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GENOTOXICITY OF ESENTAI AND ULKEN ALMATY RIVERS WATER ON ANIMAL TEST-SYSTEMS

Ecological monitoring, including water resources, is included in a set of measures for the rational use and protection of the environment. Currently, the assessment of water resources' genotoxicity and mutagenicity is an essential component of ecological monitoring. This research aimed to investigate water's genotoxicity from the Esentai and Ulken Almaty rivers flowing in Almaty. It was established that the content of Fe, Cu, Co, Ni, Pb, and Cd in the Almaty rivers water does not exceed the maximum permissible concentration (MPC). In contrast, zinc content in the Esentai and Ulken Almaty rivers’ water exceeded the MPC, respectively, by 1.1 and 7.7-folds, manganese – 2.1, and 1.9-folds. It was found that water samples from the Esentai and Ulken Almaty rivers showed a DNA-damaging effect on the studied cells (bone marrow, liver, kidneys, and spleen) of laboratory mice. DNA damage was determined using the Comet assay. In the cells of the studied organs in the experimental groups, the frequency of single-strand DNA breaks a statistically significant increase compared to the control group’s animal. The genotoxic action’s organspecificity of the studied river waters on laboratory mice was established. According to their sensitivity to water's genotoxic effect, experimental mice’s organs can arrange in the following order: spleen and bone marrow > liver > kidneys. An increase in lipid peroxidation products was found in the laboratory mice’ liver that drank water from the Esentai and Ulken Almaty rivers. Thus, the results of the physicochemical, molecular-genetic, and biochemical analysis of water on animal test objects indicate the presence in the investigated natural surface waters of chemicals with genotoxic and toxic activity.

Key words: surface waters; heavy metals; DNA comet assay, organ specificity, lipid peroxidation.
биохимиялық талдау нәтижелері зерттелетін табиғи жер үсті суларында генотоксикалық және уытты белсенділік бар химиялық заттардың болуын көрсетеді.

Түйін сөздер: жер үсті сулары, ауыр металдар, ДНҚ-комет әдісі, органның ерекшелігі, липидтердің тотығуы.

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Генотоксичность воды рек Есентай и Улкен Алматы на животных тест-системах

Экологический мониторинг, в том числе водных ресурсов, входит в комплекс мер по рациональному использованию и охране природной среды. В настоящее время оценка генотоксичности/мутагенности вод является важной составляющей экологического мониторинга водных ресурсов. Целью настоящего исследования явилось изучение генотоксичности воды из рек Есентай и Улкен Алматы, протекающих в г. Алматы. Показано, что содержание тяжелых металлов, таких как Fe, Cu, Co, Ni, Pb и Cd, не превышало предельно допустимые концентрации (ПДК) в реках Есентай и Улкен Алматы, в то время как содержание цинка превышало ПДК в 1,1 и 7,7 раза, марганца – в 2,1 и 1,9 раза, соответственно. С помощью метода ДНК-комет установлено, что образцы воды рек Есентай и Улкен Алматы проявили ДНК-повреждающий эффект в клетках костного мозга, печени, почек и селезенки лабораторных мышей. В клетках исследуемых органов опытных групп мышей частота однонитевых разрывов ДНК статистически значимо увеличивается по сравнению с животным из контрольной группы. Установлена органоспецифичность генотоксического действия воды изученных рек на лабораторных мышах. По чувствительности к генотоксическому действию воды органы экспериментальных мышей можно расположить в следующем порядке: селезенка и костный мозг>печень>почки. Также было выявлено увеличение продуктов перекисного окисления липидов в печени лабораторных мышей, принимавших воду из рек Есентай и Улкен Алматы. Таким образом, результаты физико-химического, молекулярно-генетического и биохимического анализа воды на животных тест-объектах свидетельствуют о наличии в исследуемых природных поверхностных водах химических веществ, обладающих генотоксической и токсической активностью.

Ключевые слова: поверхностные воды, тяжелые металлы, метод ДНК-комет, органоспецифичность, перекисное окисление липидов.

Introduction

Environmental pollution with household and industrial waste is one of the severe ecological problems since waste can be genotoxic, mutagenic, carcinogenic, embryotoxic activities. Most pollutants can disrupt living organisms’ genetic structures, including humans, increasing the population’s genetic burden. The danger of mutagenic effects lies in the manifestation of the occurred harmful mutations in subsequent generations. Ultimately, this can decrease the number of biodiversities, the species disappearance, and increase the birth frequency with hereditary pathology [1]. In this regard, the problem of identifying and assessing genotoxic and mutagenic factors in the environment is acute.

Surface natural waters are a natural habitat for many species of living organisms and a vital resource for humans. There are 3,000 chemical pollutants in water resources, and about 700 of them can get into drinking water, according to the World Health Organization [2]. Priority water pollutants include heavy metals, persistent organic pollutants, phenols, pesticides, and fertilizers, which lead to the subsequent death of all life in the aquatic environment.

In recent years, interest in studying the genotoxicity of surface natural waters has significantly increased. A complex of substances in natural waters can cause gene and chromosomal mutations, DNA breaks, micronuclei, and other disorders [3-11]. There is a demand for a comprehensive study of aquatic ecosystems, including the genotoxic activity, subject to anthropogenic impact. Studying the genotoxic activity of chemical substances helps determine environmental threats and form current normative actions to mitigate their impact. However, environmental pollutants’ molecular influence may transcend their interaction with the DNA sequence [12, 13]. Industrial waste impurities can cause a weakening of the human immune and reproductive systems, kidney failure. The spread of infectious gastroenteritis diseases, diarrhea, vomiting, skin, and kidney problems occurs through contaminated...
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Increasing pollutants’ content in water resources, the concentration of which goes beyond the maximum permissible concentration (MPC), results in a deterioration of the sanitary and epidemiological situation, a reduction in their use for household needs, changes of environment, mainly adverse changes in the aquatic ecological system, among them deterioration of health of living beings [14]. Xenobiotics of mutagenic action increase the “genetic load” - a complex of detrimental mutations in the population due to gametes’ mutations can be inherited [15].

On the territory of Almaty, there are rivers Esentai and Ulken Almaty. There is a reinforced concrete mudflow protection dam with mudflow storage, a cascade of hydroelectric power plants, a water supply system in Almaty, a recreation area, and the Sairan reservoir in the River Ulken Almaty valley. Almaty’s territory along the river Esentai riverbed, an embankment, and recreation area have been created, including the KazNUgrad University complex, the amusement park, and attractions “Fantasy World,” the park near Auezov theater. The water of the River Esentai is used for household purposes and irrigation. According to monitoring data for December 2020, the Ulken Almaty river belongs to the 4th class of pollution: water is suitable for irrigation and industry; for household and drinking water supply, intensive water treatment is required; Esentai - to class 2: water is suitable for all categories of water use; for domestic and drinking water supply, simple water treatment is required [16].

From the above, the research aimed to study the genotoxic effects of the Esentai River and the Ulken Almaty River on laboratory mice.

Materials and Methods

The Esentai River and Ulken Almaty River’s water, which flows in the Almaty city, was selected as the study’s object. Figure 1 shows the location of the sampling points.

Collection, filtration, and conservation of water samples were carried out according to standard recommendations [17]. The water’s physical parameters were measured using portable analyzers pH-meter PH-009(I) (Barry Century, China), TDS&EC-meter (Barry Century, China), ORP-meter ORP169E (Barry Century, China), DO-pen type DO-meter (Alvin Instrument, China). The content of...
heavy metals (Fe, Pb, Cu, Zn, Mn, Co, Ni, Cr, Cd) in water samples was determined using an MGA-915MD atomic absorption spectrophotometer (Lumex, Russia) [18].

The research test object was laboratory mice. Mice were with an average weight of 25 grams and two to three months of age. Laboratory mice drank the test water for seven days. The aqueous solution of mutagen cyclophosphamide at a 50.0 mg/kg dose was used as the positive control and was injected intraperitoneally. Drinking water was used as the negative control. The genotoxic potential of water samples in organs (bone marrow, liver, kidney, spleen) of laboratory mice was investigated using the DNA comet assay [19, 20]. We visually analyzed randomly at least 100 DNA-comets without overlapping “tails” for each preparation using Olympus microscope BX-43 (Olympus Corporation, Japan) with increasing the resolution 40x. DNA comets were stained with Giemsa stain to stain the preparations [21]. We visually analyzed comet assay [19, 20]. DNA comets were stained with Giemsa stain to stain the preparations [21]. We visually analyzed the DNA comets randomly at least 100 DNA-comets without overlapping “tails” for each preparation using Olympus microscope BX-43 (Olympus Corporation, Japan) with increasing the resolution 40x.

The degree of DNA damage was calculated by the formula:

\[ IDC = \frac{n_1 + n_2 + n_3 + n_4 + n_5}{n} \]

where \( n \) is the number of DNA comets of each class, \( \Sigma n \) is the sum of the calculated DNA comets. The damage index (DI) was calculated using the formula:

\[ DI = \frac{IDC}{IDC_{exp}} \]

where \( IDC_{exp} \) is the index of DNA comets in the experimental group, \( IDC \) – the index of DNA comets in the studied organs, \( IDC_{exp} \) – the index of DNA comets in the experimental group.

The content of primary (LOOH - lipid hydroperoxide) and secondary (MDA - malonic dialdehyde) lipid peroxidation products was determined to assess water’s toxic properties in the hepatic tissue homogenate of the laboratory mice. The method for determining LOOH is based on measuring the absorption of light by conjugated diene structures extracted with a mixture of heptane and isopropyl alcohol (1:1). The heptane phase’s optical density for determining LOOH is based on measuring the absorption of light by conjugated diene structures extracted with a mixture of heptane and isopropyl alcohol (1:1). The heptane phase’s optical density was measured at a wavelength of 233 nm on an Apel PD-303 spectrophotometer (Japan). MDA was determined by the 2-thiobarbituric acid method. Optical density was measured at 532 nm on an Apel PD-303 spectrophotometer (Japan) [24].

Statistical data analysis was in the Data Analysis add-in Microsoft Excel program and the StatPlus®5 Pro version 5.9.9.4/Core v6.7.3 (AnalystSoft Inc., USA). Each result is expressed as mean ± standard error (SE). The distribution of data for normality was checked using the Shapiro-Wilk W-test. Differences between groups were considered significant with a confidence level of 95% and higher (\( p < 0.05 \)) and were calculated using Student’s t-test.

**Research results and discussion**

It is crucial to test the water for various physical and chemical parameters before using it for drinking, domestic, agricultural, or industrial use. Table 1 shows the physicochemical parameters of water samples. The studied water sources’ pH value was within normal values (pH = 6.0-9.0). Water from the River Ulken Almaty can be attributed to slightly acidic waters (pH 5.0-6.5), water from the River Esentai can be attributed to neutral waters (pH 6.6-7.5). Within the oxidation-reduction potential (ORP) context, all waters had values in the normative values range. The total dissolved solids (TDS) and electrical conductivity (EC) of water from the River Esentai aligned the standard, but from the River Ulken Almaty - less than the standard. The dissolved oxygen in the water of the investigated sources was 1.8-2.2 mg/L. Low levels of dissolved oxygen (<7.0 mg/L) implicitly show that the river Ulken Almaty is dirty; Esentai is very dirty. However, a decrease in dissolved oxygen in water can be related to increased ambient temperature and increased microbial activity [25]. The content of Ni, Co, Pb, Cr, Fe, Cu, and Cd in the Almaty’s rivers waters did not exceed the MPC. The excess of MPC was observed for Mn and Zn in the Esentai river water by 2.1 and 1.1-fold, respectively, in the Ulken Almaty river water was observed by 1.9 and 7.7-fold.

Laboratory mice were exposed to water samples from Esentai and Ulken Almaty rivers for seven days to study water’s genotoxic activity. The genotoxic effects of water samples were examined on bone marrow cells, liver, kidney, and spleen using the Comet assay. Table 2 shows the indexes of DNA comets (IDC) in cells of the studied organs.

In intact laboratory mice, we observed class I DNA comets; when exposed to cyclophosphamide has class III and IV; when exposed to water from the rivers Esentai and Ulken Almaty has II and III damage classes (Figure 2) [22]. Comparative analysis of the data on the index of DNA comets in the studied organs’ cells showed a statistically significant increase in DNA breaks in the experimental groups’ mice compared with the negative control (\( p < 0.05-0.01 \)). The damage index (DI), which shows the degree of genotoxicity in bone marrow cells, was 2.07 when exposed to cyclophosphamide, 2.10 - Esentai river, 2.17 - Ulken Almaty water. The DI in liver cells was 2.32 when exposed to cyclophosphamide, water from the river Esentai was 1.98, the...
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The DI in spleen cells was 2.16; 2.26; 2.19 when exposed to cyclophosphamide, water from the rivers Esentai and Ulken Almaty, respectively.

Table 1 – Physicochemical parameters of water samples from rivers of Almaty city

| Physicochemical parameter | River Esentai, (M±SE) | River Ulken Almaty, (M±SE) | Standard or MPC |
|---------------------------|-----------------------|---------------------------|-----------------|
| t (°C)                    | 11.0                  | 12.8                      | -               |
| pH                        | 6.6                   | 6.4                       | 6.0-9.0         |
| ORP (mv)                  | 208                   | 145                       | +80.0-300.0     |
| TDS (ppm)                 | 127                   | 47                        | 50.0-150.0      |
| EC (μS/m/sm)              | 246                   | 94                        | 100.0-1200.0    |
| DO(mg/L)                  | 1.8                   | 2.2                       | 7.0-9.0         |
| Fe (mg/dm³)               | 0.0783± 0.0002        | 0.0337± 0.0002            | 0.1             |
| Pb (mg/dm³)               | 0.0045± 0.0002        | 0.0040± 0.0001            | 0.006           |
| Cu (mg/dm³)               | 0.0006± 0.0001        | 0.0007± 0.0000            | 0.001           |
| Zn (mg/dm³)               | 0.0106± 0.0003*       | 0.0767± 0.0001*           | 0.01            |
| Mn (mg/dm³)               | 0.0212± 0.0011*       | 0.0185± 0.0014*           | 0.01            |
| Ni(mg/dm³)                | 0.0013±0.0002         | 0.0019±0.0003             | 0.01            |
| Cr (mg/dm³)               | 0.0054± 0.0001        | 0.0052± 0.0001            | 0.02            |
| Co(mg/dm³)                | 0.0031± 0.0003        | 0.0025± 0.0004            | 0.01            |
| Cd(mg/dm³)                | 0.0004± 0.0000        | 0.0004± 0.0000            | 0.005           |

Note: MPC - maximum permissible concentration, t - temperature, ORP – oxidation-redox potential, TDS - total dissolved solids, EC – electrical conductivity, DO – dissolved oxygen in water
* - exceeds or at the level of MPC
M±SE - mean ± standard error

Table 2 – The Indexes of DNA-comets in the organs’ cells of laboratory mice that received water from the Almaty rivers

| Experimental groups       | Index of DNA comets in the cell (M±SE) |
|---------------------------|----------------------------------------|
|                           | bone marrow | liver | kidney | spleen   |
| drinking water (negative control) | 1.08 ± 0.05 | 1.03 ± 0.02 | 1.17 ± 0.10 | 1.06 ± 0.05 |
| cyclophosphamide, 50 mg/kg (positive control) | 2.24 ± 0.04*** | 2.39 ± 0.06*** | 2.27±0.07*** | 2.29 ± 0.06*** |
| river Esentai             | 2.27 ± 0.50* | 2.04 ± 0.42* | 1.77 ± 0.23* | 2.40 ± 0.38** |
| river Ulken Almaty        | 2.34 ± 0.28** | 2.00 ± 0.35* | 1.67 ± 0.19* | 2.33 ± 0.41* |

Note: * p<0.05; **p<0.01; ***p<0.001 in comparison with negative control
M±SE - mean ± standard error
Comparative analysis of the data obtained that under the cyclophosphamide action at a dose of 50 mg/kg, DNA breaks increased statistically significant (p<0.01) compared to the negative control. The studied organs can be arranged by their sensitivity to cyclophosphamide’s DNA-damaging action in the following order: liver> spleen> bone marrow> kidneys. Under the influence of the water from the Esentai and Ulken Almaty River, there is also a statistically significant increase in DNA breaks compared (p<0.05-0.01) with intact mice. The studied organs can be arranged by their sensitivity to water samples’ DNA-damaging action in the following order: spleen> bone marrow> liver> kidneys.

Thus, the organ-specificity of the studied rivers water’s genotoxic action on laboratory animals was established. The studied water samples induced single-strand DNA breaks in the cells of the mice’ organs.

The lipid peroxidation product levels in laboratory mice’s liver were studied using the extraction-spectrometric method. The lipid hydroperoxide (LOOH) accumulation in the cell occurs at the first stage of free radical oxidation of polyunsaturated fatty acids. Hydroperoxides have mutagenic and pronounced cytotoxicity. They inhibit glycolysis and oxidative phosphorylation activity, inhibit the synthesis of protein and nucleic acids, disrupt the secretion of triglycerides by hepatocytes, and inhibit various membrane-bound enzymes. LOOHs then metabolize to malondialdehyde (MDA). Malondialdehyde crosslinks lipid molecules and reduces membrane fluidity, as a result of which the processes associated with phagocytosis, pinocytosis, and cell migration are disrupted [24, 26].

Figure 3 shows the primary and secondary LPO product’s content exposed to Esentai and Ulken Almaty’s water samples. The LOOHs level in the mice liver exposed to the Esentai and Ulken Almaty rivers’ water samples was 8.71±0.76 and 8.60±0.86mMol/mg, respectively. These data were statistically significantly higher, respectively, in 3.93 (p=0.0011) and 3.88-fold (p=0.0019) than the control. The malondialdehyde levels in the liver of experimental animals are statistically significantly higher in 5.48-fold (p=0.0003, Esentai), 5.02-fold (p=0.0083, Ulken Almaty), and 5.67-fold (p=0.0001, cyclophosphamide) than the control values.

Population growth in large cities and the expansion of old cities have significantly increased domestic wastewater flow into surface water. These runoffs have become a source of pollution of rivers and lakes with pathogenic bacteria and helminths. To an even greater extent, synthetic detergents, widely used in everyday life, pollute water bodies. They are also commonly used in industry and agriculture. The chemicals in them, getting into water bodies with wastewater, affect their biological and physical regime.

Water temperature controls the speed of all chemical reactions in aquatic ecosystems and affects fish growth, immunity, and reproduction. The main processes that ensure an organism’s vital activity are redox reactions, i.e., reactions associated with electrons’ transfer or attachment. Usually, the ORP of the human body ranges from -90 mV to -200 mV, and the ORP of ordinary drinking water is almost always much higher than zero, tap water - from +80 mV to +300 mV [27]. The Total Dissolved Solids determines the total content of inorganic salts and inorganic substances. Electrical conductivity correlates with pH, temperature, TDS, chemical oxygen demand, Cl–, Fe2+, and Ca2+ content in the water [28]. Dissolved oxygen is an indirect indicator of surface waters’ quality and correlates with bacterial activity, photosynthesis, nutrient availability, and stratification [25, 28].
Urbanization has led to the deterioration of aquatic ecosystems and introduced many pollutants in water resources. Heavy metals, metalloids, and hydrocarbons, organic compounds can cause genotoxic damage to different organisms [5-7]. In many chemical elements entering water bodies, metals are essential because of their association with domestic and industrial wastewater, vehicle emissions [29]. Metals such as Pb, Cr, Zn, Cu, Cd, and Ni are typical potential water and sediment pollutants in urban areas [8, 30]. Our studies found that in the water of the studied rivers flowing in Almaty, Ni, Co, Pb, Cr, Fe, Cu, Cd, Mn, and Zn are determined, while levels of Mn and Zn exceed the MPC. Several studies have shown that natural water contaminated with heavy metals may exhibit genotoxic activity in aquatic living beings [5, 31, 32]. For example, Ni acts as a mutagen on the physiology and behavior of fish [33]. Pb can suppress the enzyme’s activity interfering with the DNA repair system [34]. Excess copper in the organism inhibits the immune system, leading to anemia and non-infectious hepatitis. Zinc has mutagenic and carcinogenic activity [26].

There are numerous data on water’s genotoxicity in various rivers, including those in areas associated with large settlements and industrial centers. For example, a study of the Pitimbu River (an essential source of water supply for the city of Natal, Rio Grande do Norte, Brazil) revealed changes in the mitotic index for two samples, an increase in the frequency of chromosomal aberrations, and micronuclei in the root meristem of Allium sulfur for all water samples compared with the control group. The data show that the surface waters of the Pitimbu River contain compounds with potential genotoxicity that can affect the ecosystem. The highest genotoxic activity was noted near the industrial center [35]. Ames and SOS/umu tests indicated that organic extracts of water from Pearl River (Guangzhou city, China) induced mutagenicity and genotoxicity [36].

At present, water bodies, due to anthropogenic impact on them, can contain a complex mixture of chemicals. Physicochemical analysis of water can not always determine the content of various substances, especially in low concentrations. However, the chemicals present’ combined effect can negatively affect the body even at low concentrations [9]. Stankevičiūtė M. et al. showed that the metal mixture’s toxicity might change and be stronger or weaker than expected. Besides, it was determined that after a 10-fold decrease in the concentration of one metal in a mixture of several metals, the accumulation of some other metals in fish tissues may increase. Accordingly, a decrease in the concentration of a particular metal in a mixture does not necessarily lead to a significantly lower accumulation of metal in fish tissues compared to the amount accumulated in fish contained in mixtures with a reduced concentration of any other heavy metals. This shows that in the presence of other metals in the environment, the MPC established for a specific metal may be unsafe for aquatic organisms. Combined metals can strengthen toxic, genotoxic effects in comparison to isolated chemicals [5, 37].
In practice, conventional monitoring programs measure almost exclusively chemical/physical parameters [27, 28]. Concentrations of pollutants can provide detailed descriptions of pollution levels and indirectly indicate the potential biological effects of mixed environmental pollution. Biological analyzes, in turn, make it possible to determine the biological effects of mixtures. The combination of these two approaches makes it possible to identify the primary sources of risk that require constant monitoring; therefore, an adequate assessment of the environmental risk caused by any pollution should be based on the use of chemical methods for controlling pollutants, followed by biological tests [9, 38].

Thus, the revealed genotoxic effect of water on the laboratory mice is determined by the combined impact of pollutants’ mixed activity, such as heavy metals, organic and non-organic compounds, genotoxicants of physical and biological origin, and other anthropogenic water pollutants.

Conclusion

Thus, research results were established that the MPC was exceeded for manganese and zinc. The toxic and genotoxic activities were shown for all studied surface waters. It was shown that in animals that received water from the Esentai and Ulken Almaty rivers, the frequency of single-strand DNA breaks in all studied organs (bone marrow, liver, kidney, and spleen) was statistically significantly higher (p<0.05) compared to the control. The increased lipid peroxidation products (lipid hydroperoxide and malondialdehyde) in the liver of experimental mice were also observed compared to intact animals. The research results indicate Esentai and Ulken Almaty rivers’ pollution with environmentally hazardous factors that threaten biota and human health. Further studies are planned to identify the main inorganic and organic pollutants in the Esentai and Ulken Almaty rivers to determine the water genotoxicity mechanism.

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