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Accumulation of Macro- and Trace Elements in the "Mother–Placenta–Fetus" System in Bank Voles (*Clethrionomys glareolus*) in the Area of a Large Copper Smelter

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Abstract—The accumulation of 28 elements (Na, Ca, Sc, Cr, Fe, Co, Zn, As, Br, Rb, Sr, Ag, Sb, Cs, Ba, La, Ce, Nd, Sm, Eu, Tb, Yb, Lu, Hf, Ta, Au, Th, U) in a maternal organism (the liver), the placenta and fetuses of the bank vole (*Clethrionomys glareolus*) from the vicinity of a large copper smelter and from the background territories (Russia, the Middle Urals) has been analyzed. It was shown that the level of trace element contamination (TEC) of the environment had no significant effect on the levels of elements in different types of samples. The exception was Br: its concentrations in the liver, placenta and fetuses from contaminated territories exceeded twofold the respective background values. At the same time, the type of accumulation of most elements depended on the sample type: the maximum concentrations of Cr, Fe, Zn, Rb, Sb, La, Ce accumulated in the liver of maternal individuals; Co, Ag, Eu, Tb, Th accumulated in the placenta; Na, Ca, Br, Ba, Ta accumulated in fetuses, and the levels of As, Cs, Nd, Lu, U did not vary between the samples compared. It was concluded that the TEC components under consideration in effective concentrations had no significant negative impact on the bank vole offspring quality in the area of the copper smelter.

Keywords: industrial contamination, macro elements, trace elements, rare earth elements, small mammals, liver, placenta, fetus

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The chemical elements dispersed in the environment are part of the global and regional biogeochemical cycles [1, 2]. Living organisms play an important role in these cycles: at each trophic level, geochemical selection is determined by the content of elements in the environment, different availablity of their forms, as well as specificity of food composition and abundance. Therefore, the concentrations of chemical elements in living organisms are not identical to their levels in the environment.

The problem of elemental homeostasis maintenance of living organisms is especially pressing under the conditions of anthropogenic pollution of the environment [3], which in the past century became multicomponent and large-scale, while its consequences became disastrous [4–6]. Animals from native populations, which permanently live and/or feed on polluted territories, are exposed to a complex mixture of chemical compounds resulting in additive, nonadditive or synergic effects [7–9]. In different groups of terrestrial vertebrates, trace element contamination (TEC) can lead to deterioration of the general state and reproductive abilities of parent individuals and decreases the surivival rate of offspring, even when the acting doses are lower than the lethal ones [10–14].

In addition to the "traditional" pollutants (carbon, sulfur, chlorine, heavy metal compounds), the anthropogenic inflow of rare earth elements (REE) to ecosystems has dramatically increased in recent decades [15, 16]. In spite of their insignificant content in the Earth crust, REE are widely used in industry (including metallurgy), agriculture, medicine, novel technologies, etc. It has been established experimentally that a single entry of REE into organisms of adult mammals with food and/or water has no negative effects, while long-term consumption is accompanied by their accumulation in soft tissues, primarily in the liver [16]. Other pathways of REE entry may result in the dam-

age to separate systems and an organism as a whole, up to lethal outcome [17, 18]. The data on the REE effects on offsrping are contradictory [16, 19, 20]. Until now there is not enough information about the effects of REE on the objects of wild nature.

For mammals, the processes of translocation and redistribution of elements in the "mother–placenta–fetus" are very important, because the latter is completely dependent on the maternal supply system [21, 22]. Both insufficient and excessive inflow of trace elements (TE) during pregnancy can result in early embryonic death, impaired growth and development of a fetus, abortions and birth of weakened offspring [14, 23–25].

The most of studies on the effects of REE on reproduction in mammals have been carried out in laboratory (rats, mice) or agricultural (small and large cattle) animals, less frequently in wild rodents (voles, mice and hamsters). At the same time, simultaneous analysis is performed for a small number of elements and the duration of exposure is low. The attempts to assess the effect of the TE complex in the "mother–placenta–fetus" system have been made only in single experimental works [19, 26, 27]; there are no such data on the natural populations of mammals.

Over the past 30 years, we have carried out the annual surveys of small mammals (SM) population in the area of the Middle Urals Copper Smelter (MUCS) [28, 29]. Over this period, extensive data have been obtained on the TE levels in the body (the liver, kidneys, spleen, skeleton, reproductive organs) and the diets of small mammals inhabiting the territories with different TEC levels [14, 30–32].

Previously we estimated TE levels at the survey sites [30] and established that the food of bank voles close to the plant (1–3 km) contained much more Zn (3-fold), Cs, Cd, Co, Fe, Cu, Cr, As (4–6-fold), Br (8-fold) and Pb (13-fold). The concentrations of other elements (Ca, Rb, Sr) were, on the contrary, 1.5–2.5 times lower than the background values (30 km from the plant). A conclusion was made about considerable TEC of the territories in the vicinity of the plant.

The accumulation of pollutants of top priority for the survey region (Cu, Zn, Cd and Pb) in the "mother–placenta–fetus" system has been analyzed in bank voles inhabiting the territories with contrasting levels of pollution [14]. It has been shown that the placental barrier limited the entry of Cu, Zn and Cd into fetuses but was permeable for Pb. In addition, the increased concentrations of toxic elements (Pb and Cd) in fetuses during prenatal development on the polluted areas were accompanied by a decrease in the relative (compared to background values) weight of fetuses, which may result in the weakened offspring and its increased elimination in the early postnatal period. Probably, such negative effects in the "mother–placenta–fetus" system in animals from the previously surveyed natural populations were also determined by the effects of other TEC components.

The present work was aimed at the analysis of accumulation of essential (Na, Ca, Fe, Co, Zn, Cr, Br, Rb) and nonessential elements, including REE* (Sc*, Sr, Ag, As, Sb, Cs, Ba, La*, Ce*, Nd*, Sm*, Eu*, Tb*, Yb*, Lu*, Hf, Ta, Au, Th, U), in a maternal organism (the liver) and offspring (fetuses) of bank voles in the area of exposure to copper smelting production. The following two hypotheses have been verified: (1) on contaminated territories, the concentrations of analyzed elements in the liver, placenta and fetuses exceed the background values; and (2) the placental barrier protects (completely or partially) offpring from TEC due to selective transport and increased TE accumulation in placenta.

MATERIALS AND METHODS

Source of Emission

The Middle Urals Copper Smelter (MUCS) situated 50 km to the west from Yekaterinburg is the largest enterprise for the primary copper smelting and sulfur acid production in Russia. The major components of emissions throughout many years (since 1940) have been S, F and N-containing gases, as well as dust with the particles of adsorbed metals (Cu, Zn, Cd, Pb, Fe, Hg) and metalloids (As). As a result of modenization of production (completed in 2010), the amount of gross atmospheric emissions in the past 30 years has decreased more than 50 times (from 141 000 tons/year in 1989 to 2500 tons/year and less after 2010). There is an especially marked decrease in the amounts of SO_2 (80 times), Cu (3000 times), Zn (15 times) and Pb (8.5 times). However, the levels of heavy metals in soil [33], food and organisms of bank voles [14, 31] in this period changed insignificantly.

Experimental Animals

The model object was a bank vole (*Clethrionomys glareolus* Schreber 1780), the typical inhabitant of south taiga coniferous forests, which has held the dominant position in the composition of SM communities of the background (about 75% of inhabitants) and contaminated (more than 50%) territories throughout the period of observations [28]. The species is characterized by a high ecological plasticity and a broad range of food objects, the bulk of which are the vegetative parts of herbaceous plants, seeds, berries, mushrooms, mosses, lichens, different invertebrates, and sometimes very small vertebrate animals [34].

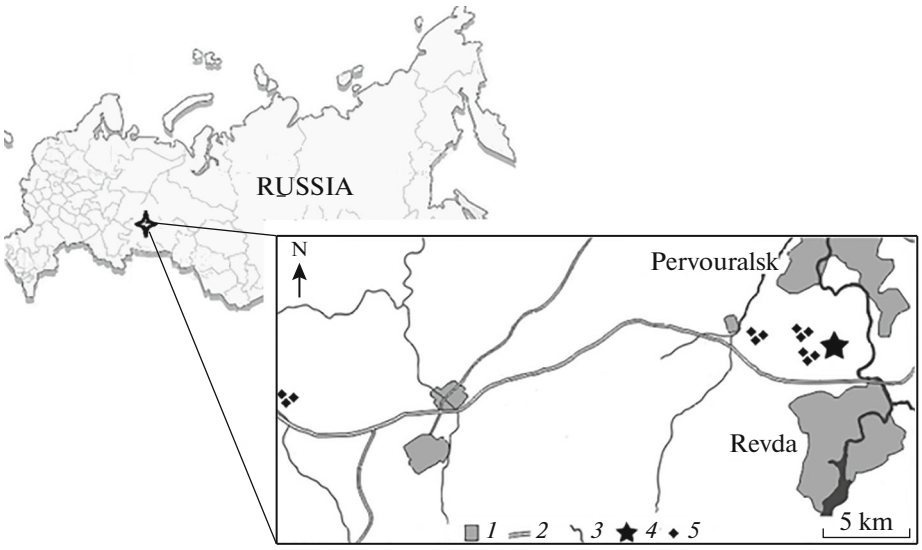

Fig. 1. The scheme of the region of research: *1*, settlements; *2*, roads; *3*, rivers; *4*, the Middle Ural Copper Smelter (MUCS); *5*, the sites of trapping animals.

Animal Trapping

The survey sites were located to the west of MUCS: in the vicinity of the plant (1–4 km, contaminated zone) and at a considerable distance from it (30 km, background zone), in spruce–fir forests with an admixture of pine, birch and aspen (Fig. 1). The background and contaminated territories had significantly different TE levels in the natural depositing environments (snow cover, soil, forest litter) and degrees of degradation of forest ecosystems [33, 35]. Animals were trapped in July of 2006 and 2007 by span trap lines (25 traps every 5–7 m, 5-day exposure with checking once a day) simultaneously in the background (3 lines) and polluted (9 lines) territories. The number of lines in the vicinity of the plant was increased due to the low number of animals (1.6 individuals per 100 trap/days) compared to the background areas (6.3 individuals per 100 trap/days). The main criteria for sample formation were the age and reproductive status of females. The work involved 18 reproducing females from the offspring of the same year (9 from each site) in the late (>17 days) pregnancy period, which did not coincide with lactation. The stage of fetus development was determined by size–weight and morphological characteristics [36, 37].

Chemical Analysis of the Samples

For chemical analysis, the liver, fetuses and the respective placentas (4 from each brood) were taken from each maternal individual. The liver was chosen among other tissues as it accumulates the maximum amounts of many TEs, including REE [38]. Fetuses and placentas were released from fetal membranes and amniotic fluid. The samples were placed onto slide, dried at 75°C to air-dry mass, and packed individually into sealed plastic bags with the respective marking.

For analysis, the samples were weighed on an analytical balance (accurate within 0.0001 g) and incinerated in a muffle furnace at 600°C. Element concentrations (μg/g dry weight) were determined by instrumental neutron activation analysis (INAA). The analysis was performed with an IRT-T nuclear research reactor at the Nuclear Geochemical Laboratory of the National Research Tomsk Polytechnic University accredited for technical competence (certificate no. PA.RU.21AB27). This method is used for the direct nondestructive assay without chemical decomposition of the samples and for the measurement of REE, U and Th levels in these samples. The thermal neutron flux density in the irradiation channel was 21013 neutrons/(cm^2 s); the time of sample exposure was 20 h. The measurements were made with a gamma-ray spectrometer equipped with a DGDK-63A germanium–lithium detector. Measurement quality was assessed by the EC-1 reference standard sample (State Standard Sample 8921-2007). The analysis was performed in 18 liver samples, 72 fetuses and 72 placenta samples.

Statistical Analysis

The distribution of element concentrations in most cases was close to log-normal; therefore, the data for further analysis were pre-logarithmized (Log_{10}). Descriptive statistics were calculated (the geometric mean, the minium and maximum values of TE concentrations). A sample was taken as a statistical unit.

The differences in TE accumulation between the sites and sample types were assessed by the two-way ANOVA. The data on fetuses and placentas of each brood depended on maternal individuals and, hence, nested models were used. Multiple comparisons were made by Tukey's test. The relationship between TE concentrations in different types of samples was estimated by Pearson's correlation coefficient. In statistical test, the differences were considered as significant at $p < 0.05$. The calculations were performed with JMP v.11. [39] and programming environment Rv.4.1 [40].

RESULTS

Element Concentrations in Samples from Different Sites

All analyzed elements were discovered in the liver of maternal individuals, placentas and fetuses (Table 1). The accumulation of most elements did not depend on the level of TEC (Table 2). Only Br concentrations in all types of samples on polluted areas were 1.9–2.3 times higher than the background values. The level of six TEs: Sc, Sr, Yb, Sm, Hf and Au, were below the detection limit (DL) in more than 30% of the samples and therefore were excluded from further statistical analysis.

Element Concentrations in Different Types of Samples

The pattern of distribution of most elements depended on the sample type, with the maximum concentrations of: Cr, Fe, Zn, Rb, Sb, La, and Ce in the liver of maternal individuals; Co, Ag, Eu, Tb, and Th in placentas; Na, Ca, Br, Ba and Ta in fetuses. The concentrations of As, Cs, Nd, Lu and U did not vary between the samples compared (Table 2).

Interrelationship between TE Concentrations in Different Types of Samples

The results of correlation analysis of the concentrations of elements under study for the generalized samples are given in Table 3. The liver–placenta pair demonstrated a strong positive relationship for Na, Br, Rb, Cs and Ag and a negative relationship for U. The liver–fetus pair showed a positive correlation for Br, Rb, Cs and Co and a negative correlation for Fe. In placenta and fetus, there were significant positive relationships for Na, Co, Br, Rb and Cs and negative relationships for Sb and Th. The samples differentiated with respect to the areas has shown significant relationships ($r = 0.36–0.74$; $p < 0.05–0.001$) for Br, Rb, Cs (all combinations), as well as for Na and Ag (liver–placenta), Ca (liver–fetus), Cr and Th (placenta–fetus) on the background site. On contaminated sites, the changes are closely related ($r = 0.35–0.96$; $p < 0.05–0.001$) to the concentrations of Br, Rb, Cs (all combinations); Fe, Sb, Co, Eu and Ba (placenta–fetus); Ca, Zn, Eu, Tb and Lu (liver–fetus); as well as Ag (liver–placenta). Figure 2 shows the typical examples of changes in element concentrations in the pairs of samples.

DISCUSSION

Mammalian organisms were shown to contain more than 80 chemical elements, the most of them being essential for vital activity [41]. Three categories are distinguished depending on the quantitative content. *Macro elements* (O, C, H, N, K, Na, Ca, Mg, S, P, Cl, F) comprise more than 99% of total mass; *trace elements* (Cu, Zn, Cr, Co, Fe, Mn, Mo, B, I, Br, etc.) are contained in organisms in minor amounts (from 1×10^{-3} to 1×10^{-6}% weight) and are components of enzymes, hormones and other vitally important substances, being essential, conditionally essential, or conditionally toxic. The third category includes the elements (As, Ag, Cs, Sb, Be, Au, U, REE) with low levels in the body (less than 1×10^{-6}% weight) and an insufficiently studied physiological role (they are often considered as conditionally toxic). As a rule, under the conditions of chemical contamination of the environment by elements of the second and third categories, they are shown to accumulate in living organisms permanently inhabiting or feeding on the polluted territories [30, 31, 41, 42].

Elemental Composition of Liver

Liver is traditionally used for quantitative assessment of the long-term effects of xenobiotics on vertebrates [5, 31, 42–45], taking into account its role in mineral metabolism and detoxification of metabolites [38]. In addition, liver is suitable for comparative studies, because there is much less information about the content of TEs in other organs and tissues of animals from native and laboratory populations.

Since TE accumulation in an organ depends on many factors: species, age, sex, reproductive condition, migration mobility of individuals, trophic level, etc. [5, 30, 31, 42–45], the maximally close groups of animals should be taken for correct comparison. In the present work, we have considered a homogeneous sample consisting of females of the same species, similar in age and physiological conditions, but inhabiting the territories with different levels of TEC. It was shown that the concentrations of elements under consideration in the bank vole liver did not depend on the TEC level ($p > 0.05$). The exception was Br: its content in voles from the vicinity of the plant was twice higher

Table 1. Element concentrations in the liver (L), placenta (Pl) and fetus (F) of bank voles from background and polluted territories

Element	Sample type	Measurement unit	Geometric mean (min–max)			
			background area		polluted area	
			Macro elements			
Na	L	mg/g	4.651 (2.903–8.797)	c	3.524 (0.402–6.198)	c
	Pl	mg/g	9.967 (6.810–13.522)	b	9.222 (6.203–12.747)	b
	F	mg/g	16.498 (8.354–36.807)	a	15.640 (7.881–28.012)	a
Ca	L	mg/g	0.372 (0.100–4.003)	b	0.310 (0.100–0.703)	b
	Pl	mg/g	0.448 (0.100–3.052)	b	0.538 (0.100–2.072)	b
	F	mg/g	1.390 (0.100–27.739)	a	1.711 (0.100–17.069)	a
			Trace elements			
Cr	L	μg/g	3.128 (2.390–6.540)	a	3.218 (2.360–4.980)	a
	Pl	μg/g	3.050 (0.500–42.392)	a	1.334 (0.099–34.448)	a
	F	μg/g	0.475 (0.053–1.180)	b	0.922 (0.049–4.298)	a
Fe	L	mg/g	2.282 (1.660–4.767)	a	1.681 (0.090–4.054)	a
	Pl	mg/g	0.504 (0.031–7.874)	b	0.500 (0.090–1.627)	ab
	F	mg/g	0.189 (0.010–1.462)	c	0.393 (0.090–1.719)	b
Co	L	μg/g	0.471 (0.390–0.840)	a	0.458 (0.400–0.600)	b
	Pl	μg/g	0.409 (0.100–1.099)	a	0.779 (0.317–1.274)	a
	F	μg/g	0.443 (0.100–1.050)	a	0.654 (0.327–2.333)	ab
Zn	L	mg/g	0.108 (0.085–0.128)	a	0.116 (0.098–0.149)	a
	Pl	mg/g	0.066 (0.024–0.210)	b	0.064 (0.020–0.144)	b
	F	mg/g	0.083 (0.037–0.340)	ab	0.091 (0.052–0.159)	a
Br	L	μg/g	8.057 (2.300–16.800)	b	18.511 (8.600–34.100)	c
	Pl	μg/g	16.648 (3.038–36.911)	a	31.623 (8.899–54.522)	b
	F	μg/g	23.102 (7.045–73.714)	a	53.094 (17.820–97.462)	a
Rb	L	μg/g	39.142 (23.100–75.300)	a	25.157 (5.500–60.100)	a
	Pl	μg/g	35.402 (6.877–63.812)	a	22.199 (5.138–60.863)	a
	F	μg/g	22.340 (2.675–56.696)	b	11.906 (0.403–41.968)	b
Ba	L	μg/g	6.400 (<DL–10.000)	a	2.400 (<DL–10.000)	b
	Pl	μg/g	6.772 (0.391–32.0.76)	ab	8.943 (0.540–23.538)	a
	F	μg/g	8.164 (1.301–21.778)	b	7.186 (0.351–19.758)	a
As	L	μg/g	0.581 (0.010–1.310)	a	0.624 (0.050–1.370)	a
	Pl	μg/g	0.541 (0.020–1.661)	a	0.722 (0.151–4.073)	a
	F	μg/g	0.628 (0.033–2.609)	a	0.771 (0.116–2.784)	a
Sr	L	μg/g	20.000	a	20.000	a
	Pl	μg/g	19.044 (3.60–20.00)	a	19.731 (6.22–79.70)	a
	F	μg/g	18.623 (2.40–65.42)	a	19.336 (6.99–77.64)	a
Ag	L	μg/g	0.062 (0.020–0.340)	a	0.073 ((<DL–0.130)	b
	Pl	μg/g	0.094 (0.007–0.461)	a	0.144 (0.026–1.031)	a
	F	μg/g	0.101 (0.003–1.188)	a	0.132 (0.029–0.691)	a
Sb	L	μg/g	0.276 (0.178–0.425)	a	0.289 (0.242–0.338)	a
	Pl	μg/g	0.043 (0.009–1.713)	b	0.030 (0.001–0.308)	b
	F	μg/g	0.026 (0.002–0.387)	b	0.055 (0.001–0.419)	ab
Cs	L	μg/g	0.090 (<DL–0.430)	a	0.110 (<DL–0.600)	a
	Pl	μg/g	0.077 (0.007–0.451)	a	0.062 (0.001–1.058)	a
	F	μg/g	0.080 (0.004–0.299)	a	0.048 (0.002–0.298)	a

Table 1. (Contd.)

Element	Sample type	Measurement unit	Geometric mean (min–max)			
			background area		polluted area	
Hf	L	μg/g	0.015 (0.009–0.063)	a	0.013 (0.009–0.046)	a
	Pl	μg/g	0.017 (0.002–0.189)	a	0.038 (<DL–0.267)	a
	F	μg/g	0.016 (0.009–0.111)	a	0.011(0.009–0.202)	a
Ta	L	μg/g	0.010 (<DL–0.020)	a	0.013 (0.109–0.046)	a
	Pl	μg/g	0.013 (0.002–0.030)	a	0.020 (<DL–0.056)	a
	F	μg/g	0.020 (<DL–0.089)	a	0.020 (<DL–0.067)	a
Au	L	μg/g	0.002 (0.001–0.006)	a	0.002 (<DL–0.003)	a
	Pl	μg/g	0.002 (<DL–0.004)	a	0.002 (0.001–0.004)	a
	F	μg/g	0.002(<DL–0.075)	a	0.002 (<DL–0.020)	a
Th	L	μg/g	0.014 (<DL–0.040)	b	0.025 (0.010–0.040)	a
	Pl	μg/g	0.045 (0.008–0.206)	a	0.035 (0.002–0.170)	a
	F	μg/g	0.038 (0.004–0.189)	a	0.031 (0.004–0.083)	a
U	L	μg/g	0.129(<DL–0.320)	a	0.124(0.100–0.270)	a
	Pl	μg/g	0.169(0.039–0.966)	a	0.099(0.007–0.787)	a
	F	μg/g	0.121(0.022–0.786)	a	0.099(0.005–0.622)	a
Rare earth elements						
Sc	L	μg/g	<DL	a	<DL	a
	Pl	μg/g	0.009 (0.002–0.045)	a	0.008 (0.001–0.046)	a
	F	μg/g	0.010 (<DL–0.023)	a	0.010 (0.005–0.024)	a
La	L	μg/g	0.173 (0.070–0.290)	a	0.174 (0.100–0.230)	a
	Pl	μg/g	0.096 (0.021–1.042)	b	0.083 (0.004–0.491)	b
	F	μg/g	0.077 (0.020–0.189)	b	0.105 (0.015–0.232)	ab
Ce	L	μg/g	0.776 (0.200–3.830)	a	0.490 (0.100–1.900)	a
	Pl	μg/g	0.175 (0.009–1.900)	b	0.109 (0.003–0.700)	b
	F	μg/g	0.128 (0.083–0.594)	b	0.230 (0.041–4.057)	a
Nd	L	μg/g	0.250 (0.040–0.720)	b	0.500 (<DL–0.850)	a
	Pl	μg/g	0.550 (0.008–1.517)	ab	0.432 (0.001–3.256)	a
	F	μg/g	0.582 (0.059–5.541)	a	0.435 (0.005–2.062)	a
Sm	L	μg/g	0.101 (0.040–0.540)	a	0.139 (0.090–0.570)	a
	Pl	μg/g	0.059 (0.002–0.311)	a	0.060 (<DL–0.090)	b
	F	μg/g	0.061 (0.006–0.090)	a	0.120 (<DL–1.703)	a
Eu	L	μg/g	0.004 (0.003–0.007)	b	0.003 (0.002–0.005)	b
	Pl	μg/g	0.018 (0.004–0.235)	a	0.011 (0.001–0.127)	a
	F	μg/g	0.006 (0.001–0.036)	b	0.006 (0.002–0.027)	b
Tb	L	μg/g	0.013 (<DL–0.020)	a	0.010 (<DL–0.020)	a
	Pl	μg/g	0.018 (0.001–0.159)	a	0.018 (0.001–0.186)	a
	F	μg/g	0.014 (0.001–0.113)	a	0.020 (<DL–0.064)	a
Yb	L	μg/g	0.116 (<DL–0.200)	a	0.103 (0.010–0.200)	ab
	Pl	μg/g	0.062 (0.001–0.200)	a	0.030 (<DL–0.200)	b
	F	μg/g	0.090 (0.003–0.200)	a	0.020 (<DL–0.200)	a
Lu	L	μg/g	0.004 (<DL–0.010)	a	0.003 (<DL–0.010)	a
	Pl	μg/g	0.008 (<DL–0.015)	a	0.007 (<DL–0.012)	a
	F	μg/g	0.008 (<DL–0.028)	a	0.005 (<DL–0.014)	a

<DL, the values below the detection limit; statistical unit, a sample. The same letters show the absence of significant differences in concentrtaions of the element between the types of samples within the same surveyed area (Tukey test).

Table 2. The results of analysis of variance of the differences in element concentrations in different types of samples depending on the level of trace element contamination of the territories (significant differences ($p < 0.05$) are in bold)

Dependent variable	Variability factors	dF1	dF2	F	*p*-Value
Na	Sample type	**2**	**130.12**	**248.40**	**0.000**
	Area	1	18.71	0.69	0.418
	Sample × Area	2	130.12	0.34	0.712
Ca	Sample type	**2**	**130.02**	**23.40**	**0.000**
	Area	1	21.61	0.07	0.800
	Sample × Area	2	130.02	1.12	0.329
Cr	Sample type	**2**	**130.18**	**19.19**	**0.000**
	Area	1	21.43	0.01	0.937
	Sample × Area	**2**	**130.18**	**5.84**	**0.004**
Fe	Sample type	**2**	**130.02**	**23.09**	**0.000**
	Area	1	20.40	0.65	0.430
	Sample × Area	**2**	**130.02**	**3.38**	**0.037**
Co	Sample type	**2**	**129.82**	**4.38**	**0.015**
	Area	1	16.447	2.10	0.166
	Sample × Area	**2**	**129.82**	**6.03**	**0.003**
Zn	Sample type	**2**	**145.0**	**11.94**	**0.000**
	Area	1	145.0	0.22	0.640
	Sample × Area	2	145.0	0.31	0.731
As	Sample type	2	130.22	0.04	0.965
	Area	1	24.65	0.14	0.712
	Sample × Area	2	130.22	0.29	0.752
Br	Sample type	**2**	**129.99**	**119.69**	**0.000**
	Area	**1**	**15.40**	**8.79**	**0.009**
	Sample × Area	2	129.99	1.73	0.181
Rb	Sample type	**2**	**92.15**	**31.15**	**0.000**
	Area	1	16.27	2.20	0.157
	Sample × Area	2	92.15	0.93	0.398
Ag	Sample type	**2**	**130.20**	**5.98**	**0.003**
	Area	1	21.73	0.52	0.480
	Sample × Area	2	130.20	0.88	0.417
Sb	Sample type	**2**	**129.31**	**13.24**	**0.0001**
	Area	1	25.61	0.73	0.393
	Sample × Area	2	129.35	3.10	0.051

Table 2. (Contd.)

Dependent variable	Variability factors	dF1	dF2	F	*p*-Value
Cs	Sample type	2	84.75	1.77	0.177
	Area	1	16.21	0.12	0.732
	Sample × Area	2	84.75	2.65	0.076
Ba	Sample type	**2**	**53.67**	**9.18**	**0.000**
	Area	1	66.36	0.94	0.335
	Sample × Area	**2**	**53.67**	**3.64**	**0.033**
La	Sample type	**2**	**130.21**	**7.384**	**0.001**
	Area	1	26.60	0.343	0.563
	Sample × Area	**2**	**130.21**	**3.574**	**0.031**
Ce	Sample type	**2**	**129.95**	**16.23**	**0.000**
	Area	1	28.68	0.17	0.686
	Sample × Area	**2**	**129.95**	**5.39**	**0.006**
Nd	Sample type	2	130.23	2.27	0.108
	Area	1	23.09	0.75	0.396
	Sample × Area	2	130.23	0.20	0.818
Eu	Sample type	**2**	**88.42**	**37.34**	**0.000**
	Area	1	20.05	0.68	0.420
	Sample × Area	2	88.42	2.01	0.140
Tb	Sample type	**2**	**85.28**	**3.15**	**0.048**
	Area	1	27.86	0.020	0.997
	Sample × Area	2	85.28	0.162	0.850
Lu	Sample type	2	86.79	0.67	0.513
	Area	1	20.61	0.64	0.431
	Sample × Area	2	86.79	0.88	0.419
Ta	Sample type	**2**	**84.90**	**7.24**	**0.001**
	Area	1	121.03	0.01	0.971
	Sample × Area	2	84.90	0.49	0.611
Th	Sample type	**2**	**145.00**	**19.32**	**0.000**
	Area	1	145.00	1.56	0.213
	Sample × Area	**2**	**145.00**	**4.66**	**0.011**
U	Sample type	2	145.00	0.51	0.603
	Area	1	145.00	0.36	0.552
	Sample × Area	2	145.00	2.59	0.078

Table 3. The relationship between element concentrations in the bank vole liver, placenta and fetus (Pearson linear correlation coefficient (*r*) was used to estimate the strength of connection)

Element	Liver–Placenta		Liver–Fetus		Placenta–Fetus	
	r	*p*	*r*	*p*	*r*	*p*
Na	**0.32**	*	0.03		**0.26**	*
Ca	–0.14		–0.15		–0.16	
Cr	0.15		0.19		–0.21	
Fe	–0.15		**–0.24**	*	0.02	
Co	0.16		–0.03		**0.41**	*
Zn	–0.02		–0.11		0.15	
As	0.04		0.10		0.02	
Br	**0.83**	**	**0.85**	**	**0.84**	**
Rb	**0.87**	**	**0.57**	**	**0.60**	**
Ag	**0.38**	*	0.09		0.15	
Sb	0.23		0.21		**–0.32**	*
Cs	**0.58**	**	**0.54**	**	**0.58**	**
Ba	–0.07		–0.07		0.15	
La	0.10		0.01		0.01	
Ce	–0.09		–0.04		–0.21	
Nd	0.09		–0.10	0	–0.09	
Eu	–0.07		–0.03		**0.28**	*
Tb	–0.16		0.06		0.11	
Lu	0.18		0.10		0.18	
Ta	0.09		–0.17		0.06	
Th	–0.07		–0.03		**–0.30**	*
U	**–0.24**	*	–0.12		0.03	

Significance of differences between the sample pairs liver–placenta, liver–fetus, placenta–fetus: *, $p < 0.05$, **, $p < 0.001$.

than the background values (see Table 1). According to D.C. Adriano [1], under the conditions of environmental pollution by industrial and motor vehicle emissions, the entry of Br with food considerably increases, though there are no data on toxicity of this element in case of oral intake.

Analogous results were obtained for another bank vole sample from the vicinity of MUCS, which included males of different age, though with a fourfold excess of the background levels [30]. The most probable cause of these differences is precisely sample composition, because the material was taken at the same sites within the same period of time. It is known that male voles are characterized by high migration mobility [46]. Therefore, under the conditions of highly mosaic pattern of contamination fields in the vicinity of the plant, they could more often visit the areas with elevated levels of this element. Indirect evidence of our assumption is the higher (3–7 times) concentrations of Cr, As and Fe in the liver of males.

In some cases, element concentrations in the liver of animals, on the contrary, decreased with approaching the plant. The level of Rb in bank voles of both sexes in contaminated areas (15–25 μg/g) were twice lower than in the background areas (30–39 μg/g). Such tendencies are in complete agreement with the changes in the Rb level in bank vole food [30].

The comparison of element compositions in the liver of small mammals from different trophic levels, living together under contrasting conditions, has shown similar levels of Zn, Co and As for phytophages (bank vole) and zoophages (Laxmann's shrew, *Sorex caecutiens*), whereas the concentrations of Cr and Fe were higher in the liver of zoophages. With increasing contamination,

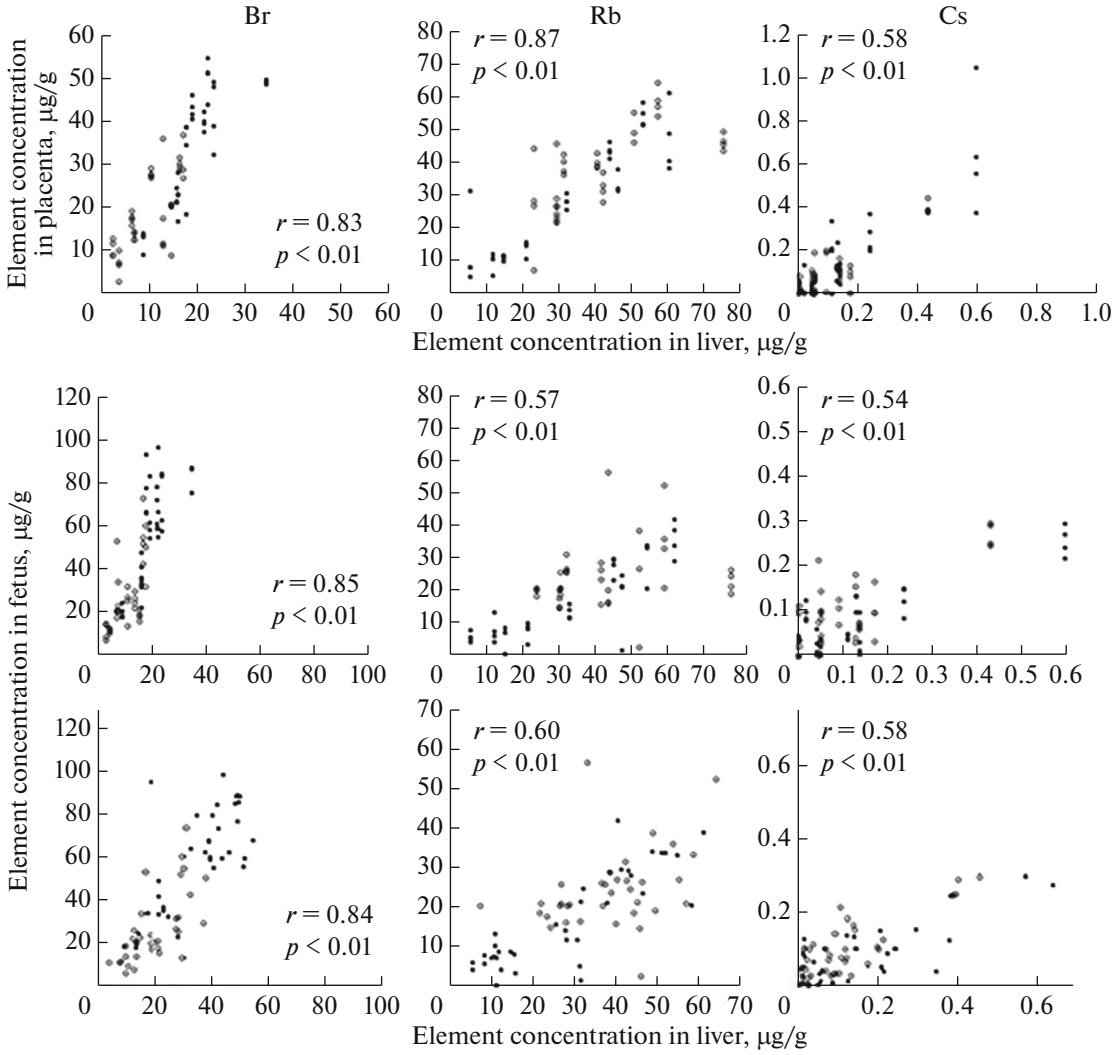

Fig. 2. The relationship between TE concentrations in the liver, placenta and fetus of bank voles from background (white circles) and contaminated (points) areas. Pearson correlation coefficient (*r*) demonstrates the strength of connections between concentrations in the same pairs.

the Br and Rb levels in the liver of shrews changed similarly, but the absolute values were lower [30].

The comparison of our results with extensive data on element composition in the liver of herbivorous of other systematic groups (rodents, hoofed mammals) [25, 43–48] has shown that the concentrations of some elements (Ca, Na, Fe, Zn, Rb, Cs, Ba) were similar, while those of other elements (Cr, Co, Ba, As, Ag) were significantly (2–25 times) different. The main causes of such differences are the sex and age composition of the samples, species and trophic specificity, TEC levels in the territories, etc.

The analysis provided the data on the content of nine REE in the bank vole liver (see Table 1). The literature data on the REE accumulation in the organisms of mammals from natural populations are few up to now. The content of eight REEs in the liver of bank voles from unpolluted monitoring sites in Sweden has been studied most thoroughly [47]. The total concentration of REE in the sample of breeding females (mature and over-wintered individuals are combined) was up to 1.693 μg/g; in our study, it varied within a range from 1.431 (close to the plant) to 1.446 μg/g (background sites) and did not depend on the TEC level, while the mean values for Ce, La, Nd, Sm and Tb were comparable. Let us note that the concentrations of separate elements in our sample varied within a broad range: from 0.004 μg/g for Lu and Eu to 0.778 μg/g for Ce (see Table 1). We believe that the relatively high content of Ce in the liver of animals from contrasting sites is due to its use as a marker of emissions in motor fuel [49], while the twofold differences in its concentrations between the

sites are due to different distances of the territories from highways with intensive traffic.

Element Composition of Placentas

The literature data on the element composition in placentas of animals from natural populations are scarce [15, 43]. In addition, the analyzed samples are usually few and considered without the connection with fetuses, while parent individuals vary in age, terms of pregnancy and other characteristics, which does not allow them to the correctly compared with our data.

In a mammalian organism, placenta performs an important barrier function, selectively transfering macro and trace elements from a mother to a developing fetus. Therefore, placenta is the optimal biological matrix for assessing the ecological risk of exposure to various elements in the "mother–offspring" system [50, 51]. In the study, particular attention is traditionally focused on either toxic (Cd, Pb, Hg) or essential (Cu, Zn, Fe) TEs, while the studies of a wide range of elements are few [19, 26, 50–52]. Such composition has been analyzed most completely (up to 37 in a single report) only for humans [51, 52]. It has been shown that, in addition of essential (Ca, Na, Fe, Cu, Zn) and toxic (Pb, Cr, Hg and Cd) elements, placenta is partially permeable for Be, Ag, Ba, Sr, Mg, Mn, Sn, Sb, Te, Tl, As, Co and Se accumulating in fetal tissues and/or amniotic fluid [19, 26, 50, 51, 53]. At the same time, the concentrations of Mg, Ag, Tl, Ba, Be, Sb could be rather high. On the contrary, Pd, Ni, V, Zr, Tc, Rh, Ru, as well as REE, did not overcome the placental barrier [16, 19].

The levels of accumulation of a broad range of elements (Na, Sc, Fe, Zn, Br, Rb, Ag, Sb, Cs, La, Ce, Au) in the placenta of humans [51, 52] and bank vole individuals from the present study were similar or had the overlapping ranges. At the same time, the placenta of bank voles contained more (sometimes by an order of magnitude) Co, Cr, and Ba. In addition to these elements, the placental barrier was also shown to be permeable for Br and Ta (Fig. 2).

Element Composition of Fetuses

The developing fetuses and newborns are highly susceptible to the effects of various chemicals due to immature detoxification system. The data on element compositions in the fetuses of mammals from natural populations, as well as laboratory animals, are few. At the same time, different species demonstrated different patterns of accumulation of separate elements.

Previous data [14] demonstrate the enhanced (2–4 times) accumulation of Pb and Cd in the developing offspring of bank voles from the vicinity of MUCP. Irrespective of the level of chemical pollution, the concentration of Cd in an fetus was always lower than in the respective placenta; for Pb, there was an inverse dependence. At the same time, the concentrations of essential Cu and Zn in fetuses varied insignificantly and increased in proportion to their levels in placentas.

Among the available literature sources, we can mention the work by M. Zakrzewska et al. [53], where the accumulation of Cu, Zn and Fe in bank vole fetuses under the conditions of chronic Cd contamination of the diet of parent individuals has been studied experimentally. The authors have shown that, irrespective of the level of contamination, Zn accumulated mainly in fetuses, while Cu and Fe accumulated in placentas. The comparison of these data and our results demonstrates similar trends.

The data on the levels of 19 TEs in the fetuses of the red-bellied tree (Pallas's) squirrel (*Callosciurus erythraeus*) show that Zn, Rb, Ag and Cs concentrations are comparable with those that we have found in the bank vole fetuses (see Table 1); other concentrations were much lower: As, 10 times; Co, 30 time; Ba, 80 times [43]. Unfortunately, it concerns small samples (4 fetuses, 2 maternal individuals of different age) obtained from the sites with different levels of anthropogenic load.

The analysis of element composition (26 TEs) in the organs of the small Indian mongoose (*Herpestes auropunctatus*) in two "mother–fetus" pairs has shown that Ca and Ba concentrations in maternal individuals were twice lower than in the respective organs of fetuses, while Co, Cr, Sb, and As accumulated more intensively in the liver of adult individuals. The Rb, Zn, Fe concentrations were similar in both types of samples [54]. In the bank vole from out research, such trends were shown for Ca, Ba, Cr and Sb.

We have no data on REE accumulation in the fetuses of mammals from natural populations, while single data on newborn laboratory animals are contradictory. Some authors [20, 55] has shown that cubs accumulated up to 90% of lanthanides taken up with maternal milk in the early postnatal period and transition intensity depended on the atomic mass of an element (Ce < Nd < Sm < Eu < Tb). According to other authors [56], no REE absorption from the gastrointestinal tract of cubs was recorded in the period of milk feeding. Our data are in good agreement with the notions of the partial barrier role of placenta: at the existing REE levels, concentrations of none of these elements in fetuses exceeded the values in the respective liver and placenta samples (see Table 1).

Peculiarities of Element Accumulation in Different Types of Samples

The specific behavior of chemicals in successive links of the biological system (e.g., trophic chains) is usually expressed by the bioaccumulation coefficient being a ratio of element concentrations in the analyzed samples [57]. Here, the role of histohematic barriers manifests itself in decreasing or increasing values of the parameter, respectively.

Depending of the pattern of accumulation in the "mother–placenta–fetus" system, all elements under study can be divided into 4 groups. Group I includes the elements with the maximum concentrations in the liver of maternal individuals: Cr, Fe, Zn, Rb, Sb, La, and Ce. The bioaccumulation coefficient in this group varied within a range from 1.1 to 12.1. The elements of group II (Co, Ag, Eu, Tb, and Th) accumulated mainly in the placenta, exceeding their content in other types of samples 1.1–4.5 times. The elements of group III (Na, Ca, Br, Ba, and Ta) penetrated through the placenta and accumulated mainly in fetuses. The bioaccumulation coefficient in this group varied within a range from 1.2 to 5.5. The concentrations of the elements of group IV (As, Cs, Nd, Lu, U) in the studied samples had no significant differences (see Table 1).

Since the concentrations of group I elements in fetuses and placentas are lower than in the liver of maternal individuals, it can be supposed that there are mechanisms for discrimination of these elements in the "mother (liver)–placenta–fetus" system. At the same time, placenta is a connecting link between maternal organism and developing offspring, providing the selective transport of elements [51].

For essential elements, the mechanisms of active regulation with the involvement of histohematic barriers are well-known. For example, the concentration of Cu in the liver of bank vole maternal individuals living close to MUCS is 7 times lower compared to the diet (on the background site, 1.4 times lower). At the same time, the differences between concentrations of this element in the liver at contrasting sites are only two-fold ($p < 0.0001$), while those in placenta and fetus do not depend on the level of contamination at all [14].

For toxic elements, such homeostatic mechanisms are usually not manifested in mammalian organisms. For example, Cd concentration in the liver of maternal individuals in contaminated areas is 3-fold higher compared to the diet (in the background area, by 30%). At the same time, the element concentration in placenta is 20 times lower compared to liver and 8 times lower compared to fetus (at the background site, 12 and 4 times, respectively). At the same time, the levels of Cd accumulation in fetuses and placentas in contaminated areas are 2–6 times higher compared to the background values [14].

The elements of groups II and III are characterized by enhanced accumulation in placenta and fetus compared to maternal organism (see Tables 1, 3, Fig. 2). The presence among them of essential elements necessary for the development of a new organism is evidence of their active transport across the placenta. Similar tendencies revealed by other researchers in direct comparison of the levels of accumulation of some TEs in the liver of parent individuals and fetuses [54] suggest the absence or limited action of the mechanisms for discrimination of elements of these groups.

More than half of group IV elements (as well as the elements excluded from further analysis due to analytical reasons) are REE, the concentrations of which in the studied samples are insignificant and the physiological role is little studied.

CONCLUSIONS

The data of neutron activation analysis were a basis for studying the levels of 28 macro and trace elements in maternal individuals (liver) and offspring of bank voles from natural populations under the conditions of TEC by emissions from a large copper smelter. It was demonstrated that element composition in the liver of adult animals, fetuses and placentas did not depend on the level of contamination. The exception was Br, as its concentrations exceeded two-fold the background values in all types of samples from the vicinity of MUCS. The original hypothesis of enhanced accumulation of elements in the analyzed substrates of animals from polluted territories was confirmed only with respect to Br.

Depending on the pattern of accumulation in the samples, the elements under study were divided into 4 groups: group I included the elements (Cr, Fe, Zn, Rb, Sb, La, and Ce) accumulating mainly in the liver of maternal individuals; the elements of group II (Co, Ag, Eu, Tb, Th) accumulated in the placenta, and those of group III (Na, Ca, Br, Ba, Ta) accumulated in fetuses. The concentrations of group IV elements (As, Cs, Nd, Lu, U) in the samples under study were not different. One can suggest the presence of mechanisms for discrimination of group I elements during their transition from a mother to a fetus; for the elements of groups II and III, such mechanisms seem to be limited or absent. It has been shown for the first time that Br and Ta easily overcome the placental barrier. Our assumption of the barrier role of the placenta was confirmed for Co, Ag and REE.

Based on the above, it can be concluded that the organisms of small mammals have mechanisms for selective discrimination of TEs, which limit their entry

to the developing offspring. At the existing TEC levels in the vicinity of the large copper smelter, the TEC components under consideration had no direct toxic effect on the bank vole offspring quality.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict 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.

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