

Mercury Content in Tissues of Amphibians of Northwest Russia (Vologda Region)

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Abstract— The content of mercury in the tissues of amphibians from Northwest Russia (Vologda region) varies within 0.001 to 0.370 mg/kg wet weight. The average values of Hg content in the organs and tissues of studied amphibian species decrease in the order: liver > kidneys > heart > intestines > muscles > skin. The mercury content in the tissues of genus *Pelophylax* is higher than that in the tissues of samples of genus *Rana* and species *Bufo bufo*. The content of mercury in the tissues of *Rana* spp. collected in the Western part of the Vologda region is significantly higher than that in samples from the Eastern part.

Keywords: mercury, amphibia, tissues

I. INTRODUCTION

Mercury entering the atmosphere due to natural processes (volcanic eruptions, evaporation from the surface of the world ocean) and human economic activity (burning of fossil fuels, metallurgical and chemical production, etc.), is carried over long distances by air and accumulates at a considerable range from the very source of the emission [1], [2].

When mercury accumulates in the organism, it produces a neurotoxic effect, negatively affects reproductive function and leads to disorders of embryonic development in vertebrates [3].

Aquatic ecosystems where mercury becomes bioavailable (becoming involved in food chains) under the influence of microorganisms play the key role in the

biogeochemical cycle of mercury [4], [5]. All forms of mercury are toxic, but methylated mercury – CH₃Hg⁺ is the most toxic of them [6]. Mercury accumulates in living organisms (mainly in the form of methylmercury).

Currently, the presence of lateral correlations in the exchange of matter and energy between aquatic and terrestrial ecosystems is beyond any doubt [7]. Amphibious organisms play an important role in the mutual exchange of organic matter between aquatic and terrestrial ecosystems.

The chemical composition of animals is a key indicator of the relation between them and the environment. The importance of studying the chemical composition of living organisms increases in the situation when background contents of elements are different in different ecosystems.

The number of studies on the behavior of mercury in the environment has increased in recent decades. However, the issues considered in these researches are usually utilitarian or social in nature, focused mostly on the potential harm to human and animal health [8], [3]. The accumulation of mercury in amphibians and the way it is stored inside their organisms are insufficiently studied, presently despite the fact that amphibians play a unique role in ecosystems, connecting the aquatic and terrestrial biocycles. Previous studies have demonstrated unequal accumulation of mercury in mammals from different parts of the Vologda region territory [9], [10]. At the same time, high concentrations of mercury in perch muscles (>0.5 mg HG/kg) have been

repeatedly found in fish from lakes of the Vologda region [11], [12], [13].

II. MATERIALS AND METHODS

The material for determining the mercury content in amphibian tissues was collected in 2014-2018 on the territory of 11 administrative units of the Vologda region, which differ in natural and climatic conditions (Fig. 1): Site 1-Babaevsky, Site 2-Vytegorsky, Site 3-Vashkinsky, Site 4-Vozhegodsky, Site 5-Cherepovets, Site 6-Kaduysky, Site 7-Kirillovsky, Site 8-Totemsky, Site 9-Velikoustyugsky. In total there were 2,046 samples from 420 individuals of 5 amphibian species: moor frog (*Rana arvalis*), common frog (*Rana temporaria*), pool frog (*Pelophylax lessonae*), marsh frog (*Pelophylax ridibundus*), common toad (*Bufo bufo*). The amphibians were caught with a hydrobiological net. Tissue samples (liver, kidneys, heart, intestines, muscles, skin) were placed in plastic bags, frozen and stored at 16°C. The mercury presence in all animal tissues was determined on the mercury analyzer RA-915M with PYRO add-on attachment.

The measurement accuracy was controlled using the certified biological material DOLT-5 (Institute of environmental chemistry, Ottawa, Canada).

The results as arithmetic means. The arithmetic means, the standard error of mean, minimal and maximal values are all given in mg/kg of wet weight. The results were statistically processed, the significance of differences in mercury content values was estimated by non-parametric method (Kruskal-Wallis) [14]. To determine the correlation between the amount of metal in different pairs of animals' organs, as well as the correlation between the amount of metal in amphibians and the natural and climatic features of their habitat (the values of which are distributed non-normally) the nonparametric Spearman's correlation coefficient (r_s , $p \leq 0.05$) was used [15].

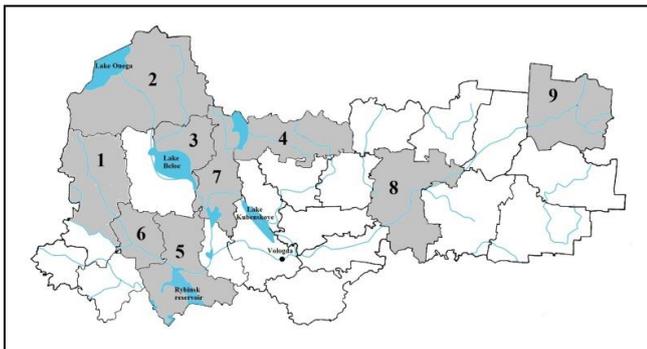


Fig. 1. Study areas (Vologda Region): Site 1 - Babaevsky, Site 2 - Vytegorsky, Site 3 -Vashkinsky, Site 4 - Vozhegodsky, Site 5-Cherepovetsky, Site 6 - Kaduysky, Site 7 - Kirillovsky, Site 8 - Totemsky, Site 9 - Velikoustyugsky.

III. RESULTS AND DISCUSSION

The research revealed that the content of mercury in the tissues of the studied amphibian species varied from 0.001 to

0.37 mg/kg of wet weight. Maximum values of mercury content were observed in the liver and kidneys, where it significantly exceeded the Hg content in other organs (Fig.2, Table I). Minimal values were found in the skin of animals. Therefore, the average values of Hg content in the organs of the studied amphibian species decrease in the following order: liver > kidneys > heart > intestines > muscles > skin.

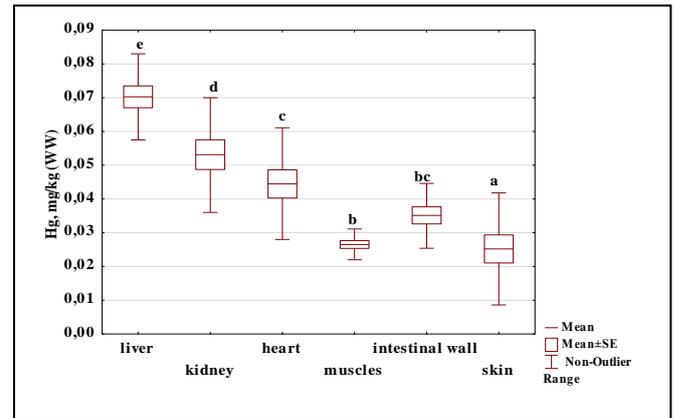


Fig. 2. The content of mercury in the tissues of Amphibia

Obtained data on the distribution of mercury in amphibians matches with data from the literature on this issue: the maximum contents of the metal are found in the liver and the kidneys, the minimal is seen in the muscles and skin [16]. Studies on mercury accumulation in organs and tissues of mammals also show the maximal mercury content to be found in the liver and kidneys, in other words, in the organs that perform the function of detoxification [17], [18], [19].

The content of mercury in the organs of amphibians caught in the study area is comparable with the data available from the literature, on animals whose habitats are significantly removed from the sources of pollution. The mercury content in the liver and kidneys of *Rana arvalis*, *Pelophylax lessonae* and *Pelophylax ridibundus* caught in the study area, is about 2-2.5 times lower than that in frogs of the same species caught in the outskirts of Temirtau city in Central Kazakhstan, where large mining and metallurgical industrial structures are deployed [20]. The average content of metal in the muscles and skin of the *Pelophylax lessonae* from the study area is 0.037 ± 0.004 in the muscles, 0.024 ± 0.003 in the skin and are comparable to the mercury content in the organs of individuals of the same species caught in the nature reserve of Serbia [21]. Content of mercury in organs of *Bufo bufo* and *Rana temporaria* caught in the study area are comparable with the data obtained after the analysis of organs of individuals of these species caught in forests of Finland. Finnish samples were not affected by industrial activity, and the metal content identified are several orders of magnitude lower than they are for individuals of the same species from areas of a large mercury mining site in Yugoslavia ($2.33-22.77$ mg / kg) [16].

TABLE I. THE CONTENT OF MERCURY IN THE TISSUES OF AMPHIBIA (MG/KG WET WEIGHT)

Species	Mean	SE	Min	Max	K-W
liver					
<i>B. bufo</i>	0.057	0.004	0.001	0.337	a
<i>R. arvalis</i>	0.057	0.005	0.001	0.305	a
<i>P. lessonae</i>	0.104	0.013	0.021	0.32	b
<i>R. temporaria</i>	0.08	0.007	0.001	0.371	a
<i>P. ridibundus</i>	0.101	0.003	0.089	0.123	b
kidney					
<i>B. bufo</i>	0.04	0.003	0.001	0.183	a
<i>R. arvalis</i>	0.046	0.006	0.001	0.24	a
<i>P. lessonae</i>	0.077	0.012	0.001	0.275	b
<i>R. temporaria</i>	0.051	0.004	0.001	0.257	a
<i>P. ridibundus</i>	0.074	0.003	0.048	0.088	b
intestinal wall					
<i>B. bufo</i>	0.027	0.002	0.001	0.151	a
<i>R. arvalis</i>	0.03	0.003	0.001	0.153	ab
<i>P. lessonae</i>	0.058	0.021	0.001	0.764	c
<i>R. temporaria</i>	0.041	0.004	0.001	0.109	bc
<i>P. ridibundus</i>	0.03	0.002	0.019	0.041	abc
heart					
<i>B. bufo</i>	0.029	0.003	0.001	0.207	a
<i>R. arvalis</i>	0.034	0.004	0.001	0.193	ab
<i>P. lessonae</i>	0.037	0.005	0.001	0.156	b
<i>R. temporaria</i>	0.047	0.005	0.001	0.326	b
<i>P. ridibundus</i>	0.058	0.004	0.042	0.078	c
muscles					
<i>B. bufo</i>	0.022	0.002	0.001	0.186	a
<i>R. arvalis</i>	0.021	0.001	0.001	0.108	a
<i>P. lessonae</i>	0.037	0.004	0.001	0.091	bc
<i>R. temporaria</i>	0.033	0.003	0.001	0.27	b
<i>P. ridibundus</i>	0.05	0.002	0.037	0.059	c
skin					
<i>B. bufo</i>	0.014	0.001	0.001	0.055	a
<i>R. arvalis</i>	0.019	0.001	0.001	0.093	ab
<i>P. lessonae</i>	0.024	0.003	0.001	0.074	b
<i>R. temporaria</i>	0.03	0.003	0.001	0.26	c
<i>P. ridibundus</i>	0.027	0.003	0.014	0.043	c

Note. AM – arithmetic average; SE – standard error; KW - Kruskal-Wallis test (a, b, c – values differ significantly in tissues of different species of amphibians (in the column) at $p < 0.05$)

The concentration of mercury in the tissues of the studied amphibian species was found to be different. The concentration of mercury in the liver and kidneys of *Pelophylax* species is significantly higher than in the same organs *Rana* and *Bufo* species, which do not differ considerably statistically (Fig. 3). The maximal level of metal concentration in the muscles was determined in the organisms of *Pelophylax* species, the average level was seen in the organisms *Rana* species, the minimal was detected in *Bufo* species, these differences are statistically significant (Fig. 3).

Apparently, this is explained by differences in the ecology of these species: *Pelophylax* are semi-aquatic, while *Rana* and *Bufo* are terrestrial. Terrestrial species stay in water only during the breeding season and a short period after metamorphosis [22]. Thus, mercury accesses the *Pelophylax* organisms mainly with food objects (insects and hydrobionts), while the rest of the studied amphibian species mainly feed on land where food objects generally contain less Hg [Необходимое уточнение, на мой взгляд].

A strong correlation of mercury content was revealed in several pairs of the studied tissues of the *Rana arvalis*: liver – intestinal wall, liver – muscles, kidney – muscles,

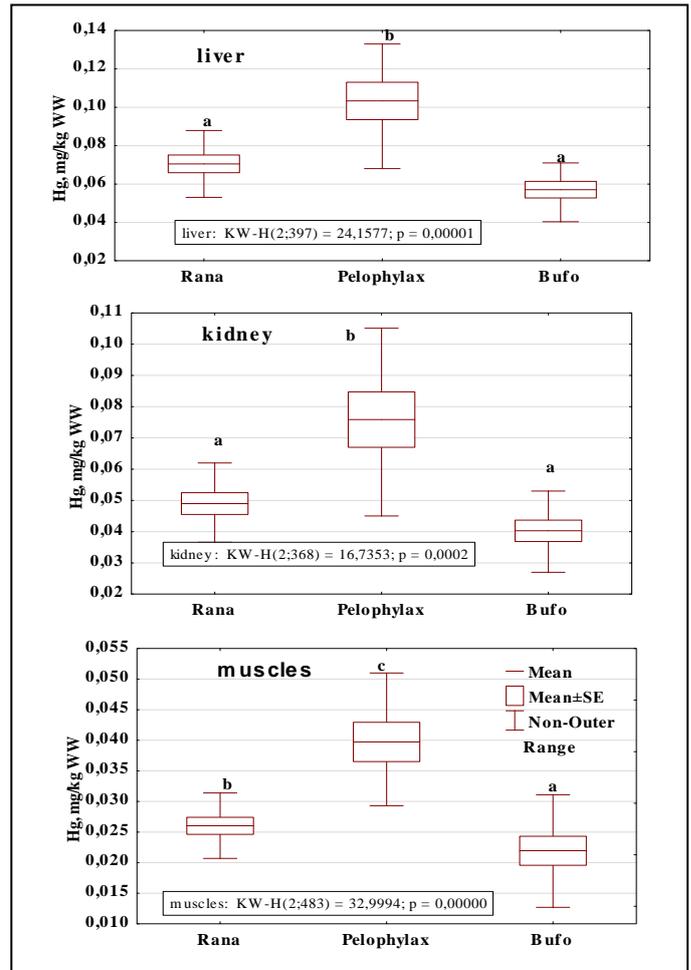


Fig. 3. The content of mercury in the tissues of Amphibia

intestinal wall - muscles ($r_s = 0.24 - 0.43$, $p \leq 0.05$, $n = 140$). A correlation of mercury content in all pairs of *Rana temporaria* tissues was revealed ($r_s = 0.44 - 0.63$, $p \leq 0.05$, $n = 178$) (Table II).

TABLE II. CORRELATION OF MERCURY CONTENT IN AMPHIBIANS OF THE GENUS

	liver	kidney	intestinal wall	muscles
<i>Rana arvalis</i>				
liver			$r_s = 0.43$ $p \leq 0.05$	$r_s = 0.24$ $p \leq 0.05$
kidney				$r_s = 0.41$ $p \leq 0.05$
intestinal wall	$r_s = 0.43$ $p \leq 0.05$			$r_s = 0.24$ $p \leq 0.05$
muscles	$r_s = 0.24$ $p \leq 0.05$	$r_s = 0.41$ $p \leq 0.05$	$r_s = 0.24$ $p \leq 0.05$	
<i>Rana temporaria</i>				
liver		$r_s = 0.63$ $p \leq 0.05$	$r_s = 0.60$ $p \leq 0.05$	$r_s = 0.48$ $p \leq 0.05$
kidney	$r_s = 0.63$ $p \leq 0.05$		$r_s = 0.44$ $p \leq 0.05$	$r_s = 0.47$ $p \leq 0.05$
intestinal wall	$r_s = 0.60$ $p \leq 0.05$	$r_s = 0.44$ $p \leq 0.05$		$r_s = 0.54$ $p \leq 0.05$
muscles	$r_s = 0.48$ $p \leq 0.05$	$r_s = 0.47$ $p \leq 0.05$	$r_s = 0.54$ $p \leq 0.05$	

No significant differences in metal content in organs and tissues from males and females of the investigated species were detected.

The species of *Rana* genus were taken as an example to reveal the correlation between mercury content in amphibians' organs and the natural and climatic features of their habitat, since their number in the General sample was the maximal, and there were no significant differences in the content of metal in the organs of species (*Rana temporaria* and *Rana arvalis*).

The mercury content in the corresponding tissues of amphibians of the genus *Rana* captured in different parts of the studied territory is not the same. The maximal values were found in the Western administrative units (districts) of the Vologda region: Site 1, Site 2, Site 3, Site 4. The mercury content in amphibians' organs from Site 5, Site 6, Site 7, Site 8 is given below. Minimal Hg content values for all organs were seen in the samples of *Rana* from Site 9 (Table III, Fig.1).

The habitats of studied amphibians differ in natural and climatic parameters. The Western regions (Sites 1-4) are characterized by the presence of a large number of lakes and marshland, while there are no large waterbodies nor large marshlands in the East of the region (Sites 8 and 9) [23]. It is known that the aquatic environment is considered to be the main source of organic mercury chemical combinations in trophic networks due to the fact that it is found in waterbodies and wetlands that are favourable in terms of conditions needed for the bacterial process of converting of less toxic organic metal combinations to metalorganic ones [24].

TABLE III. MERCURY CONTENT IN THE TISSUES OF INDIVIDUALS OF THE GENUS RANA FROM DIFFERENT DISTRICTS OF THE VOLOGDA REGION (MG/KG WET WEIGHT)

Site	liver	kidney	muscles
1 (n=15)	0.193±0.031 ^e 0.001-0.371	0.109±0.011 ^f 0.047-0.197	0.044±0.007 ^c 0.001-0.105
2 (n=19)	0.071±0.010 ^{cd} 0.019-0.129	0.049±0.006 ^{de} 0.010-0.086	0.031±0.005 ^{cde} 0.003-0.063
3 (n=7)	0.082±0.005 ^d 0.058-0.094	0.057±0.004 ^{de} 0.042-0.071	0.038±0.003 ^c 0.027-0.048
4 (n=7)	0.083±0.013 ^d 0.046-0.153	0.050±0.011 ^{cde} 0.013-0.102	0.035±0.005 ^{de} 0.019-0.056
5 (n=197)	0.069±0.007 ^{bcd} 0.001-0.358	0.048±0.006 ^{bcd} 0.001-0.257	0.025±0.002 ^{abcd} 0.001-0.137
6 (n=21)	0.044±0.009 ^b 0.001-0.167	0.027±0.004 ^{bc} 0.009-0.067	0.017±0.002 ^{abcd} 0.001-0.033
7 (n=7)	0.031±0.007 ^b 0.014-0.067	0.022±0.005 ^{bc} 0.012-0.054	0.017±0.004 ^{abc} 0.008-0.039
8 (n=9)	0.034±0.002 ^{bc} 0.021-0.045	0.022±0.002 ^b 0.015-0.031	0.049±0.028 ^d 0.016-0.270
9 (n=8)	0.017±0.002 ^a 0.010-0.026	0.011±0.001 ^a 0.008-0.018	0.012±0.001 ^{ab} 0.009-0.016

Note. Above the line the average values and their possible measurement errors are given (mean ± SE); below the line are given the minimal and maximal values (Min-Max); a, b, c, d, e, f – values differ significantly in tissues of amphibians from different regions (in the column) at p < 0.05).

The statistical analysis revealed correlation between the mercury content in some organs of frogs and the natural and climatic features of their habitat (where they were actually captured) (Table IV).

TABLE IV. THE CORRELATION BETWEEN MERCURY CONTENT IN AMPHIBIANS' ORGANS AND THE NATURAL AND CLIMATIC FEATURES OF THEIR HABITAT

Tissues	Area occupied by wetlands, %	Precipitation, mm/year	Area occupied by lakes, %
liver	r_s = 0.22 p ≤ 0.05	r _s = 0.13 p > 0.05	r_s = 0.36 p ≤ 0.05
kidney	r_s = 0.25 p ≤ 0.05	r_s = 0.18 p ≤ 0.05	r_s = 0.38 p ≤ 0.05
heart	r_s = 0.21 p ≤ 0.05	r _s = -0.02 p > 0.05	r_s = 0.37 p ≤ 0.05
Intestinal wall	r_s = 0.23 p ≤ 0.05	r _s = -0.02 p > 0.05	r_s = 0.18 p ≤ 0.05
skin	r _s = -0.02 p > 0.05	r_s = -0.19; p ≤ 0.05	r _s = 0.04 p > 0.05
muscles	r _s = 0.06 p > 0.05	r _s = 0.28; p > 0.05	r_s = 0.27 p ≤ 0.05

Note. Reliably significant indicators are highlighted in bold

The moderate positive correlations between the condition of liver, kidneys, heart and intestines of frogs (*Rana*) and the degree of marshiness were revealed (r_s = 0.21-0.25 при p ≤ 0.05).

The average annual rainfall affects mercury content only in the kidneys (r_s = 0.18 at p ≤ 0.05) and in the skin (r_s = -0.19 at p ≤ 0.05) of amphibians.

The reliable positive correlations between the mercury content and the proportion of lakes in the area of their habitats (r_s = 0.18-0.38 at p ≤ 0.05) are revealed in all the organs of frogs except the skin.

The reliable positive correlation between the content of mercury in the organisms of amphibians on one side and the average annual rainfall, the lake coefficient, the percentage of the area (%) covered by wetlands (marshlands) at the other side shows that the content of metal in biotic components of ecosystems largely depends on the availability of wetlands in animals' habitats.

IV. CONCLUSION

The studied amphibian species of the Vologda region differ in values of absolute mercury concentration in organs. The maximum concentrations of Hg were registered in the liver and kidneys of animals, the average was registered in the heart, intestines and muscles, and the minimal was found in the skin.

The concentration of metal in the liver and kidneys of *Pelophylax lessonae* and *Pelophylax ridibundus* exceeds the concentration of metal in the same organs of *Bufo bufo*, *Rana arvalis* and *Rana temporaria*.

The mercury concentration level in the organs of *Rana* species Western and Northwest part of the Vologda region is much higher than it is for the population from the Central and Eastern part of the region. The significant positive correlations between the mercury concentration in all the studied organs of *Rana*, including the skin, and the lake coefficient of their habitats were revealed, as well as the correlation between the mercury concentration in the liver, kidneys, heart and intestines of these animals and the degree of marshiness of the site.

Apparently, the variability of mercury concentrations in organs of the studied species is associated not only with

differences in the way of life, features of diet, but also with natural and climatic conditions of their habitats.

ACKNOWLEDGMENT

The reported study was funded by RFBR according to research project No. 18-34-00569

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