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# INTEGRATED HYDROCHEMICAL AND BIOCHEMICAL EVALUATION OF ENVIRONMENTAL POLLUTION IN LAKE BALKHASH

Lake Balkhash is one of the largest inland water bodies in Central Asia. The regional water supply plays an important role in fisheries and biodiversity conservation. In recent decades, the lake has been subjected to intense anthropogenic impact due to industrial activities, agricultural runoff, and hydrological changes. This has led to an increase in pollution by pollutants. This study presents a comprehensive assessment of heavy metal contamination in surface waters, bottom sediments, and commercial fish species in the northern part of Lake Balkhash (Karaganda region), combining chemical analysis with biochemical biomarkers of oxidative stress from fish liver. Surface water and sediment samples were collected in the Torangalyk Bay near the Balkhash mining and metallurgical complex and in the Bertys Bay near the city of Balkhash. The results revealed significant excess of permissible concentrations of Cu, Co, Pb, Ni, Cr and Zn in surface waters, with the highest levels recorded near the Balkhash mining and metallurgical complex and Bertys Bay. At all study sites, the concentration of heavy metals in bottom sediments exceeded the permissible limits, indicating long-term accumulation. Elevated levels of Cu, Co, Pb and Ni were found in fish tissue, especially in the gills and liver. Biochemical analysis revealed increased lipid peroxidation and decreased activity of antioxidant enzymes in fish taken from contaminated areas, indicating severe oxidative stress. The results of the research confirm that industrial pollution poses a significant threat to the ecological health of Lake Balkhash and its ichthyofauna.

**Keywords:** Lake Balkhash, heavy metals, fish, oxidative stress, malondialdehyde, superoxide dismutase, catalase.

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## Балқаш көліндегі қоршаған ортаның ластануын кешенді гидрохимиялық және биохимиялық бағалау

Балқаш көлі – Орталық Азиядағы ең ірі ішкі су айдындарының бірі. Аймақтық сумен жабдықтау балық шаруашылығында және биоәртүрлілікті сақтауда маңызды рөл атқарады. Соңғы онжылдықтарда көл өнеркәсіптік қызметке, ауылшаруашылық ағындарына және гидрологиялық өзгерістерге байланысты қарқынды антропогендік әсерге ұшырады. Бұл ластаушы заттармен ластанудың артуына әкелді. Бұл зерттеу Балқаш көлінің солтүстік бөлігіндегі (Қарағанды облысы) жер үсті суларындағы, түбіндегі шөгінділердегі және коммерциялық балық түрлеріндегі ауыр металдардың ластануын кешенді бағалауды ұсынады, химиялық талдауды балық бауырынан тотығу стрессінің биохимиялық биомаркерлерімен біріктіреді. Балқаш тау-кен металлургиялық кешенінің маңындағы Торанғалық шығанағында және Балқаш қаласының маңындағы Бертыс шығанағында жер үсті сулары мен шөгінділердің үлгілері алынды. Нәтижелер жер үсті суларында Cu, Co, Pb, Ni, Cr және Zn рұқсат етілген концентрацияларының айтарлықтай асып кеткенін көрсетті, ең жоғары деңгей Балқаш тау-кен металлургиялық кешені мен Бертыс шығанағы маңында тіркелді. Барлық зерттеу орындарында түптік шөгінділердегі ауыр металдардың концентрациясы рұқсат етілген шектен асып кетті, бұл ұзақ мерзімді жинақталуды көрсетеді. Балық тінінде, әсіресе желбезектер мен бауырда Cu, Co, Pb және Ni деңгейінің жоғарылағаны анықталды. Биохимиялық талдау ластанған жерлерден алынған балықтарда липидтердің асқын тотығуының жоғарылауын және антиоксидантты ферменттердің белсенділігінің төмендеуін анықтады, бұл ауыр тотығу стрессін көрсетеді.

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ластанудың Балқаш көлінің және оның ихтиофаунасының экологиялық денсаулығына айтарлықтай қауіп төндіретінін растайды.

**Түйін сөздер:** Балқаш көлі, ауыр металдар, балық, тотығу стресі, малониальдегид, супероксиддисмутаза, каталаза.

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**Комплексная гидрохимическая и биохимическая оценка загрязнения окружающей среды в озере Балхаш**

Озеро Балхаш – один из крупнейших внутренних водоемов Центральной Азии. Региональное водоснабжение играет важную роль в рыболовстве и сохранении биоразнообразия. В последние десятилетия озеро подвергается интенсивному антропогенному воздействию из-за промышленной деятельности, сельскохозяйственных стоков и гидрологических изменений. Это привело к увеличению загрязнения. В данном исследовании представлена комплексная оценка загрязнения тяжелыми металлами поверхностных вод, донных отложений и промысловых видов рыб в северной части озера Балхаш (Карагандинская область), сочетающая химический анализ с биохимическими биомаркерами окислительного стресса в печени рыб. Пробы поверхностных вод и отложений были отобраны в заливе Торангалык вблизи Балхашского горно-металлургического комплекса и в заливе Бертыс вблизи города Балхаш. Результаты показали значительное превышение допустимых концентраций Cu, Co, Pb, Ni, Cr и Zn в поверхностных водах, при этом самые высокие уровни были зафиксированы вблизи Балхашского горно-металлургического комплекса и залива Бертыс. На всех участках исследования концентрация тяжелых металлов в донных отложениях превышала допустимые пределы, что указывает на долговременное накопление. Повышенные уровни Cu, Co, Pb и Ni были обнаружены в тканях рыб, особенно в жабрах и печени. Биохимический анализ выявил повышенное перекисное окисление липидов и снижение активности антиоксидантных ферментов у рыб, взятых из загрязненных районов, что указывает на сильный окислительный стресс. Результаты исследования подтверждают, что промышленное загрязнение представляет собой значительную угрозу для экологического здоровья озера Балхаш и его ихтиофауны.

**Ключевые слова:** озеро Балхаш, тяжелые металлы, рыба, окислительный стресс, малоновый диальдегид, супероксиддисмутаза, каталаза.

## Introduction

Currently, hydrogeological and environmental problems are very pressing for one of the largest reservoirs of Kazakhstan – Lake Balkhash. As is known, these problems are associated with a decrease in the water level in the lake as a result of irrational use of the waters of the transboundary Ile River, which is the main source of hydroresources, as well as with significant pollution by wastewater from industrial enterprises and farms (Burlybaev et al., 2009; Smolyar et al., 2007). Until recently, Lake Balkhash (413 thousand sq. km.) was the main reservoir for drinking and domestic water supply, the basis for the development of the fisheries industry of Kazakhstan (Aleynik et al., 2011). However, due to accumulated problems: the discharge of industrial and agricultural wastewater into the lake, significant shallowing as a result of uncontrolled water intake both in China and Kazakhstan, the ecological state of the reservoir leaves much to be desired (Sultanova et al., 2012). The waters of Lake Balkhash are polluted by various pollutants: heavy metals, pesticides, radionuclides, oil products (Ministry of Ecology, Geology and Natural Resources of Kazakhstan, 2019; Nurtazin et al., 2016). From the water, they settle to the bottom and, accumulating in sediments, enter the bodies of the reservoir's inhabitants through the food chain (Amirgaliev et al., 2012; Lopareva et al., 2016). The lake, once unique in its ichthyological composition, is currently irrevocably losing its biodiversity. Previously, Lake Balkhash was home to more than twenty species of fish, six of which were native: the Ili and Balkhash marinka, Balkhash perch, spotted and one-colored sloth, and Balkhash minnow, which are now very rare to catch. Of the introduced species, carp, barbel, eastern bream, Aral barbel, Siberian dace, carp, tench, pike-perch, catfish, osman, and crucian carp have disappeared. Catches of commercial fish, such as carp, pike-perch, asp, and bream, are becoming increasingly rare. Due to their diet and life in the aquatic environment, fish are particularly vulnerable and susceptible to severe pollution, as they cannot

escape the harmful effects of pollutants (Canli et al., 2018; Mansour et al., 2023). Fish, compared to invertebrates, are more sensitive to many toxicants and are a convenient object for assessing the state of the ecosystem. Many researchers have noted a decline in the number of native and valuable fish species, as well as other aquatic organisms in Lake Balkhash (Canli et al., 2018; Kumar et al., 2023). Due to pollution of the aquatic environment, fish are constantly exposed to toxic substances, which leads to metabolic disorders that can manifest themselves in changes in various biochemical parameters of organs. Heavy metals are also known to cause oxidative stress by mediating free reactive oxygen species (Balali-Mood et al., 2021). Fish accumulate pollutants primarily in their fatty tissues, such as the liver, and the effect becomes apparent when the concentration in such tissues reaches a threshold level (Wang et al., 2011). However, this accumulation depends on their intake, storage, and excretion from the body (Aralova et al., 2016; Moldakhanova et al., 2023). Thus, the state of fish biodiversity reflects the ecological state of the reservoir.

## Materials and methods

### *Study area and objects of study*

The samples were collected along the route from Almaty to the northern shore of Lake Balkhash:

1. Torangalyk Bay near the village of the same name near the city of Balkhash (620 km from Almaty, coordinates: 46°76′35″ N 74°83′72″ E) (Figures 1-2). There are no industrial enterprises in the village of Torangalyk, so the coastline in this area is popular with Kazakhstan and is home to recreation centers and tourist attractions. Vacationers swim and fish in the lake.

2. Lake Balkhash near the Balkhash Mining and Metallurgical Plant (BMMK) of Kazakhmys Corporation (630 km from Almaty, coordinates: 46°82′54″ N 74°95′43″ E) (Figures 3-4). The Balkhash site of Kazakhmys Corporation has six main industrial facilities and auxiliary workshops, including three mines, one processing plant, one copper smelter, and one thermal power plant.

3. Bertys Bay, the city of Balkhash (635 km from Almaty, coordinates: 46°80′06″ N 75°03′46″ E) (Figures 5-6). The city of Balkhash is a major industrial region in Central Kazakhstan with a population of over 87,662. The city is located on the southern, flat outskirts of the Kazakh Uplands, with a gentle slope toward the lake. Over 400 enterprises are located within the city, including the Balkhashtsvetmet metallurgical plant and mining organizations.

During the field trips, juveniles of common species were collected: asp (Aspius aspius), roach (Rutilus rutilus), and carp (Cyprinus carpio). A total of 60 adult specimens were collected. For bioassays of water and sediment collected during expeditions to the northern part of Lake Balkhash (Aktogay District, Karaganda Region), water and sediment samples from sampling sites: Torangalyk Bay, the bay near the Balkhash Mining and Metallurgical Plant, Bertys Bay, and the city of Balkhash served as incubation media.

**Figure 1**

*Map of material collection in the Torangalyk Bay near the city of Balkhash (620 km from Almaty, coordinates: 46°76′35″ N 74°83′72″ E)*

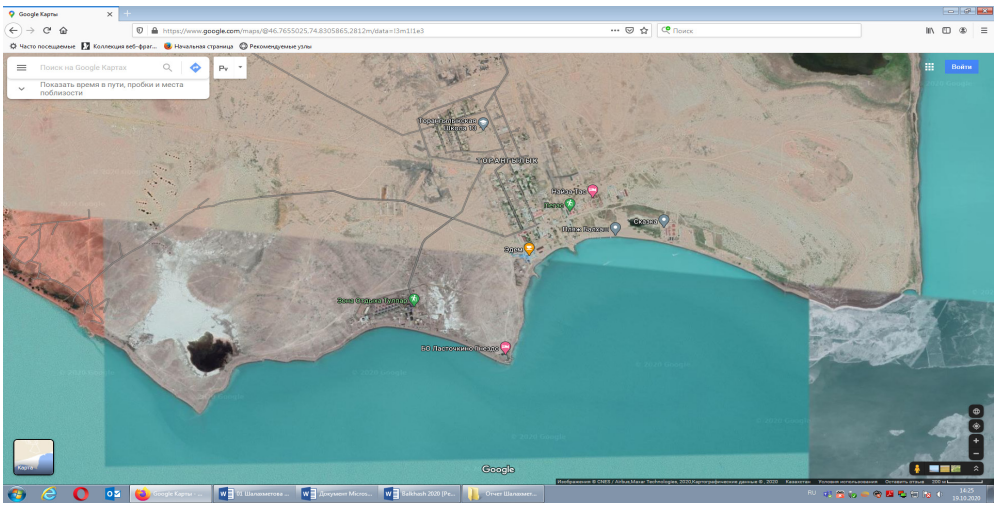


**Figure 2**
*Torangalyk Bay, altitude = 335 mBS, water temperature = 24.5°C; pH = 8.65-8.68; mineralization 1015-1016 mg/l. The soil is sandy and rocky-sandy*



**Figure 3**
*Map of material collection in the Bertys Bay near the Balkhash copper smelter of the Kazakhmys Corporation (630 km from Almaty, coordinates: 46°82'54" N 74°95'43" E)*

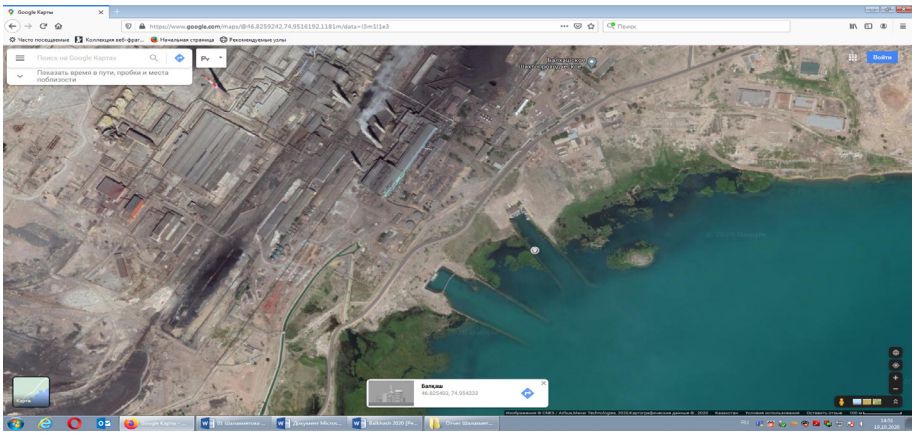


**Figure 4**
*Bertys Bay near the Balkhash copper smelter of Kazakhmys Corporation: water temperature = 24.4o C; pH = 8.67-8.72; mineralization 1062-1065 mg/l. The soil is sandy*



**Figure 5**
*Map of material collection in Bertys Bay, the city of Balkhash (635 km from Almaty, coordinates: 46°80'06" N 75°03'46" E).*

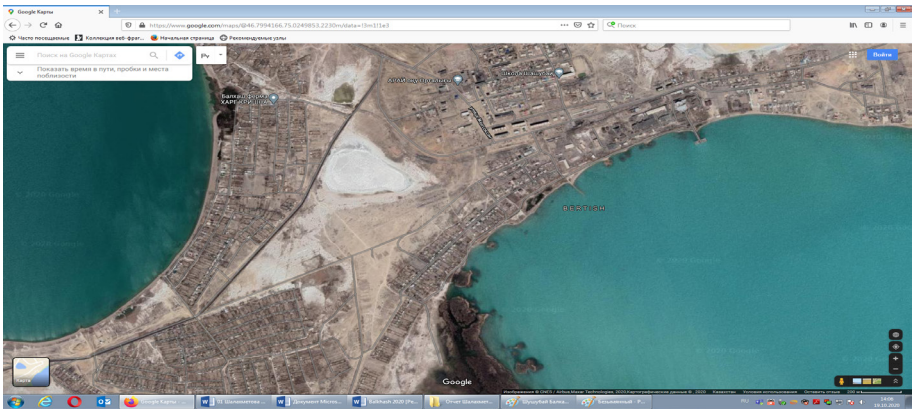


**Figure 6**
*Bertys Bay, Balkhash city: water t=24.4 oC; pH= 8.67-8.72; mineralization 1062-1065 mg/l. The soil is sandy*



***Water and sediment collection methods***

Surface water samples were collected, preserved, and stored at 30 control points in the northern part of Lake Balkhash in accordance with international standards and approved GOSTs (GOST 31861-2012, 2012; International Organization for Standardization, 1982). Water samples were collected at a depth of 0.5-1.0 m. Chemically resistant, clean 1-5 liter plastic containers were used as samplers. Water samples were stored and transported to the laboratory at a low temperature of 0°C in cooler bags. Before storage and preservation, algae were removed from the water through a fine-pored sieve. Before collecting water and sediment samples, a number of water parameters were determined using the following instruments: temperature, total mineralization (salts dissolved in water), and electrical conductivity were measured using a portable TDS&EC meter (Barry Century, China); pH was measured using a PH-009(I) portable pH meter (Barry Century, China); oxidation-reduction potential was measured using an ORP169E portable ORP meter (Barry Century, China); and dissolved oxygen content was measured using a DO-pen type portable DO meter (Alvin Instrument, China).

**Figure 7**
*Collection of material at control points (northern part of Lake Balkhash) by project participants*



At the same 30 survey points, also in accordance with international standards and approved GOSTs (Alam et al., 2001; De Smet et al., 2001), bottom sediments were sampled at a depth of 1 to 2 meters using grabs, grapnels, and stratimeters (tubes of various diameters). Depending on the pollutants analyzed and the properties of the bottom sediments, various preservatives (acids) were added to the samples before storage. The bottom sediment samples were stored refrigerated (from 0 to -3°C).

***Methods for chemical analysis of water, sediments, and fish organs for heavy metal content***

The most important indicator of the quality of the habitat of aquatic organisms is the purity of surface waters and bottom sediments for heavy metal content (Łuczyńska et al., 2018). Therefore, all collected water and sediment samples were analyzed for heavy metals in accordance with practical guidelines and GOST standards. Heavy metal content was determined after appropriate sample preparation using an MGA-915 MD atomic absorption spectrophotometer (St. Petersburg) (Bao et al., 2020; Chatta et al., 2016; Nishita et al., 2023).

In addition to chemical analysis of water and sediment samples, physicochemical analysis was performed on samples of various fish and amphibian tissues and organs (gills, lungs, muscles, and liver) brought back from the expedition. The collected fish and amphibian tissue and organ samples were stored in a freezer according to GOST 7631-85 after delivery from the expedition. Heavy metal content was determined in biological samples using atomic absorption spectrophotometry, using the above-mentioned instrument. To determine heavy metal concentrations, organ and tissue pieces were weighed and dried at room temperature. The organ pieces were placed in a fluoroplastic beaker containing a mixture of 2 ml of concentrated HNO3 and 1.5 ml of concentrated HClO4. The fluoroplastic beaker was then placed in a hermetically sealed steel beaker, which was then placed in a drying oven for 3

hours at 105°C. After cooling, the resulting solution was poured into a test tube, bringing the volume to 10 ml with distilled water (Chopra et al., 2020; Kumar et al., 2020).

High metal concentrations were measured using an MGA-915 MD atomic absorption spectrophotometer (St. Petersburg) in atomic absorption mode at wavelengths corresponding to each metal being studied.

The metal content was expressed as μg/g wet tissue using formula (1):

$$C = \gamma Vk/P \quad (1)$$

where C is the metal content; γ is the concentration; V is the solution volume, ml; k is the dilution factor, a multiple of 10; P is the tissue weight, g.

***Method for determining malondialdehyde content***

The thiobarbituric acid method (Abuid et al., 2021) was used to determine malondialdehyde content in the supernatant of animal liver homogenate. Tissue pieces were thawed for 5 minutes at room temperature, then placed in a cold 0.05 M Tris-HCl buffer (pH 7) supplemented with 0.1 M KCl and 0.9 mM EDTA. A 10% homogenate was then prepared using a Potter homogenizer. 0.5 ml of 0.1 M potassium phosphate buffer pH 7.4 was added to 2 ml of supernatant, and 1 ml of 30% trichloroacetic acid solution was added to the mixture, followed by thorough mixing. The samples were then centrifuged for 12 minutes at 4000 rpm.

To 2.0 ml of supernatant, 1.0 ml of 0.8% thiobarbituric acid solution was added, and the tubes were placed in a boiling water bath for 10 minutes. The samples were then cooled to room temperature, and their optical density was measured at 535 nm against a control sample (negative control) using an Apel PD-303UV digital spectrophotometer (Apel, Japan). The control sample was prepared similarly to the test samples, but with an equal volume of 0.1 M potassium phosphate buffer, pH 7.4, added instead of the biological material.

The malondialdehyde content was calculated using formula (2) and expressed as nmol per milligram of protein in the liver homogenate supernatant:

$$A/Cx16{,}83 \quad (2)$$

where A is the optical density of the test sample; C is the protein concentration of the liver homogenate supernatant (mg/ml).

***Superoxide dismutase activity determination method***

The method is based on the quantitative measurement of red formazan. It is formed by the reduction of nitroblue tetrazolium and superoxide radicals generated by xanthine oxidase. A 50% inhibition of this system corresponds to 1 arbitrary unit of enzyme activity (Coimbra et al., 2024).

SOD activity was determined by treating liver pieces with a 2:1 mixture of ethanol and chloroform cooled to 4°C. They were then triturated with a glass rod containing 600 mg of KH2PO4 for 15 minutes. The resulting homogenate was centrifuged at 12,000 g. Enzyme activity was determined in the supernatant. To do this, 10 μl of the supernatant was added to a 200 μl substrate solution containing xanthine and tetrazolium salt, and the reaction was initiated by adding 20 μl of xanthine oxidase. The reaction was incubated for 20 minutes in the dark at room temperature. The optical density of the samples was then measured using a Stat fax-2100 plate spectrophotometer at a wavelength of 450 nm.

SOD activity was calculated by determining the percentage inhibition (T%) of the nitroblue tetrazolium reduction reaction in the test sample. 50% inhibition of this system corresponds to 1 arbitrary unit of enzyme activity.

***Catalase activity determination method***

H. Luck's method (Cobanoglu, 2022) was used to determine catalase activity. The maximum absorption value of hydrogen peroxide solution is in the ultraviolet region of the spectrum at a wavelength of 230–240 nm. Under the influence of catalase, H2O2 decomposes, causing the optical density of the solution to decrease over time. This process occurs proportionally to the decrease in hydrogen peroxide concentration. Accounting for the time and difference in extinction changes allows one to calculate the catalase activity in the sample. For this purpose, 3 ml of a 1.25 x 10–2 M hydrogen peroxide solution in 1/15 M phosphate buffer was placed in a cuvette, and the reaction was initiated by adding 0.02 ml of previously centrifuged liver hemolysate to the solution. The time (t) required for the optical density of the test solution to decrease from 0.45 to 0.4 at a wavelength of 240 nm was then calculated. The amount of catalase enzyme that destroys half of the hydrogen peroxide in 100 seconds was taken as a conventional unit for calculations. The number of conventional units was recalculated taking into account the dilution per 1 g of liver.

***Statistical analysis***

Statistical analysis was performed using IBM SPSS 25 statistics (IBM Inc., Chicago, USA). The means and standard deviations of means of the data were calculated. Homogeneity of variance was tested using Levene's test, one-way analysis of variance and Tukey's HSD test were used to evaluate the significance of differences in multiple comparisons. The significance level was set at $p < 0.05$.

**Results and their discussion**

***Hydrochemical analysis of water from the northern part of Lake Balkhash***

The results of the chemical analysis of surface water collected during field trips to the northern part of Lake Balkhash in the Aktogay district of the Karaganda region are presented in Table 1. The following heavy metals were detected in the surface water samples: lead (Pb), chromium (Cr), cobalt (Co), manganese (Mn), nickel (Ni), cadmium (Cd), copper (Cu), zinc (Zn), and iron (Fe) in varying concentrations. The concentrations of heavy metals such as Mn, Cd, and Fe did not exceed the maximum permissible concentrations for fishery waters, but all other metals (Cu, Pb, Zn, Co, Ni, and Cr) exceeded sanitary standards by several times. Especially the excess of the content of these HM was observed in the waters of Lake Balkhash near the BKMK and the bay near the city of Balkhash, which is apparently associated with the influence of industrial and other enterprises. If, for example, the content of Zn, Cr, Ni, Pb, Cu and Co in the surface waters of the Torangalyk Bay, where there are no large industrial enterprises nearby, exceeded the MAC by, respectively: 1.1; 1.7; 1.9; 2.8; 3.7 and 7.9 times. In the surface waters of Lake Balkhash near the BMMC, the MAC for Zn, Cr, Ni, Pb, Cu and Co exceeded the sanitary level by, respectively: 3.6; 3.5; 3.2; 6.2; 13.6 and 8.2 times. From this numerical series it is evident that in the surface waters of Lake Balkhash near the BMMC the content of Cu is also significant and exceeds the MAC by 13.6 times. In the surface waters of Bertys Bay near the town of Balkhash, excess MACs for the above-mentioned heavy metals were also detected. Concentrations of Zn, Ni, Pb, Cu, and Co in Bertys Bay near the town of Balkhash exceeded sanitary standards for fishery waters by 2.9; 3.9; 4.4; 5.9; 10.5, and 6.9 times, respectively.

These results show that the MACs for heavy metals are exceeded in the following order: Cu > Co > Pb > Ni > Cr > Zn. A similar trend was observed in the bays and estuaries of Lake Balkhash (Zhambyl Region) in 2019. Apparently, the production activities of the Balkhash Mining and Metallurgical Plant have a negative impact on ecosystems in both the northern and western parts of Lake Balkhash. As is well known, emissions from the Balkhash Mining and Metallurgical Plant are a significant factor affecting the overall ecology of the Ili-Balkhash Basin. It has been established that since the early 1990s, the volume of emissions from Balkhash Non-Ferrous Metals Plant has amounted to 280,000–320,000 tons per year, while 76 tons of copper, 68 tons of zinc, and 66 tons of lead have settled on the bottom of Lake Balkhash (Ge et al., 2024). The volume of emitted pollutants nearly doubles each year. Harmful substances also enter the lake through groundwater, leaks in the tailings pond, and during dust storms, which are frequent in this region.

***Chemical analysis of sediments from the northern part of Lake Balkhash***

The results of the chemical analysis of bottom sediments collected during field trips to the northern part of Lake Balkhash (Aktogay district, Karaganda region) are presented in Table 2. It is evident that, compared to surface waters (Table 2), the content of all the studied heavy metals in the bottom sediments exceeds the maximum permissible concentrations. For example, in the bottom sediments of Torangalyk Bay, the heavy metal content increases in the order Cu > Zn > Co > Pb > Fe > Ni > Cr > Mn > Cd, which corresponds to exceeding the maximum permissible concentrations for soils by: 9.5; 6.3; 5.8; 5.5; 4.6; 4.0; 3.5; 1.2, and 1.1 times. In the bottom sediments of Lake Balkhash near the Bashkir Mining and Metallurgical Plant, the concentration of heavy metals significantly exceeded the MAC and pollution levels in the sediments of the Torangalyk Bay. Moreover, the excess of the MAC for heavy metals at the studied control points in the bay near the Bashkir Mining and Metallurgical Plant was as follows: Cu by 20.8 times, Zn by 18.6 times, Co by 15.7 times, Pb by 12.9 times, Fe by 11.9 times, Ni by 10.7 times, Cr by 8.5 times, Mn by 6.8 times, and Cd by 1.2 times. It is alarming that in the bottom sediments of Bertys Bay near the town of Balkhash, practically next to the town beach, the concentrations of the studied heavy metals also significantly exceeded the maximum permissible concentrations (MPCs): Cu by 14.6, Zn by 10.2, Co by 9.9, Pb by 8.9, Fe by 7.8, Ni by 5.9, Cr by 4.5, Mn by 3.8, and Cd by 1.1 times.

Thus, at all the points studied, the concentrations of heavy metals in the bottom sediments ex-

**Table 1**

*Content of heavy metals in surface waters of Lake Balkhash (Aktogay district, Karaganda region)*

| HM | Pb | Cr | Co | Mn | Ni | Cd | Cu | Zn | Fe |
|---|---|---|---|---|---|---|---|---|---|
| MPc, mg/L (for fishery waters) | 0,006 | 0,02 | 0,01 | 0,01 | 0,01 | 0,005 | 0,001 | 0,01 | 0,1 |
| Hazard class | II | III | III | IV | III | II | III | III | IV |
| Torangalik Bay | 0,0168 ± 0,0003 | 0,0344 ± 0,0008 | 0,0393± 0,0009 | 0,0044 ± 0,0009 | 0,0324 ± 0,0015 | 0,0018 ± 0,0005 | 0,0037 ± 0,0005 | 0,0112 ± 0,0009 | 0,0364 ± 0,0009 |
| > MPC | >2,80 | >1,70 | >3,93 | | >1,94 | | >3, 70 | >1,10 | |
| BGMK | 0,0375 ± 0,0015 | 0,0708 ± 0,0009 | 0,0823 ± 0,0011 | 0,0059 ± 0,0008 | 0,0325 ± 0,0008 | 0,0042± 0,0010 | 0,01364 ± 0,0009 | 0,0362 ± 0,0010 | 0,0645 ± 0,0012 |
| > MPC | >6,23 | >3,54 | >8,23 | | >3,25 | | >13,64 | >3,62 | |
| Bertys Bay | 0,0354 ± 0,0009 | 0,0392 ± 0,0009 | 0,0690 ± 0,0011 | 0,0060 ± 0,0012 | 0,00444 ± 0,0010 | 0,0039 ± 0,0007 | 0,01053 ± 0,0012 | 0,0294 ± 0,0010 | 0,0480 ± 0,0009 |
| > MPC | >5,90 | >3,92 | >6,90 | | >4,44 | | >10,53 | >2,94 | |

**Table 2**

*Content of heavy metals in bottom sediments of Lake Balkhash (Aktogay district, Karaganda region)*

| HM | Pb | Cr | Co | Mn | Ni | Cd | Cu | Zn | Fe |
|---|---|---|---|---|---|---|---|---|---|
| MPC, mg/kg (for soils) | 32,0 | 6,0 | 5,0 | 1500,0 | 4,0 | 0,5 | 3,0 | 23,0 | 1000,0 |
| Hazard class | II | III | III | IV | III | II | III | I | IV |
| Torangalik Bay | 176,26 ± 5,32 | 21,20 ± 1,32 | 29,24 ± 1,95 | 1800,20 ± 25,80 | 16,23 ± 1,34 | 0,55 ± 0,04 | 28,50 ± 1,98 | 149.22 ± 4,76 | 4624,5 ± 88,5 |
| > MPC | >5,5 | >3,5 | >5,8 | >1,2 | >4,0 | >1,1 | >9,5 | >6,3 | >4,6 |
| BGMK | 412,80 ± 5,99 | 51,20 ± 3,20 | 78,52 ± 3,42 | 10200,0 ± 120,4 | 42,44 ± 1,03 | 0,64 ± 0,05 | 68,44 ± 3,42 | 427,80 ± 8,26 | 11900,4 ± 118,6 |
| > MPC | >12,9 | >8,5 | >15,7 | > 6,8 | >10,7 | 1,2 | >20,8 | >18,6 | >11,9 |
| Bertys Bay | 284,82 ± 9,60 | 27,00 ± 2,50 | 49,55 ± 3,85 | 5700,3 ± 12,82 | 23,57 ± 1,43 | 0,55 ± 0,07 | 43,80 ± 2,50 | 234,40 ± 5,60 | 7800,4 ± 90,8 |
| > MPC | > 8,9 | >4.5 | >9,9 | > 3,8 | >5,9 | >1,1 | >14,6 | >10,2 | >7,8 |

ceeded the maximum permissible concentrations for soils. Particularly significant excesses were noted in the bay of Lake Balkhash near the Bashkir Mining and Metallurgical Plant (BMMC) facilities. Apparently, the observed excess of the MPCs for the studied metals in surface waters may be related not only to dust storms and smoke pollution from industrial enterprises, but also to the release of accumulated heavy metals from the bottom sediments via currents. The accumulation of heavy metals in water and sediments is known to pose a great danger to aquatic organisms and humans.

***Heavy metal content in fish organs and tissues from bays and a cove in the northern part of lake Balkhash***

As established before, the concentrations of a number of heavy metals exceeded the MAC in surface waters and bottom sediments in the northern part of Lake Balkhash (Aktogay District, Karaganda Region). Therefore, these metals were specifically determined in the organs and tissues of aquatic organisms (fish). The average content of the abovementioned heavy metals was determined in gills, muscles, and liver—organs that accumulate trace elements and pollutants to a greater extent. Since we were unable to catch bitterlings in the northern part of Lake Balkhash, heavy metal determinations were performed in the organs of commercial fish such as carp, asp, and roach. The results of determining the average HM content in fish tissues and organs are presented in Table 3. The table shows that the average Cu content in the organs of asp, roach, and carp exceeded the MAC standards by 3.2 to 6.9 times, especially in the bay of Lake Balkhash near the Bashkir Hydrometallurgical Plant and Bertys Bay near the city of Balkhash. In asp and carp, Cu accumulated most in the gills and liver and to a lesser extent in the muscles. In asp, roach, and carp, Cu accumulated most in the gills and liver and to a lesser extent in the muscles. In the studied organs of asp, roach, and carp, the excess of Co content ranged from 2.3 to 13.8 MAC. Moreover, like Cu, this HM accumulated most in the gills and liver, especially in fish from Lake Balkhash near the Bashkir Hydrometallurgical Plant and Bertys Bay (Balkhash). The average Co content in the organs of asp and roach from Lake Balkhash near the Bashkir Mining and Metallurgical Plant exceeded the permissible limits by 4.7 to 12.7 times, and in Bertys Bay (Balkhash City) by 4.0 to 9.2 times. Pb content in the gills and muscles of asp and roach from biotopes in the northern part of Lake Balkhash exceeded the MAC by 2.2 to 6.9 times, and in carp by 2.0 to 5.8 times. In the studied organs of asp, roach, and carp, Ni exceeded sanitary standards by 1.6 to 6.7 times, with the metal content being lowest in the muscles. The average Ni content in the gills and liver of asp, roach, and carp exceeded the MAC by 2.5 to 8.24 times. Thus, the highest accumulation of the studied HMs: Cu, Co, Pb, and Ni, was observed in the gills and liver of asp and carp from Torangalyk Bay, Bertys Bay (Balkhash Mountains), and Lake Balkhash (near the Bashkir Mineral Resources Complex). Roach had lower levels of these HMs in their gills and liver, but they still exceeded the maximum concentrations accepted by GOST (Table 3).

***MDA Content, SOD Activity, and Catalase Activity in the Liver of Juvenile Asp (Aspius aspius), Roach (Rutilus rutilus), and Carp (Cyprinus carpio) from Biotopes in the Northern Part of Lake Balkhash***

The results of a study of the liver biochemical parameters of juvenile commercial fish species from the northern part of Lake Balkhash (Aktogay District, Karaganda Region) are shown in Figures 8–10. Figure 8 shows that the livers of juvenile asp, roach, and carp from Torangalyk Bay had the lowest MDA content. The MDA content in the livers of juvenile asp from Bertys Bay (Balkhash City) was 1.5 times higher, while that of juvenile asp from Lake Balkhash (near the Bashkir Mining and Metallurgical Complex) was 2 times higher than that in the livers of juvenile asp from Torangalyk Bay. A similar increase in liver MDA levels was found in juvenile roach from the Bertys Bay (Balkhash Mountains) and Lake Balkhash (near the Bashkir Hydrometallurgical Complex), where this indicator was also 1.4 and 1.9 times higher, respectively. Liver MDA levels in juvenile carp from Torangalyk Bay were also the lowest, while those from Bertys Bay (Balkhash Mountains) and Lake Balkhash (near the Bashkir Hydrometallurgical Complex) were 1.4 and 2.1 times higher, respectively.

Biochemical analysis of liver samples also revealed decreased activity of antioxidant enzymes in juveniles of background fish species caught in Lake Balkhash (Karaganda Region). As Figure 9 shows, relatively high SOD activity was observed in the livers of juvenile asp, roach, and carp caught from Torangalyk Bay. In juvenile asp from Bertys Bay (Balkhash Mountains), SOD activity in the liver was reduced by 1.6 times compared to that in juveniles from Torangalyk Bay. SOD activity in juvenile asp from Lake Balkhash (near the Bashkir Hydrometal-

**Table 3**

*Content of heavy metals in organs and tissues of fish from Lake Balkhash (Aktogay district, Karaganda region)*

| Research points | View | *Aspius aspius* | | | | *Rutilus rutilus* | | | | *Cyprinus carpio* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HM | Cu | Co | Pb | Ni | Cu | Co | Pb | Ni | Cu | Co | Pb | Ni |
| | MPC mcg/g* | 10,0 | 0,5 | 1,0 | 0,5 | 10,0 | 0,5 | 1,0 | 0,5 | 10,0 | 0,5 | 1,0 | 0,5 |
| Torangalik Bay | gills | 48,40± 2,35 | 1,68± 0,23 | 2,65± 0,45 | 1,80± 0,05 | 38,43± 2,33 | 1,44± 0,12 | 3,67± 0,22 | 1,26± 0,09 | 45,34± 2,22 | 1,99± 0,08 | 1,99± 0,11 | 1,90± 0,09 |
| | muscle | 19,86± 0,08 | 1,23± 0,06 | 2,86± 0,16 | 1,52± 0,08 | 16,90± 1,12 | 1,16± 0,08 | 2,20± 0,11 | 1,12± 0,06 | 20,15± 2,15 | 1,28± 0,05 | 2,63± 0,16 | 0,82± 0,05 |
| | liver | 52,24± 4,66 | 2,80± 0,34 | 4,50± 0,32 | 1,90± 0,09 | 50,66± 2,26 | 2,43± 0,34 | 4,42± 0,25 | 1,48± 0,10 | 56,75± 5,55 | 2,99± 0,09 | 2,90± 0,11 | 2,52± 0,09 |
| BGMK | gills | 58,65± 6,40 | 3,62± 0,10 | 5,99± 0,18 | 2,90± 0,07 | 42,88± 2,39 | 2,86± 0,34 | 4,76± 0,19 | 2,55± 0,10 | 56,90± 4,50 | 3,98± 0,05 | 5,49± 0,18 | 2,98± 0,09 |
| | muscle | 36,20± 5,06 | 2,44± 0,18 | 3,90± 0,25 | 2,50± 0,16 | 30,42± 4,32 | 2,34± 0,09 | 2,99± 0,32 | 1,98± 0,07 | 36,24± 4,16 | 2,82± 0,09 | 3,86± 0,15 | 1.82± 0,04 |
| | Liver | 69,34± 5,32 | 6,35± 0,15 | 6,90± 0,50 | 3,34± 0,11 | 58,68± 6,05 | 4,95± 0,10 | 6,18± 0,56 | 2,65± 0,07 | 66,99± 4,32 | 6,29± 0,13 | 5,76± 0,23 | 4,12± 0,12 |
| Bertys Bay | gills | 54,88± 5,12 | 2,96± 0,08 | 4,65± 0,17 | 2,45± 0,12 | 40,65± 1,45 | 2,85± 0,09 | 3,56± 0,18 | 2,36± 0,09 | 50,09± 1,80 | 2,99± 0,08 | 4,40± 0,20 | 2,56± 0,06 |
| | muscle | 30,22± 2,60 | 2,00± 0,15 | 3,12± 0,15 | 2,16± 0,20 | 22,45± 1,65 | 2,25± 0,07 | 2,54± 0,26 | 1,45± 0,20 | 32,50± 1,95 | 2,05± 0,09 | 2,62± 0,14 | 1,78± 0,09 |
| | liver | 59,96± 3,22 | 4,59± 0,15 | 5,96± 0,21 | 2,65± 0,15 | 52,35± 3,55 | 3,28± 0,12 | 4,77± 0,19 | 2,11± 0,19 | 60,02± 4,32 | 3,89± 0,09 | 3,25± 0,15 | 3,08± 0,04 |

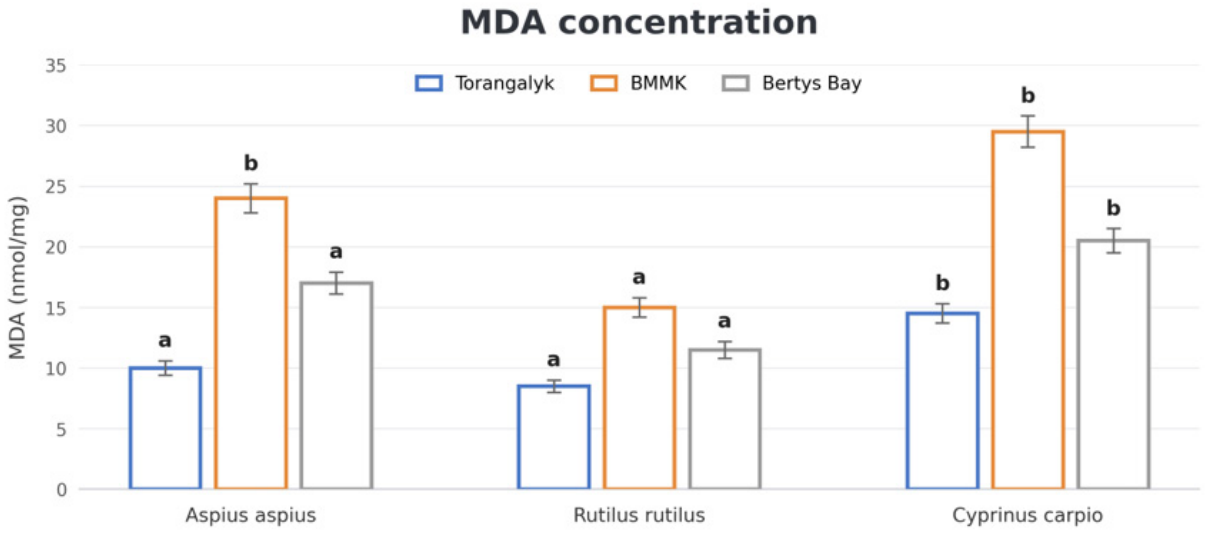
*Note: *MPV in raw fish*

lurgical Complex) was the most reduced – by 1.9 times. Among roach, the highest level of SOD activity was also recorded in juveniles from Torangalyk Bay. Compared to this biotope, SOD activity in juvenile roach from Bertys Bay (Balkhash Mountains) and Lake Balkhash (near the Bashkir Hydrometallurgical Complex) was 1.5 and 1.8 times lower, respectively. The most significant changes were observed in the liver of juvenile carp. A 1.7- and 2.2-fold decrease in SOD activity was noted in the liver of juvenile carp from Bertys Bay (Balkhash Mountains) and Lake Balkhash (near the Bashkir Hydrometallurgical Complex), respectively. A similar pattern was observed when studying catalase activity in the liver of the studied fish species. Figure 10 shows that juvenile fish from Torangalyk Bay demonstrated higher activity of this enzyme compared to the other two sites in Lake Balkhash (Karaganda Region). A 1.5-fold decrease in catalase activity was observed in juvenile asp from Bertys Bay (Balkhash City) compared to the enzyme activity in juveniles from Torangalyk Bay. Compared to the same site, the decrease in catalase activity in juvenile asp from Lake Balkhash (near the Bashkir Hydrometallurgical Complex) was 1.7 times. Catalase activity in the liver of juvenile roach from Bertys Bay (Balkhash City) and Lake Balkhash (near the Bashkir Hydrometallurgical Complex) was 1.4 and 1.5 times lower compared to the enzyme activity in juveniles from Torangalyk Bay, respectively. In juvenile carp from Bertys Bay (Balkhash Mountains) and Lake Balkhash (near the Bashkir Mining and Metallurgical Complex), suppressed catalase activity was also observed, 1.6- and 1.8-fold, respectively, compared to this value in the liver of juvenile carp from Torangalyk Bay.

Thus, biochemical analyses revealed that the livers of juvenile asp, roach, and carp caught in various biotopes in the northern part of Lake Balkhash (Karaganda Region), as well as juvenile commercial fish caught in the southwestern Balkhash region (Almaty and Zhambyl Regions), exhibited activation of lipid peroxidation processes, as evidenced by the accumulation of malondialdehyde, as well as suppression of antioxidant enzymes. This may indicate increased oxidative stress in the studied fish species under conditions of water and sediment pollution with heavy metals. Moreover, the most pronounced changes were observed in the liver of juvenile carp.

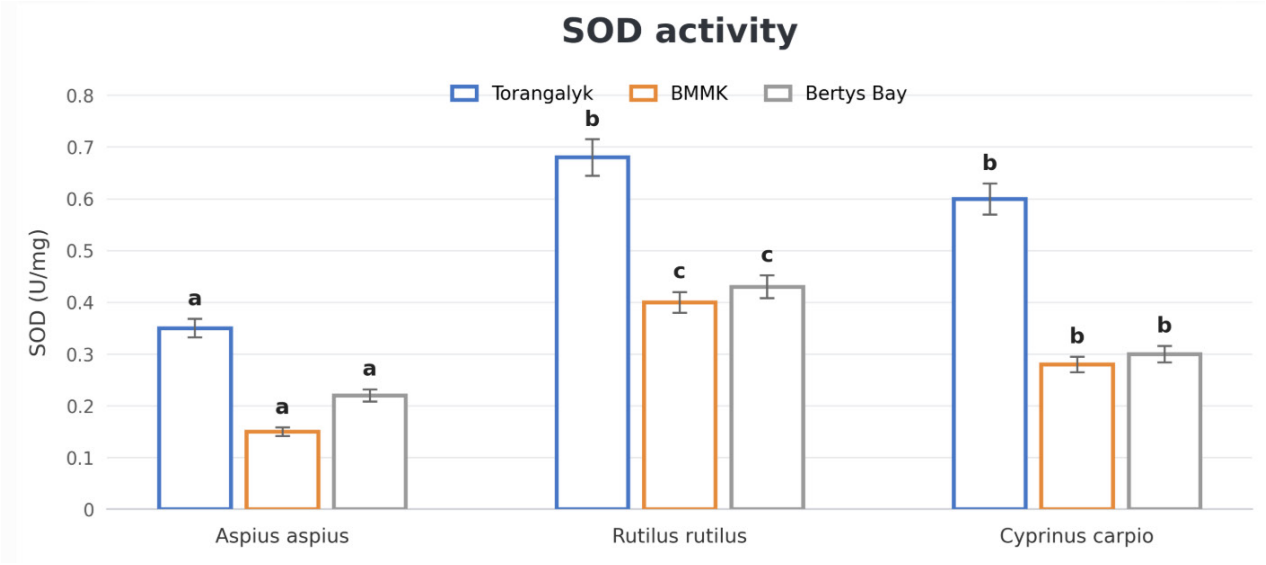
**Figure 8**

*MDA content in the liver of juvenile asp (Aspius aspius), roach (Rutilus rutilus) and carp (Cyprinus carpio) from biotopes of the northern part of Lake Balkhash (Karaganda region)*



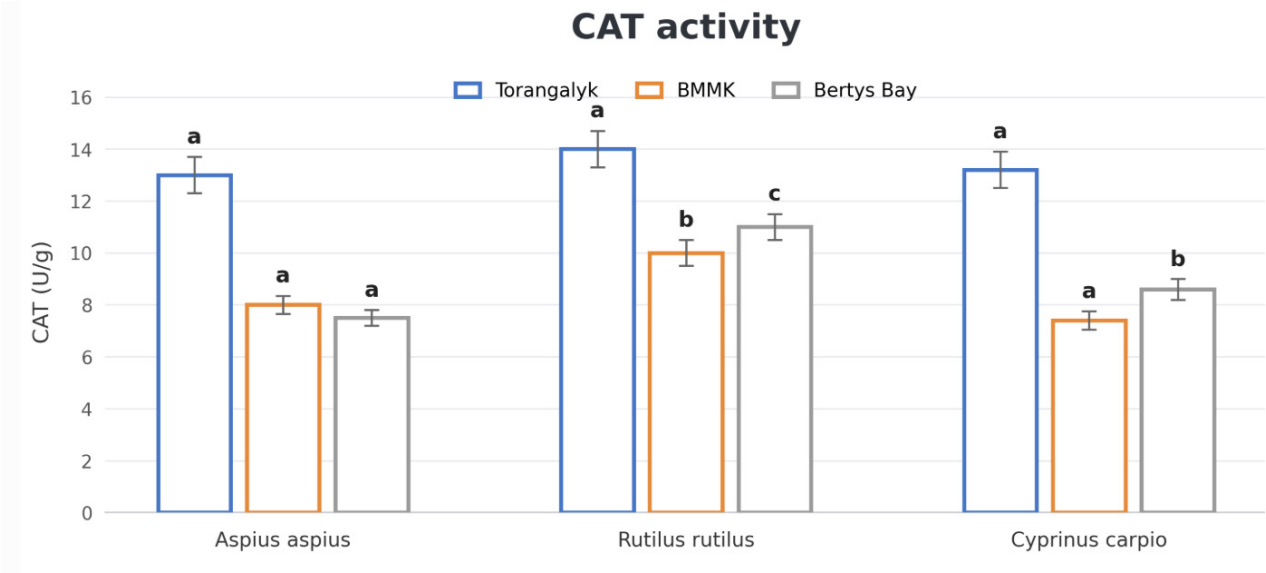

*Tukey's HSD test was used to evaluate the significance of differences in multiple comparisons. The significance level was set at $p < 0.05$*

**Figure 9**
*SOD activity in the liver of juvenile asp (Aspius aspius), roach (Rutilus rutilus) and carp (Cyprinus carpio) from biotopes of the northern part of Lake Balkhash (Karaganda region)*




*Tukey's HSD test was used to evaluate the significance of differences in multiple comparisons. The significance level was set at $p < 0.05$*

**Figure 10**
*Catalase activity in the liver of juvenile asp (Aspius aspius), roach (Rutilus rutilus) and carp (Cyprinus carpio) from biotopes of the northern part of Lake Balkhash (Karaganda region)*




*Tukey's HSD test was used to evaluate the significance of differences in multiple comparisons. The significance level was set at $p < 0.0$*

The results of the present study demonstrate a high level of anthropogenic pressure on the northern part of Lake Balkhash, as evidenced by elevated concentrations of heavy metals in surface waters, bottom sediments, and fish tissues of vital organs. The most pronounced contamination was recorded in areas adjacent to the Balkhash Mining and Metallurgical Complex, as well as in Bertys Bay near the city of Balkhash, confirming the dominant role of industrial activity as the primary source of metal inputs into the regional aquatic ecosystem. The observed pattern of heavy metal distribution in surface waters (Cu > Co > Pb > Ni > Cr > Zn) is consistent with previous investigations conducted within the Ili–Balkhash basin, which identified copper smelting and mining enterprises as the main contributors to environmental contamination (Abd El-Ghany et al., 2025). Although the concentrations of certain metals in the water column did not consistently exceed maximum permissible limits, their substantial accumulation in bottom sediments indicates chronic environmental contamination and long-term retention of pollutants in the aquatic ecosystem and in aquatic organisms (Zhang et al., 2026). Bottom sediments act both as sinks and secondary sources of heavy metals, releasing them back into the water column under changing physicochemical conditions and thereby increasing their bioavailability to aquatic organisms (Singh et al., 2022). Analysis of fish tissues revealed considerable bioaccumulation of Cu, Co, Pb, and Ni, with the highest concentrations detected in the gills and liver. This distribution reflects the physiological roles of these organs: gills are directly exposed to dissolved contaminants, whereas the liver serves as the primary organ for detoxification and metal sequestration (Gashkina, 2024). Lower metal concentrations in muscle tissue agree with findings from other freshwater ecosystems; however, even these levels exceeded sanitary and hygienic standards in some cases, posing a potential risk to human health through fish consumption (Abass, 2024). Biochemical responses in fish liver provided further evidence of toxic stress induced by heavy metal exposure. Elevated malondialdehyde levels indicate enhanced lipid peroxidation and membrane damage, while reduced activities of superoxide dismutase and catalase suggest depletion or inhibition of the antioxidant defense system. Such oxidative imbalance is a well-documented mechanism of heavy metal toxicity and may disrupt metabolic processes, growth, and survival of fish (Petrova et al., 2025). Among the studied species, the most pronounced biochemical alterations were observed in common carp, which may be attributed to species-specific differences in metal uptake, metabolic capacity, and habitat preferences (El-Bahy et al., 2025).

Overall, the combined chemical and biochemical evidence indicate that heavy metal contamination in the northern part of Lake Balkhash has reached levels capable of inducing sublethal physiological stress in fish populations. Chronic oxidative and genotoxic stress may lead to deterioration of fish health, reduced reproductive potential, and loss of biodiversity, which is consistent with the long-term decline of native and commercially valuable fish species observed in the lake over recent decades. These findings emphasize the urgent need for enhanced environmental monitoring, stricter regulation of industrial emissions, and the implementation of remediation measures to preserve the ecological integrity of Lake Balkhash and ensure the sustainable use of its aquatic resources.

## Conclusion

As a result of the study, samples of surface water and sediments, eggs, juveniles, and adults of common fish species were collected and analyzed in Torangalyk, Lake Balkhash near the Bashkir Mining and Metallurgical Combine (BMMC), and Bertys Bay near the city of Balkhash. Bioassay of the main pollutants (heavy metals) in Lake Balkhash (Karaganda Region) revealed that surface waters exceeded the MAC for fishery waters in the following order: Cu > Co > Pb > Ni > Cr > Zn, and sediments exceeded the MAC for Cu > Zn > Co > Pb > Fe > Ni > Cr > Mn > Cd. Moreover, near the BMMC, both water and sediments showed the highest concentrations. Bioassay of polluted waters and sediments revealed their embryotoxic effects in the following order: Lake Balkhash near the BMMC < Bertys Bay < Torangalyk Bay. Significant accumulations and excesses of MAC levels for Cu, Co, Pb, and Ni were detected in the gills and liver of asp and carp from Lake Balkhash near the Bashkir Mining and Metallurgical Plant (BMMC) and Bertys Bay. These HMs accumulated more in the muscles and liver of the fish, with a greater concentration in the marsh frog. These accumulations are due to the activation of lipid peroxidation processes and a decrease in the activity of antioxidant enzymes. The decrease and appearance of reactive radicals may likely be the cause of their death. Cytogenetic analysis revealed increased chromosomal instability in fish from impacted sites (Bertys Bay and BMMC) compared to

the control site (Torangalyk Bay). The frequency of aberrant cells and chromatid-type aberrations was significantly higher in affected populations, and polyploid cells were occasionally observed. These findings indicate genotoxic stress associated with heavy metal contamination, complementing the observed biochemical and toxicological effects.

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**Ethics Statement** This is a review study. No ethical approval is required.

**Conflicts of Interest** The author declares no conflicts of interest.

**Data Availability** Statement Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

***Author contributions***

*Tlenshieva A.: conceived of the presented idea, developed the theory, performed the computations. Kozhakhmetova D. and Checimbayeva D.:verified the analytical methods. All authors discussed the results and contributed to the final manuscript.*

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