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Accumulation of Radionuclids in Amphibians (*Pelophylax ridibundus* Pall.) in the Middle Urals

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Abstract—The accumulation levels of anthropogenic ^{90}Sr and ^{134}Cs and ^{137}Cs radionuclides in the marsh frog have been studied in the areas of the Beloyarskii water-storage reservoir (an industrial storm-water discharge channel of the nuclear power station) and the Verkhnii Tagil water-storage reservoir (the Tagil River downstream of the dam). No significant distinction in the radionuclide accumulation (^{90}Sr and ^{137}Cs) depending on the amphibian sex and age is detected. Comparable levels of the accumulation of radionuclides in the marsh frog, when compared to the other representatives of the water ecosystem, are estimated. An assumption of the presence of some unidentified source of radioactive contamination of marsh frogs has been made; cesium-137 may be transferred from it to the Tagil River by the frogs.

Keywords: marsh frog, discharge channel, Beloyarskii water-storage reservoir, Verkhnii Tagil water-storage reservoir, Tagil River, ^{90}Sr, ^{137}Cs, accumulation

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INTRODUCTION

The mash frog (*Pelophylax ridibundus* Pall.), one of the most widespread species of amphibians, has occasionally appeared in the water reservoirs in the Ural region and inhabited a considerable part of its territory (Toporkova et al., 1979; Ivanova, 1995). The ecological features of this species were sudied and described in the scientific works (Ivanova, 1002; Vershinin and Ivanova, 2006; Ivanova and Zhigalski, 2011). It was reported that marsh frogs are rather tolerant to chemical contaminations and increased temperatures of water environments. In addition, the larval amphibians contribute to the purification of water from organic and inorganic pollutants entering with industrial wastewater (Misyura et al., 1986; Misyura, 1989; Vershinin, 2007). Preferred marsh-frog habitats are zones of heated water discharge in the cooling reservoirs at thermal and nuclear power stations, where favorable conditions for the frogs are created to live and to spawn year-round. However, the frog can safely live and spawn at lower temperatures under conditions of temperate latitudes.

Up to the present time, the problem of radionuclide accumulation in frogs in the zones of nuclear fuel cycle enterprises has not been practically studied yet.

The work that presents the data on the $^{134, 137}Cs$ accumulation in some frog species across the 20-km zone around the Fukushima Nuclear Power Station (NPP) after the accident in 2011 should be noted among the low-detail data available. The radionuclide concentration varied from 68 to 750 Bq/kg wet weight in mature frogs and the current-year juvenils inhabiting the lake. There are works (Stark et al., 2004; Stark, 2006) devoted to research into the accumulation and and the assessment of the radiation doses to the *Rana arvalis* frogs and the current-year juvenils inhabiting the marsh ecosystems in the central eastern part of Sweden 17 years after the Chernobyl accident. According to the survey results, the average ^{137}Cs concentration in frogs comprised 1.7 ± 1.1 Bq/kg wet weight. In addition, the highest values are indicated in the smallest amphibian specimens (3.5 Bq/kg of raw mass). The coefficients of radionuclide accumulation in frogs for the soil and water categories were estimated. Despite the greater ^{137}Cs content in the soil than in the water, the coefficients of radionuclide accumulation in frogs were higher for the water category (2500–19100) than for the soil category (0.006–7).

The objective of this work is to study the accumulation levels of the anthropogenic ^{90}Sr and $^{134, 137}Cs$ radionuclides in the marsh frog inhabiting in the areas of the Beloyarskii and Verkhnii Tagil water-storage reservoir locations in Sverdlovsk oblast.

The water-storage reservoir is a cooling reservoir at the Beloyarskii nuclear power plant (BNPP), which was formed by regulating the stream of the Pyshma River at a distance of 75 km from its source. The length of the water reservoir is approximately 20 km; the width at the level of the NPP is approximately 3 km. The water depth of the navigation channel reaches 15–20 m and the average depth is 8–9 km. The reservoir surface is approximately 47 km^2. The Beloyarskii nuclear power plant is situated at the left bank of the reservoir at a distance of 7 km from the dam. It was put into operation in 1964. Up to present, the first and second power units of the plant have already been decommissioned. The third BN-600 power unit of a tank type and the fast neutron system with the liquid-crystal heat carrier was put into operation in 1980; it is still running. The fourth BN-800 power unit located at the right bank of the cooling reservoir and upstream of it is at the stage of starting the operation. The staff of the Institute of Plant and Animal Ecology, Russian Academy of Sciences, Ural Branch, has studied the detailed ecological characteristics of the cooling reservoir and has revealed the regularities in the accumulation and distribution of the anthropogenic radionuclides in different components of the Beloyarskii water-storage reservoir affected by the Beloyarskii NPP over its long-term operation period. The results of these surveys are described in a range of articles and monographs (Chebotina et al., 1992, 2002; Chebotina and Nikolin, 2005; Trapeznikov et al., 208; Trapeznikov and Trapeznikova, 2012; Chebotina et al., 2014).

At present, the main way radionuclides transfer into the BNPP Beloyarskii water-storage reservoir is the NPP industrial storm-water discharge channel (ISWDC), where the NPP debalance water is discharged. The latter includes the wastewater from the special purification facilities, laundries and showers, water from melted snow, and torrential rain water from the plant territory; in addition, the water from the neighboring enterprise of the Institute of Reactor Materials (IRM), where the experimental reactor is in operation, arrives there. The periodic monitoring for the radilogical safety status of ISWDC confirmed the fact of ^{90}Sr and ^{137}Cs contamination of the water over a background level, the hydrobionts, and the substrates (0.028 and 0.008 Bq/L dry weight in water, 2011; 10–30 and 3000–6000 Bq/kg dry weight in substrate, 2003; 104 and 95000 Bq/kg dry weight in comb pondweed; and 1980 for ^{90}Sr and ^{137}Cs, respectively). The ISWDC—unfrozen year-round—was chosen by marsh frogs, whose population is visually rather great. The reason to perform this survey was the absence of any literature data on the accumulation of the radioactive pollutants in the indicated species of amphibians within the zones of the permanent radionuclide discharge into the water environments by the nuclear fuel cycle enterprises, particularly by the Beloyarskii NPP.

The area of the Verkhnii Tagil water-storage reservoir location, which is sufficiently distant (>100 km northwestwards) from the Beloyarskii NPP and charactrized by the mass accumulation of the marsh frogs, was selected to estimate the comparable levels of the radionuclide accumulation in the marsh frog. The water reservoir was formed in 1960 in the area of the confluence of the Tagil River and the Vogulka River. The reservoir surface is 3.5 km^2; the average depth is 3.8 km and the maximum depth is 5 m. The water-storage reservoir serves as a cooling reservoir for the Verkhnii Tagil Hydro Power Plant (1500 MW). The heated water is used to supply the urban population and the enterprises of the city of Verkhnii Tagil with hot water. With respect to the thermal balance, the Verkhnii Tagil water-storage reservoir is categorized as a water reservoir with strong overheating, since the water temperature in it constantly exceeds the water temperature in natural water reservoirs by 6°C and more. The temperature indicator is ranging within 30°C in the middle of the vegetative period, while in the coldest period it lowers to 8–10°C and not below this range. The marsh frogs along with the grass carp were populated into the water-storage reservoir from Krasnodar krai in the 1980s (Toporkova et al., 1979; Vershinin, 2007); since then, they have been widely distributed in the ecosystems of the communicating rivers and water reservoirs.

MATERIALS AND METHODS

The work was carried out in 2013–2014. The survey sites were chosen in the area of the industrial storm water discharge channel (ISWDC) connecting the BNPP and the IRM with the Beloyarskii nuclear power plant (BNPP) (lat. 56°50′ N, long. 61°18′ E) and in the area of the Tagil River downstream of the dam (lat. 57°22′ N, long. 59°57′ E), which is the place of the water discharge from the Verkhnii Tagil water-storage reservoir into the river (Fig. 1). In the ISWDC area, the samples were selected in the common channel connecting the NPP and IRM. Marsh frogs, including mature specimens, current-year juveniles, and tadpoles, as well as fishes, hatchlings, aquatic plants, plankton, and substrates, served as survey objects.

Frogs, young frogs, tadpoles, and hatchlings were caught with landing nets and euthanized. The numbers of samples from the ISWDC and the Tagil River comprised 43 and 15 mature frog specimens, respectively. The samples of young frogs, hatchlings, and tadpoles were 4 specimens (25 g), 530 specimens (375 g), and 95 specimens (130 g), respectively. Plants, fish, and sandy–silt substrate were sampled in three sampling repeatabilities with 2–3 kg per repeatability. The plankton was sampled from the 0- to 1-m layer from the water surface with landing nets made of the bolting cloth in an aperture size of 0.067 mm. After selection, all of the samples were transported to the laboratory, dried up, and ashed at a temperature of

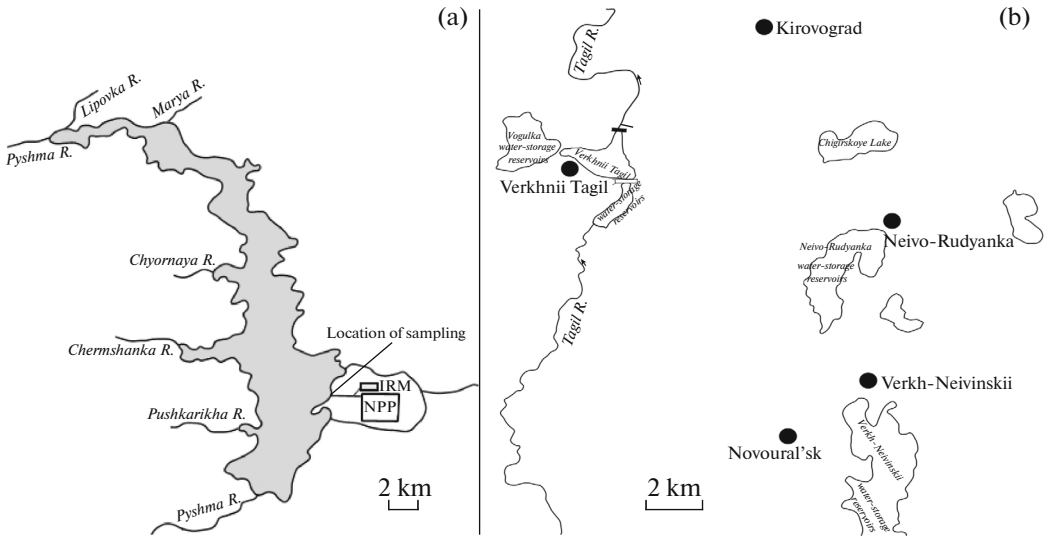

Fig. 1. Arrangement of sample locations in the areas of the Beloyarskii (a) and Verkhnii Tagil (b) water-storage reservoirs. Scale: 2 km (a) Location of Sampling, Institute of Reactor Materials (IRM), and NPP; Rivers: Pyshma, Lipovka, Marya, Chyornaya, Chermshanka, Pushkarikha, and Pyshma. (b) Location of Sampling; Tagil River; Chigirskoye Lake; Water-Storage Reservoirs: Vogulka, Verkhnii Tagil, Neivo-Rudyanka, and Verkh-Neivinskii; Towns: Verkhnii Tagil, Kirovograd, Neivo-Rudyanka, Verkh-Neivinskii, and Novoural'sk.

450°C; after that, the radionuclide content was determined in them.

In the laboratory, the frog body length was measured and the sex and age were determined. The cross section of the second phalange of the right hind limb was used to define the age in amphibians. The cross-section thickness was 15–18 μm. Decalcified limbs with 5% HNO_3 for 5 h were cut into sections with a freezing microtome, stained with hematoxylin for 3 min, and mounted in glycerol to preserve the material. The external diameter of the cross section and the average diameters of all of the lines of arrested growth, i.e., the lines formed during hibernation, were defined within data processing. Therefore, the age was defined as the number of survived hibernation periods (18–20).

The ^{90}Sr concentration in the ash samples was determined with radiochemical methods based on leaching the chemical elements with 6 N HCl, hydrochloric acid, and both the subsequent sedimentation of oxalates of alkaline-earth elements and the isolation of ^{90}Sr as carbonates. The ^{90}Sr content was determined by means of daughter ^{90}Y after their separation by noncarbon ammonia. The radiometry of the obtained sediments was performed with a UMF-2000 device of minimum ambient level in three repitabilities with the 10–15% statistical uncertainty.

The ^{134}Cs and ^{137}Cs concentrations were determined with a multichannel γ analyzer made by Canberra-Packard and ORTEC (United States) within the measuring error in the range of no more than 10–20%. The frog ash was mixed according to the sex and body-length categories to improve the efficiency of calculations. When analyzing the samples, cesium-134 of significant content was detected only in two specimens of amphibians from the ISWDC and the Tagil River containing ^{137}Cs at an abnormally high level for these populations. With respect to the other samples of amphibians from the ISWDC and the Tagil River, ^{134}Cs was registered in microconcentrations below the level of the reliable determination.

RESULTS AND DISCUSSION

Table 1 displays the results of a survey for the population of frogs from the ISWDC. The sample represents the specimens at the age of 1 to 5 years. Ten amphibians of them were females; the other 32 specimens were males. The body length varied in the range of 57–93 mm. According to the individual measurements, the ^{90}Sr concentration varied from 2 to 25 Bq/kg of dry weight with an average value of 10 ± 1 Bq/kg. The ^{137}Cs content varied from 8 to 26 Bq/kg with an average value of 18 ± 2 Bq/kg.

The results of the survey for the population of frogs from Tagil River are present in Table 2. The age indicators for this sample varied from 1 to 6 years; for the sex attribute, the sample contained five female and nine male specimens. The body length varied from 63 to 106 mm. The ^{90}Sr concentration varied from 2 to 10 Bq/kg, with an average value of 5 ± 1 Bq/kg. The ^{137}Cs content varied from 11 to 100 Bq/kg, with an average value of 48 ± 10 Bq/kg.

A comparison of two samples of frogs from the ISWDC and River Tagil populations made it possible

Table 1. Characteristics of individual indicators for frogs in the NPP industrial storm water discharge channel (ISWDC)

Age, year	Sex	Body length, mm	^{90}Sr, Bq/kg dry weight	^{137}Cs, Bq/kg dry weight
1	♀	74.0	6.6	26.2
1	♀	68.2	24.9	21.5
1	♀	59.4		
1	♀	83.4	5.2	11.3
2	♀	85.2	9.7	17.9
2	♀	83.0	6.4	
3	♀	92.5	7.7	16.7
2	♀	89.0	5.9	
2	♀	93.5	6.9	
3	♀	93.0	7.0	
2	♂	70.0	17.0	22.6
1	♂	67.0		
2	♂	75.0	12.0	23.9
1	♂	57.0	7.3	
1	♂	71.0		
3	♂	71.0	22.4	
1	♂	69.5	17.1	
1	♂	70.5		
2	♂	72.8	8.4	
2	♂	79.5	12.9	8.0
1	♂	74.0	10.5	
1	♂	77.0	6.4	
3	♂	84.0	5.3	10.7
2	♂	81.0	7.9	
5	♂	81.3	2.0	
2	♂	81.0	6.4	18.4
3	♂	78.6	8.4	
3	♂	79.0	13.9	
3	♂	83.5	14.2	
2	♂	84.6	1.4	
3	♂	79.0	6.0	
4	♂	84.5	18.5	18.7
3	♂	78.0	7.0	
2	♂	77.0	7.4	
3	♂	79.5	3.0	
2	♂	79.5	7.8	
3	♂	84.0	20.3	
3	♂	85.4	11.6	
5	♂	88.5	9.1	21.2
1	♂	87.6	10.3	
2	♂	90.0	6.3	
3	♂	91.7	3.0	
Average value			9.6 ± 0.9	18.1 ± 1.7

Table 2. Characteristics of individual indicators for frogs in the Tagil River

Age, year	Sex	Body length, mm	Concentration, Bq/kg dry weight	
			^{90}Sr	^{137}Cs
2	♀	84.0	Not available	20.8
2	♀	79.0	2.2	
6	♀	106.0	4.1	
2	♀	85.0	10.4	
2	♀	92.0	5.2	
4	♂	85.0	2.2	74.2
2	♂	73.0	5.3	39.0
2	♂	78.5	2.4	11.2
3	♂	80.0	5.1	51.8
Not identified	♂	90.5	3.4	40.4
2	♂	78.0	6.9	44.3
3	♂	88.5	9.8	98.5
1	♂	75.5	8.0	61.9
1	♂	63.5		39.7
Average value			5.9 ± 0.7	48.2 ± 9.4

to conclude that both samples were predominantly made up of males (~70%); in addition, the major mass of the caught specimens (80–90%) was 1–3 years old. With respect to the body length, the frog samples practically have no difference. As was expected, the average ^{90}Sr concentration in the amphibians from the ISWDC located in the NPP zone appeared to be two times higher than that in the Tagil River. The ^{137}Cs content in the population of frogs from the Tagil River turned out to be unexpectedly higher than that in the ISWDC. Two data aggregations presented in Tables 1 and 2 were statistically processed using the STATISTICA software program by the Student's criterion. It has been confirmed that the frogs in the ISWDC population can accumulate ^{90}Sr significantly more than similar animals in the Tagil River population ($p = 0.004$). In addition, ^{137}Cs is accumulated significantly less by frogs in the ISWDC than in Tagil River ($p < 0.005$).

Except as indicated above, there was an amphibian specimen per each population which could not be present in the tables due to its ^{137}Cs level exceeding a number of times the highest values displayed in the tables. Particlarly, one of the frogs from the ISWDC contained 45000 Bq/kg ^{137}Cs, while a frog from the Tagil River contained 53000 Bq/kg. It should be noted that a similar result was obtained in the survey (Matsushima et al., 2015), in which a frog (*Buergeria bergeri*) inhabiting the forest in the 20-km zone around the Fukushima NPP after the accident in 2011 had an especially high cesium concentration

Table 3. Levels of ^{90}Sr and ^{137}Cs concentrations in different components of the ecosystem in the NPP industrial stormwater discharge channel (ISWDC)

Syrvey object	^{90}Sr, Bq/kg dry weight	^{137}Cs, Bq/kg dry weight
Frogs	9.7 ± 0.9	18.1 ± 1.7
Young frogs	27.5 ± 2.9	397.5 ± 86.0
Tadpoles	14.8 ± 7.2	486.3 ± 61.0
Fish hatchlings	0.8 ± 0.5	5.0 ± 2.3
Crucian	1.5 ± 0.1	17.3 ± 0.8
Seabream	1.7 ± 0.6	8.8 ± 1.8
Comb Pondweed	15.1 ± 0.8	1228 ± 179
Cladophora	21.6 ± 2.2	1156 ± 150
Plankton	43.7 ± 2.3	3428 ± 178
Sandy-silt substrate	6.4 ± 1.5	81 ± 9

(160000 Bq/kg wet weight) compared to the average values for the other frogs.

The analysis of the ^{90}Sr and ^{137}Cs contents in the marsh frogs of different sexes in the ISWDC population did not reveal any differences in the radionuclide accumulation between male and female specimens. Similar data on ^{90}Sr were obtained for the frog population in the Tagil River. In this case, it was impossible to make any comparable assessment for ^{137}Cs because of the insufficient data available.

The lack of a significant correlation between the ^{90}Sr concentration in the organism and the amphibian age of 1 to 4 years old (correlation coefficient is 0.043) was ascertained. In addition, a trend toward a decrease in this radionuclide accumulation by the frogs along with an increase in the dry mass of the body (correlation coefficient is 0.314) was indicated, which might be related to the decreasing intensity in assimilation processes as the organism ages.

The levels of accumulation of the analyzed radionuclides in mature frogs, current-year-juveniles, and tadpoles were compared to the other representatives of the water ecosystem in the ISWDC (plankton, fishes, plants, and substrate), where a greater hydrobiont diversity than that in the Tagil River was registered. Table 3 shows that both radionuclides are more accumulated in the plankton (^{90}Sr: 44 Bq/kg, ^{137}Cs: 3428 Bq/kg dry weight) and less accumulated in the representatives of the ichthyofauna (^{90}Sr: 0.8–1.7 Bq/kg, ^{137}Cs: 5–17 Bq/kg dry weight). Among amphibians, young frogs and tadpoles accumulate more of both radionuclides than mature frogs, which may be related to the greater plankton contribution into the ration of the young amphibian specimens compared with the mature frogs.

With respect to the Tagil River, the ^{90}Sr and ^{137}Cs concentrations in the sedentary aquatic plants turned out to be approximately two to four times lower and two orders of magnitude lower, respectively, than that in the ISWDC. Particularly in the Elodea, Myriophyllum, and Ceratophyllum, the ^{90}Sr content comprised 4.8, 6.4, and 6.0 Bq/kg dry weight, respectively, while the ^{137}Cs concentration made up 9.1, 7.3, and 11.1 Bq/kg dry weight, respectively. The latter can indicate that the ecosystem in the Tagil River downstream of the dam may be considered radio-ecologically cleaner than that in the ISWDC. The relatively higher ^{137}Cs content in the frogs from the Tagil River makes us assume that they might accumulate this radionuclide in some other place and carry it to this location in their bodies. It can confirm the fact that some amphibians contained unusually high concentrations of the radionuclide.

CONCLUSIONS

The survey findings presented above produce an unusual result indicating that the levels of the ^{137}Cs concentrations in the frogs in the Tagil River at the exit point of the Verkhnii Tagil water-storage reservoir appeared higher than that in the industrial stormwater discharge channel of the Beloyarskii reservoir, where this radionuclide enters from the Beloyarskii NPP. In addition, a frog containing an abnormally high ^{137}Cs concentration was found in each sample from these habitats. The cesium-134 (with a half-life of 2.07 years) was detected in the same specimens, frogs from the ISWDC and the Tagil River, containing 441 and 320 Bq/kg dry weights, respectively. The ^{134}Cs occurrence in the environmental objects is associated with the acivities of the nuclear-fuel-cycle enterprises. This means that the frogs in the Tagil population may accumulate cesium-134 and -137 only on the area contaminated with these radionuclides and transfer them outwards from this area, which is limited by the patterns of migration of a certain species of amphibians. The data indicate the presence of some unidentified source of radioactive ^{134}Cs and ^{137}Cs contamination from which the pollutant it is transferred into the Tagil River by the marsh frog.

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