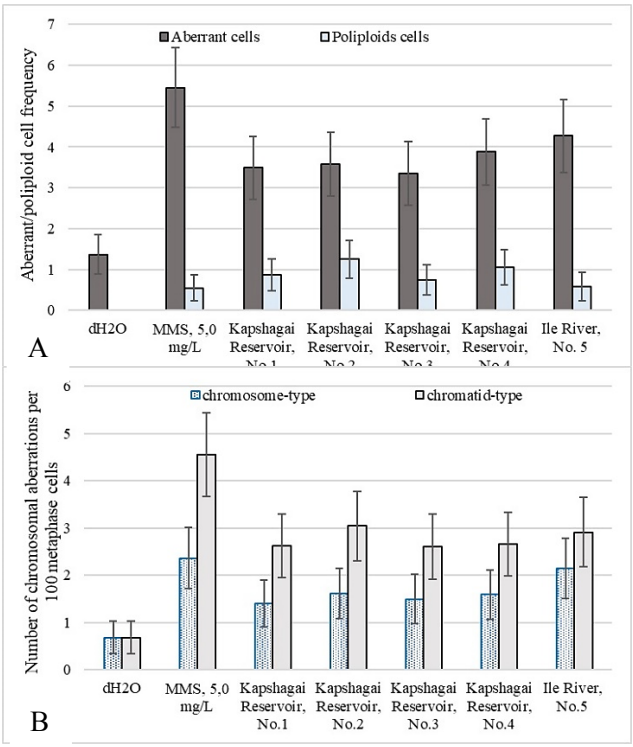


**Fig. 2.** Variations in the frequency (A) and nature (B) of chromosome abnormality in barley seeds triggered by water samples from the Kapshagai Reservoir and the Ile River.

The chromosomal aberrations encompassed both chromosome (paired centric rings and acentric fragments, isochromatid breaks, terminal deletions) and chromatid (interstitial deletions, single acentric rings, terminal breaks) types across all test variants (Figure 3).

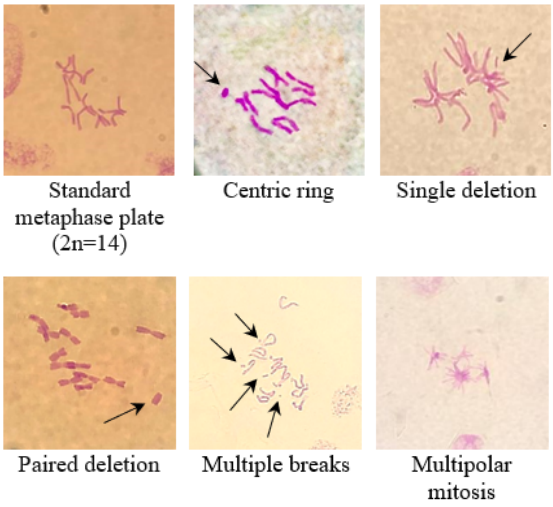


**Fig. 3.** Magnified view of structural mutations in barley seeds exposed to water from various Kapshagai reservoir locations, x 1000.

Additionally, numerous multipolar mitoses (Figure 3) indicative of the cytotoxic effects of the water samples were recorded, along with the presence of polyploid cells, which were not observed in the negative control.

The mitotic index (MI) within the meristematic zone of primary barley roots was also examined (Figure 4). Exposure to water from Kapshagai Nos. 1-4 and the Ile River No. 5 reduced MI by 1.3, 1.3, 1.4, 1.7, and 1.8 times, respectively, against the negative control. However, a statistically significant decrease in cell proliferation was only observed with samples from Kapshagai No. 4 ($p<0.01$) and the Ile River No. 5 ($p<0.001$).

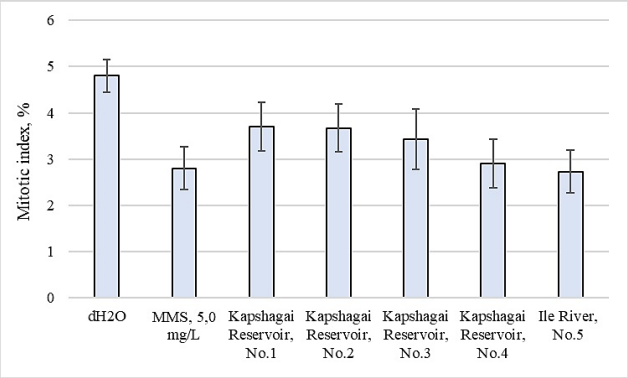


**Fig. 4.** Mitotic index of the cell population in the root germ meristem of barley germinated in water from the Kapshagai Reservoir and the Ile River.

One potential explanation for the observed mutagenic activity in the water samples from the Kapshagai reservoir and the Ile River may be the presence of household chemicals, heavy metals, fertilisers, pesticides, etc. It is noteworthy that various chemical pollutants, even at concentrations within permissible limits, can adversely affect biological processes over prolonged exposure and, when combined with other environmental factors, potentially impact genetic material. In all experimental scenarios, the statistically significant rise in induced chromosomal aberrations was predominantly attributed to chromatid-type rearrangements. This significant uptick in the frequency of chromatid-type aberrations suggests the presence of chemical compounds in the water with pronounced genotoxic activity, impacting cell cycle phases over an extended period. These findings also hint at the water containing mutagenic agents capable of a broad spectrum of action.

The observed elevation in the natural mutation rate and the reduction in mitotic activity within the germ root meristem of barley, after exposure to water samples from both the Kapshagai reservoir and the Ile River, may stem from the genotoxic effects of a complex array of pollutants, including heavy metals [12]. Heavy metals notably interfere with the ordinary course of mitosis by halting the cell cycle at the interphase stage, thus preventing the cell from progressing to mitosis [13].

The buildup of pesticides, heavy metals, petroleum derivatives, surfactants, synthetic pharmaceuticals, and other contaminants is linked to increased human morbidity, reduced biodiversity, and the destabilisation of natural ecosystems [1, 14, 15]. Water pollution poses a significant worldwide issue. Human activities can lead to a complex array of chemical substances in natural waters which might not be identified through conventional physicochemical methods. The aggregated impact of these chemicals, even at minimal concentrations, may pose a risk to living organisms [3]. Assessing the environmental risk associated with various forms of pollution requires the simultaneous application of chemical and biological methodologies to monitor xenobiotics [3, 4]. This approach ensures a comprehensive understanding of the potential impacts of pollution on the environment, facilitating the identification and mitigation of risks with precision and efficacy.

## 4 Conclusion

Cytogenetic investigations on the barley species *Hordeum vulgare* L. have revealed mutagenic and cytotoxic effects of water from different sampling sites within the Kapshagai Reservoir. Through the employment of chromosomal aberration counting tests, it was determined that water samples, irrespective of their point of collection, prompted structural mutations at a rate markedly exceeding the baseline of spontaneous mutations. Noteworthy was the observation of polyploid cells, absent in the control group, indicating a deviation from natural genetic stability. Additionally, a reduction in the proliferative activity within the root germ meristem of barley seeds was documented.

This preliminary evidence underscores the necessity for expanded research into the genotoxic influence of water from both the Kapshagai Reservoir and the Ile River, extending to laboratory animals and cytogenetic stability within natural background populations. Mutagenic agents can potentially elevate mutation rates beyond the evolutionary standards established for various species, thus augmenting the genetic load on populations, including humans. This predicament highlights the imperative need for genetic monitoring across diverse natural environments, with a specific emphasis on aquatic ecosystems, to detect mutagenic activities. Such initiatives are directed towards mitigating pollution emanating from environmentally detrimental factors, thereby safeguarding the health of both biotic communities and human populations. These measures are essential in preserving species' genetic integrity and ensuring ecosystems' sustainability amidst escalating environmental challenges.

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