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AQUATIC TOXICOLOGY

Radioecological Study of the Marsh Frog *Pelophylax ridibundus* in the Lakes Alabuga and Kuyash

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Abstract—The levels of accumulation of long-lived radionuclides ^{90}Sr and ^{137}Cs were studied in the marsh frog *Pelophylax ridibundus* Pall., 1771, inhabiting the lakes Alabuga and Kuyash located in the peripheral zone of the East Ural Radioactive Trace (EURT). The concentration of ^{90}Sr in the frogs from Lake Alabuga varies from 50 to 200 Bq/kg; in Lake Kuyash, from 100 to 320 Bq/kg of dry weight of animals. The content of ^{137}Cs in the frogs from lakes Alabuga and Kuyash changes from 5 to 35 and from 35 to 145 Bq/kg of dry weight, respectively. The levels of concentrations of the studied radionuclides in the same type of components of the reservoirs of the EURT and the reservoirs of the Middle Urals are compared. It has been shown that the content of 90Sr in frogs of lakes Alabuga and Kuyash per dry body weight exceeds the intervention level for fish according to accepted standards (SanPiN dated April 15, 2003).

Keywords: marsh frog, lakes Alabuga and Kuyash, radionuclides ^{90}Sr and ^{137}Cs, concentrations, components of the reservoir

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INTRODUCTION

Marsh frogs, one of the widespread species of amphibians in lakes and rivers, inhabit a vast territory of the world (Vershinin, 2007a). They are quite tolerant of chemical pollutions and elevated temperature of the aquatic environment; however, heating zones of thermal and nuclear power plants, where favorable conditions for life and reproduction throughout the year are created, are their favorite habitats. The frogs can be considered as indicators of the ecological state of natural environment in the regions with a high anthropogenic load on natural complexes due to the presence of heavy metals, natural and artificial radionuclides in them, consequences of radioactive and chemical pollution of the territories under conditions of urbanization and emergency situations (Jagoe et al., 2002; Stark et al., 2004; Matsushima et al., 2015).

An interest in the ecological specifics and chemical composition of frog tissues is partly caused by their use for food production in a number of countries in the world (China, Vietnam, France, Belgium, Portugal, Italy, Spain, Holland, etc.), where they are actively cultivated in artificial and natural reservoirs; at the same time, a global catch of animals is hundreds of thousands of tons per year (Mirzaj, 2003; Din', 2014, 2015).

Radioecological studies of frogs are limited by a small number of studies. In the work (Matsushima et al., 2015), data on the accumulation of $^{134,\,137}$Cs by some frog species in a 20-kilometer zone of the Fukushima Nuclear Power Plant after the accident 2011 are given. In adult frogs and yearlings living in the lake, the concentrations of radionuclides varied from 68 to 750 Bq/kg wet weight. In the works (Stark et al., 2004; Stark, 2006), the accumulation of radionuclides in moor frogs *Rana arvalis* Nilsson, 1842, living in wetland ecosystems of central–eastern part of Sweden 17 years after the Chernobyl accident, was studied. It was demonstrated that the average concentration of ^{137}Cs in frogs was 1.7 ± 1.1 kBq/kg wet weight; at the same time, the highest values were noted for the smallest individuals (3.5 kBq/kg wet weight). Changes in the population structure, physiological and genetic differences of frogs living in radioactively polluted territories in the zone of PO Mayak as compared with the control region were detected (Pyastolova and Vershinin, 1996; Vershinin, 2007b).

The aim of the present work was to study modern levels of ^{90}Sr and ^{137}Cs accumulation by the frog *Pelophylax ridibundus* in the lakes Alabuga and Kuyash located in the peripheral part of the EURT.

MATERIALS AND METHODS

Adult individuals and yearlings of marsh frogs and other components of the aquatic ecosystem were stud-

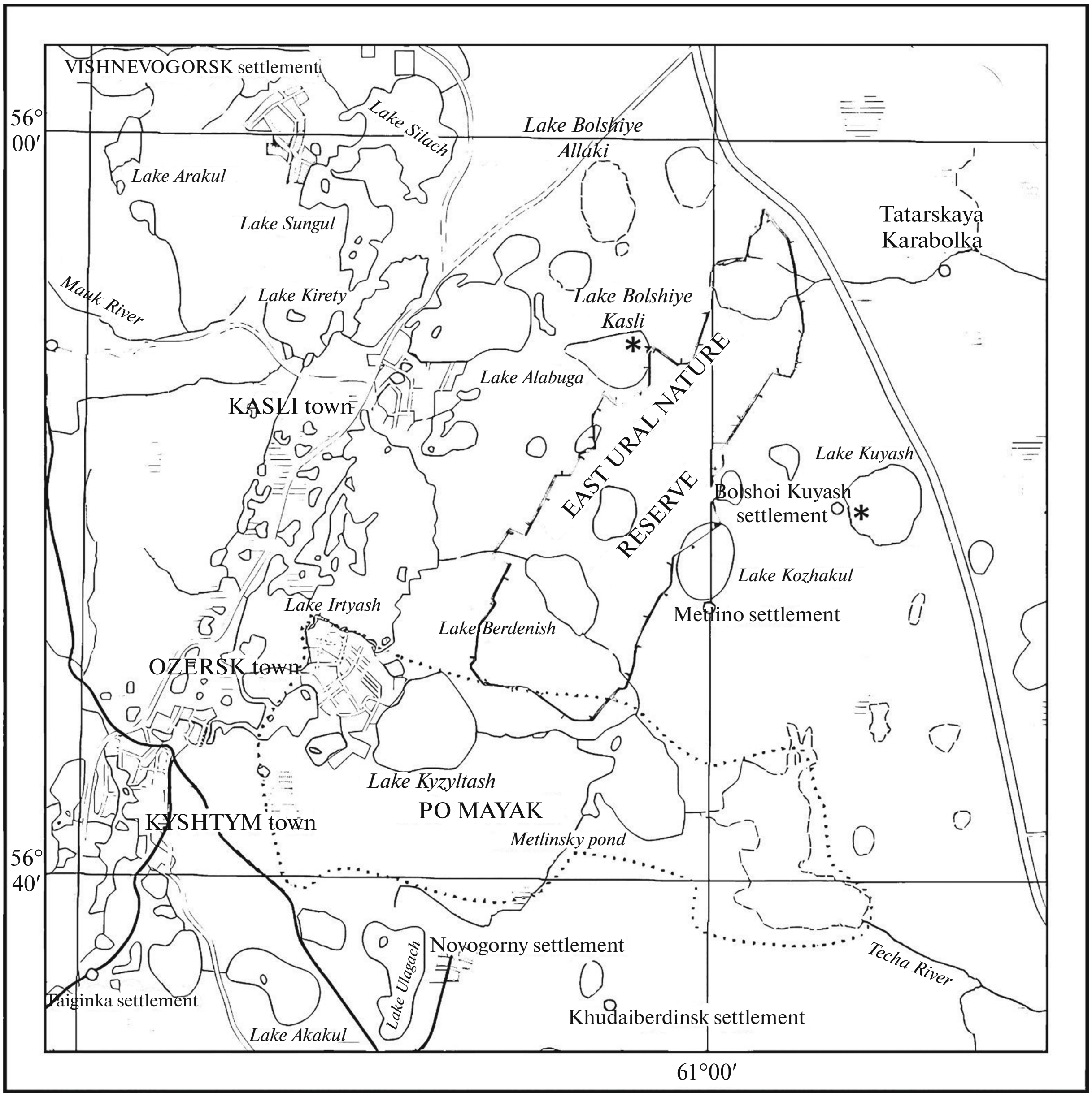

Fig. 1. Map of the location of the lakes Alabuga and Kuyash relative to the East Ural Nature Reserve and Karachay Trace. *, sampling locations.

ied: water, plants, fish, ground from the lakes Alabuga and Kuyash. The location of the lakes and sampling points relative to the territory of East Ural Radioactive Trace is given in Fig. 1.[1]

The Lake Alabuga is located on the western border of the EURT zone, which runs along the eastern shore of the lake. The lake basin is confined to the border of a piedmont fault on the eastern edge of the Trans-Ural Peneplain, the southwestern part of the lake basin is located among Tertiary deposits, it is adjoined by granites in the northwest. The reservoir is a part of the chain of Kasli–Kyshtym system of piedmont Trans-Urals lakes; by origin, it belongs to the group of erosional–tectonic ones. It has a drop shape, with a narrow part facing west, and a channel connects it to the Lake Bolshiye Kasli. The shores are gentle, slightly indented, and mostly covered with the forest. The lake bottom is level, grounds in the coastal zone are sandy, transitioning to the peaty silt with a mass of semi-decomposed plant remains. The area is 8.6 km^2, depth 3–5 m, water transparency 1.5–2 m. A part of the lake is swampy. Plants include reeds, cattails, cane, water lilies, pondweed, moss, and Cladophora; fish include

[1] https://travelask.ru/blog/posts/15203-radioaktivnyy-zapovednik-na-urale-pochemu-turisty-syuda-popa. Cited June 2, 2025.

Table 1. Characteristics of marsh frog at sampling sites

Lake	Coordinates		*n*		*L*	m	
	N	E	♀	♂		wet	dry
Alabuga	55°54′59″	60°55′53″	18	22	75.1 ± 1.8 / 55–104	43.1 ± 3.5 / 7–111	12.5 ± 1.1 / 4.8–31.8
Kuyash	55°49′11″	61°07′40″	45	37	62.6 ± 1.0 / 48–91	23.1 ± 1.4 / 11–72	5.2 ± 0.4 / 2.2–18.1

n, number of individuals; *L*, body length, mm; m, weight, g; above the line, mean value and its error; below the line, *min–max*.

carp, grass carp, silver carp, crucian carp, perch, roach, and pike (Smagin et al., 2011).

The Lake Kuyash is located on the eastern side of the reserve, at a distance of ~36 km from the epicenter of the explosion. The lake has a round shape with a diameter of ~4 km. The basin the reservoir is of erosional and tectonic origin, the shores are gentle. The surrounding terrain is flat, the landscape is forest–steppe. The catchment area is represented by meadows, pastures, and deciduous groves. The reservoir is fed by groundwater and atmospheric precipitation, there are many springs at the lake bottom. During the flood period, the lake has periodic drainage through a stream, which dries up during low water, into the Temryas River, which flows into the Karabolka River. The surface area of the reservoir is 12 km^2, the water volume is 42.5 million m^3, the average depth is 3–4 m. The bottom sediments are represented by sand, silt, and sapropelic grounds. Plants in the lake include cattails, cane, pondweed, reeds, etc.; the ichthyofauna is represented by diverse fish species (perch, ruff, roach, crucian carp, pike, tench, ide, carp, whitefish), crayfish are also found.[2]

The material was collected in July 2023. Adult frogs and yearlings were caught using a net, after which animals were euthanized using tricaine methane sulfate (buffered MS-222). Plants and fish were collected in triplicate, each of which was 2–3 kg. The ground was collected using a sampler to a depth of 0–5 cm from the surface. Water samples were collected in duplicate, 70 L at each observation point, and acidified with hydrochloric acid. In the laboratory, the frogs were weighed, the length, sex, and striata morph were determined. After drying and ashing at a temperature of 450°C, all samples were analyzed for the content of radionuclides in them. Ash concentration in the samples was determined by radiochemical method (Trapeznikov et al., 2008). To determine ^{90}Sr concentration, radiometry of the obtained sediments was performed using a UMF-2000 low-background device (Russia) with a statistical counting error of 10–15%. The concentration of ^{137}Cs was determined using multichannel γ analyzers of Canberra-Packard and ORTEC companies (United States) with a measurement error of ≤10–20%. During radiometry, some frog samples were united by sex and body weight to improve the accuracy of determinations.

In statistical processing of data, software package Statistica v. 6.0, StatSoft, 2001 (United States), license no. AXXR003A622407FAN8, was used. The obtained primary data were tested for normal distribution using the Shapiro–Wilk, Lilliefors, Kolmogorov–Smirnov criteria. In most cases, the distribution differed from normal or the sample was insufficient to test for normality; therefore, nonparametric Mann–Whitney (U) criterion was used for data processing. When conducting statistical processing of all data using the Mann–Whitney criterion, the z criterion value was given to recalculate U to a standard normal distribution. Differences were considered significant at $p < 0.05$.

RESULTS

Data on the body size and weight of lake frogs in the studied water bodies were obtained (Table 1). In Lake Alabuga, frogs have a significantly greater body weight and length compared to those from Lake Kuyash ($z = 4.89$, $p < 0.001$ and $z = 5.31$, $p < 0.001$, respectively). In both water bodies, the overwhelming majority of frogs belong to the striata morph (72% in Lake Alabuga and 91% in Lake Kuyash).

Individual values of concentrations of ^{90}Sr and ^{137}Cs radionuclides in adult frogs of the studied lakes demonstrated (Fig. 2) that the concentrations of each radionuclide are characterized by a noticeable variability. The content of ^{90}Sr in frogs of the lakes Alabuga and Kuyash varies from 50 to 200 and from 100 to 320 Bq/kg dry weight; the concentration of ^{137}Cs, from 5 to 35 and from 35 to 140 Bq/kg, respectively. The average content of each of the studied radionuclides in frogs living in the Lake Kuyash (216 ± 10 and 73 ± 7) is noticeably higher than in the Lake Alabuga (107 ± 9 and 20 ± 5 Bq/kg) for ^{90}Sr and ^{137}Cs, respectively. Statistical processing of the results obtained demonstrated a high level of significant differences in the accumulation of ^{90}Sr ($z = 4.99$, $p < 0.001$) and ^{137}Cs ($z = 4.38$, $p < 0.001$) by the marsh frog in the Lake

[2] http://reki-ozera.ru/rybalka_v_chelyabinskoy_obl/ozer/109275-kuyash.html. Cited June 2, 2025.

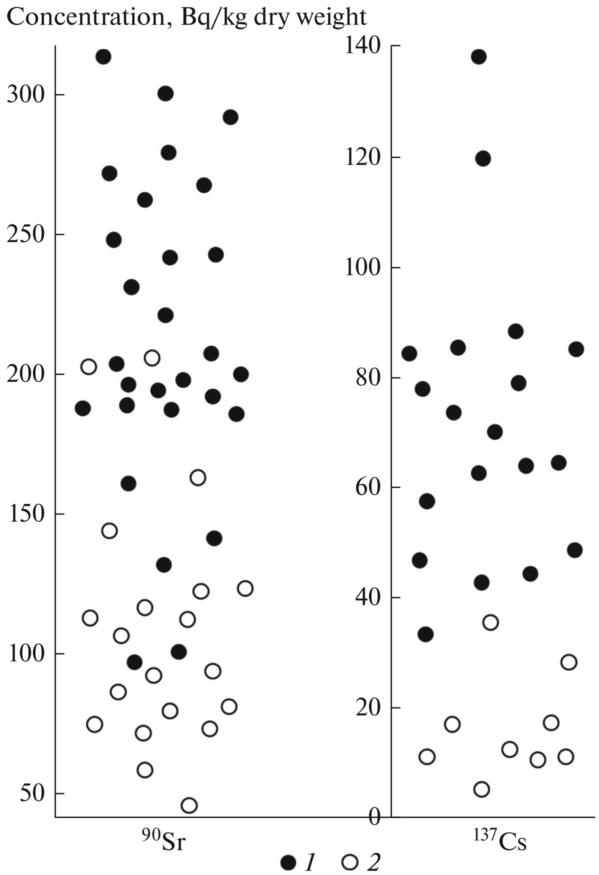

Fig. 2. Levels of concentrations of ^{90}Sr and ^{137}Cs in adult frogs from the lakes Kuyash (*1*) and Alabuga (*2*).

Kuyash as compared with the Lake Alabuga. No differences in the accumulation of radionuclides by males and females in total for both lakes were found (by ^{90}Sr: $z = -1.02$, $p = 0.31$; by ^{137}Cs: $z = 0.68$, $p = 0.49$). No ^{134}Cs radionuclide was detected in all samples.

Data on concentrations of ^{90}Sr and ^{137}Cs in the marsh frog and other components of its habitat in the Lake Alabuga were obtained (Table 2). It was detected that concentrations of the studied radionuclides in water are orders of magnitude lower than the level of intervention (5 Bq/L by ^{90}Sr and 11 Bq/L by ^{137}Cs). Among all the examined components of the reservoir, an increased content of ^{90}Sr was noted for the pond snail, swan mussel, moss, Cladophora, and chebak; ^{137}Cs, for the moss, Cladophora, and silty ground. Statistical processing demonstrated a significantly greater accumulation of ^{90}Sr relative to ^{137}Cs in all studied components of this reservoir, except for the ground, in which differences in the accumulation of these radionuclides are insignificant.

In the Lake Kuyash, the content of ^{90}Sr and ^{137}Cs in water was also significantly lower than the level of intervention (Table 3). Increased concentrations of ^{90}Sr were observed in Charophyta and Cladophora; ^{137}Cs, in Cladophora, pond snail shell, and ground. In other components of the reservoir (including frogs), the content of this radionuclide did not exceed 110 Bq/kg. Statistical processing demonstrated that there is more ^{90}Sr than ^{137}Cs in all studied components of this lake.

A comparison of the content of radionuclides in the same type of components of the studied lakes (Tables 2 and 3) detected a significant excess of the concentrations of ^{90}Sr in the Lake Kuyash as compared with the Lake Alabuga by water ($z = 2.32$, $p = 0.02$), frogs ($z = 4.99$, $p < 0.001$), Cladophora ($z = 2.5$, $p = 0.012$), and sago pondweed ($z = 2.5$, $p = 0.012$); ^{137}Cs, by water ($z = 1.73$, $p = 0.008$), frogs ($z = 4.38$, $p < 0.001$), Cladophora ($z = 1.96$, $p = 0.040$), sago pondweed ($z = 1.97$, $p = 0.041$). In other similar components of the lakes (perch, chebak, ground), no significant differences in the accumulation of radionuclides were detected.

In Fig. 3, averaged data on concentrations of ^{90}Sr and ^{137}Cs in the components of the lakes Alabuga and Kuyash, located at the periphery of the East Ural Radioactive Trace, as compared with previously obtained data on the reservoirs of the Middle Urals (not subject to local radioactive pollution) are presented (Chebotina et al., 2024). The results of statistical processing of these data (Table 4) demonstrated that the concentration of ^{90}Sr in the components of both lakes significantly exceeded that in the reservoirs of the Middle Urals. The concentration of ^{137}Cs in the components of the Lake Kuyash (except for the ground, $p = 0.056$) was significantly higher as compared with the reservoirs of the Middle Urals. However, differences in the concentrations of ^{137}Cs for the compared components of the Lake Alabuga and reservoirs of the Middle Urals were insignificant, except for the ground ($p = 0.012$).

DISCUSSION

As noted above, the studied reservoirs are located in the impact zone of PO Mayak, at the territory exposed to two radiation accidents. The first accident occurred in 1957, as a result of the explosion of tanks storing highly radioactive waste (Kyshtym accident). The spread of radioactive substances in the north—east direction led to the formation of the East Ural Radioactive Trace with the length of 300 km and width 20—40 km. The main portion of radioactivity was made up of short-lived radionuclides ^{144}Ce, ^{95}Zr, ^{106}Ru with a relatively small contribution of long-lived ^{90}Sr. The second accident (1967) was a consequence of the wind dispersal of dust-like radioactive substances from the surface of the Lake Karachay, which led to the formation of the Karachay trace with a predominant content of ^{137}Cs (48%) (Snakin et al. 2012; Mansurova et al.,

Table 2. Concentrations of ^{90}Sr and ^{137}Cs radionuclides and results of statistical data processing in the components of aquatic ecosystem of the Lake Alabuga

Object	^{90}Sr	^{137}Cs	*z*	*p*
Frogs:				
adult	107 ± 9	20 ± 5	4.66	0.001
yearlings	57*	37*	–	–
Water	0.077 ± 0.002	0.009 ± 0.002	1.73	0.050
Moss fontinalis	329 ± 17	75 ± 5	1.73	0.050
Cladophora	153 ± 4	39 ± 3	1.96	0.050
Cane	15*	9*	–	–
Sago pondweed	85 ± 8	8 ± 1	1.96	0.050
Perch	130 ± 7	13 ± 2	2.5	0.010
Chebak	174 ± 31	17 ± 2	2.32	0.020
Pond snail	1178 ± 26	21 ± 3	1.73	0.040
Swan mussel	417 ± 29	10 ± 4	1.73	0.030
Ground	120 ± 39	143 ± 5	0.73	0.460

Units of measurement of indices: water, Bq/L; other components of reservoirs, Bq/kg dry weight. Mean values and their errors are given. * United samples; "–", no data available.

Table 3. Concentrations of ^{90}Sr and ^{137}Cs radionuclides and results of statistical data processing in the components of aquatic ecosystem of the Lake Kuyash

Object	^{90}Sr	^{137}Cs	*z*	*p*
Adult frogs	216 ± 10	73 ± 7	5.58	0.001
Water	0.143 ± 0.006	0.067 ± 0.009	2.32	0.020
Cladophora	749 ± 11	155 ± 11	2.53	0.011
Charophyta	1180 ± 24	107 ± 7	1.93	0.045
Sago pondweed	259 ± 3	97 ± 9	1.96	0.049
Perfoliate pondweed	238 ± 10	110 ± 14	–	–
Perch	145 ± 6	103 ± 11	2.32	0.020
Chebal	181 ± 5	59 ± 14	1.96	0.049
Pond snail shell	Not defined	425 ± 61	–	–
Ground	119 ± 9	170 ± 11	–2.49	0.012

Units of measurement of indices: water, Bq/L; others, Bq/kg dry weight; "–"—no data available.

2021; *Atlas* ..., 2013). Radioactive pollution of the Lake Alabuga occurred mainly due to the Kyshtym accident, the pollution of the Lake Kuyash was affected by both traces. In more than 60-year period after the accident, radioactive pollution of the territory decreased due to the processes of radioactive decay and migration of radioactive elements to adjacent territories. At present, the issue of returning a part of the territories to economic use arose.

Our data indicate that at the moment of the study, the components of the lakes Alabuga and Kuyash were enriched to a greater extent with ^{90}Sr relative to ^{137}Cs. The silt ground, characterized by a slight excess of the concentration of ^{137}Cs relative to ^{90}Sr, was an exception. By the content of both radionuclides, the water of the lakes Alabuga and Kuyash is significantly lower than the intervention level (5 and 11 Bq/L for ^{90}Sr and ^{137}Cs, respectively) according to the standards of radiation safety,[3] which allows to use it for domestic and household purposes. At the same time, as a result of the study of the content of radionuclides in adult frogs, an increased radioactive pollution of these amphibians in both reservoirs was registered. Particularly, adult frogs from the Lake Kuyash contain at the average 28 times more ^{90}Sr and 5.5 times more ^{137}Cs as compared with similar animals inhabiting the reservoirs of the

[3] Radiation Safety Standards (NRB-99/2009): Sanitary and Epidemiological Rules and Regulations. 2009. Moscow: Federal Center for Hygiene and Epidemiology of Rospotrebnadzor.

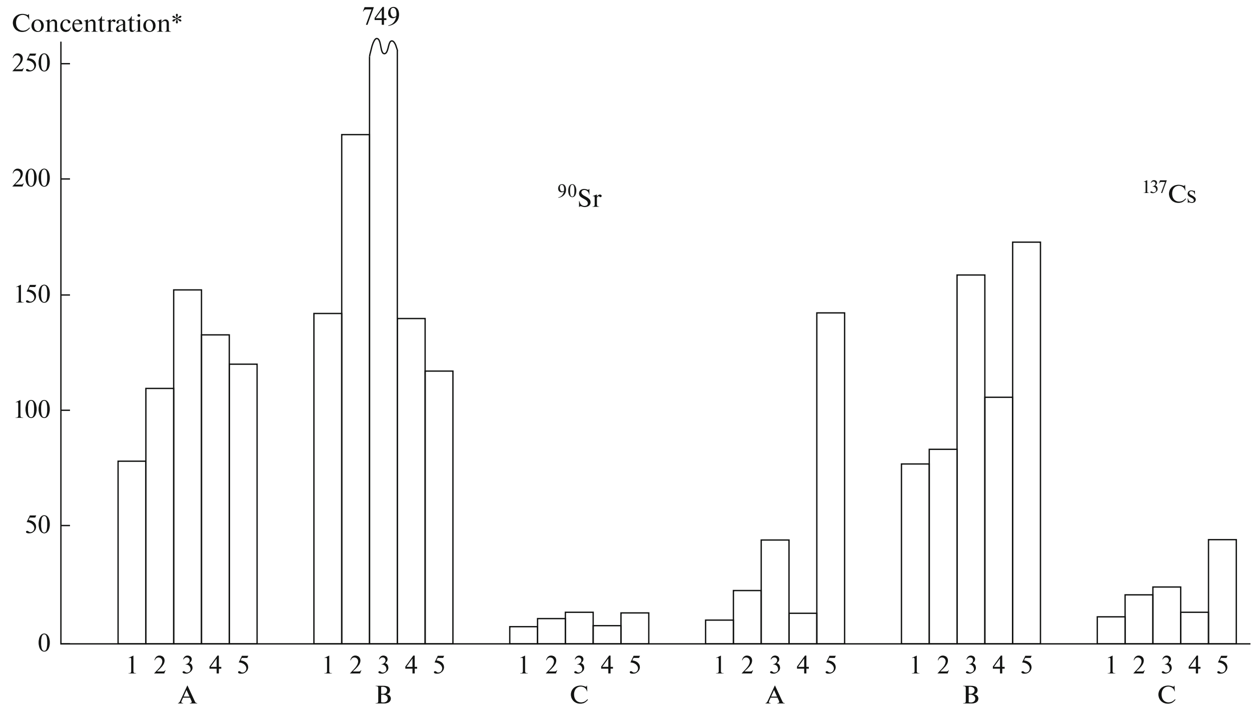

Fig. 3. Levels of radionuclide concentrations in the components of the lakes Alabuga (A) and Kuyash (B) as compared with averaged values of concentrations for similar components of reservoirs in the Middle Urals (C). Components of reservoirs: (1) water; (2) adult frogs; (3) Cladophora; (4) perch; (5) ground. *Concentrations: water, Bq/m^3; other components, Bq/kg dry weight.

Middle Urals, not subject to the additional pollution from nuclear power plants. In the Lake Alabuga, similar differences are 14 and 1.3 times, respectively. At the same time, it was established that adult frogs in the Lake Kuyash contain more ^{90}Sr and ^{137}Cs than those in the Lake Alabuga. This can be caused by the additional contribution of the Karachay trace, which covered the southern part of the Lake Kuyash, while the Lake Alabuga is located outside it.

Since marsh frogs are widely used all over the world for food purposes, and there are no standards for a permissible level of radioactive pollution for them, we consider it possible to compare data we obtained on the accumulation of ^{90}Sr and ^{137}Cs in them with the appropriate indices for fish. According to the decree,[4] at a certain level of food consumption, specific activity of a radionuclide in it, and in compliance with the standards, a permissible level of the content of ^{90}Sr in fish is 100 Bq/kg; ^{137}Cs, 130 Bq/kg wet weight. According to (Smagin et al., 2011), exceeding the permissible level of content of these radionuclides in some fish species was detected in the Lake Kozhakul, located on the southeastern border of the EURT. In our work, the average concentration of ^{90}Sr and ^{137}Cs in the frogs from the Lake Alabuga (calculated per wet weight) is 31 (15–58) and 6 (2–10) Bq/kg; in the Lake Kuyash, 48 (22–71) and 16 (8–31) Bq/kg, respectively. These indices are below the permissible level (according to the established standards).

[4] SanPiN of November 14, 2001 SanPiN 2.3.2.1078-01 "Hygienic Requirements for Food Safety" approved by: Decree of the Chief State Sanitary Doctor of the Russian Federation No. 36 dated November 14, 2001 (as amended on April 15, 2003). Appendix 1.

CONCLUSIONS

It was established that the concentrations of ^{90}Sr in the frogs of the lakes Alabuga and Kuyash, located at the peripheral border of the EURT, vary from 50 to 200 Bq/kg and from 100 to 320 Bq/kg; ^{137}Cs, from 5 to 35 and from 35 to 145 Bq/kg dry weight, respectively. Statistical processing of the results obtained demonstrated an excess in concentrations of ^{90}Sr ($p < 0.001$) and ^{137}Cs ($p < 0.001$) in the frogs of the Lake Kuyash as compared with the Lake Alabuga. No differences in the accumulation of radionuclides by males and females were detected in total for both lakes (by ^{90}Sr: $p = 0.31$; by ^{137}Cs: $p = 0.49$). By the content of both radionuclides, water of the lakes Alabuga and Kuyash is significantly lower than the level of intervention according to the radiation safety standards,[3] allowing it to be used for household purposes. Increased concentrations of ^{90}Sr were detected in adult frogs in the lakes Alabuga and Kuyash as compared with these animals inhabiting the reservoirs of the Middle Urals that are not subject to the additional radioactive pollution.

Table 4. Results of statistical processing of data on the content of ^{90}Sr and ^{137}Cs in the components of the lakes Alabuga (above the line) and Kuyash (below the line) relative to their content in reservoirs of the Middle Urals

Radionuclide	Ecosystem component	*z*	*p*
^{90}Sr	Water	$\frac{-2.81}{3.89}$	$\frac{0.004}{0.001}$
	Adult frogs	$\frac{-7.7}{8.81}$	$\frac{0.001}{0.001}$
	Cladophora	$\frac{-2.83}{4.55}$	$\frac{0.047}{0.001}$
	Perch	$\frac{-4.28}{3.67}$	$\frac{0.001}{0.002}$
	Ground	$\frac{-3.82}{4.50}$	$\frac{0.001}{0.001}$
^{137}Cs	Water	$\frac{0.95}{2.53}$	$\frac{0.340}{0.011}$
	Adult frogs	$\frac{-1.59}{6.78}$	$\frac{0.110}{0.001}$
	Cladophora	$\frac{-1.81}{2.45}$	$\frac{0.070}{0.014}$
	Perch	$\frac{-1.71}{1.96}$	$\frac{0.090}{0.049}$
	Ground	$\frac{-3.21}{2.77}$	$\frac{0.012}{0.056}$

The levels of concentrations of the studied radionuclides in the frogs from both lakes are significantly lower than the permissible limit for the fish products, according to established standards.

ABBREVIATIONS AND NOTATION

EURT East Ural Radioactive Trace

FUNDING

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

When working with animals, the applicable ethical standards were followed that were approved by the Bioethics Commission of the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences (protocol no. 15 dated October 11, 2023).

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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