ISSN 1995-0829, Inland Water Biology, 2022, Vol. 15, No. 2, pp. 189–194. © Pleiades Publishing, Ltd., 2022.
Russian Text © The Author(s), 2022, published in Biologiya Vnutrennykh Vod, 2022, No. 2, pp. 192–197.

AQUATIC TOXICOLOGY

Accumulation of Trace Elements in the Marsh Frog *Pelophylax ridibundus* in Cooling Ponds of the Middle Urals

M. Ya. Chebotina[a, *], V. P. Guseva[a], D. L. Berzin[a, b], I. V. Volkov[c], and E. V. Polyakov[c]

[a] *Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, Yekaterinburg, Russia*
[b] *Ural Federal University named after the first President of Russia B.N. Yeltsin, Yekaterinburg, Russia*
[c] *Institute of Solid State Chemistry, Ural Branch, Russian Academy of Sciences, Yekaterinburg, Russia*
**e-mail: Chebotina@ipae.uran.ru*

Received February 25, 2021; revised October 24, 2021; accepted October 29, 2021

Abstract—A study of the accumulation of trace elements in the marsh frog *Pelophylax ridibundus* Pall., 1771 in the heating zones of the cooling ponds of the Beloyarsk nuclear power plant and Reftinsk heating electric power plant has been carried out. For the entire set of data on the concentrations of elements in frogs, no statistically significant differences are found between the studied water bodies. Using the example of both water bodies, no differences in the accumulation of elements by males and females of amphibians are revealed. A significant correlation between the concentration of elements in adult frogs with their content in water and plankton is found. An excess of the maximum permissible concentrations of some elements in the water of both reservoirs, as well as an excess of the concentrations of a number of elements in frogs, compared with background values, are revealed.

Keywords: marsh frog, pollution, heavy metals, Beloyarsk Reservoir, Reftinsk Reservoir, trace elements, plankton

DOI: 10.1134/S1995082922020043

INTRODUCTION

The problem of environmental pollution with trace elements (heavy metals and rare and dispersed elements) is considered one of the key issues in ecological studies (Vinogradov, 1957; Koval'skii, 1974; Saet et al., 1990). One important feature of trace elements when they enter a body is the existence of threshold concentrations, within which the body is able to regulate metabolic processes. Exceeding the limits of these concentrations in the direction of their decrease or increase in the environment may cause the dysfunction of metabolic processes and lead to various types of diseases and even death (Koval'skii, 1974).

In the Ural region, mining and metallurgical industries are the main sources of heavy metal pollution for water and land ecosystems. The migration of elements and their dispersion from emission sources caused a high level of pollution of soils, rivers, air, groundwater, wild plants, and agricultural products (Mahonina, 1987; *Gosudarstvennyi*..., 1999, 2000, 2008, 2009, 2012). Animals zoned in contaminated areas exhibit a disturbance in their immune systems and the development of pathological processes (Donnik and Smirnov, 2002; Donnik and Khaibullin, 2002).

The high load on the natural ecosystems of the Ural region caused by the presence of heavy metals and other technogenic emissions is exacerbated by an increased radiation background. This background originates from the operation of nuclear industry enterprises in the region and the consequences of radioactive contamination of territories as a result of radiation accidents and human production activities (the use of materials with an increased content of natural radionuclides, medical procedures, etc.) (*Vostochno-Ural'skii*..., 2000; Klimshin et al., 2011; Chebotina et al., 2017).

In a number of papers, frogs are considered indicators of pollution of freshwater ecosystems by various pollutants, including heavy metals (Sharygin, 1980; Pyastolova et al., 1996; Misyura, 2006; Stark, 2006; Zaripova et al., 2009; Smalling et al., 2019; Berzin et al., 2020; Beresford et al, 2020). However, at present, despite the wide distribution of these amphibians in the aquatic ecosystems of various regions of the country and their resistance to negative environmental factors, they are insufficiently studied.

The goal of the present paper is to study the content of trace elements in marsh frogs *Pelophylax ridibundus* Pall. inhabiting cooling ponds of the Middle Urals. To achieve the goal, the following tasks are necessary: study the concentrations of trace elements in water

Abbreviations: MPC, maximal permissible concentration.

and frogs of the Beloyarsk and Reftinsk reservoirs; comparatively assess the concentrations of trace elements in female and male frogs from the studied water bodies; and study the relationship between the content of trace elements in frogs, water, and plankton.

MATERIALS AND METHODS

The marsh frogs and water from the Beloyarsk and Reftinsk reservoirs were studied. Beloyarsk Reservoir, impounded in 1959–1963 by damming the Pyshma River, serves as cooling pond of the Beloyarsk Kurchatov nuclear power plant (NPP). The ecological and geographical characteristics of the reservoir are given in (Trapeznikov et al., 2008). During its existence, the ecosystem of the Beloyarsk reservoir was affected by four nuclear power units, two of which were decommissioned in 1981 and 1989; the third and fourth have been operating since 1980 and 2014, respectively. The Reftinsk Reservoir is a cooling reservoir of one of the largest power plants in Russia; it was formed in 1968 on the Reft River, the left tributary to the Pyshma River. The station runs on coal, which is why ~6 million t of ash and slag are thrown into ash dumps every year. The Reftinsk Poultry Farm, the largest enterprise in the Urals for the production and processing of broiler meat, is located on the shore of the reservoir. Both reservoirs are subject to thermal pollution due to the discharge of heated water.

This study was carried out in 2019. The frog sampling sites were located in the sites of water bodies adjacent to areas of heated water discharge. Five male and five female frogs were caught in each reservoir and put down with ether. In the laboratory, the frogs were weighed, dried, and ashed at 500°C. Water was sampled in triplicate, 5 L per repetition, filtered through a blue ribbon filter to remove mechanical impurities, and dried at 105°C to a dry residue. The samples of amphibians and water prepared in this way were dissolved in a mixture of nitric, hydrochloric, and hydrofluoric acids of chemical purity grade in a ratio of 2 : 1 : 1 and kept in an autoclave for 15–30 min at a temperature of 150–160°C, followed by calcination. The elements were quantitatively determined on an ELAN 9000 quadrupole mass spectrometer (PerkinElmer SCIEX, United States–Canada) using a calibration standard solution with the required range of concentrations of the studied elements. The relative standard error of determinations did not exceed 10–20%. All data given in the figures are calculated on the dry weight of the substance.

The data were processed statistically applying STATISTICA software package (Mann–Whitney, Student, and Pearson tests).

RESULTS

The concentrations of various elements in amphibians varied within five orders of magnitude (Fig. 1). The content of Fe in the frogs of both reservoirs was the highest: $(6–10) \times 10^3$ μg/g. Along the decrease in concentrations, there are rows of elements characteristic of both reservoirs: Ti, V, Mn, Cu, Zn, Br, and Sr (10^2–10^1 μg/g); Li, B, Cr, As, Rb, Ba, and Th (10^1–10^0 μg/g); and Sc, Ga, Y, Zr, Ce, Pb, and U (10^0–10^{-1} μg/g and less). Co and Ni in detectable amounts were found only in the frogs from the Reftinsk Reservoir; Mo, Cd, and Cs were found only in the Beloyarsk Reservoir. The concentrations of these trace elements are below the detection limits of this method. For the rest of the elements, the differences between the frogs of both water bodies turned out to be statistically insignificant ($p > 0.05$).

The mean values of the concentrations of the studied elements in the frogs of the Beloyarsk and Reftinsk reservoirs depending on the sex of the animals are given in Figs. 2 and 3. The male and female amphibians accumulate trace elements in approximately the same amounts. This is confirmed by the results of statistical processing of the totality of data for all the studied elements for both the Beloyarsk and the Reftinsk reservoirs ($p < 0.001$).

The water is an important source of elements in the body of frogs. This is why the relationship between the concentrations of trace elements in the bodies of the animals and the water of the Beloyarsk and Reftinsk reservoirs was studied (Fig. 4). For both reservoirs, a highly significant correlation between their concentration in frogs and in water was revealed for the totality of the studied elements (correlation coefficient 0.78; $p < 0.001$). For the Beloyarsk Reservoir, a significant correlation was also found between the concentrations of trace elements in frogs and plankton (correlation coefficient 0.78; $p = 0.001$) (Fig. 5). In the latter case, we used the data on plankton given in (Polyakov et al., 2012) for analysis.

DISCUSSION

The Beloyarsk and Reftinsk reservoirs in the Middle Urals are water bodies subject to constant anthropogenic pressure. Weakly radioactive and chemical effluents from two nuclear industry enterprises (Beloyarsk NPP and the Institute of Reactor Materials) enter the Beloyarsk Reservoir. The Reftinsk Reservoir receives the washouts of chemicals from ash dumps and a poultry farm. The reservoirs serve as recreation areas for the population and are used for fishing and the industrial farming of caged fish. They are also exposed to thermal pollution due to the discharge of heated water. An analysis of water quality showed an excess of the content of a number of trace elements in the heated area of the sanitary protection zone of reservoirs in comparison with the MPCs established for water bodies of fishery importance (*Perechen'*..., 1999). At the time of the study, the MPCs in the Beloyarsk and Reftinsk reservoirs were exceeded 12 and 14

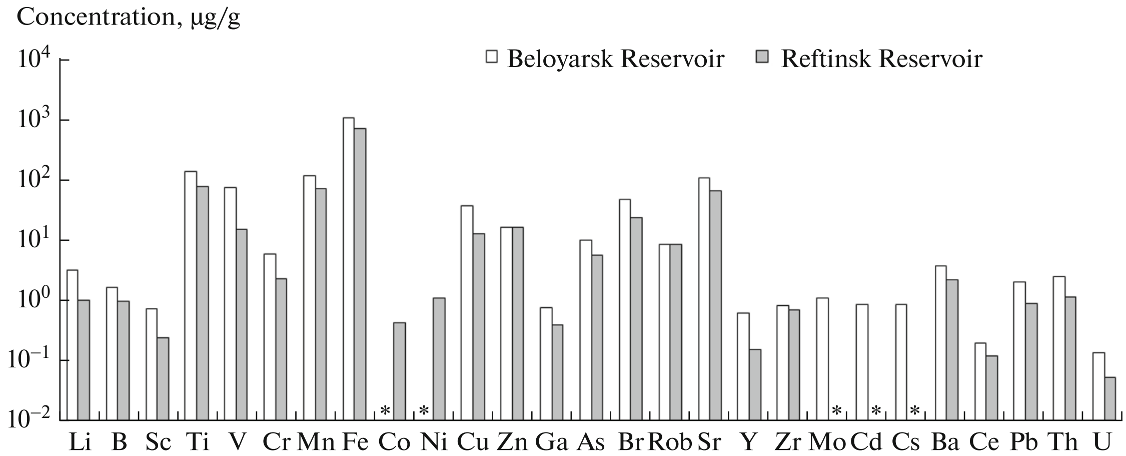

Fig. 1. Mean concentration of trace elements in the marsh frogs of the Beloyarsk and Reftinsk reservoirs. *Concentrations below the detection limit.

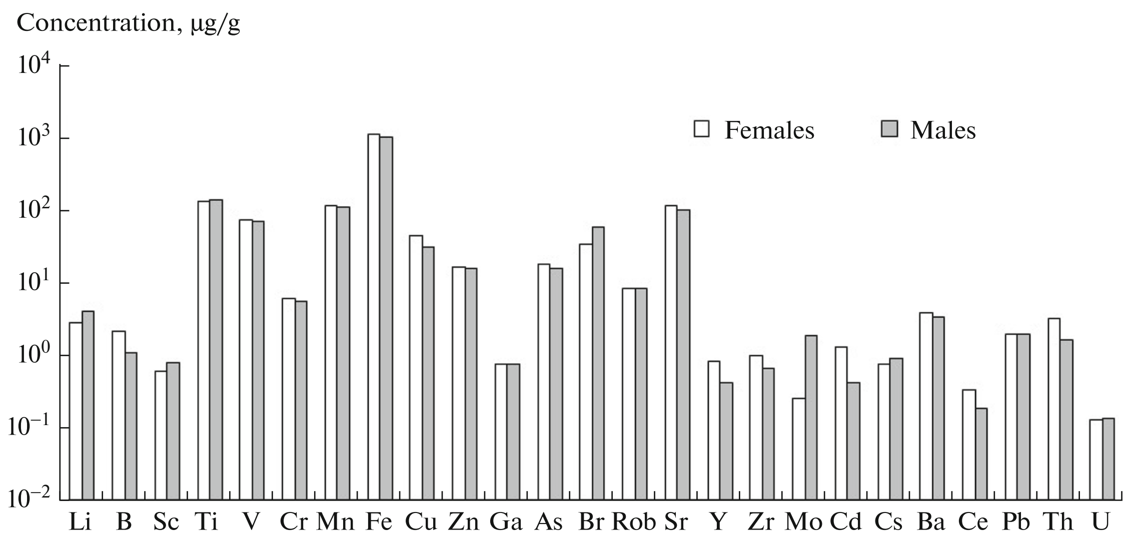

Fig. 2. Concentrations of trace elements in female and male marsh frogs of the Beloyarsk Reservoir.

times for V, 9 and 17 times for Mn, 5 and 12 times for Fe, and 5 and 22 times for Cu, respectively. In addition, the MPCs were exceeded 4 times for Ni and 1.6 times for Zn in the Reftinsk Reservoir.

It has been found that the concentrations of various trace elements in frogs from the heating zones of these reservoirs vary within five orders of magnitude (from 10^{-2} to 10^4 μg/g dry body weight). Frogs of both habitats accumulate iron in the greatest amounts, which is consistent with the data of (Misyura et al., 2004). With small fluctuations in the content of microelements in the frogs of the Beloyarsk and Reftinsk reservoirs, no significant differences were found between animals from different habitats. Similar data on the variability of trace element concentrations in amphibians from different habitats are given in (Sharygin, 1980; Misyura, 2006).

Because frogs are consumed by humans as food in a number of countries around the world (Mirzaj, 2003; Ding, 2015), it was interesting to compare the levels of accumulation of trace elements by amphibians in our studies with background indicators. Due to the absence of MPCs for freshwater animals, for frogs in particular, we used the results of a paper by Nikanorov and Zhulidov (1991), which shows the concentrations of chemical elements in freshwater organisms conventionally taken as background. The excess of these values for some elements reached from 2 to 21 times. The frogs of the Beloyarsk Reservoir contain 2–4 times more Li, Sc, Ti, Ga, and Br, while those of the Reftinsk Reservoir contain Li, Ti, and V. In the Beloyarsk Reservoir, the concentration of V in amphibians from the heating zone exceeded the background values by 18 times, and that of As exceeded it 17 and 7 times for

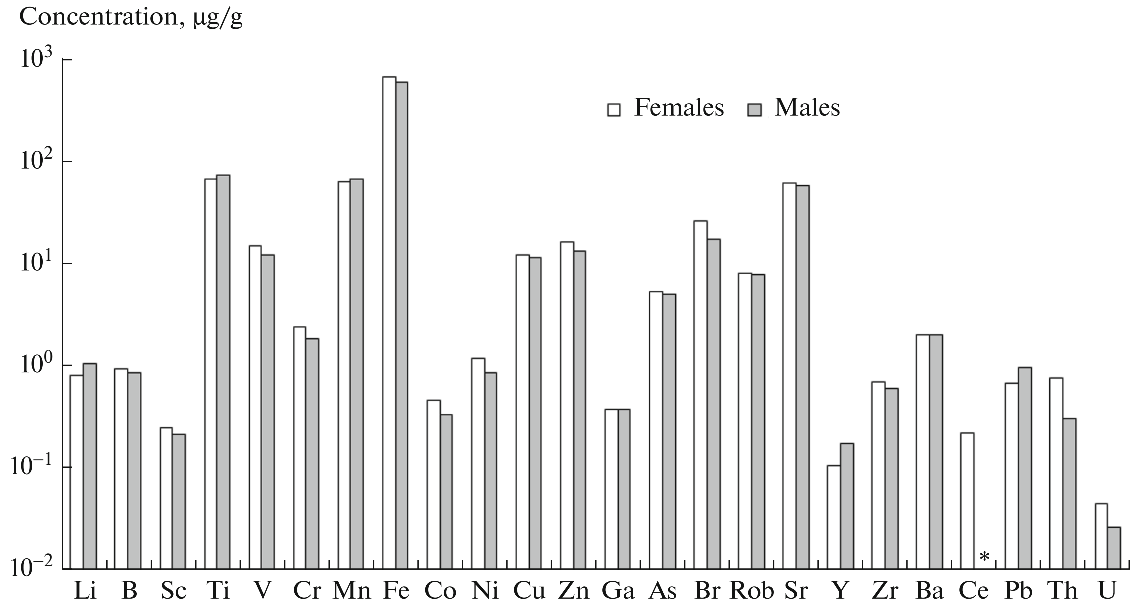

Fig. 3. Concentrations of trace elements in female and male marsh frogs of the Reftinsk Reservoir.
*Concentrations below the detection limit.

the Beloyarsk and Reftinsk reservoirs, respectively. Cooling systems at nuclear and thermal power plants, as well as ash dumps at Reftinskaya GRES (Mahonina, 1987; Trapeznikov et al., 2008), may be sources of increased concentrations of trace elements in cooling ponds.

The present study revealed that the accumulation of the totality of the studied elements by the marsh frog does not depend on the sex of the animals. Earlier, we revealed the absence of differences in the accumulation of ^{90}Sr and ^{137}Cs radionuclides by frog males and females of the Beloyarsk Reservoir (Berzin et al., 2020).

Water serves as a necessary habitat for marsh frogs, providing their vital activity. The absorption of trace elements from water is possible through the skin and with food. Together with water, frogs swallow food (zoobenthos, juvenile fish, spiders, caddisflies, dragonflies and their larvae, beetles, and others) containing various trace elements (Vershinin, 2007). In addition, the aquatic environment is inhabited by a huge number of plankton species of plant and animal origin. As a rule, marsh frogs feed on consumers of two or

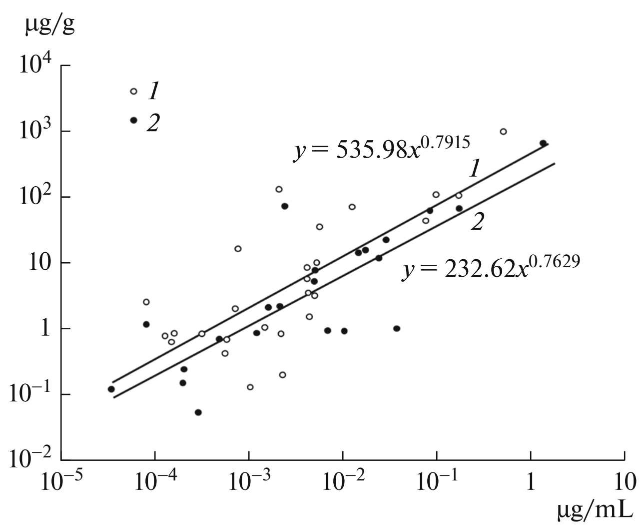

Fig. 4. Correlation between concentrations of trace elements in the marsh frogs (ordinate) and in water (abscissa) of the Beloyarsk (*1*) and Reftinsk (*2*) reservoirs.

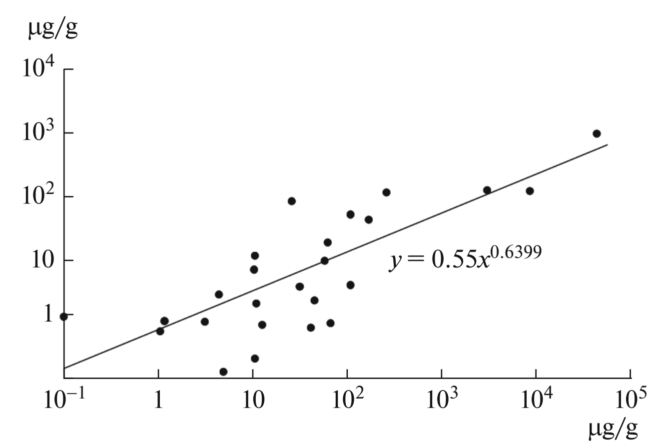

Fig. 5. Correlation between concentrations of trace elements in the marsh frogs (ordinate) and in the plankton (abscissa) of the Beloyarsk Reservoir.

three orders, which are directly part of the plankton or its consumers. Plankton, which has a huge biosorption activity, contains a wide variety of heavy metals and rare and trace elements (Polyakov et al., 2012). This explains the significant correlation we found between the content of trace elements in marsh frogs and water, on the one hand, and between frogs and plankton, on the other.

These results may be used for a comparative assessment of the content of trace elements in marsh frogs of other water bodies around the country and the development of appropriate standards for them.

CONCLUSIONS

Marsh frogs inhabiting the heated zone of the Beloyarsk and Reftinsk reservoirs accumulate various trace elements in concentrations within the limits of five orders of magnitude. For the entire set of trace elements found in frogs, significant differences between the studied water bodies have not been revealed. It has been shown that the accumulation of trace elements by the amphibians as a whole does not depend on the sex of the animals. A significant correlation was revealed between the concentration of trace elements in adult frogs and their content in water and plankton. An excess of MPCs of some trace elements in the water of the heating zone of both reservoirs, as well as the concentrations of a number of trace elements in frogs of the studied habitats in comparison with their background values, was revealed.

FUNDING

This study was carried out as part of the State Assignment of the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, project no. AAAAA19-119032090023-0.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

REFERENCES

Beresford, N.A., Barnett, C.L., Gashchak, S., et al., Radionuclide transfer to wildlife at a "Reference site" in the Chernobyl Exclusion Zone and resultant radiation exposures, *J. Environ. Radioact.*, 2020, vol. 211, p. 1.

Berzin, D.L., Chebotina, M.J., and Guseva, V.P., Accumulation of radionuclides in the marsh frog *Pelophylax ridibundus* in the zone of a nuclear plant, *Inland Water Biol.*, 2020, vol. 13, no. 4, p. 709.
https://doi.org/10.1134/S1995082920060048

Chebotina, M.Ya., Ponomareva, R.P., and Trapeznikov, A.V., *Dispersnost' chastits plutoniya v proizvodstvennykh protsessakh i okruzhayushchei srede* (Dispersion of Plutonium Particles in Production Processes and Environment), Yekaterinburg: AkademNauka, 2017.

Din', V.Kh., Rational processing technology of marsh frog *Rana ridibunda, Cand. Sci.* (*Eng.*) *Dissertation*, Astrakhan, 2015.

Donnik, I.M. and Khaibullin, R.R., The health status assessment of cattle from farms with different ecological situation, *Trudy Nauchno-prakticheskoi konferentsii "VURS-45"* (Proc. Sci.-Pract. Conf. "VURS-45"), Ozersk: VRB, 2002.

Donnik, I.M. and Smirnov, P.N., *Ekologiya i zdorov'e zhivotnykh* (Ecology and Animal's Health), Yekaterinburg: Ural. Otd., Ross. Akad. Nauk, 2002.

Gosudarstvennyi doklad o sostoyanii i ob okhrane okruzhayushchei sredy Sverdlovskoi oblasti (Governmetnal Report on the State and Protection of Environment of Sverdlovsk Oblast), Yekaterinburg: Ural. Gos. Univ., 1999, 2000, 2008, 2009, 2012.

Klimshin, A.V., Mindubaev, M.G., and Kolotukhin, A.Z., Radon transfer in the surface layer of soil under free thermal convection, *Ural. Geofiz. Vestn.*, 2011, no. 1 (18), p. 23.

Koval'skii, V.V., *Geokhimicheskaya ekologiya* (Geochemical Ecology), Moscow: Nauka, 1974.

Makhonina, G.I., *Khimicheskii sostav rastenii na promyshlennykh otvalakh Urala* (The Chemical Composition of Plants on the Industrial Dumps of Urals), Sverdlovsk: Ural. Gos. Univ., 1987.

Mirzaj, A., *Biological Evaluation of the Frog Species of Rana ridibunda in Anzali Lagoon for Consumption and Export*, Tehran: Agric. Res., Educ., Ext. Org., 2003, p. 1.

Misyura, A.N., Influence of wastes from mining enterprises on the level of trace elements in organs and tissues of amphibians from reservoirs with various degree of pollution, in *Problemy ekologii i okhrany prirody tekhnogennogo regiona* (Ecology and Nature Protection in the Technogenic Region), Donetsk: Donetsk. Nats. Univ., 2006, p. 140.

Misyura, A.N., Marchenkovskaya, A.A., and Spodarets, D.A., Comparative characteristics of the content of biogenic elements in the organs and tissues of the marsh frog from reservoirs polluted by wastes from the uranium mining industry and Dnieper-Orel'skiy Nature Reserve, *Vestn. Orenb. Gos. Univ.*, 2004, no. 6, p. 62.

Nikanorov, A.M. and Zhulidov, A.V., *Biomonitoring metallov v presnovodnykh ekosistemakh* (Biomonitoring of Metals in Freshwater Ecosystems), Leningrad: Gidrometeoizdat, 1991, p. 56.

Perechen' rybokhozyaistvennykh normativov, predel'no dopustimykh kontsentratsii (*PDK*) *i orientirovochno bezopasnykh urovnei vozdeistviya* (*OBUV*) *vrednykh veshchestv dlya vody vodnykh ob"ektov, imeyushchikh rybokhozyaistvennoe znachenie* (List of Fishery Standards, Maximum Permissible Concentrations (MPC) and Approximate Safe Levels of Impact (SLI) of Harmful Substances in Water Reservoirs Proposed for Fishery), Moscow: VNIRO, 1999.

Polyakov, E.V., Chebotina, M.Ya., Khlebnikov, N.A., et al., Patterns of the accumulation of chemical elements in the plankton of a fresh water body, *Russ. J. Ecol.*, 2012, vol. 43, no. 5, p. 378.

Pyastolova, O.A., Vershinin, V.L., Trubetskaya, E.A., et al., Utilization of amphibians in bioindication research on territories of the Eastern Urals radioactive trace, *Russ. J. Ecol.*, 1996, vol. 27, no. 5, p. 361.

Saet, Yu.E., Revich, B.A., Yanin, E.N., et al., *Geokhimiya okruzhayushchei sredy* (Geochemistry of Environment), Moscow: Nedra, 1990.

Sharygin, S.A., The influence of anthropogenic factors on dynamics of trace elements in the body of some amphibians and reptiles, *Cand. Sci.* (*Biol.*) *Dissertation*, Sverdlovsk, 1980.

Smalling, K.L., Anderson, C.W., Honeycutt, R.K., et al., Associations between environmental pollutants and larval amphibians in wetlands contaminated by energy-related brines are potentially mediated by feeding traits, *Environ. Pollut.*, 2019, vol. 248, p. 260.
https://doi.org/10.1016/j.envpol.2019.02.033

Stark, K., Risk from radionuclides: a frog's perspective: accumulation of ^{137}Cs in a riparian wetland, radiation doses, and effects on frogs and toads after low-dose rate exposure, *PhD Thesis*, Stockholm: Dep. Syst. Ecol., Stockholm Univ., 2006.

Trapeznikov, A.V., Chebotina, M.Ya., Trapeznikova, V.N., et al., *Vliyanie AES na radioekologicheskoe sostoyanie vodoema-okhladitelya* (Influence of NPP on the Radioecological State of the Cooling Pond), Yekaterinburg: AkademNauka, 2008.

Vershinin, V.L., *Amfibii i reptilii Urala* (Amphibians and Reptiles of the Urals), Yekaterinburg: Ural. Otd., Ross. Akad. Nauk, 2007.

Vostochno-Ural'skii radioaktivnyi sled (East Ural Radioactive Trace), Yekaterinburg: Inst. Prom. Ekol., Ural. Otd., Ross. Akad. Nauk, 2000.

Vinoradov, A.P., *Geokhimiya redkikh i rasseyannykh khimicheskikh elementov v pochvakh* (Geochemistry of Rare and Trace Chemical Elements in Soils), Moscow: Akad. Nauk SSSR, 1957.

Yushkova, E.A., Bodnar, I.S., Shadrin, D.M., et al., Cytogenetic and molecular genetic indexes in populations of anura (*Rana arvalis* Nilsson) under conditions of radioactive and chemical pollution of an aquatic environment., *Inland Water Biol.*, 2018, vol. 11, no. 3, p. 349.
https://doi.org/10.1134/S1995082918030239

Zaripova, F.F., Faizzulin, A.I., and Yumagulova, G.R., The content of heavy metals in the liver of marsh frog *Rana ridibunda* Pallas, 1771 in Bashkir Trans-Ural region, *Vestn. Orenb. Gos. Univ.*, 2009, no. 6, p. 145.

Translated by D. Pavlov