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TOXIC AND MUTAGENIC ACTIVITY OF THE SPRING WATERS OF ALMATY

Spring waters, as natural discharges of groundwater to the surface, are an essential component of the natural environment. Spring water is considered by nature to be exceptionally pure, transparent, tasty, and balanced in its chemical composition, which makes it attractive to humans. However, the quality of spring water in urban areas has deteriorated significantly due to the high risk of chemical and bacterial contaminants entering groundwater. Therefore, studies were carried out of spring waters, actively consumed by the population of Almaty, for toxic and mutagenic activity. Spring 1 is located near the river Prohodnaya, bus stop number 28; spring 2 - on the road to the Big Almaty Lake, in front of the bridge over the river Ulken Almaty; spring 3 - near the river Batareika on the road to Kok-Zhailau; spring 4 at the Prosveshchenets stop on the road to Medeu. The excess of MPC for lead in the water of springs 1, 3, and 4 has been established. Following the established MPC for heavy metals, water from spring 2 belongs to quality class 1 and is suitable for all types of water use; water from springs 1, 3 and 4 belongs to quality class 2. The waters of this water use' class are suitable for all categories of water use, except for domestic. A bioluminescent test revealed high toxicity of spring waters 1-3, which significantly reduced the bioluminescent response of RecA and CoID biosensors (p < 0.05). In the Allium test, the water of all the springs have phytotoxic activity, water from springs 1 and 3 also have a genotoxic effect. In the chromosomal aberration assay in H. vulgare, it was found that the water of spring-3 had a mutagenic activity. There was a statistically significant increase in the frequency of chromosomal aberrations in barley seeds (p < 0.01). The remaining studied waters also increased the frequency of aberrant cells compared with the control; however, the difference was not statistically significant. Besides, polyploid cells (more than 1.5%) were identified with high frequency in the root meristem of barley seeds germinated in water from springs 1 and 3. The research indicates the presence of mutagenic factors in the waters of these springs. A comparative analysis of the level of contamination of the studied springs with heavy metals, the level of toxic and mutagenic activity of water allows ranking the investigated water objects in the following order: spring 3> spring 1> spring 2> spring 4.

Key words: spring water, toxicity, mutagenicity, biosensor strains, heavy metals.

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Алматы бұлақтарының уытты және мутагендік белсенділігі

Бұлақтар, жер асты суларының табиғи ағындары ретінде, табиғи ортаның маңызды құрамдас бөлігі болып табылады. Табиғи бұлақтың суы таза, мөлдір, дәмді және химиялық құрамы жағынан үйлестірілген болып саналған соң, бұл оны адамдар үшін тартымды етеді. Алайда, қалалық жерлерде бұлақтардың суының сапасы химиялық және бактериялық ластаушы заттардың жер асты суларына түсу қаупі жоғары болғандықтан айтарлықтай нашарлады. Сондықтан осы зерттеуде Алматы қаласының тұрғындары пайдаланатын бұлақ суларының уытты және мутагендік белсенділігі аңықталды. 1-ші бұлақ Проходная өзеннің жанында орналасқан, № 28 автобустың аялдамасы; 2-ші бұлақ — Үлкен Алматы көліне барар жолында, Үлкен Алматы өзен үстіндегі көпірдің алдында; 3-ші бұлақ — Көкжайлауға барар жолында, Батарейка өзеннің жанында; 4-ші бұлақ Медеуге барар жолында, Просвещенец аялдамасында. 1, 3 және 4 бұлақ суларында қорғасынның мөлшері ШРК көрсеткішінен асып кетті. Ауыр металдар үшін белгіленген

ШРК-ға сәйкес, 2-ші бұлақтың суы 1 сапа класына жатады және оның суы пайдаланудың барлық түрлеріне жарамды; 1, 3 және 4 бұлақ сулары 2 сапа класына жатады. Суды пайдалану бойынша осы кластағы сулар ішуге және ішуге арналған суды қоспағанда, суды пайдаланудың барлық санаттарына жарамды. Биолюминесценттік сынақ көмегімен 1-3 бұлақтардың су жоғары уыттылығы анықталды, осы бұлақтардың суы RecA және ColD биосенсорларының биолюминесценттік реакциясын статистикалық маңызды төмендеткен (р <0.05). А. сера сынағында барлық зерттелген бұлақтардағы судың фитотоксикалық белсенділігі, сондайақ 1 және 3-ші бұлақтардағы судың генотоксикалық әсері анықталды, H. vulgare сынағында 3-ші бұлақ суының мутагендік белсенділігі бар екендігі анықталды, осы суда өсірілген арпа тұқымдарының тамыр жасушаларында хромосомалық аберрациялардың жиілігі статистикалық маңызды артты (р < 0.01). Қалған зерттелген сулар бақылаумен салыстырғанда аберранттық жасушалардың жиілігін арттырды, алайда олардың айырмашылығы статистикалық маңызды емес. Сонымен қатар, полиплоидты жасушалар (1,5%-дан астам) 1 және 3-ші бұлақтардан суда өсетін арпа дәндерінің тамырлық меристемасында жоғары жиілікпен анықталды, бұл осы көздердің суларында мутагендік факторлардың болуын көрсетеді. Зерттелетін бұлақтардың ауыр металдармен ластану деңгейіне, судың уытты және мутагендік белсенділігінің деңгейіне салыстырмалы талдау зерттелетін су нысандарын келесі ретпен саралауға мүмкіндік береді: 3-ші бұлақ > 1-ші бұлақ > 2-ші бұлақ > 4-ші бұлақ.

Түйін сөздер: бұлақ суы, уыттылық, мутагенділік, биосенсор штаммдар, ауыр металдар.

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Токсическая и мутагенная активность родников г. Алматы

Родники как естественные выходы подземных вод на поверхность являются важнейшим компонентом природной среды. Родниковая вода считается от природы исключительно чистой, прозрачной, вкусной и сбалансированной по своему химическому составу, что делает ее привлекательной для человека. Однако качество родниковой воды на урбанизированных территориях значительно ухудшилось вследствие большого риска поступления в грунтовые воды химических и бактериальных загрязнителей. Поэтому были проведены исследования родниковой воды, активно потребляемой населением г. Алматы, на токсическую и мутагенную активность. Родник 1 расположен близ р. Проходной, конечная остановка автобуса №28; родник 2 – по дороге на Большое Алматинское озеро, перед мостом над р. Улкен Алматы; родник 3 – близ р. Батарейка по дороге на Кок-Жайляу; родник 4 – у остановки Просвещенец по дороге на Медео. Установлено превышение ПДК по свинцу в воде родников 1, 3 и 4. В соответствии с установленными ПДК по тяжелым металлам вода из родника 2 относится к 1 классу качества и пригодна для всех видов водопользования; вода из родника 1, 3 и 4 относится ко 2 классу качества. Воды этого класса водопользования пригодны для всех категорий водопользования за исключением хозяйственнопитьевого назначения. С помощью биолюминесцентного теста выявлена высокая токсичность воды родников 1-3, которая значительно снижала биолюминесцентный ответ биосенсоров RecA и ColD (p<0,05). В тесте на А. сера установлена фитотоксическая активность воды из всех изученных родников, а также генотоксический эффект воды из родников 1 и 3. В тесте по учету хромосомных аберраций на H. vulgare установлено, что вода родника-3 обладала мутагенной активностью, проявившейся в статистически значимом увеличении частоты хромосомных аберраций в семенах ячменя (p < 0,01). Остальные изученные воды также увеличивали частоту аберрантных клеток по сравнению с контролем, однако разница была статистически не значимой. Кроме того, выявлены с высокой частотой полиплоидные клетки (более 1,5%) в корневой меристеме семян ячменя, проращиваемых на воде из родников 1 и 3, что указывает на наличие мутагенных факторов в водах данных источников. Сравнительный анализ уровня загрязненности исследованных родников тяжелыми металлами, уровня токсической и мутагенной активности воды позволяет ранжировать исследованные водные объекты в следующем порядке: родник 3 > родник 1 > родник 2 > родник 4.

Ключевые слова: родниковая вода, токсичность, мутагенность, биосенсорные штаммы, тяжелые металлы.

Introduction

Natural aquatic ecosystems, being the habitat of many organisms, a source of drinking water and a resource for human economic activity, are currently subjected to powerful anthropogenic pressures. Most environmentally hazardous factors have not only toxic but also mutagenic and carcinogenic activity. Identification of potential mutagens and carcinogens for humans is the main task of bioassay. In the complex effects of pollutants, genetic consequences are essential. The increase in the spectrum and the number of environmental pollutants with a mutagenic activity creates a real basis for increasing the genetic load and changing the rate of the mutation process. In the medicalgenetic attitude, this can be expressed in increased intrauterine death of individuals, the appearance and spread of congenital malformations, hereditary diseases, and a genetically determined predisposition to diseases [1]. The test for the genotoxicity and mutagenicity of various pollutants in model organisms under laboratory conditions is aimed at finding opportunities to prevent adverse genetic consequences if introduced into the environment. However, not a single model test-object and not a single test system individually can obtain data based on which it would be possible to conclude about the degree of genetic danger of the substance to humans. The specificity of the biological object on which the effect of mutagen is studied, as well as the specificity of mutational events, dictate the need for several biological models and testing methods [1-3].

Recently, interest in springs has grown unusually. The springs represent the natural discharges of groundwater to the surface and are an essential component of the natural environment and geographical environment [4]. It is considered that spring water, passing through sand or gravel, undergoes natural and almost perfect treatment. Therefore, people believe that natural spring water is naturally pure, transparent, delicious, and balanced in its chemical composition. However, there is an excellent risk of chemical and bacterial pollutants entering groundwater, and therefore the quality of spring water in urban areas has deteriorated significantly lately. This is confirmed by the results environmental-hydrochemical, toxicological studies, and sanitary-hygienic monitoring [4-8].

In view of the foregoing, this study aimed to study the toxic and mutagenic activity of spring water, actively consumed by the population of Almaty.

Material and methods

The objects of the study were water from springs located in the territory of Almaty. Four springs, often used by the population, were selected: Spring 1 is located near the river Prohodnaya, bus stop number 28; spring 2 – on the road to the Big Almaty Lake, in front of the bridge over the river Ulken Almaty; spring 3 – near the river Batareika on the road to Kok-Zhailau; spring 4 – at the Prosveshchenets stop on the road to Medeu. The location of the springs is shown in Figure 1.

The test objects of the study were the biosensor strains of *Escherichia coli* (MG 1655 (pSoxS-lux), MG1655 (pKatG-lux), MG1655 (pColD-lux), MG1655 (pRecA-lux), barley (*Hordeum vulgare* L.) variety Baiseshek, onion (*Allium cepa*). The positive controls were 4-nitroquinoline 1-oxide (4-NQO, C₉H₆N₂O₃); methyl methanesulfonate (MMS, C₂H₆O₃S), as mutagens; 1,1'-dimethyl-4,4'-dipyridylium dichloride (paraquat, C₁₂H₁₄C₁₂N₂) and hydrogen peroxide (H₂O₂), as oxidants.

Sampling, filtration, and preservation of water samples were carried out according to GOST 31861-2012 [9]. At the place of water sampling, physical parameters of water quality were measured: temperature, pH, Total Dissolved Solids (TDS), oxidation-reduction potential (ORP), dissolved oxygen (DO), electrical conductance (EC) [10]. Temperature, Total Dissolved Solids and electrical conductivity were measured using a portable TDS & EC meter (Barry Century, China), pH values a portable pH meter analyzer PH-009(I) (Barry Century, China), oxidation-reduction potential a portable analyzer of the ORP-meter ORP169E (Barry Century, China), the content of dissolved oxygen – a portable analyzer of the DO-meter DOpen type (Alvin Instrument, China).

The heavy metals were determined by atomic absorption on an MGA-915MD atomic absorption spectrophotometer (Lumex, Russia) according to PND F 14.1: 2.214-06 [11].

To determine the toxic, genotoxic, and oxidative activity of water using a bioluminescent test, genetically modified *E. coli* strains were used: *E. coli* MG 1655 (pSoxS-lux), *E. coli* MG1655 (pKatG-lux), *E. coli* MG1655 (pRecA-lux) [12, 13]. The pCoD and pRecA promoters detect substances that induce DNA damage. 4-nitroquinoline 1-oxide (4-NQO) was used to activate these promoters at a concentration of 75.0 μg/mL. PkatG and PsoxS promoters detect substances inducing oxidative stress in the cell. The PkatG promoter (OxyR activator protein) specifically reacts

to hydrogen peroxide, organic peroxides, and the PsoxS promoter (SoxR activator protein) to specific superoxide ion radicals [8, 12, 13]. Hydrogen peroxide was used to activate the pKatG promoter at a concentration of 0.01 μg/mL; paraquat was used to activate the PSoxS promoter at a concentration of 10.0 μg/mL. Negative control was distilled water. Bacteria grew in Luria-Bertani broth (LB) containing 100 μg/mL ampicillin. The overnight culture was diluted to a concentration of 107 cells/mL in fresh LB-broth and grown at 37° C for 2-3 hours. Aliquots of this culture (160 µL each) were transferred to sterile cells (located in the well of the microplate) and added 40 µL of test water samples. 40 μL of distilled water or a standard mutagen/oxidant was added to control wells. Incubated at specific time intervals: for pColD-lux – 90 minutes, pRecAand pSoxS-lux - 60 minutes, for pKatG-lux - 45minutes. The bacterial luminescence level was measured on a LuMate 4400 microplate luminometer (Awareness Technology, USA) and expressed in relative light units (RLU). The criterion of the toxic effect is the change in the bioluminescence intensity of the test object in the sample compared to that for the sample with a solution that does not contain toxic substances. The change in the intensity of bioluminescence is proportional to the toxic effect. A quantitative assessment of the test reaction parameter is expressed as the dimensionless value of the toxicity index (IT): IT <20% - allowable degree of toxicity; IT = 15-20% with a statistically significant difference between experience and control and IT = 20-50% – the sample is toxic; IT> 50 – the sample is highly toxic. A measure of genotoxicity is induction factor (I), which was defined as the ratio of the luminescence intensity of a suspension of a lux biosensor containing the test compound (Lc) to the luminescence intensity of a control suspension of a lux biosensor (Lk). With a significant difference between the experiment and control I <2, the detected genotoxic effect was evaluated as "weak"; when 2 <I <10 – as "average"; at I> 10 – as a "strong" effect. All experiments were performed in 3-5 independent replicates [8].



Spring 1 – coordinates: 43°6'47.79" north, 76°54'49.70" east, near the river Prohodnaya, bus stop number 28; spring 2 – coordinates: 43°5'45.13" north, 76°57'51.65" east, on the road to the Big Almaty Lake, in front of the bridge over the river Ulken Almaty; spring 3 – coordinates: 43°9'33.96" north, 77°2'8.95" east, near the river Batareika on the road to Kok-Zhailau; spring 4 – coordinates: 43°9'43.79" north, 77°2'27.47" east, at the Prosveshchenets stop on the road to Medeu

Figure 1 - The location of the sampling points of water samples of the springs of Almaty

Bulbs of onions (Allium cepa L.) were used to determine phytotoxicity. Standard mutagen (positive control) was methyl methanesulfonate (MMS) at a concentration of 10.0 mg/L [14]. Negative control was distilled water containing residual amounts of nitric acid. Bulbs of equal size (4 cm in diameter) were cleaned of tunics, weighed, and placed in filtered water samples so that the bottom came into contact with the test water (5 replicates). The experiment was carried out at room temperature, protecting the onion from direct sunlight. Phytotoxicity of water samples was determined by the growth of the root bundles of the bulb. On days 7 and 14, the roots' length of each bulb was measured, and after the end of the experiment, the bulb and its root part were weighed [15].

The test object for determining the cytotoxicity and mutagenicity of water was used seeds of spring two-row barley (*Hordeum vulgare* L.) variety Baiseshek. Standard mutagen (positive control) was methyl methanesulfonate (MMS) at a concentration of 5.0 mg/L. Negative control was distilled water containing residual amounts of nitric acid. Treatment with each water sample was carried out for 4 hours. Germination and fixation were carried out according to the generally accepted method [16]. To determine

the mutagenic activity of the studied samples, we used chromosomal aberration assay (metaphase method) [16]. Staining was carried out with *fuchsin*-sulfurous acid. Cytological preparations were analyzed on an Olympus BX 43F series microscope (Olympus, Japan).

Data were evaluated using The Analysis ToolPak add-in of Excel, StatPlus, and WINPIPI. In all cases, mean values and standard errors of the mean were determined. The significance of differences in means was evaluated using the Student's test. The differences were considered significant, with a confidence level of 0.95 (p <0.05).

Results and Discussion

Physicochemical analysis of the composition of water samples

Table 1 presents the results of the physicochemical parameters of the studied spring waters. In the studied springs, the temperature values ranged from 2.8 to 9.8°C, and the pH values in the water samples were 5.8-6.4 so that waters are slightly acidic. The redox potential (ORP) in the studied waters fluctuated mainly in the range of 153-167 mv.

Table 1 - Physicochemical parameters of water samples from the springs of Almaty

_	Water samples					
Parameters	Spring 1	Spring 2	Spring 3	Spring 4		
Temperature	9.8	4.2	4.4	2.8		
pН	5.8	6.4	6.5	6.0		
ORP (mv)	155.0	153.0	158.0	167.0		
TDS (ppm)	365.0	45.0	181.0	191.0		
EC (μS/cm)	580.0	90.0	362.0	398.0		
DO (mg/L)	2.6	2.3	2.8	2.7		
Ni (mg/L)	0.0026±0.0002	0.0009±0.0001	0.0030±0.0001	0.0029±0.0001		
Mn (mg/L)	0.0095± 0.0003	0.0096± 0.0001	0.0094± 0.0001	0.0067± 0.000		
Co (mg/L)	0.0018± 0.0002	0.0014± 0.0001	0.0005± 0.0001	0.0004± 0.0000		
Pb (mg/L)	0.0096± 0.0002	0.0050± 0.0001	0.0078± 0.0002	0.0106± 0.0003		
Cr (mg/L)	0.0093± 0.0001	0.0037± 0.0001	0.0104± 0.003	0.0077± 0.0002		
Fe (mg/L)	0.0106± 0.0003	0.0040± 0.0001	0.0034± 0.0001	0.0125± 0.000		
Zn (mg/L)	0.0083± 0.0003	0.0025± 0.0002	0.0065± 0.0001	0.0257± 0.000		
Cu (mg/L)	0.0006± 0.0000	0.0004± 0.0001	0.0006± 0.0001	0.0007± 0.000		
Cd (mg/L)	0.0009± 0.0001	0.0004± 0.0001	0.0006± 0.0001	0.0007± 0.000		

The total salinity (ppm) of water varied in the range of 45-365. The electrical conductivity (μS/cm) of water from source Spring 1 was 580, Spring 2 – 90, Spring 3 – 362 and Spring 4 – 398. The increase in TDS and EC in water samples can be associated with a high concentration of dissolved mineral salts and ions: Na⁺, K⁺, Ca²⁺, Cl⁻, SO₄²⁻, HCO₃⁻. The dissolved oxygen content (mg/L) ranged from 2.3 to 2.8.

The content of the following heavy metals was determined in water samples: nickel, manganese, cobalt, lead, chromium, iron, zinc, copper, cadmium. According to the Unified system for classifying water quality in water bodies, springs 1, 3, 4 can be attributed to the 2nd class of water quality according to the lead content in water [17]. For other metals, water quality can classify as class 1, which is the best quality.

The results obtained indicate that, according to the content of heavy metals, the water from spring 2 is of the best quality and is suitable for all types of water use: for drinking water, fishery purposes, domestical use. Water from spring 1, 3, and 4 belongs to quality class 2. The waters of this class of water use are suitable for all categories of water use, except for domestic. For domestic use, simple water treatment methods are required.

The study of the mutagenicity of water samples using a bioluminescent test

The spring water samples were studied to detect responses to DNA-tropic agents and oxidative stress using a bioluminescent test (luxbiosensors). The genotoxic activity of water samples was studied on strains of E. coli MG1655 (pRecA-lux) and E. coli MG1655 (pColD-lux), and the prooxidant activity was studied on strains of E. coli MG 1655 (pSoxS-lux) and E. coli MG1655 (pKatG-lux). The pRecA-lux and pColD-lux biosensors respond by increasing the level of bioluminescence to DNA damaging substances, the pKatG-lux biosensor by the oxidative stress caused by the appearance of hydrogen peroxide, and the pSoxS-lux biosensor by the appearance of a superoxide anion.

When using the RecA biosensor, no increase in the bioluminescent response was recorded in the studied waters. Moreover, in samples of springs 1 and 2, high values of the toxicity index were noted, amounting to 19.73% (p <0.05) and 21.03% (p <0.01), respectively (Table 2). Perhaps this is due

to the high toxicity of the samples, which inhibits the growth of the culture and reduces the level of bioluminescence.

When using the CoID biosensor, a similar pattern was observed with low values of the induction factor with simultaneous high values of toxicity (Table 2). Water from all the studied springs was toxic (toxicity index was 21.4-26.7%). Moreover, a statistically significant decrease in bioluminescence was observed in water samples of springs 1-3 (p <0.05).

When using biosensors KatG and SoxS, a decrease in the bioluminescent response was also observed; however, these indicators were not statistically significant (Table 2).

In this series of experiments, the bioluminescent test did not reveal DNA tropic or oxidative stress effects in any of the water samples. However, the revealed toxicity in spring water may have led to the death of microorganisms, which led to a significant decrease in the bioluminescent response of RecA and ColD biosensors.

The study of phytotoxic and mutagenic activity of spring water using plant test systems

The water phytotoxicity was evaluated by measuring the root's length of onion (*Allium cepa*) when germinating it on various water samples. An effect is phytotoxic if not only the inhibitory but also the eutrophic (stimulating) effect is detected.

The average length of the onion root system in the negative control (distilled water) at 7 and 14 days of germination was 2.83 cm and 5.14 cm, respectively. The average length of the onion root system in the positive control (MMS; 10.0 mg/L) on days 7 and 14 of germination was 1.50 cm and 2.49 cm, respectively. The inhibitory effect of MMS was 46.85% and 51.66%. When germinating bulbs in spring water, an inhibitory effect was not detected, but a stimulation effect was observed. After seven days of onion germination, the stimulating effect was exerted by water samples from spring 2 (181.99%), and after 14 days by water samples from spring 1 (155.43%), spring 3 (187.31%) and spring 4 (173.90%).

On the roots of onions, germinated in the water of springs 1 and 3, on day 14, such a morphological change as branching was observed (Figure 2). Some authors have found that increasing doses of mutagenic substances in plants cause primarily morphological changes in the roots [15].

Table 2 - Effect of water samples on the luminescence of bacteria of strains E. coli MG1655 (pRecA-lux) and E. coli MG1655 (pColD-lux) (genotoxic activity), E. coli MG1655 (pKatG-lux) and E. coli MG 1655 (pSoxS-lux) (prooxidant activity)

			T				
xS-hα)	toxici-ty index, %	,	•	11.01	13.05	10.81	11.03
E. coli MG 1655 (pSoxS-lux)	induction factor		6.93	0.89	0.87	68.0	0.89
E. coli N	lumine- scence, RLU	5316.81± 317.61	36830.14± 14221.39***	4731.38± 1285.16	4623.20± 1541.93	4742.25± 501.50	4730.50± 237.50
tG-lux)	toxici-ty index, %		•	2.09	7.94	13.18	10.87
E. coli MG1655 (pKatG-lux)	induction factor		30.88	86.0	0.92	0.87	68.0
E. coli]	lumine- scence, RLU	1679.23±	51858.93± 3762.52***	1644.15± 86.59	1545.95± 99.40	1457.82± 255.52	1496.64± 251.19
ID-lux)	toxici-ty index, %	•	-	26.70	21.40	25.69	24.21
E. coli MG1655 (pColD-lux)	induction factor	•	37.75	0.73	0.79	0.74	0.76
E. coli l	lumine- scence, RLU	533.84 ± 38.35	20151.74 ± 4152.37***	391.29 ± 40.27*	419.63 ± 36.00*	396.69 ± 42.06*	404.59 ± 57.10
cA-lux)	toxici-ty index, %	•		19.73	21.03	10.37	6:36
E. coli MG1655 (pRecA-lux)	induction	•	5.66	0.80	0.79	06:0	0.91
E. coli I	lumine- scence, RLU	19742.37 ± 967.28	111819.60 ± 4601.19***	15847.13 ± 965.23*	15591,25± 746.46**	17695.00± 2656.14	17887.88± 2704.90
	Water	Distilled	Positive control*	Spring 1	Spring 2	Spring 3	Spring 4

Note: RLU - relative light units;
- Positive control for the pCoD and pRecA promoters is 4-nitroquinoline 1-oxide, 75.0 µg/mL; for PkatG promoter - hydrogen peroxide, 0.01 µg/mL; for PSoxS promoter - paraquat,

* - p<0,05; ** - p<0,01; *** - p<0,001 in comparison with distilled water



Figure 2 – Branching on the roots of onions, germinated in the water of the spring 3

The results obtained in this series of experiments indicate the toxicity of water from all the springs and the possible mutagenic activity of water from springs 1 and 3.

We also studied the mutagenic activity of spring water in chromosomal aberration assay. Table 3 presents the results of the cytogenetic analysis of barley seeds treated with water from the studied springs.

Table 3 - Frequency and spectrum of structural chromosome abnormalities induced by spring water in barley seeds

Experimantal variant	1. Total studied cells	The aberrant cell frequency (M ± m%)	The number of chromosomal aberrations per 100 metaphase cells			
			2. total aberrations	1. chromosome type	3. chromatid type	
Distilled water	490	1.63 ±0.57	2.04 ±0.64	0.82 ± 0.41	$1.22 \pm 0,50$	
MMS, 5.0 mg/L	530	5.66 ± 1.00***	6.98 ± 1.11***	2.83± 0.72*	4.15 ± 0.87**	
Spring 1	487	2.05±0.64	2.26±0.67	1.44±0.54	0.82±0.41	
Spring 2	450	1.78±0.62	2.67±0.76	1.56±0.58	1.11±0.49	
Spring 3	529	4.54±0.90**	7.75±1.16***	4.35±0.89***	3.40±0.79*	
Spring 4	448	1.79±0.63	2.46±0.73	1.34±0.54	1.12±0.50	

Cytogenetic analysis showed that water from spring 3 has mutagenic activity and induces structural chromosome abnormalities with a frequency that is statistically significantly higher than the spontaneous mutation level. An excess of the frequency of aberrant cells and the number of chromosome aberrations per 100 metaphases were observed. The frequency of aberrant cells was 2.8 (p <0.01) times higher than the control level, and the number of chromosomal aberrations per 100 metaphases was 3.8 (p < 0.001) times. The mutagenicity of water from spring 3 was at the level of positive control (MMS). MMS, being a classical mutagen, statistically significantly increased the frequency of aberrant cells and the number of chromosome aberrations per 100 metaphases in the cells of the root meristem of barley seeds compared to the negative control (distilled water) in 3.5 (p < 0.001) and 3.4 (p < 0.001) times, respectively. A comparative analysis of cytogenetic parameters in variants with MMS and spring water, which showed genotoxic activity, did not reveal statistically significant differences. It indicates the presence of mutagenic factors in spring water that induce structural mutations. The water of springs 1, 2, 4 have not mutagenic activity.

The spectrum of structural mutations induced by water with mutagenic activity was aberrations of both chromosomal and chromatid types. Aberrations of the chromosome type were represented mainly by paired terminal and interstitial deletions. Among The chromatid-type aberration were represented single terminal deletions, single acentric rings, and microfragments. The revealed spectrum of chromosomal aberrations indicates the presence of mutagenic factors of a wide range of activities in the spring water 3.

It is important to note that in almost all variants of the experiment where seed germination was carried out on samples of spring water, anaphases with structural chromosomal abnormalities were observed: bridges, chromosome lag, single and paired fragments, multipolar anaphases that were absent in the negative control (distilled water). Besides, polyploid cells were identified in variants where seed germination was carried out on the water from springs 1 and 3.

Conclusion

With the increase in the number and rate of emission of environmentally hazardous factors into the environment, among which various chemicals with mutagenic and genotoxic activity, ecological and genetic monitoring of the environment is of particular the relevance and the importance [1-3, 18]. Identification of potential mutagens and carcinogens for humans is the main task of testing. In the late 60s and early 70s, the methods for studying mutagenic and carcinogenic activity did not overlap and were based on different principles. The priority tasks were to control environmental and to develop scientific areas that would allow a comprehensive assessment of the impact of these processes on humans.

The test for the mutagenicity of various pollutants at model organisms in the laboratory aims at finding opportunities to reduce human contact with mutagenic factors and prevent adverse genetic consequences if introduced into the environment. However, none of them individually allows us to obtain data based on which it would be possible to conclude the degree of genetic danger of the test substance to humans. The specificity of the biological object on which the effect of mutagen is studied, as well as the specificity of mutational events, dictate the need for several biological models and testing methods [3].

Natural aquatic ecosystems, being the habitat of many organisms, a source of drinking water and a resource for human economic activity, are currently subjected to powerful anthropogenic pressures. In this regard, the conduct of toxicological and genotoxicological studies of natural waters exposed to environmental pollutants, especially in areas of human residence, is critical.

Using various physicochemical and biological methods of analysis, test systems, and test objects, we studied the physicochemical parameters of spring water, their toxic, genotoxic, and mutagenic activities.

An analysis of the physicochemical parameters of the studied spring waters showed that they are slightly acidic (pH 5,8-6,5). For drinking water according to the standards, the pH should be 6.5-8.5 [17]. Lower pH values in the waters are probably due to the presence of weak organic acids and weak base cations. The total dissolved solids (ppm) of the water varied in the range of 45-365, and the electrical conductivity was 90-580 μS/cm. An increase in the values of total dissolved solids and electrical conductivity in water samples can be associated with a high concentration of dissolved mineral salts and ions: Na⁺, K⁺, Ca²⁺, Cl⁻, SO₄²⁻, HCO₃⁻.

Following the established MPC for drinking water, spring water 2 is suitable as a source of

drinking water supply, water from other springs can use for drinking purposes only after simple water treatment methods.

The studied spring waters have no DNA-tropic and oxidative effects using the bioluminescent test. However, toxicity was established for it, which caused a significant decrease in the bioluminescent response of RecA and ColD biosensors.

On plant test systems, the phytotoxic activity of all the studied springs was established, and the mutagenic activity for spring 1 and 3. It indicates the presence of toxic and mutagenic factors in the water of these sources.

Natural waters prone to anthropogenic pollution, as a rule, may contain a complex mixture of chemicals, the physical and chemical analysis of which may not always give accurate knowledge about the composition and concentrations of various components. Besides, individual chemicals may be in deficient concentrations and not detected by analytical methods. However, the combined action of the chemicals presents even at low concentrations can produce a variety of adverse effects on the body [19-23].

It is essential to evaluate the genotoxicity of contaminated water as a whole, and not of each component [23], since biological effects can be caused mainly by the effects of a mixture of pollutants. It is the simultaneous use of a battery of test objects and test systems (microorganisms E. coli; plants A. cepa, H. vulgare) that can assess the potential risk of water pollution for various forms of organisms.

A comparative analysis of the level of pollution of the studied spring water with heavy metals, the level of toxic and mutagenic activity allows ranking the studied water objects in the following order: spring 3> spring 1> spring 2> spring 4.

Thus, the results of studies of the toxic, genotoxic, and mutagenic potentials of the water of 4 springs on the territory near Almaty, obtained at various test systems and test objects, testify to their pollution by environmentally dangerous factors that pose a threat to biota and human health. At present, studies are being conducted on the effect of water from the studied springs on laboratory mammals (rodents) to extrapolate the data obtained to humans.

Conflict of interest

All authors have read and are familiar with the article's contents, and have no conflict of interest.

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Abbreviations

TDS – Total Dissolved Solids, ORP – oxidationreduction potential, DO – dissolved oxygen, EC – electrical conductance, 4-NQO – 4-nitroquinoline 1-oxide, MMS – methyl methanesulfonate, RLU – relative light units, IT – toxicity index, MPC – Maximum permissible concentration

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