

IRSTI 87.19.03

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THE FIRST-LINE ASSESSMENT OF TOXIC POTENTIAL OF HIGH-ALTITUDE WATER SOURCES OF ILE ALATAU GORGES (Northern Tien Shan, Kazakhstan) USING ACUTE *D.MAGNA* TOXICITY TEST

The ecotoxicological evaluation of Ile Alatau headwaters is extremely important for South-East Kazakhstan as mountain cryosphere provide gross of water sources that supply Almaty agglomeration and surrounding industrial and agricultural areas of Almaty region. In the current study, the toxic potential of surface water of two gorges, namely Kuygensay gorge located directly above Almaty city and referenced Orta Turgen gorge, including moraine lakes, alpine lake, springs and main current at altitudes 2400 – 3500 m above sea level, was assessed by acute *D.magna* toxicity test according to OECD guideline. No significant toxicity was shown for water samples from Orta Turgen high-altitude tributaries and alpine lakes, whereas Titov moraine lake (the very source of Kuygensay river), main current and tributary spring of river Kuygensay demonstrated significant toxicity toward young daphnids in acute tests. The indexation based on *D.magna* immobilization/mortality data scoring, showed that headwaters of river Kuygensay are considered to meet “moderately toxic” to “toxic” class of toxicity, and Almaty agglomeration is probably the main source of pollution. These data provide opportunity to further investigation on pollution of glaciers and high-altitude water resources of Ile Alatau mountain range.

Keywords: high-altitude water sources, *D.magna*, bioassay, toxicity assessment, Ile Alatau.

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Іле Алатауының (Солтүстік Тянь-Шань, Қазақстан) шатқалдарындағы биік таулы көздердің суларының уыттылық әлеуетін *D. magna*-ға арналған жедел уыттылық сынағы арқылы бастапқы бағалау

Таулы криосфера – Алматы агломерациясының және Алматы облысының ауылшаруашылық, өнеркәсіптік аумақтарының басты су көзі. Осыған орай, Іле Алатауының биік таулы аймақтарын экотоксикологиялық тұрғыдан бағалаудың Қазақстанның Оңтүстік-Шығысы үшін маңызы зор. Осы зерттеуде ЕҚЫҰ ұсынған *D.magna*-ға арналған жедел уыттылық тесті Алматы қаласынан жоғары орналасқан Күйгенсай шатқалы мен салыстырмалы Орта Түрген шатқалының беткі суларының уыттық әлеуетін бағалау мақсатында пайдаланылды. Зерттеуге теңіз деңгейінен 2400-3500 м биіктіктегі мореналық көлдер, биік тау көлдері, бұлақтар және өзендердің негізгі арналары енгізілді. Орта Түргеннің биік таулы көлдері мен салаларынан алынған су сынақтарын зерттеу кезінде айтарлықтай уытты әсер байқалмады, ал Титов мореналық көлінен (Күйгенсай өзенінің бастауы), Күйгенсай өзенінің негізгі ағысынан және оның биік таулы бұлақ-саласынан алынған сынақтар жас дафнияларға статистикалық тұрғыдан расталған жедел уытты әсер етті. *D.magna*-ның иммобилизациясы/өлімі деректері бойынша индекстеу Күйгенсай өзенінің бастауын «орташа уытты» және «уытты» деп сипатталатын уыттылық класына жатқызады. Осыған орай, Алматы агломерациясын биік таулы аймақтарды ластаушы негізгі көз деп санауға негіз бар. Бұл мәліметтер Іле Алатауы тау сілеміндегі мұздықтар мен биік таулы су ресурстарының ластану саласындағы зерттеулерді жалғастыруға жол ашады.

Түйін сөздер: биік таулы су көздері, биотестілеу, *D.magna*, уыттылықты бағалау, Іле Алатау.

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**Первичная оценка токсического потенциала вод
высокогорных источников ущелий Иле Алатау (Северный Тянь-Шань, Казахстан)
с использованием теста на острую токсичность для *D.magna***

Горная криосфера является главным источником водных ресурсов для Алматинской агломерации, сельскохозяйственных и промышленных территорий Алматинской области, вследствие этого экотоксикологическая оценка высокогорий Иле Алатау для Юго-Востока Казахстана крайне важна. В настоящем исследовании тест на острую токсичность вод для *D.magna*, рекомендованный ОБСЕ, был применен для оценки токсического потенциала поверхностных вод двух ущелий, Кюйгенсай, расположенного над г. Алматы, и референсного ущелья Орта Тургень, включая моренные и высокогорные озера, родники и основное течение рек на высотах 2400–3500 м над уровнем моря. При исследовании образцов воды из высокогорных озер и притоков Орта Тургень не было отмечено значительного токсического эффекта, в то время как образцы из мореного озера Титова (исток р. Кюйгенсай), основного течения р. Кюйгенсай и ее высокогорного притока-родника оказывали статистически достоверное острое токсическое действие в отношении молодых дафнид. Индексация на основе данных по иммобилизации/смертности для *D.magna* показала, что верховья реки Кюйгенсай соответствуют классу токсичности «умеренно токсичные» и «токсичные», при этом Алматинскую агломерацию можно рассматривать как основную источник загрязнения высокогорий. Полученные данные открывают возможности для дальнейших исследований в области загрязнения ледников и высокогорных водных ресурсов горного хребта Иле Алатау.

Ключевые слова: высокогорные водные источники, биотестирование, *D.magna*, оценка токсичности, Иле Алатау.

Introduction

Water pollution is currently one of the most important environmental concerns facing the world. The importance of water quality reflects a basic need for clean water for human consumption and health of aquatic and terrestrial ecosystems, as specified by the UN SDG “Clean water and sanitation for all” [1]. The issue of water resources is one of the most relevant for Central Asia especially rich with arid and semiarid areas, where water resources are limited. All Central Asian rivers are nourished by the mountain cryosphere, their headwaters are diverse and located in the glacierized mountains of the Tien Shan, Pamir and Hindu Kush. Thus, this region strongly depends on water provision from the mountains and on glacier melt. The mountain ranges of the Northern Tien Shan, namely Trans-Ili Alatau, Dzungarian (Zhetysu) Alatau and Kungey Alatau, provide the glacierized catchments for multiple rivers that feed all South-Eastern Kazakhstan and terminally take part in Balkhash-Alakol basin. It is evidenced that remote alpine ecosystems couldn't be further considered as being free from chemical pollution: long-range atmospheric transport of pollutants from industrialized and/or urbanized areas leads to the precipitation and accumulation of potentially hazardous substances in mountain

cryosphere and their subsequent long-term release into the headwater streams [2]. For these obvious reasons, the ecosystems of European Alps, Rocky Mountains, Sierra Nevada, South American Andes, European Pyrenees, and others are already under substantial ecological pressure [3-5].

Accumulation of heavy metals (Cd, Sb, Bi, Tl, Sn, Pb) in glaciers has been reported for Tien Shan, Himalayas and European Alps since 1980s, thus they were considered as “legacy pollutants” – amassed and slowly released during glaciers melting up to tens of years after accumulation [6]. Recently Co, Cu, Zn, Cd, and Mo accumulation was determined in snowpack and high-altitude rivers of Tibetan Plateau (China) [7]. The contents of several metal(oid)s was measured in soil, moss and water in Shennongjia, alpine critical zone of central China, and overall pollution level and ecological risks were calculated to be low to moderate, and As, Co, and V were the most critical potential ecological risk factors [8]. Similarly, amass due to atmospheric transport and distant in time release into the waters was evidenced for persistent organic pollutants as was researched at Tibetan Plateau [9]. Elevated concentrations of heavy metals (Sb, Cu, Cr, Ni, As, Mo) were determined in the surface snow (cryoconites) samples in the glaciers of the eastern Tibetan Plateau, the heavy metal pollutants long-range transport to the

glaciers from the surrounding densely populated area of Asia has been proposed [10]. Short-term atmospheric transport through large low-mountain cities and adjacent industrial areas was considered to be responsible for high-altitude pollution by black carbon that is a product of incomplete combustion, such as automobile exhausts, crop burning and forest fires. Black carbon accumulation was evidenced for the glaciers of the Cordillera Real (Bolivia) [5]. In the same region the accumulation of monomethylmercury was evidenced in surface and sediment pore waters of high-altitude Lake Uru-Uru (Bolivia) due to urban and mining activities [11]. The phenomenon of headwaters secondary pollution due to snowpack and glaciers melting has been proposed as “glacier hypothesis”. Researches carried out in Switzerland Alps during and immediately after the COVID-19 lockdown showed a general improvement of high-altitude water quality [12].

The bioassay is the most informative and rapid tool of environmental screening for toxic potential of surface water and sediment, the test objects of bioassay are multiple and include various bacteria, plant, invertebrates and vertebrates species. The zooplankton cladoceran *Daphnia* spp. has been used as model organism of water toxicology since 1920s as it is lab-adaptive and at the same time chemically sensitive, has rapid parthenogenetic reproductive cycle. *Daphnia* spp. toxicity tests are the part of standardized OECD protocols for assessment of the acute (OECD 202) and reproductive (OECD 211) water toxicity [13, 14]. Acute tests with *Daphnia* spp. are effectively applied in toxicological researches to establish the LC50 and LOEC of individual and/or mixed xenobiotics and calculation of environmental risk [15]. Endpoints such as mortality and/or immobilization, swimming and feeding behavior, increase or decrease in the number of neonates per adult daphnid, delay between broods are measured in acute and chronic tests [16]. Thus, the *Daphnia magna* acute immobilization test recommended by OECD is a sensitive and valid test for the first line of biological monitoring of alpine head waters.

The ecological researches in Northern Tien Shan are scarce despite their irrefutable significance for Middle Asia and Northern-west China and bioassay investigation in this region are nearly absent. Ile Alatau mountain range is considered to be appropriate area for ecotoxicology researches of alpine water resources in our region due to its relative proximity and significance for well-developed Almaty city and Almaty region. Numerous springs, small tributaries and moraine lakes under glaciers form headwater catchments of rivers Kishi Almaty and Ulken Alma-

ty, Talgar, Issyk, Shamalgan, Aksay, Turgen, Chilik, Charyn, Chong-Kemin, Lepsy, Baskan, Karatal and others that provide the only water source for nearly four million people and economic activity of Almaty and Taldykorgan cities and adjoining regions. When water qualities and yields change in headwaters, the consequences affect the lands downstream, so monitoring the toxic potential of alpine surface water resources is the key concern in determining the water quality in high-altitude region.

Therefore the aim of the current work was to assess the toxic potential of alpine headwaters of two Ile Alatau gorges: Orta Turgen (Turgen river basin) and Kuygensay (Kishi Almaty river basin) including moraine lakes and tributaries using *D.magna* acute immobilization test by OECD regulations.

Materials and methods

Study area and sample collection. In this study, the r. Kuygensay (Gorelnik) (Kuygensay gorge, Ile Alatau, Northern Tien Shan) was selected as the main study region, while the r. Orta Turgen (Orta Turgen gorge, Ile Alatau, Northern Tien Shan) was used as the reference study region for spatial comparison. (fig. 1). The sampling time was from August to September 2024, and the sampling points were arranged in the headwaters of the source region, including the very river source (moraine lake), main current and tributaries, altitudes ranged from 2421 to 3564 asl (above sea level). The surface water was collected into the sterile plastic sampling bottles (Y&K Health Care, South Korea) and delivered into the laboratory within two days, where water was filtered using PES 0.22 µm filter flasks (UltraCruz, USA) and stored at 4°C until use. The measurement of water parameters was conducted *in situ* using portable calibrated water quality tester (Jinan Huiquan Electronic, China).

Acute *Daphnia magna* bioassay. All bioassay experiments with *Daphnia magna* were conducted according to Guideline 202 on “*Daphnia* sp. Acute Immobilisation Test, OECD Guidelines for the Testing of Chemicals” [13]. Specifically, young daphnids, aged less than 24 at the start of the test, were exposed to natural water samples for 48 hours and mortality/immobilization was scored. All animals derived from a healthy stock (no signs of high mortality, ephippia, discolored animals etc.), established up to two months prior to experiments from the one female, so the culture was genetically homogenous parthenogenetic brood. Stock animals were maintained in culture conditions similar to those used in the test.

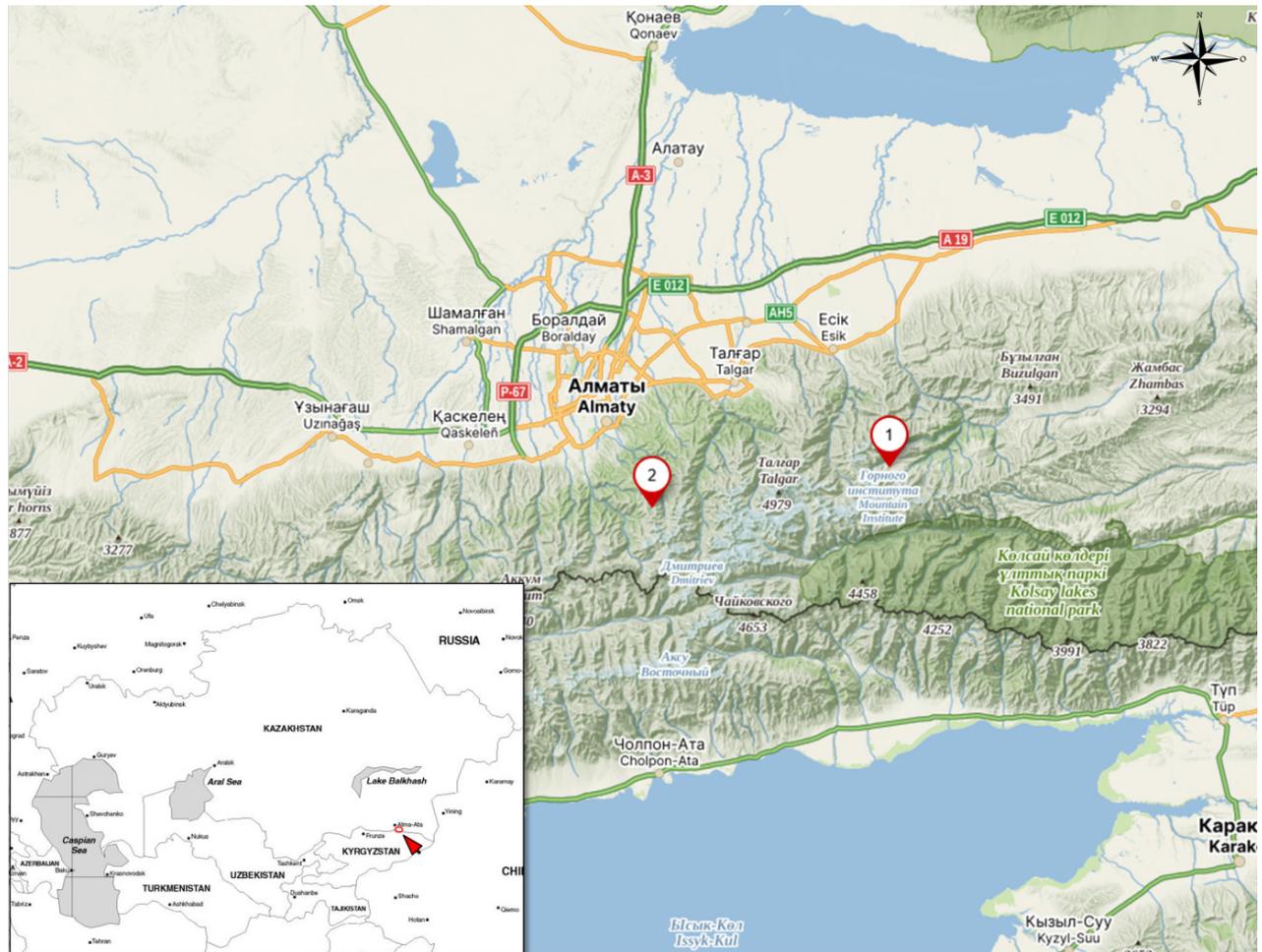


Figure 1 – Sampling sites location in the high-altitude areas of Ile Alatau range (Northern Tien Shan, Kazakhstan).
1 – r. Orta Turgen headwaters, 2 – r. Kuysgensay headwaters

Natural water samples were preliminary warmed up to experimental temperature conditions in test vessels of sterile glass beakers, media volume was 25 ml. 15 young daphnids were placed into each test vessel, in a fivefold sequence per point, thus 75 animals were used per study area bioassay. The ratio of air/water volume in the vessels was identical for test and control groups. Test was static, vessels were placed in thermostat (TS-1/20 SPU, Russia) and kept in darkness at $23 \pm 1^\circ\text{C}$ for 48 h. Vessels were not aerated and daphnids weren't fed during the test. To heavy metal salts were used during the tests as positive controls, namely cadmium (CdCl_2 , 0.8 mg/L) and lead ($\text{Pb}(\text{CH}_3\text{COO})_2$, 3.0 mg/L), in concentrations referred to a highly polluted water. The test conditions and daphnids number were similar to surface water bioassay tests.

The number of immobilized and/or dead animals was scored at 24 h and 48 h, the total mortality/immobilization

was calculated as the ratio of the difference between the number of survivors in the control and in the test groups to the number of survivors in the control, expressed as a percentage. Any signs of disease or stress such as discoloration or unusual behavior were recorded. The main validity criteria were no signs of stress and not more than 10% immobilization in the control groups throughout the test.

Statistics and data representation. All bioassay tests per study area were performed in fivefold sequence, and the data are expressed as mean \pm standard error ($M \pm SE$), a probability of $p < 0.05$ was accepted as statistically significant using a t-test. Statistical calculation and numerical data visualization were performed using StatPlus and Excel MS Office. All maps were prepared using online software mapy.com (<https://mapy.com/en>) where sample area point were placed according to precise coordinates.

Results and discussion

Study area description. Two distinct high-mountain gorges were selected for the *Daphnia magna* bioassay study – Orta Turgen and Kuygensay (Gorelnik) gorges, both attributed to the northern slope of Ile Alatau ridge and the great basin of river Ile. The gorges are at different distance from

large Almaty city: Kuygensay gorge is to the south of Almaty (directly above) at 12 km, while Orta Turgen gorge is located to the south-east of the city at about 55 km and was considered to be reference area. Location information and the main characteristics of surface waters in chosen sampling areas are represented in Table 1 and on figures 2B and 3B.

Table 1 – Sampling sites location information and parameters of surface water (Ile Alatau mountain range).

Code	Location	Coordinates	Elevation asl (m)	Type	TDS, ppm	EC, $\mu\text{s}/\text{cm}$	PH
Sp.T.#1	Orta Turgen gorge	43.12636, 77.58954	3 564	Moraine lake, source	33 \pm 3.1	66 \pm 4.3	7.5 \pm 0.04
Sp.T.#2	Orta Turgen gorge	43.15813, 77.63632	3 187	Alpine lake, main stream	136 \pm 4.3	272 \pm 1.1	7.8 \pm 0.04
Sp.T.#3	Orta Turgen gorge	43.16469, 77.64986	2 942	Tributary main stream	114 \pm 7.9	231 \pm 2.6	7.2 \pm 0.14
Sp.T.#4	Orta Turgen gorge	43.18021, 77.68755	2 421	Tributary main stream	170 \pm 9.5	352 \pm 1.8	7.4 \pm 0.07
Sp.K.#1	Kuygensay gorge	43.08904, 77.04867	3 316	Moraine lake, source	26 \pm 3.5	52 \pm 1.8	7.3 \pm 0.14
Sp.K.#2	Kuygensay gorge	43.10710, 77.04052	2 931	Main stream	40 \pm 2.9	80 \pm 2.1	7.4 \pm 0.07
Sp.K.#3	Kuygensay gorge	43.10705, 77.04047	2 934	Tributary spring	38 \pm 5.7	136 \pm 1.1	7.3 \pm 0.19
Sp.K.#4	Kuygensay gorge	43.11486, 77.04458	2 788	Tributary spring	62 \pm 2.3	124 \pm 2.6	7.7 \pm 0.14
Control	laboratory control	-	-	Negative control	137 \pm 2.1	274 \pm 1.5	7.5 \pm 0.04
Pb(CH ₃ COO) ₂ , 3.0 mg/L	laboratory control	-	-	Positive control	136 \pm 4.4	272 \pm 1.8	7.5 \pm 0.14
CdCl ₂ , 0.8 mg/L	laboratory control	-	-	Positive control	155 \pm 5.0	310 \pm 1.1	7.5 \pm 0.05

Note: asl – above sea level

Turgen gorge is located in Almaty region, in eastern part of Ile Alatau range, at about 60 km east from the Almaty city. R. Turgen is formed by the joining of several large tributaries – Orta Turgen, Shenturgen, Temirtas, Kishi Turgen and others. R. Orta Turgen that was chosen for the current study, is a main receiving stream, originates from the large Gornogo Instituta glacier. The melting glacier from a large moraine lake named simply Lake №1, this lake is the very source of Turgen river and was chosen as first sampling area – sp.T#1 (moraine lake). The torrent further follows north-eastward down the wide picturesque valley, sp.T#2 – one of the so called

“Sphagnum lakes” located downstream. This is a group of the small lakes, shallow and mossy, originated from the left Orta Turgen tributary. Sp.T#3 is an orographically left upper tributary and sp.T#4 is the lower small left tributary of r. Orta Turgen, flowing into the river above the convenience with the r. Temirtas. The Orta Turgen gorge is the touristic hiking place, but less popular than Kuygensay gorge due to its remoteness from the Almaty city.

Kuygensay gorge is a trough valley. Kuygensay (Gorelka) river is the main tributary of the r. Small Almaty, length is 5.8 km, catchment area square – 12 km², three small moraine lakes are in

the river uppers. As Kuygensay gorge is located close to Almaty city and very picturesque it is very popular touristic hiking area. Kuygensay sampling point sp.K#1 is located at lake Lower Titova, the very source of the river Kuygensay, and at sampling time moraine lake became shallow, 1-2 m from the shore the depth does not exceed 0.5m, the lakeshore as well as the wide mountain valley leading to it are alpine meadows used as mountain horse pastures (zhaylau). River further descends northward by a long wide mountain valley, sp.K#2 is the main stream sampling, located at end of this valley, river further turns eastward. Stream is 1.5m width in average, in places it spills out into many inter-boulder streams, bottom is rocky. The place is shady, and riverbed is covered with a large snow shell, powerful enough to support an adult. The surrounding area of rocky slopes and subalpine meadows are dwelled with marmots (*Marmota baibacina*). Sp.K#3 is an orographically left tributary, descending sharply down the mountainside, 0.5-0.7 m width, 3 m length spring, shadow and mossy. Sp.K#4 is an orographically right tributary, torrent and shallow, that originates from multiple mossy springs, sampling area is at the very border of the spruce forest, vegetation is mixed: spruce, juniper, subalpine grasses. Recently this area became popular hiking place and is under the direct anthropologic pressure.

As all mountain rivers of Central Asia, Kuygensay and Orta Turgen rivers are characterized by low temperatures, low channel stability and low solute concentrations (sediment flux is high during the high flow period), low productivity. The main properties of surface waters are characteristic to high-mountain reservoirs usually total concentrations of dissolved substances are low, but for Orta Turgen tributaries TDS is high and in case of Sphagnum lake comparable to laboratory control. Lake Shagnum is mossy and the bottom is partially silted, as for the tributaries, they are large and extensional, carrying huge amounts of sediment and organic matter. At the same time tributaries of r. Kuygensay are real springs and the sampling sites were no more than 2-5 m from the spring origin. The measurements of electrical conductivity support the TDS data, and by these parameters surface water samples form Orta Turgen basin (with the exception of moraine lake #1) approximate the ones for the control laboratory water. pH value is typically between 7.2 and 7.8, no signs of acidification or alkalization were recorded for any sampling points. It should be noted, that incubation of young daphnids for 48 hours greatly changes the TDS and EC indexes due to physiologi-

cal activity of animals in a limited volume of medium (data not shown).

Daphnia magna bioassay. Indeed the bioassay is the only way to evaluate the direct toxic potential of natural waters being fast, reliable and sensitive method of first-line ecotoxicological screening. OECD recommendations on water toxicity testing are referred to several key test-objects, and daphnids are among them. *Daphnia spp.* – is the most common and standardized test-object for assessing water quality, used as “sensor” of toxic pollution of aquatic environment. Due to its adaptability to laboratory cultivation, parthenogenetic reproduction (allows to establish genetically homogenous batch of test-objects), short life-cycle and great sensitivity to organic and inorganic substances *Daphnia magna* Straus is overall used in first-line bioassay researches [17].

In the current study the immobilization/mortality acute test with *D.magna* was applied to evaluate the toxic potential of surface water of high-altitude water bodies of Ile Alatau gorges. The results of acute *D.magna* larvae exposition to surface water samples are represented on figures 2A and 3A. In control groups immobilization/mortality didn't exceed 6% throughout the experiments signifying the main validity criterion for bioassay research (no more than 10% immobilization/mortality in control).

Two heavy metals were chosen as positive controls – cadmium (CdCl_2 , 0.8 mg/L) and lead ($\text{Pb}(\text{CH}_3\text{COO})_2$, 3.0 mg/L), in concentrations referred to a highly polluted water, results indicating that daphnids are susceptible to both metals (fig. 2A, 3A). At the same time cadmium exposure showed to be a highly toxic to daphnids – up to 92% of animals died nearly after 24 h. of exposure ($p \leq 0,001$), while the lead in four-fold more concentration was two-fold less toxic.

Nevertheless $\text{Pb}(\text{CH}_3\text{COO})_2$ exposure for 48 h. gained 52% immobilization/mortality index ($p \leq 0,001$). The toxic effects of heavy metals and Cd and Pb in particular, are evidenced and since widely used in various toxicological researches. These heavy metals are absorbed by young daphnids through filtration and with food, disrupt enzymatic processes, affect physiological activity (heart rate and thoracic limb activity), reduce growth and possess not only acute but long-time effects inhibiting reproductive capacity and population dynamics [18, 19]. Furthermore, studies have shown that heavy metals can interact with other contaminants, such as plastics and nanoparticles, altering their toxicity for water invertebrates [20].

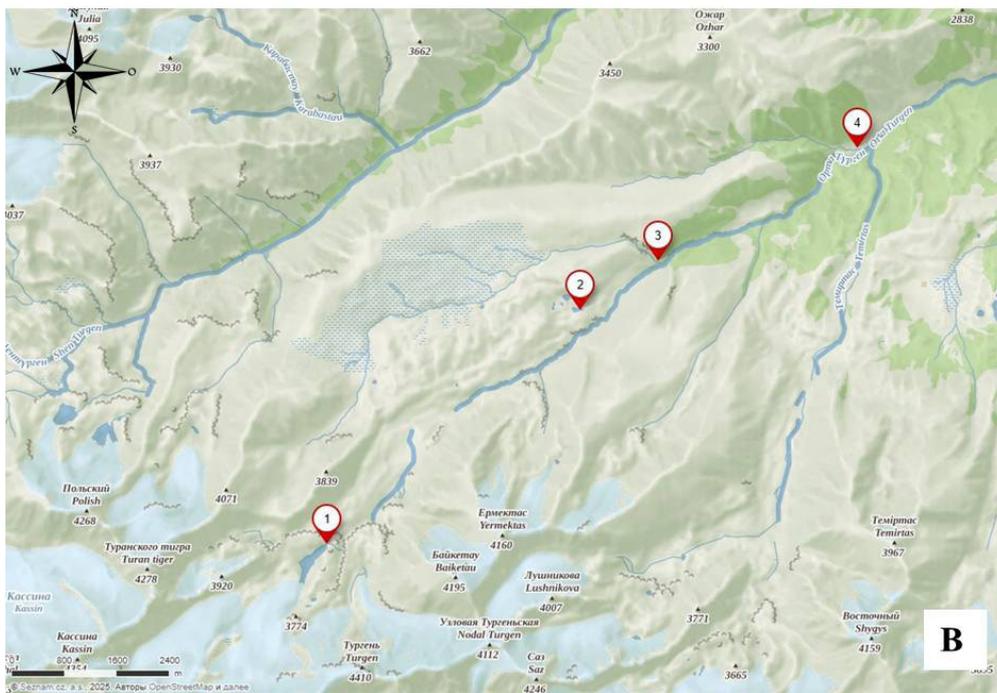
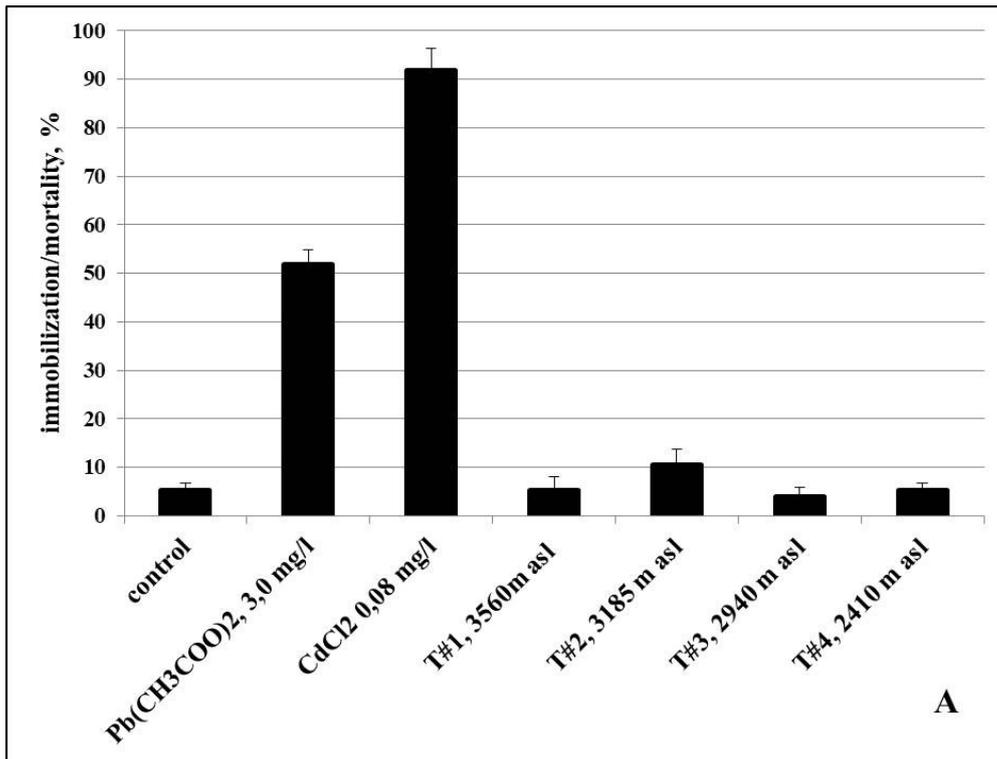


Figure 2 – Acute *D.magna* bioassay for surface waters of high-altitude r. Orta Turgen (Ile Alatau, Northern Tien Shan).

A – results of acute *D.magna* toxicity test, mean±SE; B – sampling sites location. asl – above sea level

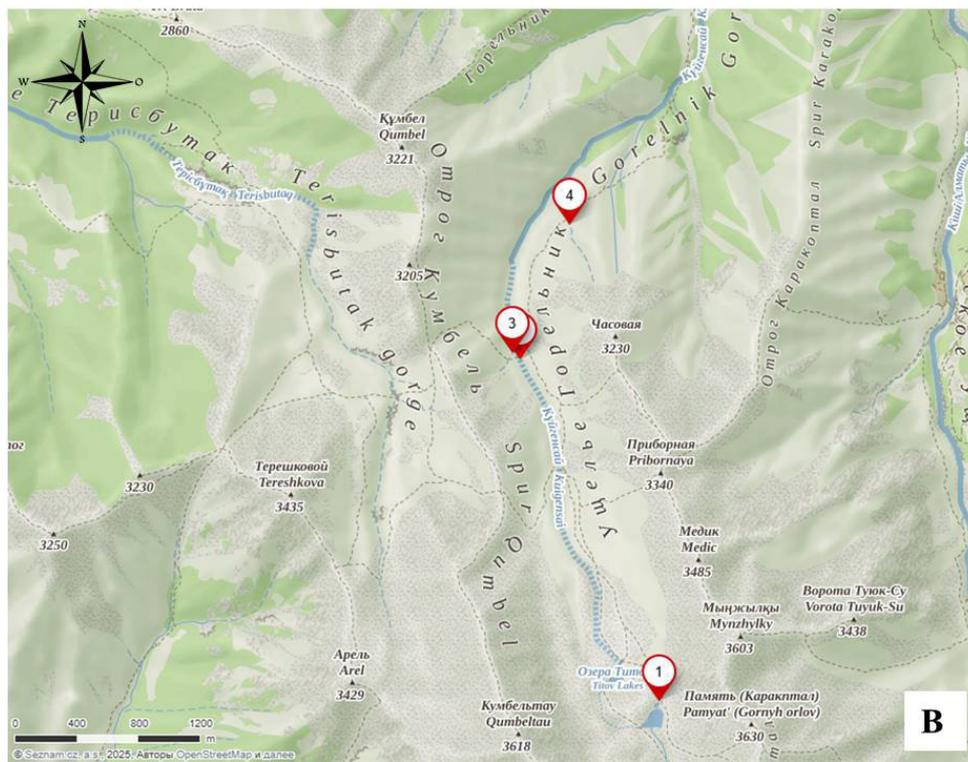
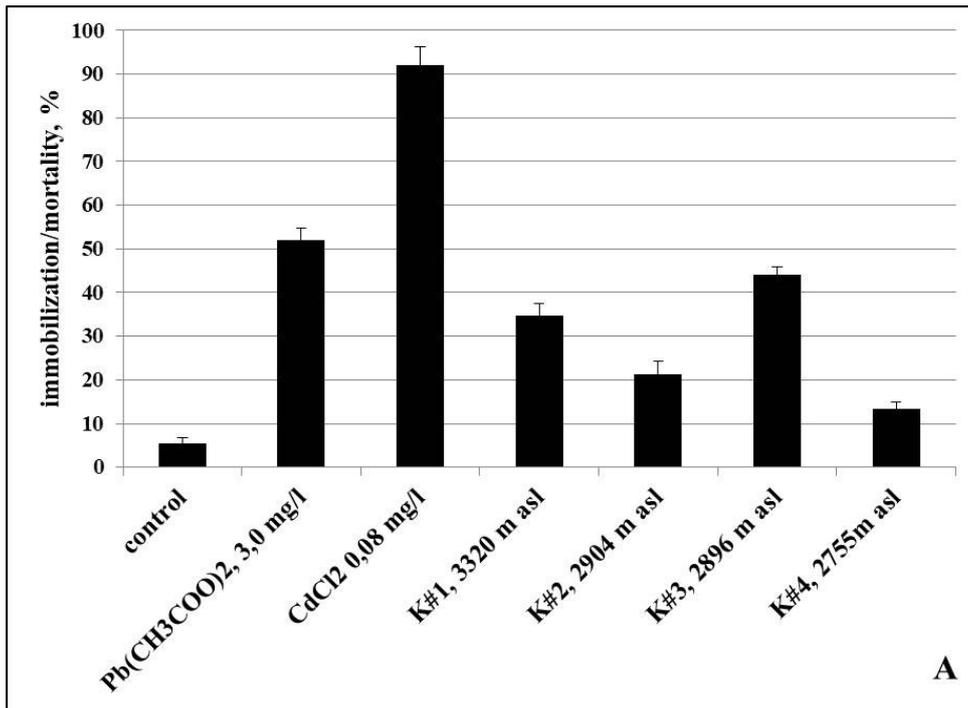


Figure 3 – Acute *D.magna* bioassay for surface water of high-altitude r. Kuysengsay (Ile Alatau, Northern Tien Shan).

A – results of acute *D.magna* toxicity test, mean±SE; B – sampling sites location. asl – above sea level

Exposure of young daphnids to high-altitude surface water evidenced the differences in toxic potential of two studied areas. For all water samples from Orta Turgen highlands the average immobilization/mortality didn't exceed the 10% referred to control value and no toxicity effect was recorded, the only exception is sp.T#2 (Sphagnum lake) – 11%, but differences were not statistically significant comparing to control. For Kuygensay gorge the situation was different. The slight mortality effect was shown for the lowest sampling point – K.#4 (2755 asl.), large tributary originating in mossy mountain slope, at the same time higher points all gained significant mortality. So, exposure to water from small tributary spring, K#2 (2904 asl.) resulted in nearly 22% mortality ($p < 0,05$) of young daphnids, whereas water samples from main current located no more than several meters ahead, K.#3 (2896 asl.) caused 44.0% of young daphnids to die after 48 h. of exposure ($p \leq 0,001$). Water samples from moraine lake Titov (K.#1, 3320 a.s.l.) were evidenced to be of moderate toxicity for *D.magna* as well – 35% immobilization/mortality score has been recorded after 48 h. of exposure ($p \leq 0,01$).

Thus the results of a current bioassay study evidence the toxic potential of r. Kuygensay headwaters and generate two issues about the reason for difference between two studied areas and about the sources of toxicity. As it was noted earlier, daphnia species showed to be very sensitive to both organic and inorganic substances. Apart from heavy metals *Daphnia* species were shown to be susceptible to persistent organic pollutants, pesticides and microplastic up to extreme toxicity [21-23]. Multiple researches have shown that high-altitude areas can receive and amass contaminants from external sources, which have been deposited onto the ice surface via processes of atmospheric transfer and precipitation. The contamination of microplastics was shown for remote high-altitude lakes by Chinese researches, the results of the risk assessment were found to be interconnected with the distribution of the local population and agricultural activities around the sampling area [24]. Alpine areas of Western Europe were also indicated to be polluted by heavy metals and persistent organic pollutants, however for some remote high-altitude areas and alpine lakes bedrock and surficial geology proved to be a major factor controlling metal concentrations in lake water [25]. The potential serious heavy metal pollution of alpine

lakes was investigated by physiochemical methods for the mountain ecosystems of Pakistan and Himalayan part of India, and lakes were observed to be more polluted than the rivers with Cd, Hg, Mn, Cu [2, 26]. Accumulation of organochlorine pesticides due to long-range atmospheric transport and subsequent cold-trapping under low-temperature condensation at higher altitude has also been shown for Tibetan Plateau [9]. Melting glaciers would result in a substantial release of persistent organic pollutants as has been evidenced by the researches at Tibetan Plateau, Alps and Antarctica. It also should be noted, that toxic effect might be modulated or caused by the algotoxins as it is established that daphnids are susceptible to toxic action of cyanobacteria, and that toxicity is increased in higher temperature conditions [27]. The water samples for the current assay were filtered from bacteria, but nevertheless the presence of algotoxins in water samples couldn't be excluded and the specific research is needed on that issue for highland waters of Ile Alatau.

Indexation by toxicity classes. The issue of water pollution indexation is of high importance due to diversity of test objects and measured endpoints especially when a battery of test is applied. The three toxicity classes were proposed to estimate the level of pollution: no toxicity, moderate toxicity and high toxicity, where for all bioassays $>50\%$ endpoint response was considered as lower boundary for “high toxicity” of water samples [28]. For invertebrates bioassay the 20-50% toxicity response is usually assigned to “moderate toxicity” and 0-20% toxicity response as “no toxicity”. However according to the OECD criteria, the threshold (validity criterion) for *D.magna* bioassay is no more than 10% toxicity in control, so that “no toxicity” index ought to be revised. In a current research the following toxicity criteria were proposed based on *D.magna* acute toxicity test: “not toxic” (0-10% mortality), “slightly toxic” (10-20% mortality), “moderately toxic” (20-50% mortality), “toxic” (50-70% mortality), “highly toxic” (70-100% mortality). The results of bioassay indexation of high-altitude waters of studied Ile Alatau gorges based on *D.magna* acute toxicity test are represented in table 2.

As mentioned above the pollution level of the tested waters from Orta Turgen gorge is considered as “not toxic” and in case of Sphagnum alpine lake is “slightly toxic”, while for Kuygensay gorge the tested pollution level is noticeably higher.

Table 2 – Toxicity indexes for high-altitude surface water bioassay based on *D.magna* acute toxicity test

High-altitude water sampling sites					
Toxicity class		Orta Turgen river basin			
		Moraine lake#1 3564m asl	Alpine “Sphagnum lake”, 3187m asl	Tributary, main stream, 2942m asl	Tributary, main stream, 2421m asl
not toxic	5				
slightly toxic	4				
moderately toxic	3				
toxic	2				
highly toxic	1				
		Kuygensay river basin			
		Morain “Titov lake” 3316m asl	Main stream 2931m asl	Tributary, spring 2934m asl	Tributary spring 2788m asl
not toxic	5				
slightly toxic	4				
moderately toxic	3				
toxic	2				
highly toxic	1				

Note: asl – above sea level

With the exception of lower tributary, water samples from moraine lake Titov and main stream of Kuygensay river are indexed as “moderately toxic”, and for one sample from small spring the level of pollution is “toxic”. Thus, there are some chemical substances in those water samples that reveal toxic potential when testing on young daphnid, whether through direct or indirect interactions with organism, including synergistic interactions and biotransformation. One ought to keep in mind that the underlying rocks themselves may be sources of toxic inorganic ions, but that is likely true in relation to springs with point sources, but not large lakes.

The pollution footprints in highland water basins depend on geographical position, orographic characteristics and climatic features, wind rose first of all, as the atmospheric transport is considered to be the main source of pollution [29]. Two studied river basins differ by the orographic position (fig. 2B and 3B). The Orta Turgen highlands extend from south-east to north-west, while Kuygensay highlands occupy less area directed nearly from south to north and located nearly above the large Almaty city. This gorge receives the wind-stream from massive wind-flow coming from the west-north, passing through dusty semi-deserts, industrial city suburbs and highly polluted Almaty city. The winds reach-

ing the Orta Turgen gorge are usually passing the Almaty city by.

Moreover main sources of runoff are different for two gorges. For Orta Turgen Moraine lake#1 the main source is glacier melting, riverbed is slope and extended, main stream and tributaries torrents dilute water significantly, and probably the sediment is the main collector of toxic substances. In Kuygensay gorge the primary source of runoff is the snow melt, the gorge is narrow and river is supplied by snowpack melting from surrounding mountain slopes. It is well known that mountain cryosphere (glaciers, snowpack, frozen ground) serve as reservoirs for contaminants, however glaciers are regarded as accumulators of pollutants releasing them slowly, but snowpack receives pollutants by precipitation immediately and they are quickly released during the hot seasons. The deposition of environmental pollutants such as persistent organic pollutants and black carbon in mountain snowpack has been shown recently [3, 6, 7]. The investigations of snowmelt samples toxicity potential got evidences for accumulation of heavy metals and PAHs in snowpack and subsequent release into the snowmelt with the ability to induce teratogenic and toxic effects when studied on embryo-larval fathead minnow [30].

Therefore the source of toxic potential observed for headwaters of r. Kuygensay remains unclear, since it can be of different origin: natural rock erosions, contamination of water with amassed pollutants or algotoxines, etc. it also should be noted that water hardness may significantly influence the bioavailability and toxicity of pollutants. Recent meta-research demonstrated that high water hardness exerted significant effect against pollutant toxicity, reducing their bioavailability through competitive mechanisms involving calcium and magnesium ions [31]. Thus the waters with low TDS as for moraine lakes and high-altitude currents, potentially may gain more toxic effect than those with high water hardness.

Conclusion

Currently there is a serious gap in water quality assessment for high-altitude regions mainly due to remoteness of research areas. The ecological researches of Northern Tien Shan and its rivers remain scarce, despite its great significance for population and industrial activity of South-East Kazakhstan. High-mountain environments are sensitive to changes in atmospheric pollution, and for regions of Ile Alatau range close to the highly polluted Almaty agglomeration the ecotoxicological studies are of great interest and urgency, as its mountain rivers are the very source of drinking water for population. The current bioassay for the first time was aimed to evaluate the toxic potential of two mountain Ile Alatau rivers by acute *D.magna* toxicity test. *D.magna* acute toxicity test, recommended by OECD is proved to be the sensitive and accurate methods for water toxicity assessment, and used as first-line indicator of pollution screening with the certain degree of approximation may provide the quantitative assessment of the level of toxicity for the natural surface waters. The headwaters of rivers Kuygensay and Orta Turgen attributed to high-altitude regions of Ile Alatau range, starting from 2400

m asl (above sea level) and up to the very sources of moraine lakes (3316 – 3564 m asl) were indexed according to toxicity level. The hotspots of water pollution were discovered for Kuygensay river headwaters, indexed as from “moderately toxic” to “toxic” starting from 2900 m asl, and rising the issue of high pollution level for Ile Alatau gorges located above Almaty city. The additional researches are needed to evaluate the level of glaciers and snowpack pollution, to assess the role and reasons for toxic potential of small springs rather than highly diluted main currents, issue of sediment pollution, role of agoflora and other open questions. The ecotoxicological researches of high-altitude Ile Alatau water sources not only provide information on water quality but should further be aimed on understanding the mechanisms of mountain cryosphere pollution and subsequent water contamination.

Acknowledgements

This research has been supported by the Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No.AP23486019 “Comprehensive ecotoxicological assessment of alpine glacierised drainage basins of main rivers of the Northern Tien-Shan”).

In preparing this work, the authors used Google AI Studio in order to translate abstract text into Kazakh language. After using this tool/service, the author(s) reviewed and edited the content as necessary and take full responsibility for the content of the publication.” We confirm that the article fully complies with the journal’s policy regarding the use of generative AI, and that all tools used were applied in accordance with the guidelines of publishing ethics. We also understand that in case of serious violations of publishing ethics related to the use of AI, the process of retraction may be initiated, and we accept full responsibility for adhering to all norms and standards established by the journal.

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Поступила 30 октября 2025 года

Принята 25 декабря 2025 года