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STUDY OF THE TOXIC AND GENOTOXIC ACTIVITY OF WATER OF LAKE KOLSAI, LOCATED ON THE TERRITORY OF THE ALMATY REGION OF THE REPUBLIC OF KAZAKHSTAN

An important step in assessing the state of natural environment is the identification of various environmentally hazardous factors that possess toxic, genotoxic and mutagenic activity. The appearance in the environment of a new pollutants and an increase in the concentration of already present physicochemical components that exhibit toxic and genotoxic properties contribute to environmental mutational load on biota. In recent decades, due to the intensification of economic activity and the development of the tourism sector, the anthropogenic load has significantly increased not only on terrestrial, in particular mountain ecosystems, but also on natural water reservoirs. In this regard, the purpose of this study was to investigate the genotoxic, mutagenic and toxic activity of surface water of the freshwater lake Nizhny Kolsai, located on the territory of national park "Kolsai kolderi" in the Kegen district of the Almaty region, Republic of Kazakhstan. For the first time, using a number of test systems (DNA comet method, cytogenetic test for accounting for chromosomal aberrations and biochemical analysis of lipid peroxidation products), water samples taken in 2018 and 2022 (spring-summer period) were studied at the Mus musculus model facility. It was found that water samples induced DNA breaks in cells of bone marrow and spleen of experimental mice, the level of which was significantly higher than in intact animals ($p < 0.001$). Cytogenetic analysis of the bone marrow of mice treated with lake water also showed a statistically significant increase in the frequency of aberrant cells and the number of structural chromosome aberrations per 100 metaphases ($p < 0.05$). A biochemical study of lipid peroxidation products (LPO) in the liver of experimental animals also revealed a statistically significant ($p < 0.05$) increase in the level of lipid hydroperoxide (HPL) and malondialdehyde (MDA). The results obtained indicate the presence in the studied natural surface waters of chemicals with genotoxic, mutagenic and toxic activity.

Key words: natural surface waters, genotoxicity, organ specificity, chromosomal aberrations, comet DNA assay.

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Қазақстан Республикасы Алматы облысының аумағында орналасқан Көлсай көлі суының токсикалық және генотоксикалық белсенділігін зерттеу

Табиғи ортаның жай-күйін бағалаудың маңызды кезеңі – ондағы токсикалық, генотоксикалық және мутагендік белсенділікке ие әртүрлі экологиялық қауіпті факторларды анықтау. Қоршаған ортада жаңа компоненттердің пайда болуы улы және генотоксикалық қасиеттері бар физика-химиялық компоненттер концентрациясының жоғарылауы әртүрлі экожүйелердің биотасына қоршаған ортаның мутациялық қысымының жоғарылауына ықпал етеді. Соңғы он жылдықтарда экономикалық белсенділіктің күшеюіне және туризм секторының дамуына байланысты антропогендік жүктеме тек жер үсті, атап айтқанда, таулы эко жүйелерде ғана емес, сонымен қатар табиғи жер үсті суларында да айтарлықтай өсті. Осыған байланысты бұл зерттеудің мақсаты – Қазақстан Республикасы Алматы облысы Кеген ауданындағы «Көлсай көлдері» ұлттық саябағының аумағында орналасқан Төменгі Көлсай тұщы көлі суының генотоксикалық, мутагендік және токсикалық белсенділігін зерттеу. Алғаш рет Mus musculus модельдік объектісінде бірқатар сынақ жүйелерін (ДНҚ кометасы әдісі, хромосомалық аберрацияларды есепке алу бойынша цитогенетикалық тест және липидтердің асқын тотығу өнімдерінің биохимиялық талдауы) пайдалана отырып, 2018 және 2022 жылдары (көктем-жаз кезеңі) іріктелген су үлгілері зерттелді. 2018 және 2022 жылдардағы су үлгілері тәжірибелік тышқандардың сүйек кемігімен көк бауыр жасушаларында ДНҚ бұзылыстарын индукциялағаны анықталды, оның деңгейі интактты жануарлардағы аналогиялық көрсеткіштерден статистикалық тұрғыдан айтарлықтай жоғары болды ($p < 0,001$). Көл суын қабылдаған тышқандардың сүйек кемігін цитогенетикалық талдауда аберрантты жасушалардың жиілігінің және 100 метафазаға шаққанда құрылымдық хромосомалардың бұзылыстарының статистикалық маңызды жоғарылауын көрсетті ($p<0,05$). Тәжірибелік жануарлардың бауырындағы липидтердің асқын тотығу өнімдерін биохимиялық зерттеуі де липидті гидропероксид (ГПЛ) және малондиальдегид (МДА) деңгейінің статистикалық маңызды ($p<0,05$) жоғарылауын анықтады. Зерттеу барысында алынған нәтижелер зерттелген табиғи жер үсті суларында генотоксикалық, мутагендік және токсикалық белсенділігі бар химиялық заттардың бар екендігін көрсетеді.

Түйін сөздер: жер үсті табиғи сулары, генотоксикалық, органоспецификалық, хромосомалық аберрациялар, ДНҚ комета әдісі.

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Исследование токсической и генотоксической активности воды озера Кольсай, расположенного на территории Алматинской области Республики Казахстан

Важным этапом оценки состояния природной окружающей среды является выявление в ней различных экологически опасных факторов, обладающих токсической, генотоксической и мутагенной активностью. Появление в окружающей среде новых и увеличение концентрации уже присутствующих физико-химических компонентов, проявляющих токсические и генотоксические свойства, способствует усилению мутационного давления внешней среды на биоту различных экосистем. В последние десятилетия в связи с интенсификацией хозяйственной деятельности и развитием туристической сферы значительно увеличилась антропогенная нагрузка не только на наземные, в частности горные экосистемы, но и на природные поверхностые воды. В связи с этим, целью настоящего исследования явилось изучение генотоксической, мутагенной и токсической активности воды пресноводного озера Нижний Кольсай, расположенного на территории национального парка «Көлсайкөлдері» в Кегенском районе Алматинской области РК. Впервые с помощью ряда тест-систем (метод ДНК-комет, цитогенетический тест по учету хромосомных аберраций и биохимический анализ продуктов перекисного окисления липидов) на модельном объекте Mus musculus были изучены образцы воды, отобранные в 2018 и 2022 годах (весенне-летний период). Установлено, что пробы воды 2018 и 2022 г.г. индуцировали в клетках костного мозга и селезенки опытных мышей разрывы ДНК, уровень которых статистически значимо превышал аналогичные показатели у интактных животных ($p<0,001$). Цитогенетический анализ костного мозга мышей, принимавших озерную воду, также показал статистически значимое увеличение частоты аберрантных клеток и числа структурных нарушений хромосом на 100 метафаз ($p<0,05$). Биохимическое исследование продуктов перекисного окисления липидов (ПОЛ) в печени опытных животных также выявило статистически значимое ($p<0,05$) увеличение уровня гидроперекиси липидов (ГПЛ) и малонового диальдегида (МДА). Полученные в ходе исследования результаты свидетельствуют о наличии в исследуемых природных поверхностных водах химических веществ, обладающих генотоксичной, мутагенной и токсической активностью.

Ключевые слова: поверхностные природные воды, генотоксичность, органоспецифичность, хромосомные аберрации, метод ДНК комет.

Introduction

Escalating anthropogenic load leads to large-scale pollution of the environment with various xenobiotics, the number and range of which is increasing every year. This situation, of course, causes reasonable concern among specialists in various fields, environmental services, and, above all, among healthcare representatives [1-4].

Currently, the study of pollutants action on living organisms is the main goal of environmental monitoring, of which genetic monitoring is an integral part. Living organisms, including humans, are constantly exposed to a variety of environmentally hazardous factors of physical, chemical and biological nature, the vast majority of which, as has been shown in experimental studies, exhibit toxic, mutagenic and carcinogenic properties [5-9].

Of particular danger are factors that possess mutagenic activity. Environmental mutagens may cause irreversible damage to DNA, disrupt DNA replication and cause mutations leading to various hereditary pathologies, as well as cancer [10,11]. The frequency of spontaneous mutations in normal cells is estimated ranging from 10^{-8} to 10^{-11} per DNA nucleotide [12]. However, various mutagens can dramatically increase this frequency by several hundreds of times [13]. The impact of mutagenic factors on the body contributes to an increase in the genetic load in populations, which can cause a population decline, extinction of entire species and an increase in the frequency of hereditary diseases in the population.

At present, when there is a rapid increase in the world's population, the state of natural environment which is a direct habitat or a source of vital resources is deteriorating significantly. Natural surface waters located near settlements deserve special attention. Therefore, the assessment of genotoxicity and mutagenicity of water bodies is becoming increasingly important [14,15].

Different levels of anthropogenic impact significantly affect water quality and lead to pronounced gradients of environmental pollution. Ecological gradients have significantly affected the geographic distribution of zooplankton biodiversity. In addition, along with the trend towards increasing environmental pollution, indicator taxa associated with habitats have shifted in constituent parts, changing from large species (for example, arthropods) in lightly disturbed areas to small organisms (for example, rotifers and ciliates) in heavily disturbed areas. All this clearly demonstrates that water pollution as a result of anthropogenic activity significantly affects biological communities [16].

According to the surface water monitoring data of the Republic of Kazakhstan for the 1st half of 2022, out of 134 water bodies, 27 rivers belong to classes 5 and > 5 according to the Unified Water Quality Classification, that is, water is not suitable for all types of water use [17]. The main pollutants of surface water bodies in Kazakhstan include the main ions of the salt composition, biogenic and organic compounds, pesticides, oil and oil products, phenols, etc. Exceeding the quality standards for these indicators to some extent may be due to the natural and climatic features of the territory, then in most cases, the cause is anthropogenic factors, wastewater discharges from various economic enterprises and public utilities, etc. [17].

However, it should be noted that natural surface waters that are not subjected to direct human activity are also exposed to pollutants. Popular tourist and recreational areas are also experiencing anthropogenic pressure. One of such picturesque places is the Kolsai lakes (Upper, Middle and Lower), located on the territory of the national park "Kolsai Kolderi" in Kegen district, Almaty region, Republic of Kazakhstan. The national park is included in the UNESCO World Network of Biosphere Reserves. Kolsai lakes are characterized by the biodiversity of hydrobionts (phyto- and zooplakton, zoobenthos and fish resources), and are popular places of outdoor activity, there are guest houses and campsites nearby. Therefore, the purpose of this study was to study the genotoxic and mutagenic potential of the water of the Nizhny Kolsai Lake, located at an altitude of 1800 m above sea level.

Materials and methods

The object of the study was the water from Lower Kolsai Lake, located in Kungei Alatau mountains (South-Eastern Kazakhstan) on the territory of State National Natural Park "Kolsai Kolderi" at an altitude of 1800 m above sea level. Water samples were taken in 2018 and 2022 (Figure 1). Collection, filtration and conservation of water samples were carried out according to standard recommendations [18].

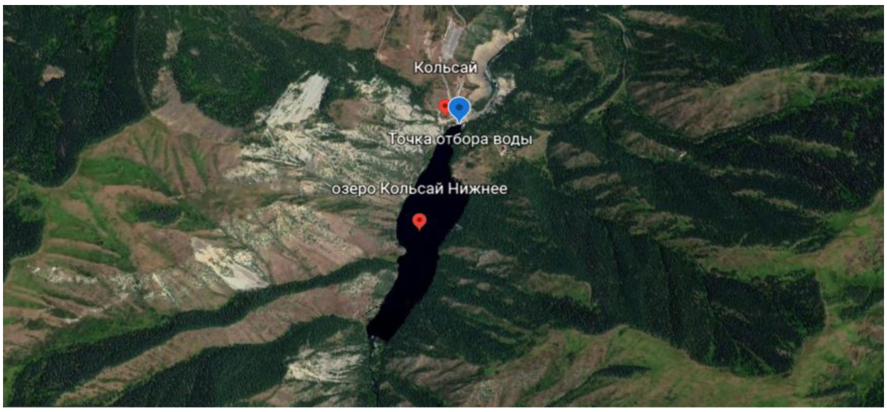

Figure 1 – Location of water sampling points:
Lake Kolsai- 42°59'29"N 78°19'36"E.

The genotoxic and mutagenic activity of water samples was studied on laboratory mice, 2-3 month age, the average weight was 25 g. Intact and experimental animals were kept in a vivarium on a standard diet. Laboratory animals were maintained in accordance with state and international principles [19]. Animals were sacrificed by cervical dislocation. Samples of internal organs (liver, kidneys, spleen, and bone marrow) were taken to study DNA breaks using the DNA comet assay, bone marrow – for cytogenetic analysis, liver – to determine the content of lipid peroxidation products (LPO).

Laboratory mice drank sampled water for seven days. An aqueous solution of standard mutagen methyl methanesulfonate (MMS) at a dose of 40.0 mg/kg was used as a positive control, which was administered intraperitoneally. Standard drinking water was used as a negative control. The genotoxic potential of water samples was studied using the DNA comet assay, which makes it possible to detect DNA breaks at the level of single cells. The following internal organs of laboratory mice were analyzed: bone marrow, liver, kidneys, and spleen [20, 21]. Giemsa stain was used for slides staining [22]. At least 100 DNA comets with non-overlapping tails for each preparation were visually analyzed using Olympus BX-43 microscope (Olympus Corporation, Japan) at 40x magnification. DNA comets were divided into five conditional types: class I: 0 – 6.0%; class II: 6.1 – 17%; class III: 17.1 – 35.0%; class IV: 35.1 – 60.0%; class V: 60.1–100.0% single- and double-strand DNA breaks in “comet tails” [23]. The index of DNA comets (IDC) as the degree of DNA damage was calculated using the following formula:

$$IDC = \frac{0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4}{\Sigma n},$$

where n_0-n_4 – number of DNA comets in each class, Σn – sum of calculated DNA comets. The damage index (DI) was calculated using the formula:

$$DI = IDC_e/IDC_c,$$

where IDC_e – index of DNA comets in the experimental group, IDC_c –index of DNA comets in negative control [24].

To assess the mutagenic potential of water samples, a metaphase method for analysis of chromosome aberrations in bone marrow cells was carried out [24]. Cytogenetic preparations were prepared 24 hours after the last injection and stained with azure-eosin. Slides were analyzed using light microscope under x1000 magnification. Cells with aberrations of chromosome and chromatid types (single and paired fragments of chromosomes, acentric and centric rings, point fragments) were counted.

To evaluate the toxic properties of water samples for mice liver the content of primary (HPL – lipid hydroperoxide) and secondary (MDA – malonic dialdehyde) products of lipid peroxidation (LPO) was determined in liver homogenate [25, 26]. The method for HPL determining is based on measuring the absorption of light by conjugated diene structures extracted with a mixture of heptanes:isopropyl alcohol (1:1). The optical density of the heptane phase was measured at a wavelength of 233 nm on Apel PD-303 spectrophotometer (Japan). MDA was determined by the 2-thiobarbituric acid method. Optical density was measured at 532 nm on Apel PD-303 spectrophotometer (Japan).

Statistical data processing was performed using StatPlus®5 Pro version 5.9.9.4/Core v6.7.3 software (AnalystSoft Inc., USA). In all cases, mean values (M) and mean errors (m) were calculated. The statistical significance of the differences between groups was analyzed using Student’s t-test. Differences were considered statistically significant with a confidence level of 95% or more ($p<0.05$).

Research results and discussion

In recent decades, natural aquatic ecosystems, being a habitat for many organisms, source of drinking water and human economic activity, have been subjected to powerful anthropogenic pressure. Most environmentally hazardous factors possess not only toxic, but also mutagenic and carcinogenic activity. Identification of potential mutagens and human carcinogens in terms of genetic consequences is a priority for biological assays. An increase in the range and number of environmental pollutants with mutagenic activity creates a real basis for enhancing genetic load and contributes to the rate of mutation process. The current research is intended on the investigation of genotoxic, mutagenic, and toxic effects of water samples from Lake Kolsai on laboratory mice *Mus musculus*.

Assessment of water samples genotoxicity using DNA comet assay. Water samples from Kolsai Lake were orally administered to mice for seven days. To detect genotoxic activity, DNA comets were analyzed in cells of bone marrow, liver, kidneys, and spleen. The indices of DNA comets (IDCs) in the cells of the studied organs are represented in Table

1. It has been found that the level of induced DNA breaks is significantly higher in cells of the bone marrow and spleen of experimental animals compared to the negative control ($p<0.001$). At the same time, in the cells of liver and kidneys, this index was at the control level. No statistically significant differences were found between similar indices in animals that received water samples collected whether in 2018 or 2022.

The DNA damage index (DI) exceeding 2 indicates pronounced genotoxic effect of the studied xenobiotics. As a result of the experimental study, it was found that in animals that received water from Lake Kolsai collected in 2018, the damage index, which reflects the degree of genotoxicity, increased by 2.7 and 3.5 times in the bone marrow and spleen cells, respectively, compared to intact animals. In animals that received water samples collected in 2022, DI increased only in the cells of the bone marrow (2.3 times), and in the cells of the spleen, liver and kidneys it was below 2 (Figure 2). Comparative analysis of DI in animals that drank water collected in 2018 and 2022 did not reveal statistically significant differences between the studied parameters, with the exception of DI in spleen cells, which was below 2 in the experimental group for 2022 water samples.

Table 1 – The level of DNA comets in the cells of mice that received water from Lake Kolsai

Experimental groups	Index of DNA comets in the cell (M±SE), %			
	Bone marrow	Liver	Kidneys	Spleen
Drinking water (negative control)	0,17 ± 0,05	0,23 ± 0,04	0,43 ± 0,03	0,22 ± 0,04
Positive control (MMS – methyl methanesulfonate)	3,38 ± 0,33*	3,1 ± 0,3*	2,7 ± 0,16*	3,14 ± 0,24*
Lake Kolsai, 2018	0,61 ± 0,17*	0,33 ± 0,04	0,48 ± 0,12	0,61 ± 0,04*
Lake Kolsai, 2022	0,42 ± 0,02*	0,32 ± 0,07	0,33 ± 0,04	0,43 ± 0,08*
Note – * – p<0.001 in comparison with the negative control				

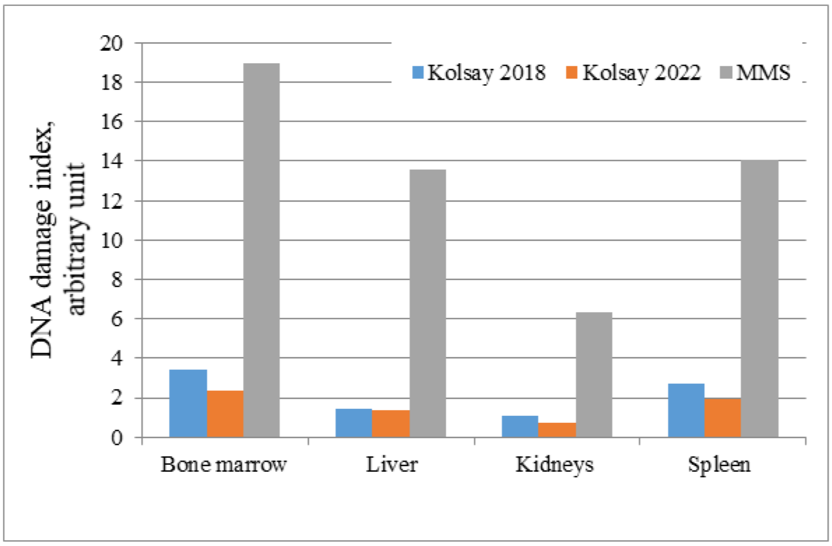

Figure 2 – Index of DNA damage in the cells of laboratory mice that received water from Lake Kolsai

In terms of sensitivity to DNA-damaging effects of water samples from 2018 and 2022, the studied organs can be arranged in the following order: bone marrow > spleen > liver > kidneys. The results obtained demonstrate the presence of genotoxic properties of the Kolsai water samples, collected in 2018 and 2022. In addition, the organ-specificity for genotoxic effect was established. The most sensitive were cells of the bone marrow and spleen.

The study of mutagenic activity of water samples from Lake Kolsai by chromosome aberration test. To study the mutagenic activity of water from Lake Kolsay, we carried out a cytogenetic analysis of bone marrow cells of laboratory mice that orally took the studied water samples. The analysis was carried out using a chromosome aberration test (metaphase method). The results of cytogenetic test for mutagenic activity of water samples in mice are presented in Table 2.

As can be seen, in intact animals (negative control), the frequency of aberrant bone marrow cells and the number of structural mutations per 100 metaphases were 1.33±0.25. The classical mutagen methyl methanesulfonate (MMS) induced aberrant cells in the bone marrow of experimental animals and the number of chromosome rearrangements per 100 metaphases, which was statistically significantly higher than in intact animals. Thus, the frequency of aberrant cells in MMS-treated mice increased by 4.3 times ($p<0.001$), and the number of chromosome aberrations per 100 metaphases increased by 5.4 ($p<0.001$) times. All types of chromosome rearrangements increased statistically significantly. The most pronounced was the frequency of chromosomal aberrations of chromatid type.

Table 2 – The frequency of structural chromosome aberrations in bone marrow cells of mice treated with water from Lake Kolsai

Experimental groups	Metaphase studied	Frequency of aberrant cells (M ± m %)	Number of chromosome aberrations per 100 metaphases			
			Total aberrations	Chromosome type	Chromatid type	Point fragments
Negative control (H_2O)	525	1,33±0,25	1,33±0,25	0,37 ± 0,25	0,59 ± 0,27	0,38 ± 0,26
Positive control (MMS – methyl methanesulfonate)	520	5,73±0,91••	7,15±0,95•••	1,35 ± 0,27•	4,06 ± 0,45•••	1,74 ± 0,42•
Water from Lake Kolsai (2018)	517	2,91±0,50•*	3,10±0,42••**	0,58 ± 0,27	2,13 ± 0,41•	0,39 ± 0,27
Water from Lake Kolsai (2022)	544	2,39±0,26•	2,58±0,38•*	0,38 ± 0,26	1,47 ± 0,26•	0,72 ± 0,20

Note: ● – $p<0.05$; ●● – $p<0.01$; ●●● – $p<0.001$ compared to the negative control (H_2O);
* – $p<0.05$; ** – $p<0.01$ in comparison with the positive control (MMS).

In an experimental study of water samples collected in 2018, the frequency of aberrant cells, as well as the number of aberrations per 100 metaphases, increased significantly compared to the negative control. Single cells containing a polyploid set of chromosomes were noted, which were absent in intact animals. The frequency of aberrant cells increased by 2.2 times ($p<0.05$), and the number of chromosome aberrations per 100 metaphases increased by 2.3 times ($p<0.01$). At the same time, these indicators were statistically lower than for the positive control (MMS). In the spectrum of chromosome alterations, rearrangements of both chromosomal and chromatid types were registered. Structural mutations of the chromosomal type were represented by paired terminal deletions, while those of the chromatid type were represented by single acentric rings, single terminal fragments, and point fragments were also found (Figure 3). A similar picture was observed in the experimental study in 2022. The frequency of aberrant cells increased by 1.8 times ($p<0.05$), and the number of chromosome aberrations per 100 metaphases increased by 1.9 times ($p<0.05$). In the spectrum of chromosome disorders, rearrangements of the chromosomal and chromatid types were also noted. In experimental animals that took water collected in 2018 and 2022, a statistically significant increase in structural mutations of the chromatid type was observed, which is typical for most mutagens of a chemical nature. There were no statistically significant differences in the frequency of induced chromosome aberrations in animals that took water samples collected in the lake in 2018 and 2022. However, in experiments conducted in 2022, there is a decrease in the absolute number of the aberrant cells and chromosome aberrations, which may be due to a decrease in the tourist number during the pandemic years (2020-2022).

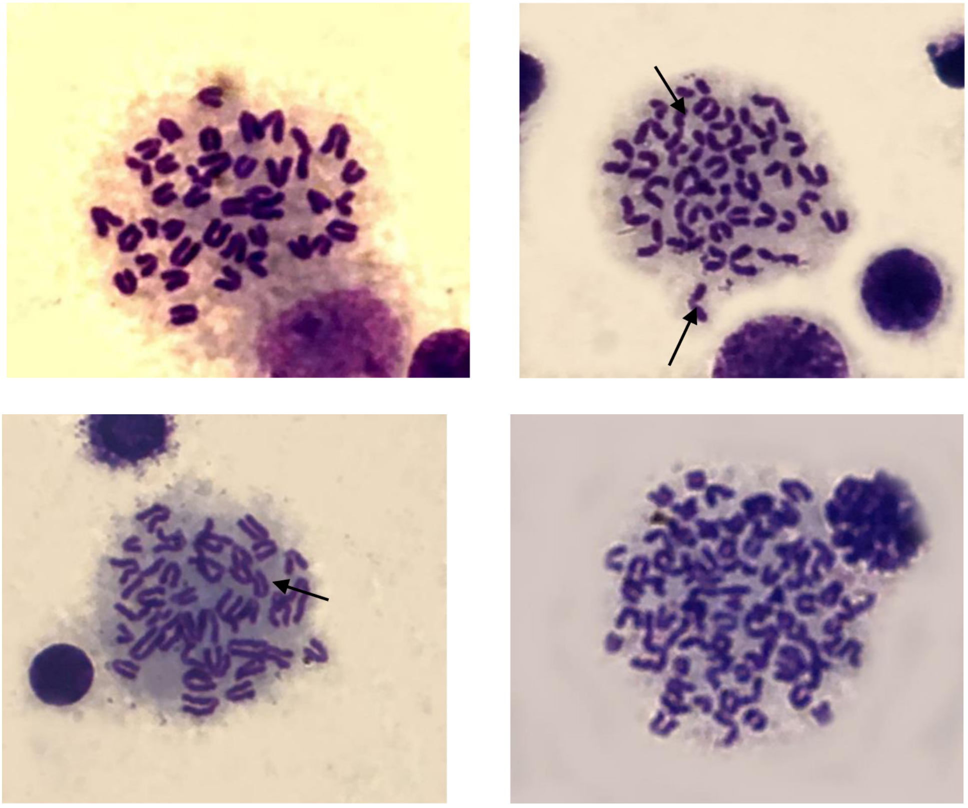

Figure 3 – Structural mutations in bone marrow cells of mice induced by water samples from Kolsai Lake in Almaty region, x1000

Because of cytogenetic studies, it was found that the water from Lake Kolsai exhibits mutagenic activity. In laboratory animals that received lake water, there was a statistically significant increase in the frequency of aberrant cells and the number of chromosome aberrations per 100 metaphases. The results obtained indicate the presence in the studied natural waters of chemicals with mutagenic and genotoxic activity.

Study of the influence of Kolsai lake water on the processes of lipid peroxidation. A free radicals of chemical nature play a crucial role in damage to the nuclear genome as is indicated by a number of works. The authors note that LPO secondary products, including MDA, can cause cross-links in biopolymers, which disrupts their structure and functions [27-31].

This paper presents the results of a spectrophotometric analysis of lipid peroxidation products in the liver of laboratory mice that took water from the Nizhny Kolsai Lake (Table 3). The level of lipid hydroperoxide (HPL) in the liver of experimental animals exposed to lake water in 2018 and 2022 was 0.52±0.18 and 1.49±0.35 mmol/mg, respectively. These data show that in animals that received native water samples in 2022, the HPL is significantly higher, by 2.59 times than in the control. The level of malondialdehyde (MDA) in the liver of animals was statistically increased by 2 times (2018) and 1.38 times (2022) compare to control values. An increase in the content of HPL and MDA in animals that received lake water indicates an enhancement of free radical processes in the liver of experimental mice.

Table 3 – The content of lipid peroxidation products in the liver of laboratory mice

Experimenta lgroups	HPL content	MDA content
Negativ econtrol (H_2O)	0,58 ± 0,14	10,16 ± 0,56
Positive control (MMS)	3,01 ± 0,58*	25,34 ± 0,84*

Continuation table

Water from Lake Kolsai (2018)	0,52 ± 0,18	20,53 ± 1,84*
Water from Lake Kolsai (2022)	1,49 ± 0,35*	14,03 ± 1,48*
Note: HPL – lipid hydroperoxide, MDA – malondialdehyde; * – p<0.05 in comparison with the negative control		

It is known that lipid peroxidation occurs under the action of free radicals, which are active charged molecules [32]. At the first stage of lipid peroxidation, diene conjugates of fatty acids are formed, when exposed to hydroxyl radicals, lipid hydroperoxides are formed. At the same time, lipid peroxidation products cause conformational changes in phospholipids and phospholipid complex, which leads to disruption of the functions of cells organelles, organs, and then the whole organism. At the sites of attachment of peroxide radicals, fatty acids break into fragments containing aldehyde groups, which are known to be highly reactive. When broken on both sides, malonic dialdehyde is formed. MDA, reacting with the SH- and CH3- groups of proteins, inhibits the activity of a number of enzymes [33].

Due to the continuous growth of the world population and the growth of consumption, all natural ecosystems are exposed to negative factors of various nature. Aquatic ecosystems, especially fluid ones (rivers, streams), are subjected to powerful anthropogenic pressure, as a result, a decrease in the diversity of aquatic organisms is observed. Water and soil pollution occurs due to industrial, agricultural, household waste, fertilizers and pesticides used by farmers, oil spills and radioactive materials [34]. Polluted water and soil pose a serious threat to living organisms, including humans, since they can have toxic, mutagenic, carcinogenic, teratogenic, and other negative effects [35]. In the complex of influence of pollutants on the body, genetic consequences are important. For any population, including humans, an increase of mutagenic factors in the environment is undesirable, since a real basis is created for increasing the genetic load and changing the rate of the mutation process.

According to the CAS (Chemical Abstracts Service, USA), by September 2018, 144 million chemical compounds were registered, and by October 2022, already 201 million [36]. All of them can end up in the air, water, soil, food. As a result, several dozens of compounds that can be found in the tissues of humans and other organisms can enter the body at the same time.

Among many chemical elements, heavy metals are of particular importance due to their association with human activities – vehicle exhausts [37], domestic and industrial wastewater discharges. Metals such as Pb, Cr, Zn, Cu, Cd, and Ni are typical potential pollutants of water and bottom sediments in urban areas [38,39]. Concern about the distribution of these elements in the environment is growing worldwide because they are persistent, bioaccumulative and toxic [40]. In addition to the fact that they contribute to the occurrence of morphological, behavioral, reproductive, genotoxic, and mutagenic damage in representatives of aquatic biota [41], they can also adversely affect human health.

The combined effect (synergy) of the chemical compounds can negatively affect the body even at low concentrations [42]. The genotoxic, mutagenic and toxic activity of the water of Nizhny Kolsai Lake (2018 and 2020), established in our studies on laboratory mice, is apparently due to the complex action of various chemical components of aquatic environment, which may include organic and inorganic compounds.

Thus, the studies of native water samples of the Nizhny Kolsai Lake carried out on the *Mus musculus* in 2018 and 2022 revealed genotoxic, mutagenic and toxic activity. Water samples collected 2018 and 2022 induced DNA breaks in cells of the bone marrow and spleen of experimental mice, the level of which was statistically higher than in intact animals ($p<0.001$). Cytogenetic analysis of the bone marrow of mice treated with lake water also showed a statistically significant increase in the frequency of aberrant cells and the number of structural chromosome disorders per 100 metaphases ($p<0.05$). Biochemical study of lipid peroxidation products (LPO) in the liver of experimental animals revealed a statistically significant increase ($p<0.05$) in the level of lipid hydroperoxide (HPL) and malondialdehyde (MDA). The results obtained indicate the presence in the studied natural surface waters of chemicals with genotoxic, mutagenic and toxic activity.

Conclusion

Environmental pollution by environmentally hazardous factors has become global in nature and is observed even in uninhabited areas where there is no economic activity. Most contaminants exhibit a potential mutagenic and carcinogenic hazard to living organisms. To solve this problem, first of all, it is necessary to take strict control over the processes of environmental pollution, prevent the expansion of pollutants, reveal the nature of their action and find ways to protect against their influence. Ecotoxicants have a negative impact on all components of natural ecosystems and lead to an increase of genetic load in natural and human populations. Therefore, the priority task is to assess the genetic consequences of the impact of pollutants on living organisms, as well as to find ways to protect against their negative effects.

In connection with the development of tourist and recreational activities in Kazakhstan in recent years, aquatic ecosystems, including high mountain lakes, have been intensively exploited. Therefore, the state of water bodies, which play an important role both in the nature water cycle and in the national economy, deserves special attention. The study of waters and bottom sediments of ecosystems of water bodies is important for studying their toxic and mutagenic effects, since the general recent trend is known – the active distribution of toxicants in the aquatic environment and their accumulation in bottom sediments [43, 44].

As noted earlier, natural environments that are not subject to direct human economic activity are also polluted. Tourist and recreational areas are also experiencing anthropogenic pressure. These include the national park "Kolsai Kolderi", located on the territory of the Almaty region, Republic of Kazakhstan. On the territory of the park, within the heights of 1800-3500 meters above sea level, there are unique landscapes rich in flora and fauna. The main attraction is the cascade of Kolsai lakes, located in a beautiful gorge, the tract of the tributary of Shilik River, the Kolsai River. These are three unique mountain lakes of tectonic origin – Upper, Middle and Lower Kolsai.

Kolsai lakes are characterized by the biodiversity of hydrobionts (phyto- and zooplakton, zoobenthos and fish resources), are a tourist attraction, there are guest houses and campsites nearby. According to the Institute of Hydrobiology and Ecology of the Ministry of Education and Science of the Republic of Kazakhstan [45], in accordance with the hydrochemical characteristics of reservoirs, water in terms of chemical composition and content of biogenic elements is a favorable habitat for hydrobionts. Nevertheless, for the first time in 2018 and 2022, cytogenetic, molecular genetic and biochemical studies of water at the model object *Mus musculus* revealed its genotoxic, mutagenic and toxic activity. Further studies are planned to identify the main inorganic and organic pollutants in Lake Kolsai and the sources of genotoxic water action.

As noted earlier, genotoxic and mutagenic factors present in the environment can increase the evolutionary level of mutation for each species and genetic load in the population of living organisms, including humans. Therefore, it is very important to carry out genetic monitoring of various natural media, including water, to detect mutagenic activity in order to reduce the level of pollution by environmentally hazardous factors that pose a threat to biota and human health.

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