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Ecological characteristics of the area and evaluation of bio indicator species condition in Altyn-Emel State National Natural Park

To assess the degree of contamination and to preserve the natural state of the Altyn-Emel State National Park (Kazakhstan), the monitoring studies were conducted. The total amount of persistent organic pollutants and heavy metals in water and soil samples was determined. Radiodosimetric research, ecological and genetic monitoring of indicator groups of animals were carried out. Chemical analysis revealed the presence of some pollutants exceeding the maximum permissible concentrations in water and soil samples. No area radioactive contamination was detected within the boundaries of the park zone, and the EDR values in general do not exceed the normative values. The exception is the local area of the "Suluchekinskoye" uranium deposit, for which EDR values above the normative values were determined. A comparative analysis of indicator animals showed that their cytogenetic homeostasis, morpho-functional indicators, habitus, and ecological characteristics are matched to the normal indexes of animals living in Kazakhstan. Thus, monitoring studies show that the ecological situation in the "Altyn-Emel" National Park is satisfactory, with some tense circumstances that require constant monitoring.

Keywords: cytogenetic analysis, genetic monitoring, genotoxicity, heavy metals, micronucleus test, morpho-functional indicators, persistent organic pollutants, species diversity.

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Introduction

The problem of pollution with POPs, heavy metals and radionuclides has recently become particularly acute among global environmental threats. These compounds are difficult to degrade and may be transported over long distances through air and water. Insects, birds, migrating warm-blooded animals can accumulate such toxicants. Moreover, they may be not only a place of accumulation but also a means of transporting these substances. As a result, conditions are formed that contribute to the destruction of biocenoses not only in areas of contamination with genotoxicants, but also in areas where they were not used. In this regard, remote or protected areas that are not directly affected by anthropogenic factors may also be contaminated [1]. Even in low doses, this contamination poses a threat to nature and humans [2].

The diversity of potential genotoxicants and their mechanisms of action on the genome, as well as interspecies differences in sensitivity to genotoxic agents, complicate the development of adequate approaches to their monitoring and testing system, as no single genetic test can detect all genotoxic effects.

Modern ecology is increasingly focusing on the use of bio indicator species for diagnosing the state of environment. The advantage of using bio indicators for the integral evaluation of biosystems of different levels of complexity is that they respond not only to individual pollutants, but to the whole complex of influencing substances by certain reactions of the organism as a whole [3]. Bio indication makes it possible to assess the degree and intensity of the impact of pollutants and reflects the dynamics of ecosystem degradation in an integral form. At the same time, cytogenetic homeostasis [4] is one of the indicators of anthropogenic factors that generate significant changes in genotype, organism condition and ecosystem as a whole. It makes it possible to detect early changes in functional systems of an organism, when there are no visible (phenotypic) manifestations of these changes yet, and to predict further state of the system under changing conditions. It can be characterized by the micronucleus test, which consists in counting the frequency of cells with micronuclei [5, 6] and other cytological disorders of blood cells.

In the Republic of Kazakhstan territories with different types and sources of environmental pollution (radiation — natural and anthropogenic, chemical-heavy metals, and persistent organic pollutants) are widely represented. It should also be noted that these territories may differ from each other by natural landscape characteristics. For monitoring studies, the problem of choosing a control area or a comparison area is acute. Considering that the assessment of the status of a monitoring area may vary depending on the selected area of comparison and not only on anthropogenic impacts, defining baseline levels of relevant criteria for specific sites is important [7]. Therefore, a natural undisturbed area with a combination of various relatively close physical-geographical and climatic features with similar flora and fauna and indicator groups is required as a reference region. Such an area combining different natural zones is the State National Nature Park (SNNP) “Altyn-Emel” (Kerbulak district, Almaty region).

The peculiarity of Altyn-Emel is that on its modern territory in the Ili intermountain hollow within the Ili uranium ore province in 1969-1980 years of the 20th century, a large strata-infiltration uranium deposit, Suluchekinskoye, was explored. Here, an area-wide exploration of uranium reserves in the Ili ore province was carried out based on the Geological Exploration Camp (GEC). At the end of the 1980s, pilot uranium production by the in-situ borehole leaching (ISL) method was carried out at the Suluchekinskoye deposit. By the end of the 1980s, this production and the activity of GEC had been stopped completely. Reclamation was carried out at the geotechnological polygon, and the wells were liquidated [8]. No negative impact on the environment, according to specialists from “Volkovgeology” opinion, was established [9]. Suluchekinskoye and all other uranium deposits of the Ili uranium province were transferred to the long-term reserve of the mining industry of Kazakhstan [8]. However, a number of studies showed that local radioactive contamination may develop over time in areas where exploration and short-term exploitation of uranium deposits and ore occurrences have been carried out. This may occur for various reasons of natural and anthropogenic origin [10]. A lot of time has passed since the conservation and liquidation of the pilot mine (PSV) at the Suluchekinsky deposit. In addition, the economic purpose of these lands and territories has changed. The previously mothballed deposit turned out to be within the boundaries of the specially protected natural area of the SNNP “Altyn-Emel”, which has now changed its boundaries (park expansion, 2019) and status into a new form of existence — the Biosphere Reserve.

In this regard, studies of the degree of water and soil pollution with persistent organic pollutants (POPs) and heavy metals, as well as a radiodosimetric study of the territory were carried out in the Altyn-Emel State National Natural Park. To assess ecological well-being, cytogenetic, and ecological-faunistic studies of indicator groups of animals (fish, amphibians, birds, mouse-like rodents) were carried out. Such an approach makes it possible to determine the degree of the impact of negative environmental factors in general and the uranium deposit in particular, on the environmental situation and animals of the territory.

Experimental

Subjects of study

Water and soil samples from “Altyn-Emel” SNNP were the material for the studies. The most common natural populations of animals found in other territories of the Almaty region were used as indicator objects from the habitat of “Altyn-Emel” SNNP. They included fish (*Triplophysa strauchii*, *Pseudorasbora parva*, *Triplophysa dorsalis*, *Triplophysa stoliczkai*), amphibians (*Pelophylax ridibundus*, *Bufotes viridis*), reptiles (*Eremias velox*), birds (*Columba livia*) and rodents (*Microtus arvalis*, *Mus musculus*, *Apodemus sylvaticus*, *Cricetulus migratorius*).

Separate territories of the Almaty region Talgar (Belbulak, Enbekshi), Enbekshi Kazakh (Taukaraturyk) districts, Alakol Lake with similar natural and climatic conditions without special anthropogenic stress were used as comparison regions.

Water, soil sampling and chemical analysis

Water sampling was conducted in accordance with State Standard 17.1.5.05-85 “General Requirements for Surface and Marine Water Sampling” in spring. Soil sampling was conducted according to State Standard 14.4.4.0284, State Standard 29269-91 using envelope method from soil horizons 1 and 2 (0-5 and 5-25 cm deep).

Quantitative analysis of water and soil samples for the content of POPs and their decay products (24 names: Hexachlorobenzene; α,β,γ,δ – isomers of hexachlorocyclohexane; heptachlor; aldrin; heptachloropoxide; chlordane; endosulfan 1, 2; 2,4'-DDE; deldrin; DDT; 4,4'-DDE; DDD; chlorobenzylate; endrin; endrin aldehyde; endosulfanesulfate; dibutylendan; methoxychlor; hexabromobenzene) was carried out. Also the content of 8 heavy metals: Zn, As, Cd, Pb, Cu, Co, Ni, Cr was determined in all samples. The chemical reagents used for assessing the content of the substances under investigation had a purity grade of “pure for analysis”. The methods, measuring and testing equipment were certified. To determine POPs in soil and water samples, gas chromatography method (Agilent 6890N chromatograph with MSD 5975C (USA), Fluorate — 02 liquid analyser) and mass spectrometry (ACME 9000 HPLC with UV/VIS Detector) were used. Determination of heavy metals was carried out by atomic absorption method [11–14].

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Radiodosimetry of the area

Radiometric survey of the surveyed areas was carried out in accordance with the recommendations set out in the regulatory documents and guidelines for the use of SRP-88H and RKS-01-SOLO instruments. A pedestrian search and detailed γ- survey and local β- and α-indicative survey of the monitoring territories were carried out using radiometers. γ-radiation measurements were carried out at a height of 1m to 0.1m from the surface, while α- and β-radiation were measured contractually.

Collection of fauna survey objects

Expeditions to collect materials in "Altyn-Emel" SNNP and comparable separate territories of the Almaty region (Taukaraturyk, Enbekshy, Amangeldy, Belbulak) were conducted from April to September 2019-2020. During the expeditions, traditional methods of field ecological-zoological research were used. Diagnostics of the main vertebrate groups (fish, amphibians, reptiles, birds, mammals) are based on the study of a set of ecological, morphobiological, and genetic indicators. The desktop study of indicator mammal species combines various methodological techniques, which include the determination of morpho-functional characteristics for comparative ecological characterization of the surveyed areas. Determination of morpho-functional characteristics was carried out according to the scheme of metric (exterior) indicators of animals: p – weight (g), l – length of body, c – tail, a – height of ear, pl – length of foot, (all in mm).

Physiological features were determined by (interior) indices of internal organs: il – liver, ih – heart, ik – kidneys, is – spleen. All indices were expressed in ppm – ‰. Comparative analysis of field and faunistic characteristics was performed according to the summary "Mammals of Kazakhstan" [15–17].

Micronucleus test of indicator animals

Blood sampling and preparation of smears were performed in the field according to suggested recommendations [18]. Dried peripheral blood smears were fixed in 96 % ethyl alcohol for 30 minutes. They were dried and stained with 10 % Romanowsky-Giemsa solution for 5 minutes [18]. Micronuclei frequencies were counted on a Zeiss Axioscop 40 microscope under oil immersion and magnification of 10x100. Photodocumentation was made for the most characteristic abnormalities of erythrocytes. Depending on the subject, 10,000 to 20,000 erythrocytes from each examined individual were examined.

Results and Discussion

Determination of POPs in water and soil samples at "Altyn-Emel" SNNP

Fig. 1 demonstrates the total content of 24 POPs and 8 HMs in natural water and soil samples taken in the territory of "Altyn-Emel" SNNP and compared territories. When examining the soil samples, it was found that the soil has poly-component contamination.

The main POPs pollutants in soil and water are pesticides. Soil samples from "Altyn-Emel" SNNP revealed presence of trace amounts of DDT and its metabolites DDE and 4,4-DDE, β HCCH, as well as aldrin and deldrin, exceeding MPC. The spectrum of detected POPs in the comparison territories was much wider. In addition, in each of the comparison territories, from 3 to 9 types of POPs, exceeding the MPC, were identified. In the natural water of "Altyn-Emel" SNNP, dibutylendan and δ-HCG were detected in trace amounts, in contrast to Belbulak and Taukaraturuk territories, which have a significant spectrum and amount of POPs. Thus, to the level of soil pollution by persistent organic pollutants the territory of the National Park is much more environmentally favorable.

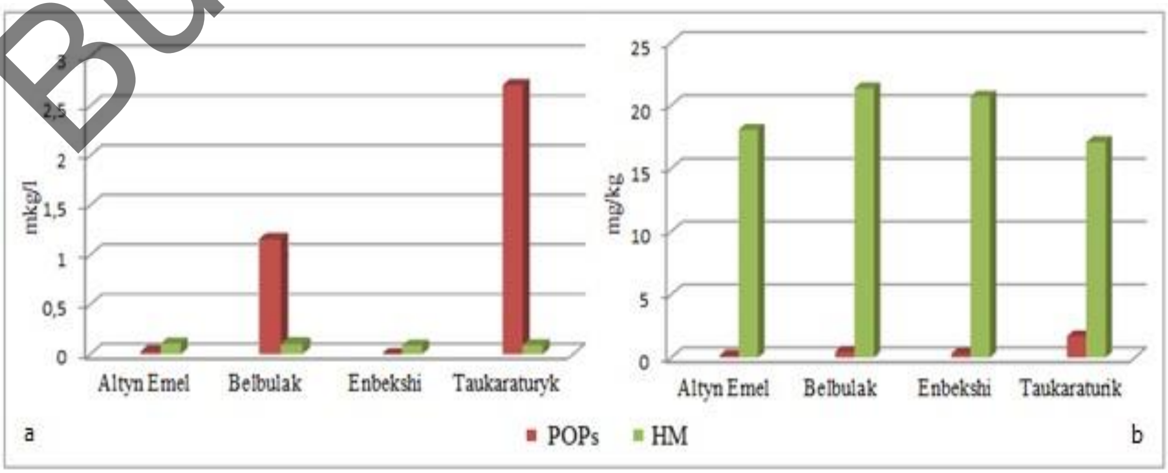

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Figure 1. Total POP and PM amounts in water (A) and soil (B) samples in “Altyn-Emel” SNNP

A different picture is observed for the detection of metals in water and soil: their total amount in the “Altyn-Emel” SNNP is approximately the same as in the regions of comparison. The presence of heavy metals such as Ni, Cd and Zn was detected in the soil of the National Park “Altyn-Emel”, with the nickel content in different samples being almost equal to or slightly exceeding the MPC. In addition to nickel, zinc, cadmium and copper exceed the MPC in the territories under comparison. The natural water contains zinc, nickel, copper and chromium in insignificant concentrations.

Radiodosimetry of the area

At present monitoring of surface layer radioactive contamination of the atmosphere on the territory of the Almaty region, which includes the territory of SNNP “Altyn-Emel”, is carried out by specialized subdivisions, “Kazgidromet” at 5 meteorological stations (Almaty, Narynkol, Zharkent, Lepsy, Taldykorgan). According to the results of the works, the average values of radiation γ-background layer of the atmosphere in the Almaty region for the settlements of the region are within 0.12-0.23 μSv/h. On average in the region, the radiation gamma background is 0.17 μSv/h, which does not exceed the natural background. Average daily density of radioactive fallout in the surface layer of the atmosphere on the territory of the region ranges from 0.7-1.8 Bq/m^2. The average deposition density in the region is 1.2 Bq/m^2, which does not exceed the maximum permissible level [19]. Measurement of beta activity, which is mainly associated with radioisotopes of man-made origin, showed permissible values of daily radioactive fallout, in accordance with approved hygienic standards [20] and does not exceed 110 Bq/m^2 a day. Analysis of available materials on the radioecological situation in the Almaty region and, specifically, in Talgar and Kerbulak districts, is safe and the accepted characteristics of equivalent dose rate (EDR) and radioactive contamination of the land surface are within normal limits. However, the radio-ecological monitoring carried out in this way cannot always characterize such large areas in detail and unambiguously. Therefore, a detailed assessment of the radiodosimetric situation in the territory of SNNP “Altyn-Emel” was carried out (Tab. 1).

Table 1

Selected EDR and flux densities — α particles on the territory of "Altyn-Emel" SNNP

Radiodosimetry areas	Min (μSv/h), (parts/min per cm^2)		Max (μSv/h), (parts/min per cm^2)	
	EDR	α	EDR	α
SNNP “Altyn-Emel”, former Geological Exploration Village	0.095	<0.2	0.156	1.23
SNNP “Altyn-Emel”, “Kalkan” Seismic Station	0.110	0.61	0.200	1.28
SNNP “Altyn-Emel”, “Kalkan” Seismic Station well	**0.462**	0.61	**4.158**	1.21
SNNP “Altyn-Emel”, Barkhan, former Geological Exploration v.	0.098	0.61	0.112	1.21
SNNP “Altyn-Emel”, water reservoir	0.120	<0.2	0.180	1.28
SNNP “Altyn-Emel”, Bashy village	0.094	<0.2	0.105	2.42
Belbulak	0.100	0.61	0.120	2.42
Taukaraturyk	0.12	<0.2	0.27	1.16

The results indicate that all areas surveyed are characterized by low EDR values. The range of minimum values is about 0.09 — 0.12 μSv/h, and rarely occurring maximum values are 0.15 — 0.27 μSv/h. The detected values of alpha particle flux density are also not high — in the range of minimum 0.11-0.61 parts/min per cm^2, and in the maximum 1.16 — 2.86 parts/min per cm^2. In general, the detected values of radiodosimetric indicators comply with the accepted sanitary standards of the Republic of Kazakhstan [20, 21]. The results of the measurements indicate that there are no natural radioactive anomalies in the Enbekshikazakh, Talgar, and Kerbulak districts. The relatively safe radio-ecological condition in the Almaty region as a whole does not exclude the probability of local radioactive impact of the former mine on environmental objects, such as soils, flora and fauna, natural waters, the surface layer of the atmosphere of SNNP “Altyn-Emel”. This determines the relevance of studying the current radiation situation in the territory of the National Park and carrying out research on morpho-functional characteristics, development features and genetic status of living indicator animals.

The surveyed territories are not located in the influence zones of uranium deposits of the Almaty region. The exception is the local area of “Altyn-Emel” SNNP within the boundaries of “Kalkan” seismic station,

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where the Suluchekinskoye uranium deposit of formation-infiltration type is located. Here, in a limited area of 7-9 m^2, in the contour of a self-discharging well, EDR values in the range of 0.46-4.16 μSv/h — (2019) and 0.26-2.99 μSv/h (2020) are set, which noticeably exceeds permissible values (calculated dose for population is not more than 0.57 μSv/h) [20, 21]. The small area of the anomalous zone in terms of the EDR level may not have a significant impact on the environmental objects on the territory of SNNP “Altyn-Emel”.

Analysis of the indicator animals of “Altyn-Emel” SNNP

About 400 species of fish, amphibians, reptiles, birds, and mammals, including 42 Red Data Book species currently inhabit the national park [22]. In addition, there are dozens of invertebrate species that are also classed as “Red Data Book”. However, their number is still unknown and needs to be studied.

To indicate the status of vertebrate fauna inhabiting the territory of “Altyn-Emel” SNNP and the comparison territories, the most typical for the survey sites species, as a rule, belonging to synanthropic group, were selected. The following species and groups of animals were identified according to ecological and faunistic features: fish (*Diptychus dybowskii*, *Triplophysa strauchi*, *Triplophysa dorsalis*, *Pseudorasbora parva*); amphibians (*Pelophylax ridibundus*, *Bufotes viridis*); reptiles (*Eremias velox*); birds (*Columba livia*); mammals, rodent group (*Cricetulus migratorius, Mus musculus, Apodemus sylvaticus, Microtus arvalis* sp). Thus, a total of 11 indicator species of vertebrates inhabiting the territory of “Altyn-Emel” SNNP were used in the study. Morpho-functional indices of animals of each indicator species were determined, which represent a set of characteristics of individuals caught in the summer season, and which lived in places relatively distant from each other.

Fish fauna

The aquatic environment is one of the main living environments, and water itself is the main component of biosystems. Fish are considered the most appropriate subject for screening for mutagenic and carcinogenic chemical compounds in water, since they metabolize, accumulate in the body the chemicals contained in the water and react to toxic compounds in a similar way to higher vertebrates [23, 24]. The use of fish in laboratory and natural habitats allows the genotoxicity of a wide range of pollutants to be assessed, including the effects of individual chemical compounds as well as cumulative effects [25–27].The fish fauna study was conducted for individuals from two locations in “Altyn-Emel” National Park. These are the Aktobe River watercourse from a reservoir located between the settlements of Nurum and Aktobe and a water body (of spring origin) of Kosbastau cordon within the boundaries of a former geological prospecting settlement, where an elevated radiation background has been identified. As the potential indicator species, 4 native (*Triplophysa strauchii*, *Triplophysa dorsalis*, *Triplophysa stoliczkai*, and *Diptychus dybowski*) and 1 non-native (*Pseudorasbora parva*) fish species were found. During the morpho-biological study of the species of this indicator group, it was found that the parameters of *Pseudorasbora parva* are within the limits of variability known for the Balkhash basin. The maximum sizes of fish in the studied 2019 sample were smaller than those known for this species. This may indicate unfavorable conditions for prolonged life span. This is also indicated by the significant within-sample variability in the fatness coefficients.

A sample of *Triplophysa strauchii* from the watercourse of the Aktobe River is represented by individuals of different sizes — both adults with developed gonads and fingerlings, which indicate favorable reproductive conditions for this species. In April, gonads were at different stages of maturity: mature and ready-to-hatch sex products, as well as spawned specimens were found. Biological and morphological parameters were within the normal range of this species, with only one individual having noticeably shorter front antennae. This abnormality may be caused by trauma or abnormalities in the ontogenetic trajectory. Phenodeviates were not detected in the sample studied.

In the water of “Kosbastau” cordon, where elevated radiation background was detected, the sample of *Pseudorasbora parva* is small and represented mainly by small specimens. Comparison of data on the composition of fish fauna and the status of indicators of individual species suggests insignificant anthropogenic transformation of the ecosystem of this water body. Habitat conditions in general do not impede reproduction of this species, but longevity and maximum size of fish are far from species limits, as representatives of native fish fauna dominate in number, represented by different-sized individuals, which morphologically do not deviate from the species norm.

As shown by the analysis of materials (Fig. 2), in general, the fish in these areas have normal indicators of condition and development [28, 29]. In plain shallow water reservoirs near Enbekshi settlement (comparison region) *Pseudorasbora parva* indicators were analyzed among indicator fish. Their relatively low abundance is shown. The morpho-biological analysis revealed small size of these fish, which is noticeably smaller than known for these species, low fatness factor and color defect in some specimens.

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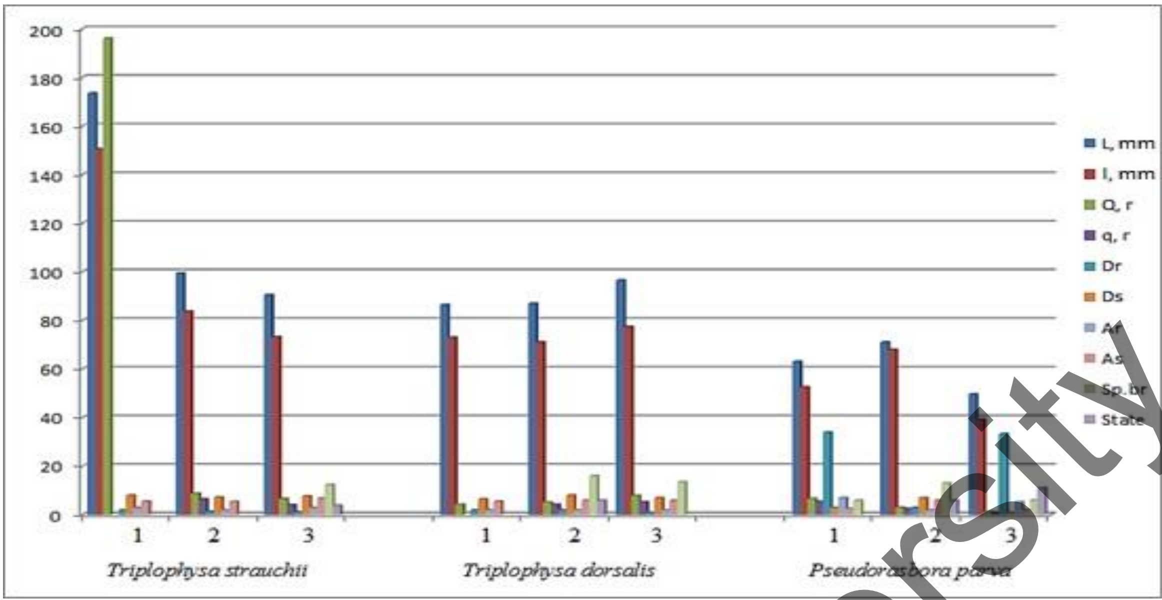

Figure 2. Morpho-biological indicators of the development of indicator fish species from the “Altyn-Emel” SNNP: 1 — average indicators of normal development; 2 — development indicators of fish from Aktobe river; 3 — development indicators of fish from “Kalkan” seismic station reservoirs

Amphibians

Amphibians meet all the requirements for the species used for bio indication, and as their main representative, the marsh frog (*Rana ridibunda*) is a widespread amphibian species that has clear and easy-to-study characteristics, is highly sensitive to pollutants and mutagens. The morpho-physiological parameters of the amphibian organism reflect the state of the local habitat since they do not have a pronounced tendency to migrate. The marsh frogs are characterized by a high level of polymorphism — they have a number of non-specific and specific responses of the population to anthropogenic impact (variability of morpho-physiological parameters, an increase in the frequency of aberrant cells and structural mutations of chromosomes) — all these factors make it possible to successfully use *R. ridibunda* as a bio indicator species for monitoring environmental pollution by mutagens [30–34].

The morphometric parameters of the mouth apparatus structure in tadpoles of *Bufotes viridis* complex caught in two reservoirs in the territory of the hydraulic fracturing, near the mothballed uranium deposit were studied (Tab. 2). The analysis of the obtained material showed the absence of any deviations in the morphology of tadpoles, except for variations in the structure of the mouth apparatus. 27.6 % of tadpoles had anomalies of the oral apparatus, which were expressed in ruptures, partial reduction, fusion, curvature and deformation of the tooth rows. In a number of cases the oral apparatus had not one type of abnormality, but a combination of two or more. For comparison, tadpoles collected in 2013 from the Akeshke reservoir (Almaty region, Yeskeldy district) showed 6.7 % anomalies in the morphology of the oral apparatus. It is likely that these abnormalities are associated with elevated radiation levels within the boundaries of the “Kalkan” seismic station.

Table 2

Deviations in the structure of the mouth apparatus of *Bufotes viridis* tadpoles from the reservoir of SNNP “Altyn-Emel”

Criterion	Water reservoir wells seismic station "Kalkan"	Reservoir Akeshke
Tadpoles examined	108	30
Frequency of tadpoles with mouth apparatus anomalies	24.1 %	6.67 %
Total anomalies	44.4 %	10 %

Ornithofauna

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Birds are a sensitive indicator of the impact of various factors. Pesticides may be part of the reason for the decline in bird numbers [35]. There were no anomalies in the development and coloration of synanthropic avifauna species from the "Altyn-Emel" SNNP during the period of observation. Visual inspection of these birds from the Talgar district in the process of ringing revealed defects of beak, paws and eyes in more than 30 specimens of *Corvus frugilegus*, *Coloeus monedula,* and *Corvus cornix*. There are also abnormalities of the characteristic coloration. Most likely the abnormalities appeared as a result of anthropogenic influences, both physical and chemical.

Mammals

Mouse rodents are sensitive bio indicators of pesticide soil contamination, and their ubiquity makes them irreplaceable objects in environmental monitoring [36]. During the 2019-2020 field season, mammal surveys from the "Altyn-Emel" SNNP and the comparison area were carried out. The indicator species (mouse-like rodents) *Cricetulus migratorius, Mus musculus, Apodemus sylvaticus* were studied.

Exterior indices were determined, the average values of which were within the limits of variation for these species of rodents in Kazakhstan. Indices of liver, heart and kidneys, spleen of animals caught on different monitoring sites were determined. The variability of indices of internal organs of rodents was revealed. This variability is an indicator of their physiological state, reflecting mainly the nature of nutrition and the level of basic metabolism for each species.

Comparative analysis of morpho-functional indices of mouse-like rodents from "Altyn-Emel" SNNP showed that their values are close to those of animals from other sites of Talgar district (comparison area) (Fig. 3). In general, the habitus, metrical, functional and ecological characteristics of the animals of all studied areas were within the norm for animals inhabiting Kazakhstan [37]. No anomalies were found in the studied animals.

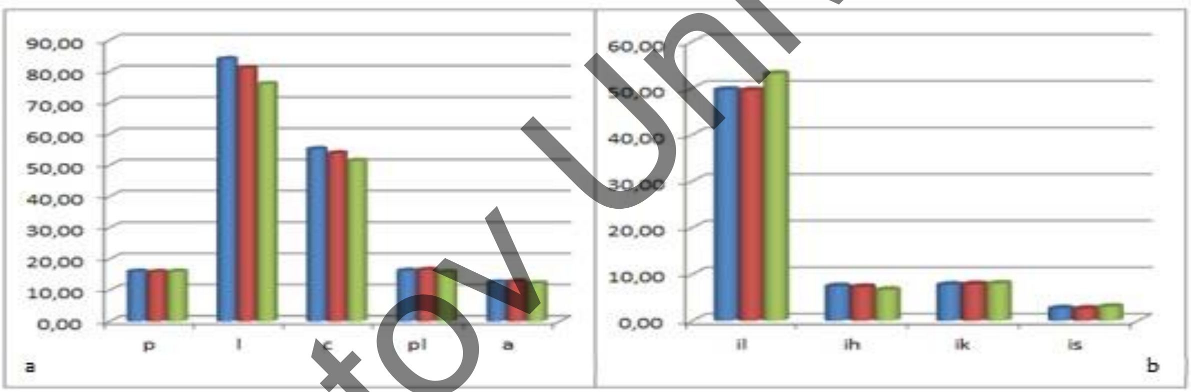

Figure 3. Comparative morpho-functional indices of mouse-like rodents (*Mus musculus*) of surveyed sites; exterior indices (a), interior indices (b); 1 column — mice from Altyn-Emel, 2 column — mice from Almaty region, 3 column — mice from East Kazakhstan region

Thus, the studied rodent individuals inhabiting the compared monitoring sites generally correspond to ecological and field characteristics given in the summary "Mammals of Kazakhstan" and in the "Book of Genetic Fund of Kazakhstan" [15–17, 38]. The identified ecological indices and values of the main metric and functional parameters of the studied animals indicate that no deviations from the norm were established and they generally correspond to similar characteristics of these animal species inhabiting Kazakhstan.

Micronucleus test of indicator animals

Bio testing and bio indication methods provide an opportunity to obtain a rapid response to a complex biological reaction of an ecosystem or population, as a result of the impact of various anthropogenic factors on it. The cytogenetic characteristics of peripheral blood were used as a biomarker to assess the genetic status of indicator animals living in "Altyn-Emel" National Park and comparison territories. Micronuclei were analyzed in peripheral blood erythrocytes. Micronuclei in a microscope can be seen as round, oval in different sizes, densely stained corpuscles with a clear outline. Additional information about the processes occurring in response to environmental stressors can be obtained by analyzing the structure of red blood cells that differ from the normal morphology characteristic of the species. Thus, amitosis indicates the development of degenerative processes in the body due to various reasons, including environmental ones. Nucleus division

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can also occur without cytoplasm rearrangement, in which case erythrocytes become binuclear [39]. Cytogenetic analysis of nuclear erythrocytes from peripheral blood of fish, amphibians and birds recorded the following disorders — erythrocytes with micronuclei; binuclear erythrocytes; amitosis; erythrocytes with cytoplasmic "tail" disorders, vacuolization and invagination of cytoplasm; erythrocytes with budding micronuclei; invagination of the nuclear membrane. The presence of cytological abnormalities in erythrocytes of peripheral blood of the studied animals indicates the presence of degenerative processes in the body due to various reasons, including environmental [40].

Micronucleus test in Pelophylax ridibundus

Amphibians meet all the requirements for species used for bio indication. Their main representative *P. ridibundus* is a widespread amphibian species, which has clear and easy to study traits, is highly sensitive to pollutants and mutagens. The morpho-physiological parameters of the amphibian organism reflect the local habitat condition, as they lack a pronounced tendency to migrate. The results of micronucleus analysis in blood erythrocytes of *P.ridibundus* are presented in Table 3. In *P.ridibundus* caught in SNNP "Altyn-Emel" and regions of comparison (Belbulak, Taukaraturyk) frequency of erythrocytes with micronuclei is practically at one level – 0.31-0.34 %. That is, there is no reliable difference in the frequency of micronuclei in frogs inhabiting "Altyn-Emel" National Park, and other areas. At the same time, it is reliably increased in comparison with the Alakol region (0.20±0.007 %).

Table 3

Results of *Pelophylax ridibundus* micronucleus test caught in and comparison regions

Place of capture	Viewed cells	Micronuclei, %	Amitosis, %	Tail, %	Nucleus invagination, %	Binuclei, %
SNNP Altyn-Emel	200 000	0.31±0.012*	0.005±0.0016	0.05±0.005	0.075±0.006	0.005±0.0016
Belbulak	50 000	0.34±0.026*	0.058±0.01	0.002±0.002	0	0.06±0.01
Taukaraturyk	300 000	0.32±0.014*	0.005±0.002	0.073±0.007	0.017±0.007	0
Alakol-region	340 000	0.20±0.070	0	0	0	0
*$p \leq 0.01$						

A high level of heterogeneity of cytogenetic indices in the studied groups of frogs should be noted. The difference in control values in different literature sources sometimes differs several times. Some show control values of 0.78±0.03 % [41], others 0.23±0.01 %. Probably, it depends on a level of purity of researched areas and, accordingly, control groups matched to them.

Micronucleus test in fish fauna

Blood samples from four fish species – *T.strauchii* (10 individuals), *P.parva* (8 individuals), *D.dybowskii* (5 individuals), *T.dorsalis* (4 individuals) were obtained in SNNP "Altyn-Emel". Comparative analysis was made with fish fauna caught in Taukaraturak and Alakol Lake areas (Tab. 4).

Table 4

Results of cytogenetic analysis of erythrocytes of fish caught in water bodies in the "Altyn-Emel"

Place of capture	Animals studied	Viewed cells	Micronuclei, %	Amitosis, %	Tail, %	Nucleus invagination, %
SNNP "Altyn-Emel"	13	190 000	0.065±0.009*	0.01±0.003	0.05±0.007	0.024±0.011
Taukaraturyk	137	364 000	0.13±0.006*	0.001±0.001	0.006±0.001	0.01±0.002
Enbekshi	13	130000	0.083±0.008*	0.023±0.002	0.006±0.001	0.069±0.007
Lake Alakol	16	160 000	0.027±0.007	0.003±0.002	0.001±0.001	0.003±0.002
*$p \leq 0.01$						

Fish caught in "Altyn-Emel" National Park (Aktobe River watercourse) have a significantly higher frequency of erythrocytes with micronuclei compared to fish caught in Lake Alakol, but half as many as fish caught in Taukaraturyk. At the same time, almost the whole spectrum of cytological disturbances described above was revealed in this group. In many cases, these changes accompany compensatory processes occurring in tissues, e.g., during functional overloading, starvation, after poisoning, or denervation [42]. In addi-

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tion, there is literature evidence that nuclear and cytoplasmic abnormalities of this nature can be the result of chemical exposure inducing gene and cytotoxicity [43].

Different fish species of the carp family have been studied by many researchers. The frequency of micronuclei in different literature sources sometimes differs by time. Proportion of cells containing micronuclei in a sample of *Carassius gibelio* (Bloch, 1782) inhabiting the Tom river was on average 0.057±0.030 %. Also, many researchers showed that the background level of micronuclei in fish is 0.5-1‰ [44]. These data correspond to the results of fish from Alakol Lake (0.015±0.005 %). At the same time, other authors demonstrate micronucleus frequency values at the level of 0.25±0.03 % in *Cyprinus carpio* (Linnaeus, 1758) [45] or even – 2.91±0.15 % in *Abramis brama* (Linnaeus, 1758) from the Volga-Caspian channel [42]. It is possible that such discrepancies are due to differences in methods of counting abnormal cells or statistical processing of results.

As shown above, within the boundaries of the mothballed "Suluchekinskoye" uranium deposit, an increased radiation background has been identified within the boundaries of the former fracking site. In this regard, a comparative analysis of the micro-nuclear test of fish inhabiting three points in the vicinity of the former GEC (Kosbastau cordon) and Aktobe River with a normal radiation background was carried out. The main sample of fish fauna of these reservoirs consisted of 2 fish species – *Triplophysa strauchii* and *Pseudorasbora parva*. In this connection, cytogenetic analysis was carried out in individuals of these species. For more objective analysis and absence of influence of species differences the results of micronucleus test of these fish species, from two reservoirs were taken into account separately (Tab. 5).

Table 5

Results of cytogenetic analysis of erythrocytes of fish living in water bodies of SNNP "Altyn-Emel"

Place of capture	Species	Animals studied	Viewed cells	Micronuclei, %	Amitosis, %	Binuclei, %
Kosbastau cordon	*T. strauchii*	10	200000	0.076±0.006*	0.034±0.006	0.019±0.003
Aktobe river	*T.strauchii*	6	60000	0.042±0.008	0.008±0.003	0.003±0.002
Kosbastau cordon	*P.parva*	8	160000	0.053±0.006*	0.03±0.004	0.023±0.004
Aktobe river	*P.parva*	3	60000	0.027±0.006	0.006±0.003	0.002±0.002
Lake Alakol	Cyprinidae	16	160000	0.027±0.007	0.003±0.002	0.002±0.002

**p≤0.01*

Analysis of the presented data shows reliable excess of frequency of cytogenetic and cytological disorders in fish (both species) caught at Kosbastau Kordon with increased radiation background. At the same time, there is a slight interspecific difference in the frequency of cytogenetic disorders in *T.strauchii* and *P.parva*. In *P.parva* (*Cyprinidae*) caught in the Aktobe River, the level of cytogenetic abnormalities is similar to that of *Cyprinidae* fish (different species) from ecologically clean Lake Alakol. This indicates that there is a relatively favorable environmental situation in the Aktobe River. The local increase of radiation background in the area of Koybastau cordon does not have a significant impact on the environmental objects in the territory of SNNP "Altyn-Emel".

Micronucleus test in Columba livia

Birds are sensitive indicators of the impact of various factors. *Columba livia* was used as an indicator species, belonging to the ornithofauna, to assess habitat quality in the territories of SNNP "Altyn-Emel" and Amangeldy (comparison region). *Columba livia* is a widespread polytypical species, the natural range of which occupies a considerable part of Eurasia and North Africa [45, 46]. The attachment of *Columba livia Gmelin* to its nesting sites creates conditions for the long-term effects of environmental factors on these animals. Micronucleus analysis revealed a significant ($p \leq 0.05$) difference in the frequency of micronuclei in *Columba livia* from the "Altyn-Emel" (0.027±0.005 %) and Amangeldy (0.05±0.07 %) regions. However, analysis of cytological abnormalities revealed no fundamental difference in the results of *Columba livia* of these territories.

The micronucleus test in rodents

One of the main animal model species in population biology studies is mouse-like rodents due to their ubiquitous distribution, abundance, habitat in a relatively limited area and close association with the soil. The micronucleus test in rodents can be carried out in cells of any proliferating tissue. The easiest way to perform a micronucleus test is using erythrocytes because blood is the most accessible tissue for examination and does not usually require killing the animal. The average life span of red blood cells is 3–4 months, which

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allows estimating completely the effects of the factors under investigation. Since mammalian peripheral blood erythrocytes are nucleus-free, the main type of cytogenetic disorders is micronuclei. Microscopically they are visible as rounded or oval, densely stained cells of different sizes with a clear contour. In the process of erythropoiesis, after the last mitosis, the main nucleus is pushed out, and micronuclei resulting from chromosomal abnormalities in erythrocyte cells remain in the cytoplasm. Abnormalities in erythrocyte shape and size – poikilocytosis are also found.

Species such as *Microtus sarvalis, Musmus culus, Apodemus sylvaticus, Cricetulus migratorius* were investigated to assess the ecological condition of “Altyn-Emel” SNNP.

A number of cytological abnormalities have been recorded, such as abnormally sized erythrocytes, usually small (microcytes) or large (macrocytes) erythrocytes, sometimes, poikilocytosis — changes in normal cell shape, thickness and volume. The results of the cytogenetic analysis of erythrocytes of rodents are presented in Table 6.

Table 6

Results of cytogenetic analysis of erythrocytes of mouse-like rodents from Altyn-Emel SNNP

Place of capture	Viewed cells	Micronuclei, %	Poikilocytosis, %
SNNP Altyn-Emel	60000	0.03±0.007	0.095±0.012
Belbulak	70000	0.41±0.024*	0.006±0.003
Enbekshi	50000	0.38±0.022*	0.1±0.012
Taukaraturyk	80000	0.054±0.008	
* p≤0.01			

The frequency of micronuclei in erythrocytes of peripheral blood of rodents from SNNP “Altyn-Emel” was significantly (p≤0.01) lower than frequency of micronuclei of rodents from the comparison regions — Belbulak and Enbekshi. This indicates considerably more favorable ecological conditions for rodents in Altyn-Emel than outside of this area. Some works show that approximately 0.025±0.011 % of micronuclei are detected in erythrocytes of white non-pedigreed mice [47, 48], which corresponds to the data obtained from the national park.

Conclusions

The assessment of the environmental status of the “Altyn-Emel” SNNP area showed the presence of some POPs and HM pollutants in water and soil. Pesticides are the main pollutants of soil among POPs. Presence of trace amounts of DDT and its metabolites, as well as aldrin, deldrin, exceeding MPC were detected. In the comparison territories, the spectrum of detected POPs was much wider, and the number of POPs items exceeding MPC was from 3 to 9. The presence of such heavy metals as Ni, Cd and Zn in addition to nickel, zinc, cadmium, and copper exceed the MPCs in the comparison territories. Thus, by the level of soil pollution, the territory of the National Park is much more favorable in environmental terms. In the natural water of “Altyn-Emel” SNNP, dibutylendan and δ-HCG in concentrations below the MPC were detected. Also Zn, Ni, Cu and chromium in concentrations below the MPC were detected. The territory of “Altyn-Emel” SNNP practically does not differ from the compared territories by the level of pollution of natural water with heavy metals.

Analysis of the radio-ecological situation in “Altyn-Emel” National Park showed that it is safe and the accepted characteristics of EDR and radioactive contamination of the land surface are within normal limits. The exception is a local area within the boundaries of seismic station “Kalkan” where the “Suluchekinskoye” uranium deposit of formation-infiltration type is located. Here, on the limited area of 7-9 m^2, in the contour of a self-discharging well, EDR values in a range of 0.26-2.99 μSv/h were detected, which noticeably exceeds permissible values (the calculated dose for the population is no more than 0.57 μSv/h). The small area of the anomalous zone in terms of EDR level probably does not have a significant impact on the environmental objects on the territory of SNNP “Altyn-Emel”.

Characterization of biodiversity of terrestrial and aquatic fauna of endemic species and indicator groups of vertebrates habiting the “Altyn-Emel” SNNP showed their significantly higher species diversity than outside the territory of the National Park. To objectively determine the levels of ecological load in terrestrial and aquatic cenoses, a detailed study of selected key and indicator vertebrate species using modern methods of morpho-functional indication, cytogenetic and toxicological analysis was conducted. The comparative analysis showed that morpho-functional indicators, habits in general, metrical, functional and ecological character-

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istics of animals of the surveyed area are within the norm for animals living in Kazakhstan. No anomalies that could be associated with anthropogenic pollution were found in these groups of vertebrate animals studied. Assessment of cytogenetic homeostasis using a micronucleus test did not reveal any significant disturbances of the genetic apparatus in indicator animals.

Thus, according to the data of chemical analysis, assessment of species diversity and state of indicator species of plants and vertebrates, we can conclude that the ecological situation in the territory of SNNP “Altyn-Emel” is satisfactory. Manifestation of morpho-functional changes in *P.parva* and tadpoles of toad complex *B.viridis* and cytogenetic disorders in fish *P.parva* and *T.strauchii* inhabiting near the well of seismic station “Kalkan” with increased radiation background is probably connected with manifestation of local radioactive pollution, which has no negative influence on the whole territory of the National Park.

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«Алтын Емел» Мемлекеттік ұлттық паркіндегі биоиндикаторлық түрлердің жай-күйін бағалау және аумағының экологиялық сипаттамасы

«Алтын-Емел» (Қазақстан) Мемлекеттік ұлттық паркі аумағының ластану дәрежесін бағалау және табиғи жағдайын сақтау мақсатында су мен топырақ үлгілеріндегі тұрақты органикалық ластаушы заттар мен ауыр металдардың мөлшері анықталды. Жануарлардың индикаторлық топтарына радиодозиметриялық зерттеулер, экологиялық-генетикалық мониторинг жүргізілді. Химиялық талдау су мен топырақ үлгілерінде шектеулі рұқсат етілген концентрациядан асатын кейбір ластаушы заттардың бар екендігін көрсетті. Парк аймағының шекараларында аумақтың радиоактивті ластануы анықталған жоқ, ал ЭДҚ (эквивалентті доза қуаты) мәндері әдеттегі стандартты мәндерден аспайтындығы дәлелденді. Сұлушеке уран өндіретін кен орнының ерекше екендігін атап кеткен жөн, себебі бұнда ЭДҚ мәндері нормативтік мәндерден жоғары екендігі анықталған. Жекелеген индикаторлық топтардағы жануарлардың жай-күйін салыстырмалы талдау олардың цитогенетикалық гомеостазы, морфофункционалды көрсеткіштері, габитусы мен экологиялық сипаттамалары Қазақстанда мекендейтін жануарлардың қалыпты дамуына сәйкес келетіндігін көрсетті. Мониторингтік зерттеулердің нәтижелері «Алтын-Емел» Мемлекеттік ұлттық паркі аумағындағы экологиялық жағдайдың қанағаттанарлық екендігін және кейбір шиеленісті жағдайлар тұрақты бақылауды қажет ететінін көрсетті.

Кілт сөздер: цитогенетикалық талдау, генетикалық мониторинг, қанға уыттылық, ауыр металдар, микроядролық тест, морфофункционалды көрсеткіштер, тұрақты органикалық ластағыштар, түрлердің әртүрлілігі.

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Экологическая характеристика территории и оценка состояния биоиндикаторных видов в Государственном национальном парке «Алтын-Эмель»

С целью оценки степени загрязнения и сохранения природного состояния территории Государственного национального парка «Алтын-Эмель» (Казахстан) определено содержание стойких органических загрязнителей и тяжелых металлов в пробах воды и почвы. Проведены радиодозиметрические исследования, эколого-генетический мониторинг индикаторных групп животных. Химический анализ показал наличие в пробах воды и почвы некоторых загрязняющих веществ, превышающих предельно допустимые концентрации. В границах парковой зоны радиоактивного загрязнения территории не обнаружено, значения МЭД, в целом, не превышают нормативных значений. Исключение составляет локальная территория уранового месторождения «Сулучекинское», для которой были определены значения МЭД выше нормативных. Сравнительный анализ состояния животных отдельных индикаторных групп показал, что их цитогенетический гомеостаз, морфофункциональные показатели, габитус и экологические характеристики соответствуют нормальному развитию животных, обитающих в Казахстане. Результаты мониторинговых исследований показали, что экологическая ситуация на террито-

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рии Национального парка «Алтын-Эмель» удовлетворительная, с некоторыми напряженными обстоятельствами, требующими постоянного мониторинга.

Ключевые слова: цитогенетический анализ, генетический мониторинг, генотоксичность, тяжелые металлы, микроядерный тест, морфофункциональные показатели, стойкие органические загрязнители, видовое разнообразие.

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