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COMPLEX STUDY OF POTENTIAL TOXICITY AND GENOTOXICITY OF WATER SAMPLES FROM NATURAL SOURCES OF THE SUBURBAN ZONE OF ALMATY

© A.V. Lovinskaya [1], S.Z. Kolumbayeva [1], M.A. Suvorova [1], A.I. Iliyassova [1], Z.M. Biyasheva [1], S.K. Abilev [2, 3]

[1] al-Farabi Kazakh National University, Almaty, Kazakhstan;
[2] Vavilov Institute of General Genetics, Moscow, Russia;
[3] Lomonosov Moscow State University, Moscow, Russia

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❀ **Background.** Natural aquatic ecosystems are the habitat of many organisms, a source of drinking water, a resource for human activities and are subjected to anthropogenic pressure. In this regard, interest in studying the genotoxicity and mutagenicity of surface waters has increased significantly. The aim of this study is to investigate the cytotoxic, genotoxic and mutagenic effects of the surface waters of the suburban area of Almaty. **Material and methods.** The research materials were water samples of the rivers Esik, Turgen and Lake Esik. The atomic absorption method, lux-test, cytogenetic tests (*Hordeum vulgare L.*), phytotoxicity test (*Allium cepa L.*) and embryotoxicity (*Danio rerio H.*) were used. **Results.** Physico-chemical water analysis revealed an excess of MPC for Mn, Pb, Cd, Zn. Using the lux-test on *E. coli* KatG strains, the pro-oxidant activity of Esik R. water. On the plant test objects revealed toxicity and mutagenicity of water samples. The results of bio-testing of natural waters with *D. rerio* revealed their high toxicity and teratogenicity for embryos at all stages of development. **Conclusion.** The results of this study obtained on various test-systems and test-objects indicate that surface waters are contaminated by environmentally dangerous factors that pose a threat to biota and human health.

❀ **Keywords:** surface water; heavy metals; cytogenetic analysis; mitotic index; chromosomal aberrations; embryotoxicity.

КОМПЛЕКСНОЕ ИССЛЕДОВАНИЕ ПОТЕНЦИАЛЬНОЙ ТОКСИЧНОСТИ И ГЕНОТОКСИЧНОСТИ ОБРАЗЦОВ ВОДЫ ИЗ ПРИРОДНЫХ ИСТОЧНИКОВ ПРИГОРОДНОЙ ЗОНЫ г. АЛМАТЫ

© А.В. Ловинская [1], С.Ж. Колумбаева [1], М.А. Суворова [1], А.И. Илиясова [1], З.М. Бияшева [1], С.К. Абилев [2, 3]

[1] Казахский национальный университет им. аль-Фараби, Алматы, Казахстан;
[2] ФГБУН «Институт общей генетики им. Н.И. Вавилова» РАН, Москва;
[3] ФГБОУ ВО «Московский государственный университет им. М.В. Ломоносова», Москва

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❀ Проведен скрининг образцов природных поверхностных вод из трех водных объектов (рр. Есик, Тургень, оз. Есик) в Енбекшиказахском районе Алматинской области. В результате физико-химического анализа состава образцов воды было обнаружено превышение предельно допустимой концентрации по марганцу, свинцу, кадмию, цинку. С помощью биолюминесцентного теста на штамме *E. coli* установлена прооксидантная активность воды для р. Есик. На растительных тест-объектах *Allium cepa* и *Hordeum vulgare* выявлена токсичность и мутагенность изученных образцов воды. Наблюдалась фитотоксическая, цитотоксическая (снижение митотического индекса) и мутагенная (статистически значимое превышение частоты аберраций хромосом) активность изученных водных объектов. Результаты биотестирования природных вод с помощью *Danio rerio* показали их высокую токсичность и тератогенность для эмбрионов на всех стадиях развития.

❀ **Ключевые слова:** поверхностные воды; тяжелые металлы; цитогенетический анализ; митотический индекс; аберрации хромосом; эмбриотоксичность.

INTRODUCTION

Natural aquatic ecosystems, which are natural habitats, sources of drinking water, and a location for human activities, are currently subject to powerful anthropogenic pressures. Recent interest in studying genotoxicity and mutagenicity of surface water has grown in many countries [1–6]. A number of studies have assessed the mutagenic potential of both natural and drinking water, particularly that of chlorinated tap water [5, 6].

Monitoring water resources is essential for the protection and rational use of nature, and assessment of water genotoxicity and mutagenicity is an important part of evaluating water sources. However, the genotoxicity dynamics in rivers and water reservoirs currently affected by contaminants are poorly understood and methodologies for the assessment of water genotoxicity have not been fully developed. Nevertheless, genotoxicity studies of water are important and required for public health and safety. Water genotoxicity studies in Kazakhstan have not been conducted previously and to our knowledge, are not currently underway. Only genotoxicity analyses of drinking water from regional centers are presently available in the scientific literature. In those studies, correlations were found between genotoxicity of the drinking water and the observed frequencies of congenital abnormalities and malignant cancers in the local population [7].

Along with physical and chemical analyses of water, studying the biological effects of harmful agents in water, including synergetic and antagonistic effects, is required. Combining these different approaches can help determine the contribution of specific contaminants to the total observed biological effects [2]. Simultaneous use of standard physicochemical analyses and analyses of mutagenicity/genotoxicity will be implemented in the programs of the water quality monitoring [2, 3].

It is important to assess the genotoxicity of water as a whole, rather than of individual components [4] because, as noted above, a mixture of contaminants can often determine biological effects. The simultaneous use of a bank of test objects and test systems can allow assessment of the risk of water contamination to different organisms.

In light of these points, the goal of this research was to study the cytotoxic, genotoxic, and mutagenic effects of surface water from the suburban areas surrounding Almaty.

MATERIALS AND METHODS

Samples assessed in the study included surface water from ten locations: the River Esik (p. No. 1: No. 1-1, 1-2, 1-3), Lake Esik (p. No. 2: No. 2-1, 2-2, 2-3, 2-4), and the River Turgen (p. No. 3: No. 3-1, 3-2, 3-3) (Fig. 1). Tests organisms included biosensor strains of *Escherichia coli* [MG 1655 (pSoxS-*lux*), MG1655 (pKatG-*lux*), and MG1655 (pColD-*lux*)], the common onion (*Allium cepa* L.), grade Baisheshek barley (*Hordeum vulgare* L.), and zebra fish (*Danio rerio* H.). As positive controls, 4-nitroquinoline 1-oxide (4-NQO, $C_9H_6N_2O_3$) and methyl-methane sulphonate (MMS, $C_2H_6O_3S$) were used as mutagens, while paraquat (1.1'-dimethyl-4.4–dipyridyl dichloride; $C_{12}H_{14}Cl_2N_2$) and hydrogen peroxide (H_2O_2) were used as oxidants.

Determination of physical and chemical properties. Sampling, filtration, and preservation of water samples complied with recommendations from GOST 31861-2012 [8]. The following physical properties were measured at the points of sampling: pH, total mineralization (Total Dissolved Solids), Oxidation-reduction potential (ORP), and water-dissolved oxygen. Total mineralization was measured using a portable analyzer TDS & EC-meter (Barry Century, China), pH was measured with a portable pH meter PH-009(I) (Barry Century, China), ORP was measured with a portable ORP meter ORP169E (Barry Century, China), and the level of dissolved oxygen was measured with a portable DO meter of DO-pen type (Alvin Instrument, China).

Heavy metal content was determined by atomic absorption using an atomic absorption spectrophotometer MGA-915MD (Lumex, Russia) according to PND F 14.1:2.214-06 [9].

Determination of genotoxic and oxidative activities using bioluminescence tests. Four genetically modified strains of *E. coli* were used in this study: MG 1655 (pSoxS-*lux*), MG1655 (pKatG-*lux*), MG1655 pColD-*lux*, and MG1655 (pRecA-lux) [10, 11]. The promoters pCoD and pRecA were used to detect substances that induce DNA damage. 4-NQO at a concentration of 75.0 μg/mL was used as a positive control to activate these promoters. Induction of oxidative stress was detected using the PKatG (protein-activator OxyR) and PSoxS (protein-activator SoxR) promoters. PKatG produces a response to hydrogen peroxide and organic peroxides, while PSoxS responds to superoxide ion radicals [11,12]. Hydrogen peroxide at a concentration of 0.01 μg/mL was used as a positive control to activate KatG and 10.0 μg/mL paraquat was used to activate PSoxS. Distilled water was used as a negative control. Bacteria were grown in Luria-Bertani broth containing 100 μg/mL of ampicillin. Overnight cultures were diluted to a concentration of 10^7 kl/mL in fresh broth and grown at 37 °C for 2–3 h. Aliquots of the culture (160 μL) were transferred to sterile wells (in plate strips) and 40 μL of each water sample was added. 40.0 μL of distilled water or control mutagen/oxidant was added to control cells. Incubations were performed for specific durations depending on the promoter: pColD for 90 min, pRecA and pSoxS for 60 min, and pKatG for 45 min. The level of bacterial luminescence was measured with a Lu-

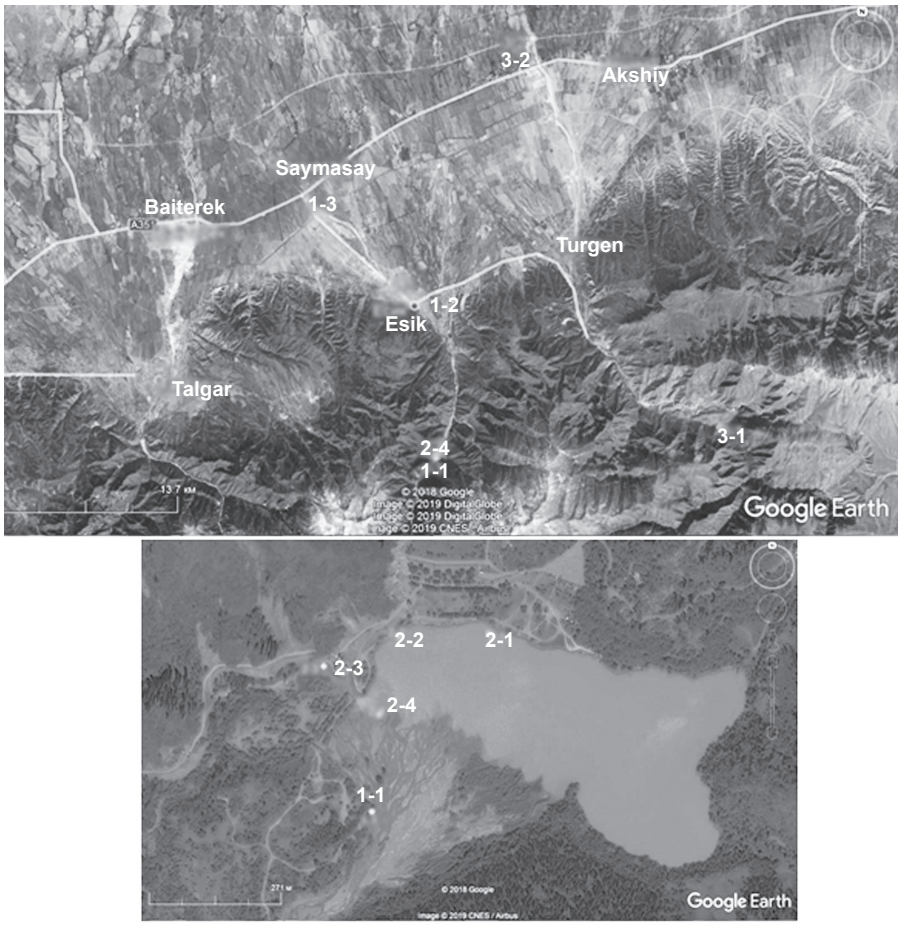


Fig. 1. The location of water sampling points in the suburban area of Almaty: No. 1 — river Esik (p. No. 1-1, 1-2, 1-3); No. 2 — Lake Esik (p. No. 2-1, 2-2, 2-3, 2-4); No. 3 — river Turgen (p. No. 3-1, 3-2)

Mate 4400 microplate luminimeter (Awareness Technology, USA) and expressed as relative light units (RLU). The level of genotoxicity was defined as the induction factor (I), the ratio of luminescence from the lux-biosensor suspension with the tested compound (L_c), to the luminescence of the control suspension (L_k). $I < 2$ was considered a weak genetoxic effect, while, $2 \leq I \leq 10$ was defined as moderate and $I > 10$ was defined as a strong effect [12].

Determination of water phytotoxicity. Common onion (*Allium cepa* L.) was used as the test object for the phytotoxicity tests. MMS was used as the standard mutagen at a concentration of 10.0 mg/L [13], while distilled water containing a residual quantity of nitric acid was used as the negative control. Onions of almost equal size (4 cm in diameter) were placed in the experimental and control water samples (5 replicates) and grown at room temperature. Phytotoxicity was assessed by the growth of the rooting tufts after 7 and 14 days [14].

Determination cytotoxicity and mutagenicity. Seeds of the spring-crop two-raw barley (*Hordeum vulgare L.*) of grade Baisheshek were used as the test object for these experiments. MMS was used as the standard mutagen at a concentration of 5.0 mg/L, while distilled water containing residual nitric acid was used as the negative control. Treatment was conducted for 4 h before seeds were grown and fixed in an alcohol-vinegar mixture. Metaphase and ana-telophase methods were used to determine the mutagenicity of the samples and mitotic index was used to determine cytotoxicity [15]. Staining was done with fuchsin-sulfuric acid. Cytological preparations were analyzed with an Olympus BX 43F microscope (Olympus, Japan).

Determination of embryotoxicity using *D. rerio*. These experiments were conducted using five water samples from three locations: River Esik (p. No. 1-2, 1-3), Lake Esik (p. No. 2-2, 2-3), and River Turgen (p. No. 3-2). Experiments were started with stage 50%–75% of epiboly. Embryo exposition was conducted in sterile Petri dishes with 25 mL of incubation medium for 72 h at 27 °C in a Binder B28 incubator (Binder, Germany). Embryos that survived after hatching of the chorion were fixed in 10% neutral buffered formalin. Embryos were analyzed at 24, 48, and 72 h post fertilization (hpf) *in vitro*. Stages of

development were determined according to the atlas of *D. rerio* normal embryonic development [16]. Alive and fixed embryos were analyzed and photographed using a MoticDM 143 digital stereo microscope. Acute embryotoxic effects of the water samples were assessed according to recommendations of OECD [17]. Morphological analyses of embryos were performed at 72 hpf. Damage to more than 50% of embryos in the exposure group was considered typical. Deviation from normal development of *D. rerio* was assessed by malformation of development of head, tail, tail tip, otic capsules, pericardial oedema, body bending, deformation of yolk, and growth retardation [18].

Statistical analyses of results. All experiments were conducted in triplicate. Statistical processing was conducted using the "Data analysis" add-in of Microsoft Excel, StatPlus, and WINPIPI. In all cases the average values and the errors of means were calculated. Mean statistical significance was assessed using Student's t-test. Differences were considered significant at the level of confidence 0.95 ($p < 0.05$).

RESULTS

Physicochemical analyses of water samples

Measurements of the water samples' physical parameters demonstrated that the sources could be characterized as faintly acidic, transitional redox (rivers Esik and Turgen), or oxidating (lake Esik) (Table 1).

The heavy metal content (see Table 1) of the samples was also examined. It was determined that the levels of Ni, Co, Cr, Fe, Cu content did not exceed maximum permissible concentrations (MPCs) in any samples. Level of Cd did not exceed the MPC in samples from the Rivers Esik and Turgen. However, Mn levels did exceed the MPC, by factors of 1.5, 2.9, and 7.0, in water samples from the River Esik, Lake Esik, and the River Turgen, respectively. Pb levels exceeded the MPC by 1.3 and 1.4 times in

Table 1

Physical parameters and chemical analysis of water samples from water bodies near Almaty

Physical and chemical parameters	River Esik			Lake Esik				River Turgen	
	No. 1–1	No. 1–2	No. 1–3	No. 2–1	No. 2–2	No. 2–3	No. 2–4	No. 3–1	No. 3–2
pH	5.5	5.0	6.5	5.0	5.0	6.0	5.5	5.6	5.7
ORP, mv	93.0	96.0	80.0	101.0	110.0	119.0	98.0	89.0	79.0
Total Dissolved Solids, ppm	91	85	79	132	130	129	105	111	87
Dissolved oxygen, mg/L	7.9	5.7	2.7	5.8	5.5	5.7	5.7	3.2	5.4
Ni	0.0071 ± ± 0.0004	0.0024 ± ± 0.0002	0.0014 ± ± 0.0003	0.0003 ± ± 0.0001	0.0006 ± ± 0.0001	0.0012 ± ± 0.0002	0.0020 ± ± 0.0004	0.0008 ± ± 0.0000	0.0014 ± ± 0.0001
Mn	0.0096 ± ± 0.0002	0.0132 ± ± 0.0002*	0.0152 ± ± 0.0003*	0.0195 ± ± 0.0013*	0.0188 ± ± 0.0002*	0.0296 ± ± 0.0003*	0.0187 ± ± 0.0002	0.0728± 0.0002*	0.0336 ± ± 0.0003*
Co	0.0017 ± ± 0.0002	0.0020 ± ± 0.0002	0.0035 ± ± 0.0003	0.0037 ± ± 0.0001	0.0015 ± ± 0.0002	0.0021 ± ± 0.0002	0.0019 ± ± 0.0001	0.0030 ± ± 0.0002	0.0022 ± ± 0.0001
Pb	0.0040 ± ± 0.0002	0.0059 ± ± 0.0002	0.0084 ± ± 0.0001*	0.0025 ± ± 0.0002	0.0047 ± ± 0.0001	0.0069 ± ± 0.0003*	0.0055 ± ± 0.0001	0.0060 ± ± 0.0003*	0.0064 ± ± 0.0001*
Cr	0.0034 ± ± 0.0002	0.0082 ± ± 0.0002	0.0034 ± ± 0.0002	0.0026 ± ± 0.0002	0.0021 ± ± 0.0001	0.0020 ± ± 0.0001	0.0030 ± ± 0.0001	0.0050 ± ± 0.0002	0.0045 ± ± 0.0002
Fe	0.0046 ± ± 0.0001	0.0338 ± ± 0.0002	0.0263 ± ± 0.0002	0.0064 ± ± 0.0001	0.0056 ± ± 0.0001	0.0058 ± ± 0.0001	0.0051 ± ± 0.0001	0.0134 ± ± 0.0001	0.0176 ± ± 0.0001
Zn	0.0102 ± ± 0.0001*	0.0350 ± ± 0.0001*	0.0371 ± ± 0.0003*	0.0058 ± ± 0.0001	0.0063 ± ± 0.0001	0.0106 ± ± 0.0001*	0.0070 ± ± 0.0001	0.0051 ± ± 0.0003	0.0153 ± ± 0.0002*
Cu	0.0003± 0.0001	0.0006 ± ± 0.0001	0.0006 ± ± 0.0001	0.0003 ± ± 0.0001	0.0009 ± ± 0.0001	0.0008 ± ± 0.0001	0.0006 ± ± 0.0001	0.0004 ± ± 0.0000	0.0007 ± ± 0.0001
Cd	0.0002 ± ± 0.0000	0.0006 ± ± 0.0001	0.0004 ± ± 0.0000	0.0003 ± ± 0.0001	0.0003 ± ± 0.0001	0.0004 ± ± 0.0000	0.0013 ± ± 0.0001•*◊	0.0004 ± ± 0.0001	0.0004 ± ± 0.0001

Note: ORP — Oxidation-reduction potential. • exceeds or at the level of MPC_{dw} for drinking water, * exceeds or at the level of MPC_{fr} for water of fishery reservoirs, ◊ exceeds or at the level of MPC_{dom} for water sources, domestic and drinking water supply, places of cultural and domestic water use.

samples from Lake Esik and the River Esik, respectively, while Pb levels in the water samples from the River Turgen were near the MPC level. Zn content exceeded the MPC by factors of 1.1, 1.5, and 3.7 in the Lake Esik, the River Turgen, and the River Esik, respectively. The Cd content in the water from Lake Esik exceeded the MPC by 1.3 times. These results indicate differential contamination of the natural waters with heavy metals, which could negatively impact living organisms.

Assessment of genotoxicity of water samples with lux-biosensors

Water samples were tested for sterility before determination of mutagenicity, and all were found to be sterile. In the bioluminescence tests, water samples were examined for detection of responses to DNA-tropic agents and oxidative stress. Genotoxic activity of water samples was studied using *E. coli* strains MG1655 (pRecA-*lux*) and MG1655 (pColD-*lux*), and pro-oxidant activity was studied using *E. coli* strains MG 1655 (pSoxS-*lux*) and MG1655 (pKatG-*lux*) (Table 2). Biosensors pRecA-*lux* and pColD-*lux* respond to DNA-damaging substances by increasing bioluminescence. Biosensor pKatG-*lux* responds by increasing bioluminescence in response to oxidative stress caused by hydrogen peroxide in the medium. Biosensor pSoxS-*lux* responds to the presence of the superoxide anion. When biosensor RecA is used, an increase in bioluminescence was detected with water samples of

Table 2

The effect of water samples on the luminescence of bacteria *E. coli* MG1655 (pRecA-*lux*) and *E. coli* MG1655 (pColD-*lux*) strains (genotoxic activity) and *E. coli* MG1655 (pKatG-*lux*) and *E. coli* MG 1655 (pSoxS-*lux*) strains (prooxidant activity)

Water samples	*E. coli* MG1655 (pRecA-*lux*)		*E. coli* MG1655 (pColD-*lux*)		*E. coli* MG1655 (pKatG-*lux*)		*E. coli* MG 1655 (pSoxS-*lux*)	
	Luminescence, RLU	Induction factor	Luminescence, RLU	Induction factor	Luminescence, RLU	Induction factor	Luminescence, RLU	Induction factor
Water	19742.37 ± ± 967.28		533.84 ± ± 38.35		1679.23 ± ± 92.70		5316.81 ± ± 317.61	
Positive control	111819.60 ± ± 4601.19***	5.66	20151.74 ± ± 4152.37***	37.75	51858.93 ± ± 3762.52***	30.88	36830.14 ± ± 14221.39***	6.93
River Esik								
No. 1-1	17066.21 ± ± 2552.58	0.86	507.71 ± ± 144.09	0.95	1375.04 ± ± 125.77	0.82	5917.72 ± ± 666.97	1.11
No. 1-2	16519.71 ± ± 2445.20	0.84	501.58 ± ± 143.12	0.94	1326.25 ± ± 111.47*	0.79	5973.96 ± ± 416.92	1.12
No. 1-3	22120.46 ± ± 1300.50	1.12	576.88 ± ± 30.89	1.08	2400.74 ± ± 258.44*	1.43	5621.46 ± ± 1365.51	1.06
Lake Esik								
No. 2-1	16893.17 ± ± 2576.22	0.86	506.54 ± ± 153.71	0.95	1362.17 ± ± 91.67*	0.81	6037.17 ± ± 393.31	1.14
No. 2-2	16838.58 ± ± 1903.63	0.85	515.58 ± ± 145.65	0.97	1344.96 ± ± 95.84*	0.80	5986.83 ± ± 586.98	1.13
No. 2-3	17529.54 ± ± 2500.47	0.89	513.58 ± ± 152.26	0.96	1442.04 ± ± 60.65*	0.86	6125.13 ± ± 350.72	1.15
No. 2-4	18830.58 ± ± 3713.55	0.95	538.00 ± ± 165.04	1.01	1526.44 ± ± 62.04	0.91	6364.58 ± ± 414.67	1.20
River Turgen								
No. 3-1	17612.63 ± ± 2710.57	0.89	535.08 ± ± 175.09	1.00	1511.00 ± ± 75.72	0.90	6131.58 ± ± 340.92	1.15
No. 3-2	17429.29 ± ± 2872.57	0.88	413.21 ± ± 55.74	0.77	1233.00 ± ± 197.92	0.73	5745.52 ± ± 205.59	1.08

Note: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ in comparison with distilled water; RLU — relative light units, relative light units; positive control: 4NQO (pRecA-*lux* and pColD-*lux*); hydrogen peroxide (pKatG-*lux*), paraquat (pSoxS-*lux*).

the River Esik (p. No. 1-3). However, statistically significant differences in the levels of bioluminescence were not detected relative to control. Low values of induction were detected for all other samples. Similar low levels of induction were observed when biosensor ColD was used.

A statistically significant increase in bioluminescence was detected from biosensor KatG in response to the sample from the River Esik taken at point No. 1-3, which indicates the presence of the substances causing oxidative stress. A statistically significant reduction in bioluminescence was detected in response to the water samples from the River Esik ($p < 0.05$) and Lake Esik ($p < 0.05$). Increases in bioluminescence were observed from biosensor SoxS in response to all tested water samples; however, these changes were not statistically significant in comparison to the control.

Assessment of toxicity and mutagenicity using plant tests objects

Phytotoxicity was assessed by measuring the root system length of the common onion (*A. cepa*) while growing on the different water samples. Effects were considered toxic if both inhibiting and eutrophing (stimulating) effects were detected. Fig. 2 shows that the positive control MMS statistically significantly inhibited root growth, with inhibition levels of 46.85% and 51.66% on day 7 and 14 of growing, respectively. After 7 days of onion growing, the inhibitory effects were assessed for water samples taken from the River Esik, p. No. 1-3; Lake Esik, p. No. 2-4; and the River Turgen. After 14 days of onion growing, the inhibitory effects were assessed for samples from Lake Esik, p. No. 2-2 and a stimulating effect was observed for sample p. No. 1-1 from the River Esik.

Cytogenetic analyses of barley seeds were conducted using metaphase and ana-telaphase methods following treatment with the experimental water samples. The metaphase method detected mutagenicity of water from Lake Esik, p. No. 2-1 (Table 3). A statistically significant increase in the number of chromosome aberrations per 100 metaphases ($p < 0.05$) was detected in the apical root meristems of barley. Experimental water mutagenicity was at the level of positive control (MMS). Rearrangements of chromosomal and chromatid types were observed in a spectrum of structural mutations (Fig. 3). Aberrations of chromosomal type were mostly paired-end and interstitial deletions, centric rings, and paired dotted fragments. Single-end (terminal) and interstitial deletions, single acentric rings, and dotted fragments were among the detected aberrations of chromatid type. The spectrum of detected chromosomes aberrations indicates the presence of a wide range of mutagenic factors in the water of Lake Esik, p. No. 2-1.

The ana-telophase method allowed detection of anaphases with bridges of high frequency, with chromosome lagging and single and paired fragments in the apical root meristem of barley seeds grown in water from the River Esik (p. No. 1-3), Lake Esik (p. No. 2-1, 2-3, 2-4), and the River Turgen (Table 4). Single multipolar mitosis, which was absent in the negative controls, occurred in all variants of the experiment. In addition, polyploid cells were detected when seeds were grown in water form the River Esik (p. No. 1-1, 1-2) and Lake Esik (p. No. 2-1) (see Fig. 3, Table 3).

Measurements of mitotic index showed significant reductions in proliferation for all cell treatments in comparison to negative control, except for p. No. 2-4 from Lake Esik and p. No. 3-1 from the River Turgen (see Table 4).

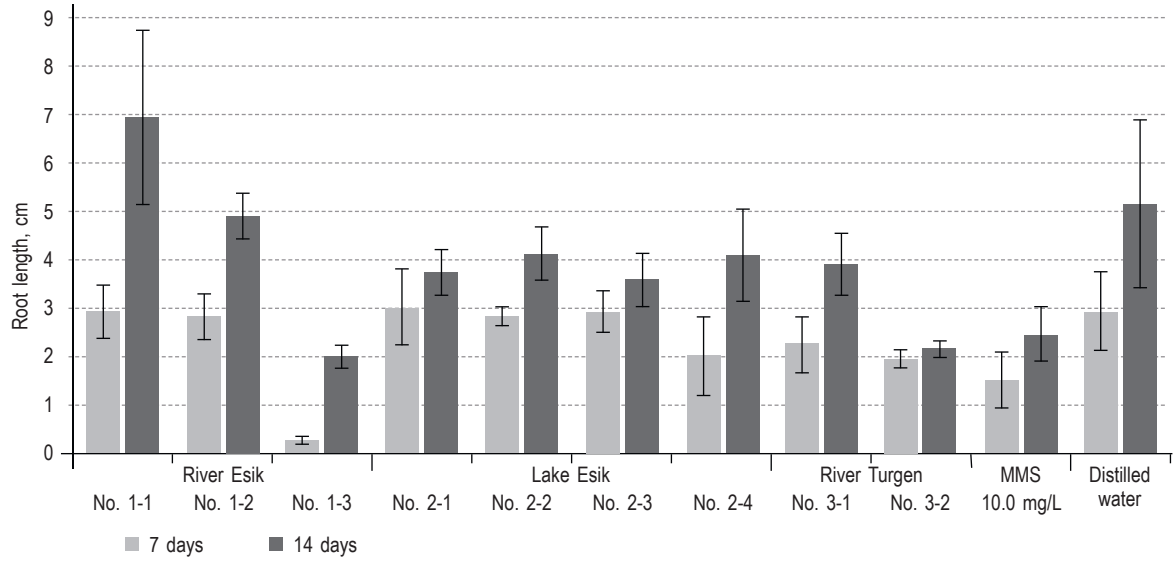


Fig. 2. The average length of the roots of *Allium cepa* exposed to water samples from water bodies near the city of Almaty for 7 and 14 days

Table 3

Frequency and spectrum of structural disorders of chromosomes induced by natural waters in barley seeds

Experimental variant	Total metaphase studied	Aberrant metaphase frequency ($M \pm m$, %)	The number of chromosomal aberrations per 100 metaphases			Polyploid cell frequency ($M \pm m$, %)
			total aberrations	chromosome-type	chromatid-type	
Water	490	1.63 ± 0.57	2.04 ± 0.64	0.82 ± 0.41	1.22 ± 0.50	0.56 ± 0.32
MMS, 5.0 mg/L	530	5.66 ± 1.00***	6.98 ± 1.11***	2.83 ± 0.72*	4.15 ± 0.87**	2.39 ± 0.66*
River Esik						
No. 1-1	456	1.97 ± 0.65	2.19 ± 0.69	1.32 ± 0.53	0.88 ± 0.44	1.72 ± 0.60
No. 1-2	487	2.46 ± 0.70	2.87 ± 0.76	1.64 ± 0.58	1.23 ± 0.50	1.02 ± 0.45
No. 1-3	490	2.04 ± 0.64	2.45 ± 0.70	1.63 ± 0.57	0.82 ± 0.41	–
Lake Esik						
No. 2-1	484	3.31 ± 0.81	4.34 ± 0.93*	2.89 ± 0.76*	1.45 ± 0.54	3.01 ± 0.76**
No. 2-2	485	2.47 ± 0.71	3.30 ± 0.81	1.86 ± 0.61	1.44 ± 0.54	–
No. 2-3	510	2.55 ± 0.70	3.14 ± 0.77	1.37 ± 0.52	1.76 ± 0.58	–
No. 2-4	510	1.57 ± 0.55	1.76 ± 0.58	0.98 ± 0.44	0.78 ± 0.39	–
River Turgen						
No. 3-1	507	1.78 ± 0.59	2.37 ± 0.68	1.58 ± 0.55	0.99 ± 0.44	–
No. 3-2	530	1.89 ± 0.59	2.45 ± 0.67	0.94 ± 0.42	1.51 ± 0.53	–

Note: MMS — methyl-methane sulphonate. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ in comparison with control.

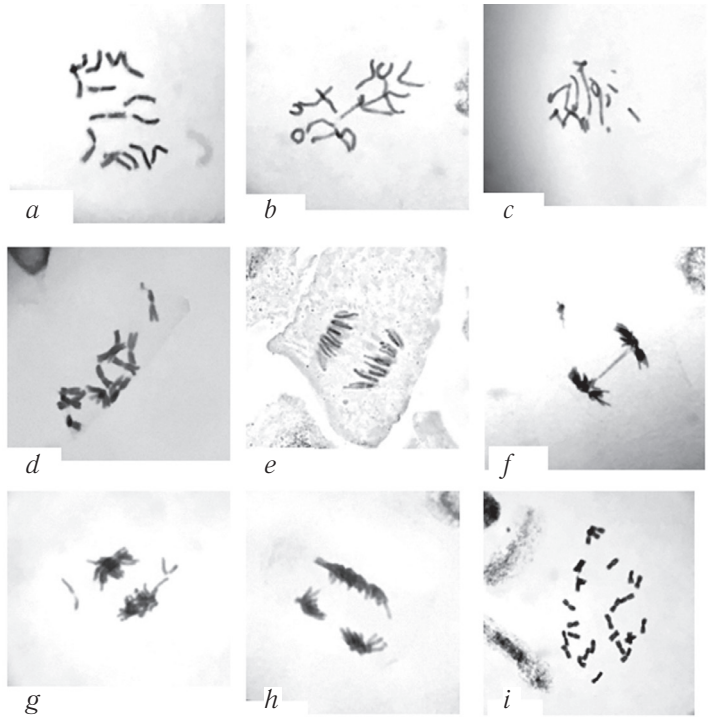


Fig. 3. Chromosome abnormalities induced by the waters of natural sources in barley seeds: *a* is the norm in metaphase ($2n = 14$); *b* — centric ring; *c* — acentric ring; *d* — chromatid terminal deletion; *e* — the norm in anaphase; *f* — bridge; *g* — lagging chromosomes; *h* — multipolar mitosis; *i* — polyploid set ($2n = 28$)

Table 4

Mitotic index and frequency of structural disorders of chromosomes at the anaphase stage, induced by natural waters in barley seeds

Experimental variant	Total cells studied	Mitotic index	Total anaphases studied	Frequency of anaphase with chromosomal aberrations
Water	4849	5.30 ± 0.14	540	1.38 ± 0.50
MMS, 5.0 mg/L	5185	2.40 ± 0.21***	511	4.57 ± 0.93**
River Esik				
No. 1-1	4710	4.82 ± 0.19*	567	1.98 ± 0.59
No. 1-2	4548	4.10 ± 0.25***	564	2.11 ± 0.61
No. 1-3	5915	4.40 ± 0.19***	592	3.09 ± 0.71*
Lake Esik				
No. 2-1	5487	3.92 ± 0.30***	589	3.19 ± 0.72*
No. 2-2	5645	4.90 ± 0.13*	557	2.14 ± 0.61
No. 2-3	4910	4.72 ± 0.19*	580	3.11 ± 0.72*
No. 2-4	5388	5.10 ± 0.25	585	3.10 ± 0.72*
River Turgen				
No. 3-1	5640	4.80 ± 0.21	591	3.09 ± 0.71*
No. 3-2	5225	3.90 ± 0.32***	583	3.11 ± 0.72*

Note: MMS — methyl-methane sulphonate. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ in comparison with control.

Assessment of embryotoxicity using *Danio rerio*

In many countries, the internationally standardized FET (Fish Embryo Toxicity) test is mandated in standard examinations of wastewater. Embryotoxicity results for more than 140 xenobiotics have confirmed the effectiveness of using fish embryos for the assessment of toxicity, suggesting that FET results can be extrapolated to mammals [19]. Acute toxicity of the water samples for Danio eggs was assessed by calculating the amount of coagulated embryos at 24, 48, and 72 hpf and was expressed as the percent of the sum of the dead embryos of the total number of embryos at the beginning of experiment (Table 5). Embryo mortality in the control was 2.67%, which met our criteria for validity. Eggs incubated in 3.4 mg/L MMS had a mortality rate of 33.3% ($p \leq 0.01$ in comparison to the control); however, mass coagulation occurred by 72 hpf as a result of serious development abnormalities.

Acute cytotoxicity of the water samples varied significantly, even within bodies of water. For example, the toxicities of the samples taken from different parts of Lake Esik were different; the water sample from p. No. 2-2 induced mortality of 24.0% ($p \leq 0.01$), while the sample from p. No. 2-3 resulted in 53.3% ($p \leq 0.01$) mortality, compared to negative control. Differences in responses to the River Esik samples were also observed. In p. No. 1-2 from the River Esik the total mortality was 16.0%, while in p. No. 1-3, it was 90.6%. In this option almost all embryos died after 72 hpf ($p \leq 0.001$ in comparison with negative control). Death of 25.3% of eggs ($p \leq 0.01$) was observed following treatment with water from the River Turgen (p. No. 3-2).

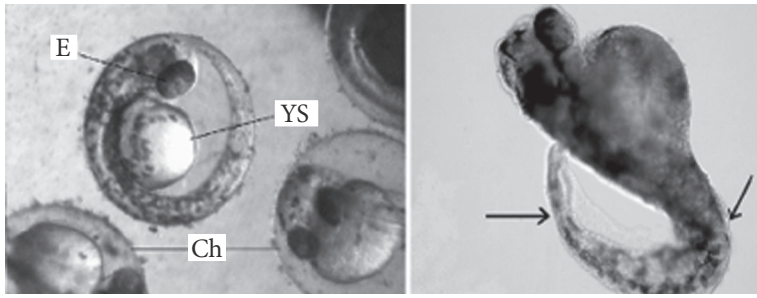
The teratogenic effects of the tested water samples was described as the percent of all *D. rerio* embryo with malformations in normal development or in the number of the living embryos to 72 hpf. During incubation of *D. rerio* in MMS, growth malformations occurred in 89.3 % of embryos ($p \leq 0.01$), which is significantly larger than control (Table 5). For all experimental samples, the fraction of embryos with abnormalities was significantly smaller in comparison to the positive control. However, water from p. No. 1-2 from the River Esik resulted in 18.7% malformation of the embryos, and 50.7% for p. No. 1-3, which were significantly exceeded the level of the negative control ($p \leq 0.05$).

Besides the fraction of embryos with abnormalities, specific malformations to the normal development of Danio embryos were taken into account, including scoliosis (Fig. 4), malformations of the tail part and tail tip, oedema of pericard and the yolk sac, and growth retardation (Table 5). The majority of embryos had malformations of different notochord parts. Malformations typical for substances with mutagenic/disruptive effects were noted, including Tail tip hypoplasia as resulting from malformations in normal cell reproduction and differentiation. Embryos with such abnomalies reached 37.3% of the total embryos with malformations resulting from incubation in MMS. Most abnomalies in tail development were accompanied

Table 5

Acute lethal effect and teratogenicity of water samples from various water sources near Almaty in FET test

Experimental variants	Mortality, % of the total number of embryos	Teratogenicity, % of the total number of embryos	Frequency of malformations, % of the number *D. rerio* in deviation of embryonic development					
			scoliosis	tail malformation	tail tip malformation	growth retardation	oedemas	tail tip hypoplasia
Control	2.67 ± ± 1.86	4.00 ± ± 2.26	66.67	33.33	–	–	–	–
MMS, 3.4 mg/L	33.33 ± ± 5.44	89.33 ± ± 3.56	13.43	14.93	25.37	86.57	20.90	37.31
River Esik								
No. 1-2	16.00 ± ± 4.23	18.67 ± ± 4.50	92.86	–	14.29	–	–	–
No. 1-3	90.67 ± ± 3.36	50.67 ± ± 5.77	–	–	–	100	–	–
Lake Esik								
No. 2-2	24.00 ± ± 4.93	16.00 ± ± 4.23	100.0	–	–	–	–	–
No. 2-3	53.33 ± ± 5.76	5.33 ± ± 2.59	91.67	8.33	–	–	–	–
River Turgen								
No. 3-2	25.33 ± ± 5.02	8.00 ± ± 3.13	66.67	50.00	–	–	–	–



a *b*

Fig. 4. Development of the *D. rerio* embryo in normality and pathology: *a* — is a normal *D. rerio* embryo in the chorion, ×40; E — eyes, YS — yolk sac, Ch — chorion; *b* — embryo with abnormal development of the caudal region and growth retardation, ×100; the arrows indicate the curvature of the axial skeleton

by morphological changes of the fin fold, including epithalization, cell vacuolization, deformation and adherence of the fin limbus, and thinning.

DISCUSSION

Contamination of water is a global issue. Water sources subject to anthropogenic pressure are able to transport contaminants over large distances from the source of contamination. Many contaminants lead to cytotoxic, genotoxic, and mutagenic effects, and can also cause sterility and abnormalities in metabolism and other body functions [1, 20]. Natural water subject to anthropogenic contamination usually contains a complicated mixture of chemicals, and physical and chemical analyses do not always produce accurate data regarding the identities and concentrations of contaminants. In addition, the concentrations of some chemicals may exist below the limit of detection for current analytical methods, yet still cause negative effects for exposed organisms [2].

Practically, standard monitoring programs provide mostly for measurement of chemical and physical parameters [21, 22]. These measurements can provide a detailed description of the levels of individual contaminants, but only indirectly indicate the potential

biological consequences of the overall contamination. Biological analyses, however, can assess the biological effects of mixtures. A combination of these two approaches allows for identification of the main risk sources, which require continuous monitoring. Therefore, adequate risk assessment for any contaminant should first be based on physicochemical methods, followed by biological tests [2, 3].

We measured the following physical parameters of surface water sampled at various locations: pH, Total Dissolved Solids, ORP, and dissolved oxygen. It was determined that the sampls under examination were all weakly acidic. Weakly acidic water, with a pH of 5.0–6.5, is typical for swamps due to their humic acid content. The pH of river water normally varies between 6.5–8.5, and the lower pH in our samples can likely be explained by the availability of weak organic acids and cations of weak bases. Transition redox detected in water from the Rivers Esik and Turgen is characterized by unstable geochemical regime and variable levels of oxygen and hydrogen sulphide, and weak oxidation and reduction of a number of metals takes place. Oxidizing water of Lake Esik is characterized by the presence of free oxygen, as well as the number of elements in their highest form of valency (Fe^{3+}, Cu^{2+}, Pb^{2+}). Low levels of dissolved oxygen indirectly indicate contamination of the water.

The measurement of the heavy metals in the water did not detect levels exceeding MPCs for Ni, Co, Cr, Fe, or Cu. MPCs were exceeded for Mn, Zn, and Pb in water from the Rivers Esik and Turgen and Lake Esik. The level of Cd in Lake Esik exceeded MPC. Our results found differential contamination of the studied water samples with heavy metals, which can cause deleterious effects in organisms.

We assessed the water samples with bioluminescent strains of *E. coli* with reporters for genotoxic (pRecA-*lux* and pColD-*lux*) and pro-oxidant (pSoxS-*lux* and pKatG-*lux*) activity. Biosensors RecA, ColD, and SoxS did not detect genotoxic activity in any samples under investigation. A statistically significant increase in bioluminescence occurred in response to water sample p. No. 9-3 from the River Esik in the KatG assay, which indicates the presence of oxidizing agents in the water.

The cytotoxic effects of the samples on *A. cepa* was assessed, and determined by the degree to which root growth slowed. Additionally, a significant reduction in proliferation of the cell population was detected. The works by I. Dimitrova et al. and M. Yildiz et al. demonstrated that heavy metals prevent growth of the vegetative organs of plants [23, 24]. Reduction of mitotic activity may be caused by cytotoxic activity of heavy metals, and levels above MPCs were detected in our study. Heavy metals can disturb mitosis by blocking the cell cycle at interphase and preventing proper cell division [24].

Cytogenetic analyses of barley seeds grown on the experimental water samples indicated mutagenic activity in the water, which could be caused by the presence of heavy metals able to damage DNA (by increasing the amount of the intracellular free radicals as a result of inhibiting fermentation), and bond to sulfydryl, carboxyl, and amine groups of proteins [25].

The FET tests with *D. rerio* detected embryotoxic effect of samples from the Rivers Esik and Turgen, and Lake Esik. As noted above, acute cytotoxic and teratogenic effects of water varied from different sampling points. Scoliosis was the most prevalent malformation observed. It is known that the embryo emerging from the chorion is accompanied by chorion thinning, fermentative damage of the inner layer, and ultimately mechanical damage and osmotic fracture. MPCs of Mn, Pb, Zn were exceeded in the tested samples. Previously it was determined that cations with negative standard electrode potential (Zn^{2+}, Cd^{2+}, Pb^{2+}) can more easily penetrate the chorion and are accumulated in the perivitelline space. On the contrary, those with positive potentials (Hg^{2+}, Cu^{2+}, Ag^{2+}) have high affinities for sulfydryl groups and are bonded with the chorion, which serves as a barrier for these ions. Metals accumulation in the chorion largely depends on pH; lower pH leads to larger amounts of metal bound with the chorion [26]. Our results indicate that the large mountain rivers (i.e., the Esik and Turgen) going through the city, and in particular through the circular highways, collect organic and inorganic substances of high toxicity and teratogenicity for fish embryos at stages of development. The high-altitude Lake Esik, though supplied by glacial melting, is accessible to tourists and is also subject to anthropogenic pressure, which may have resulted in the levels exceeding the MPCs of such toxic heavy metals as Mn, Pb, Zn, and Cd.

Comparative analysis of contamination with heavy metals, as well as of toxic, mutagenic, and genotoxic activity allowed for rating the examined water reservoirs in the following sequence: Lake Esik > River Esik > River Turgen.

Three test systems (*A. cepa*, *H. vulgare*, and *D. rerio*) out of the four used in this study detected toxicity, mutagenicity, or embryotoxicity of the water samples. However, the *lux*-test, well-proven for testing genotoxicity, oxidative, and anti-oxidative activity [10–12, 27–29], did not detect expected activities in comparison with the test systems recommended for genetic monitoring [30]. We observed suppression of biosensor luminescence, which can be caused by heavy metals and other contaminants. Suppression of the culture growth and reduction of the level of bioluminescence was probably caused by high toxicity of samples for *E. coli*. Several studies have demonstrated that mutagenicity of heavy metals is not detected in bacterial systems. E. Igonina et al. demonstrated that oxidizing

stress in cells of *E. coli* was induced only by cadmium chloride and potassium bichromate out of ten tested metal salts [27]. At the same time, cytogenetic activity of heavy metals has been detected in the cell cultures and plant test systems [23, 24, 31].

Overall, our study detected levels exceeding MPCs for a number of heavy metals. Pro-oxidant activity of water from the River Esik was detected using biosensor KatG. Phytotoxic, cytotoxic, mutagenic, and embryogenic activity was demonstrated for all the studied water samples. Our examination of the mutagenic, genotoxic, and toxic potential of the natural water sources in the Almaty region, obtained on different test systems and test objects, indicate that water contamination with ecologically hazardous factors posed threats to biota and human health. Water from the most contaminated sources will be further examined using laboratory mammals (rodents) in order to make the data more relevant to humans.

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❀ Information about the authors

Anna V. Lovinskaya – PhD, Senior Lecturer, Senior Researcher, Department of Molecular Biology and Genetics, Faculty of Biology and Biotechnology. al-Farabi Kazakh National University; Scientific Research Institute of Biology and Biotechnology Problems at al-Farabi Kazakh National University, Kazakhstan. SPIN: 5200-6734. E-mail: annalovinska@rambler.ru.

Saule Zh. Kolumbayeva – Doctor of Biological Science, professor, Chief Researcher, Department of Molecular Biology and Genetics, Faculty of Biology and Biotechnology. al-Farabi Kazakh National University; Scientific Research Institute of Biology and Biotechnology Problems at al-Farabi Kazakh National University, Kazakhstan. SPIN: 6953-7523. E-mail: saule.kolumbayeva@kaznu.kz.

Maria A. Suvorova – PhD, Senior Researcher of the Mutagenesis Laboratory. Scientific Research Institute of Biology and Biotechnology Problems at al-Farabi Kazakh National University, Kazakhstan. SPIN: 3320-0084. E-mail: maria_suvorova@list.ru.

Akerke I. Iliyassova – Master Student, Research Intern, Department of Molecular Biology and Genetics, Faculty of Biology and Biotechnology. al-Farabi Kazakh National University; Scientific Research Institute of Biology and Biotechnology Problems at al-Farabi Kazakh National University, Kazakhstan. E-mail: ailiyassova@mail.ru.

Zarema M. Biyasheva – Candidate of Biological Science, Associate Professor, Department of Molecular Biology and Genetics, Faculty of Biology and Biotechnology. al-Farabi Kazakh National University; Scientific Research Institute of Biology and Biotechnology Problems at al-Farabi Kazakh National University, Kazakhstan. E-mail: zaremabiya@gmail.com.

Serikbay K. Abilev – Doctor of Biological Science, Professor, Scientific Secretary, Vavilov Institute of General Genetics, Moscow, Russia; Professor, Department of Genetics, Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia SPIN: 4692-4311. E-mail: abilev@vigg.ru.

❀ Информация об авторах

Анна Владимировна Ловинская — канд. биол. наук, старший преподаватель, старший научный сотрудник, кафедра молекулярной биологии и генетики факультета биологии и биотехнологии. Казахский национальный университет им. аль-Фараби, Научно-исследовательский институт проблем биологии и биотехнологии при Казахском национальном университете им. аль-Фараби, Алматы, Казахстан. SPIN: 5200-6734. E-mail: annalovinska@rambler.ru.

Сауле Жанабаевна Колумбаева — д-р биол. наук, профессор, главный научный сотрудник, кафедра молекулярной биологии и генетики факультета биологии и биотехнологии. Казахский национальный университет им. аль-Фараби, Научно-исследовательский институт проблем биологии и биотехнологии при Казахском национальном университете им. аль-Фараби, Алматы, Казахстан. SPIN: 6953-7523. E-mail: saule.kolumbayeva@kaznu.kz.

Мария Александровна Суворова — канд. биол. наук, старший научный сотрудник лаборатории мутагенеза. Научно-исследовательский институт проблем биологии и биотехнологии при Казахском национальном университете им. аль-Фараби, Алматы, Казахстан. SPIN: 3320-0084. E-mail: maria_suvorova@list.ru.

Акерке Илиясовна Илиясова — магистрант, стажер-исследователь, кафедра молекулярной биологии и генетики факультета биологии и биотехнологии. Казахский национальный университет им. аль-Фараби, Научно-исследовательский институт проблем биологии и биотехнологии при Казахском национальном университете им. аль-Фараби, Алматы, Казахстан. E-mail: ailiyassova@mail.ru.

Зарема Маратовна Бияшева — канд. биол. наук, доцент, кафедра молекулярной биологии и генетики факультета биологии и биотехнологии. Казахский национальный университет им. аль-Фараби, Научно-исследовательский институт проблем биологии и биотехнологии при Казахском национальном университете им. аль-Фараби, Алматы, Казахстан. E-mail: zaremabiya@gmail.com.

Серикбай Каримович Абилев — д-р биол. наук, профессор, ученый секретарь, ФГБУН «Институт общей генетики им. Н.И. Вавилова» РАН, Москва, Россия; профессор кафедры генетики, биологический факультет, ФГБОУ ВО «Московский государственный университет им. М.В. Ломоносова», Москва, Россия. SPIN: 4692-4311. E-mail: abilev@vigg.ru.