Adaptation of Small Mammals to Conditions of Biogeochemical Province with the Increased Level of Heavy Metals

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***Abstract*- At present, the natural non-endemic geochemical anomalies impacts on biota are not studied enough. The purpose of this work was to study effects of the natural geochemical anomaly with high content of nickel, cobalt and chrome on animals. The anomalous environment accounts for low abundance of small mammals, changes in the interior weight characteristics, alterations in the adrenal cortex structure, and reproductive function modifications. Natural anomalies serve as a factor of the additional functional load upon reproductive and endocrine systems.**

Keywords- Natural Geochemical Anomaly; Adaptation; Bank Vole; Adrenal Gland; Reproductive Function

I. INTRODUCTION

Currently, the issues concerning the influence of geochemical factors on animals are widely discussed. An excess or lack of certain chemical elements disturbs the balance of metabolic processes in the organism and may result in various diseases, reduced lifetime etc [1]. In modern literature, the materials on heavy metals accumulations within geochemical anomalies of different genesis in soil [2, 3, 4, 5], water [6], plant and animal tissues [7, 8, 9], have been widely provided.

The areas with excess supply of chemical elements into the food chain, coming predominantly from the underlying rocks, are classed as natural positive geochemical anomalies [10]. By now, natural geochemical anomalies that form biogeochemical provinces and cause endemic diseases have been recorded. For example, Kashin-Bek disease (human and animal skeleton damage – spinal curvature, joint disease, bones fragility, dedentition etc) is characteristic for the areas with the low Ca content under excesses of Ba and, in particular, Sr. In the areas with increased Se contents in soil, specific contamination by this element occurs known as alkalosis [11].

Natural geochemical anomalies that provoke no endemic diseases are relatively less studied. In these areas, despite significant gross concentrations of heavy metals in soil, the food chain is supplied with such concentrations that cause no specific diseases, but set conditions for certain adaptive changes in organism regulatory systems. The Ural region in Russia provides a unique opportunity for studying impacts from anomalous geochemical condition on the biota. The Ural region is characterized by a broad distribution of natural geochemical anomalies with a surplus of Ni, Cr, Co, Cu, associated with the rocks pertained to zonal mafite and ultra mafite complexes of the Ural and Alaska type.

The purpose of our work was to study impacts from a natural geochemical natural geochemical anomaly with high content of nickel, cobalt and chrome associated with ultrabasic rock distribution on animals.

II. MATERIAL AND METHODS

The study was conducted in three locations of Sverdlovsk area (the Ural region, Russia). Two locations lay within natural geochemical anomalies with a surplus of chrome, nickel, cobalt in soil: Location 1 – the settlement of Uralets, Location 2 – the village of Anatolskaya. The maximum nickel, cobalt, chrome concentrations in soil at Location 1 are 23, 15, 100 times higher in comparison to the average Uralian clarke values, and in soil at Location 2 are 67, 20, 20 times higher, accordingly. High concentrations of these elements are determined by the ultrabasic rock occurrence. The third location was situated in the Visim State Nature Biosphere Reserve and was used as a control. Soil concentrations of chemical elements in the reserve do not exceed the average Uralian clarke values. The areas for Location 1 and Location 2 were 44 km^2 and 43 km^2, accordingly, and for the control location 36 km^2. The concentrations of heavy metals in plant and animal tissues taken from the anomalous areas are statistically and significantly different from those in the control area [12, 13]. No endemic diseases were recorded in theses areas.

Capturing Bank Voles was carried out during summer (June-August) from 2001 till 2006 with the use of Hero traps and live traps that were arranged in lines by 50 pieces [14]. Removal of animal from the population in the Sverdlovsk Province was in accordance with the recommendations of the Directorate of the Federal Service for Control in the Field of Natural Resource Use. The animals were killed by cervical dislocation and the organs removed and fixed in 10% formalin, embedded in paraffin, sectioned at 5 micron, and stained with hematoxylin-eosin.

The animal sexual maturity was determined by a set of the morpho-physiological parameters. Males with the testis length at least 8 mm, weight at least 250 mg, well developed gonads and with spermatozoids in the swabs taken from the epididymis were considered as mature animals. Pregnant females and females participating in reproduction (evidence of embryos, placentary spots in the uterine horns and yellow bodies in the ovaries) were also classified as mature individuals.

The overwintering and underyearling animals participating in reproduction have been classified as one group (mature animals) because there were no significant differences between them in terms of statistics.

Estimation of the animal morpho-physiological characteristics was carried out through the method of Shvarts's morpho-physiological indicators [15] and its modifications [16]. The method of morpho-physiological indicators enables to assess physiological characteristics of animals, including the intensity of energy balance, based on the set of indirect indicators such as the relative mass of animal visceral organs. The following parameters were estimated: the animal body mass, index of fatness, or index of condition (the body mass-length ratio), liver, adrenal, thyroid glands, testis indexes and the hepatosuprarenal coefficient (the liver mass-adrenal mass ratio) was also used. In the assessment of the animal body mass, the mass of stomach contents was excluded from the total body mass. The mass of the uterus with embryo in the breeding females was subtracted from the total body mass to exclude the influence of the embryo weight on the body mass and therefore on the indexes analyzed.

Actual (counts of the number of fetuses in the uterus) and potential (counts of the yellow bodies in the ovaries) fecundity was examined only on the pregnant females. The counts of the yellow bodies and follicles and measurement of the maximum follicle diameters were conducted on serial sections made according to the standard technique [17, 18]. The animal adrenal cortex was also examined through the use of microslides. On the sections, areas of the adrenal, glomerular, fasciculate, reticular zones, cells and their nuclei in the glomerular, fasciculate, reticular zones and also relative size of cortical zones (percentage of the organ section area) were measured. The measurements were conducted on the adrenal middle sections. The value for each karyocytometric parameter was calculated as the mean resulted from the measurement of 50 cell sizes. Morphometry on the adrenal and ovary sections was done with the use of SIAMS PHOTOLAB software.

To assess intensity of the folliculogenesis process in the ovaries of pregnant and immature Bank Vole females, the number of single-layered, secondary (compact, cavity) and tertiary follicles was counted. The maximum diameters of compact and cavity follicles were measured. To eliminate the influence of the mature animal ovarian cycle on the ovary functional activity, the assessment of folliculogenesis intensity was done only for pregnant females. To eliminate the influence of age aspects on the ovary functional activity in immature bank vole females, one-month-old individuals of similar weights (14-19 g) were selected [19].

A statistical analysis of the data was done in the software package STATISTICA 5.0 (StatSoft, Inc. 1984–2001). To test the assumption of normal distribution, we used the χ^2 and Kolmogorov-Smirnov criteria. In the statistical treatment of morpho-physiological and histological data, we use multifactor, multidimensional models of dispersion analysis and also Mann-Whitney test. The models included the following factors and their graduations: geochemical conditions (the geochemical anomalies, control area), phase of animal population cycle ('growth', 'peak'), sex (males, females), reproductive status of animals (mature, immature), and type of ovarian follicle (single-layered, secondary, tertiary). A significance level of 5% was chosen to test factor significance hypothesizes.

III. RESULTS

To assess overall physiological conditions of animals inhabiting natural geochemical anomalies an analysis of morpho-physiological indicators was done according to the method of Shvarts. The examination of morpho-physiological parameters for the Bank Vole revealed the influence of the geochemical habitat conditions, density-related mechanisms, sexual identity and reproductive status on the metabolism intensity, energy potential of animals and adrenal gland functional activity. For this purpose, four-factor, multidimensional models of dispersion analysis were used. We found statistically significant effects of the all four factors: 'geochemical conditions', 'phase of population cycle', 'sex', 'reproductive status of animals' (Table I).

TABLE I RESALTS OF DISPERSION ANALYSIS. ALL EFFECTS

Dispersion Source	λ-Uilks (6. 112)	Rao's R (6. 112)	p-level
Variability of Bank Vole Internal Parameters (Morpho-Physiological Indicators)			
Geochemical factor	0.820	4.099	0.001
Phase of animal population cycle	0.512	17.790	2.14E-14
Sex	0.452	22.597	2.61E-17
Reproductive status	0.266	51.620	5.14E-30
Variability of Bank Vole Adrenal Cortex Morpho-Metric Parameters			
Geochemical factor	0.652	4.652	1.2E-05
Phase of animal population cycle	0.366	15.128	1.3E-16
Sex	0.313	19.112	1.1E-19
Reproductive status	0.420	12.057	6.8E-14

Analysis of partial effects from the factors with the use of one-dimensional F-criterion found statistically significant changes in some vole interior parameters. This study addresses the partial effects from the factor ‘Geochemical conditions’ (Table II).

TABLE II PARTIAL EFFECTS OF GEOCHEMICAL FACTOR*

Parameters	Average unweighed location 1/location 2/background	F	Degree of freedom	p-level
Kidney index, ‰	7.93 / 8.09 / 7.28	8.84	2, 267	<0.01
Adrenal gland index, ‰	0.41 / 0.35 / 0.30	16.45	2, 267	0.02
index of fatness, g/sm	2.06 / 2.00 / 2.15	4.31	2, 267	0.01
Hepatosuprarenal coefficient	214.67 / 239.40 / 268.21	5.77	2, 267	<0.01
testis index, ‰	10.57 / 11.53 / 9.0	5.37	2, 131	0.01
Number of follicles in ovary (pregnant)	50.3 / 51.0 / 49.0	0.3	2.84	0.74
Maximum diameters of follicles in ovary, (pregnant), mcm	241 / 254 / 242	1.17	2.421	0.31
Number of follicles in ovary (immature)	36.7 / 38.3 / 31	12.01	2, 61	<0.01
Maximum diameters of follicles in ovary, (immature), mcm	214 / 219/ 184	17.57	2, 273	<0.01
Area of adrenal cortex fasciculate-reticular zone, mm^2	0.94 / – / 0.83	4.75	1, 106	0.03
Area of adrenal cortex fasciculate zone cell, mcm^2	70.7 / – / 66.7	4.46	1, 106	0.03
Area of adrenal cortex fasciculate zone cell nuclei, mcm^2	17.9 / – / 16.1	18.38	1, 106	<0.01

Note *: only statistically significant effects are resulted; the crossed out section means absence of the data.

It is shown that a statistically significant increase in animal kidney and adrenal relative masses occurs in natural geochemical anomalies (Table II), which indicates an activation (or stirring up) of these organs functions. In addition, the lowered index of fatness and lowered hepatosuprarenal coefficient were recorded in the anomalies (Table II). A comparative study of values for the kidney, adrenal indexes, index of fatness and hepatosuprarenal coefficient, by locations and with the use of LSD test of planned comparisons, showed significant differences in the following cases: Location 1 – background, Location 2 – background ($p < 0.05$). The interior features recorded in voles inhabiting natural geochemical anomalies provide evidence of increased metabolism and lowered energy potential in the animals.

Bank voles also display increased testis index under extreme geochemical conditions (three-factor model of dispersion analysis, the factors ‘geochemical conditions’, ‘phase of population cycle’, ‘reproductive status’); significant differences are recorded in the cases: Location 1 – background, Location 2 – background based on the HCP test results ($p < 0.05$). Larger testis index in young animals indicates an accelerated sexual maturation under a surplus of heavy metals in the environment.

The adrenal cortex is known to play the main part in formation of physiological defense reactions that underlie adaptation processes. Examination of the vole adrenal cortex structure was done for anomalous Location 1 and control area. Despite increased relative and absolute adrenal mass, bank voles from the anomalous location did not show a statistically significant (four-factor model of dispersion analysis, factors: ‘geochemical conditions’, ‘sex’, ‘phase of population cycle’, ‘reproductive status’) increase in the adrenal section area, i.e. the organ volume. Thus, increased adrenal index and mass in anomalies result predominantly from increase of the organ blood filling.

In the course of examination of the adrenal structure in the anomalies, the fasciculate-reticular zone hypertrophy was found. Increased size of the fasciculate and reticular zone is determined by an increase in the cell area of the fasciculate zone only. In addition, hypertrophy of the fascicular cells nuclei was found (Table II). This hypertrophy is thought to be due to an increase in the volume of nucleo synthesis products and indicates intensification of the glucocorticoids that participate in adaptive reactions and ensure increased non-specific resistance of animals in extreme geochemical conditions.

The analysis of statistically significant interactions of the variability factors for the adrenal cortex structure concluded that extreme geochemical conditions aggravate functional tension of this organ, which occurs under the influence of the other factors. Interactions of the factors ‘geochemical conditions’ and ‘sex’, and also ‘geochemical conditions’ and ‘phase of population cycle’ are demonstrated by the parameters for the cortex fasciculate zone: the area of the fascicular cells and their nuclei (Fig. 1). It was found that sexual dimorphism with respect to the cortex structure was more evident in the anomaly than in the background (Fig. 1 a, b). This is likely to mean that females in anomalies are characterized by higher increase in the functional activity of the fasciculate zone compared to the males, than females living in the background. In anomalies, the influence of population density on the adrenal cortex structure is also more evident than in the background (Fig. 1 c, d).

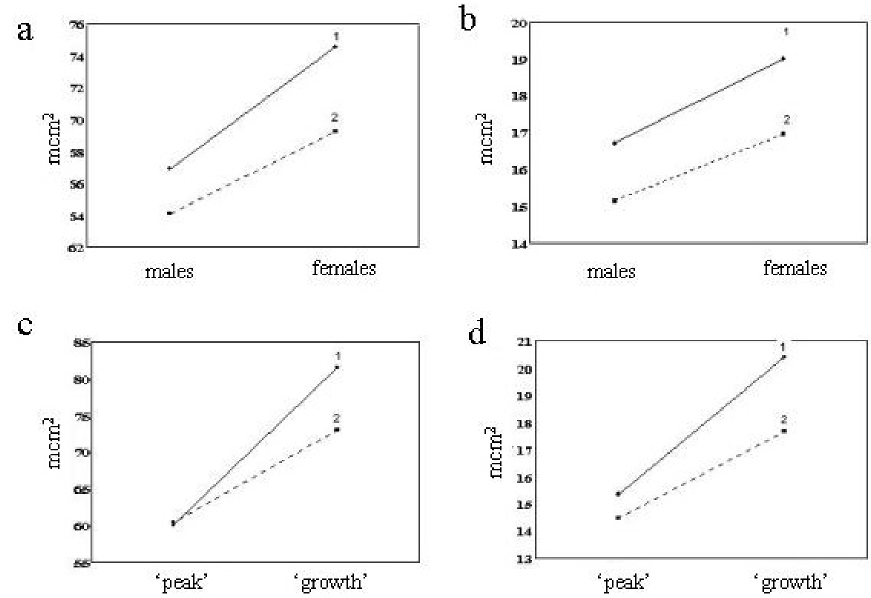

Fig. 1 Bank vole adrenal cortex morpho-physiological parameters at interaction of factors (average unweighed). 1 – anomaly (location 1), 2 – background

a- interaction of geochemical factor and factor ‘sex’: area of adrenal cortex fasciculate zone cells

b- interaction of geochemical factor and factor ‘sex’: area of adrenal cortex fasciculate zone cell nuclei

c- interaction of geochemical factor and factor ‘phase of animal population cycle’: area of adrenal cortex fasciculate zone cells

d- interaction of geochemical factor and factor ‘phase of animal population cycle’: area of adrenal cortex fasciculate zone cell nuclei

It is known that external causes can influence the fecundity in small mammals. Estimation of the bank vole female fecundity parameters found an increase in the potential fecundity in natural geochemical anomalies [5]. Statistically significant differences between values of this feature were revealed only for the case Location 2 ($X \pm m$: 6.88±0.30) — Control ($X \pm m$: 5.44±0.22) (U=19.50, Z= –2.92, p<0.05). As regards the actual fecundity, it also increased in the anomalies, the differences are significant for the both cases: Location 1 (5.74±0.22) – Control (4.83±0.23) (U=90.5, Z=2.45, p<0.05), Location 2 (6.13±0.52) – Control (4.83±0.23) (U=36.0, Z=2.0, p<0.05).

Examination of the pre-implantation mortality found its decline in anomalous areas. The pre-implantation mortality in females from Location 1 was 1.8 times lower than in those from the control, and for females from Location 2, it was 2.4 times.

Different female reproductive potencies correspond to specific morphological patterns of the ovary tissue. To analyze the ovaries histology in voles caught in anomalies, we counted the number of ovarian follicles by stages of their development and measured their maximum diameters. In analysis of variability factors for the follicles in the ovaries of the pregnant Bank Voles, we used a three-factor model of dispersion analysis (factors: ‘Geochemical conditions’, ‘Phase of population cycle’, ‘Type of follicle’). The follow-up paired comparisons were done with the use of Mann-Whitney U test.

The differences in the number of follicles in the pregnant females depending on geochemical conditions were not found (F = 0.3, p = 0.74). At the same time, triple interaction of the factors ‘Geochemical factor’, ‘Phase of population cycle’ and ‘Type of follicle’ (F = 0.88, p = 0.48, Fig. 2, a) and post hoc test, suggested that, in the background, the significant increase in the number of secondary follicles during the population ‘growth’ phase (p = 0.02, LSD test) occurred. The number of single-layered and tertiary follicles in the ovaries of females from all locations is nearly the same. The dependence of the secondary follicles number on the population density was not found in animals from anomalies (p > 0.05, LSD test, Fig. 2, a).

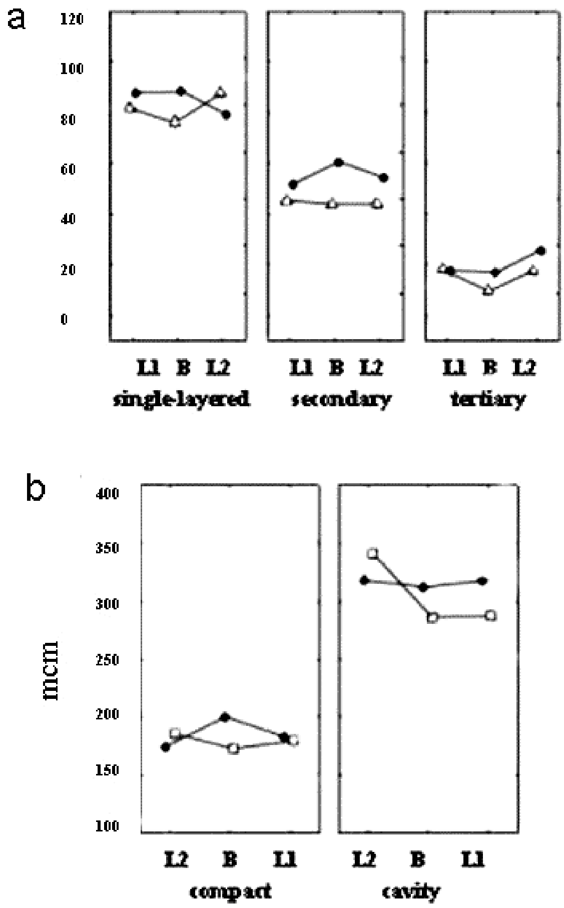

Fig. 2 Pregnant bank vole ovaries morpho-physiological parameters at interaction of factors (average unweighed). 'Growth' phase of animal population cycle is designated by black geometrical figures, 'peak' – by white geometrical figures

a - interaction of geochemical factor, factor 'phase of animal population cycle' and 'type of ovarian follicle': number of ovarian follicle, L1 – location 1, B – background, L2 – location 2;

b - interaction of geochemical factor, factor 'phase of animal population cycle' and 'type of ovarian follicle': diameters of follicles in ovary, L2 – location 2, B – background, L1 – location

In analysis of variability factors for the maximum diameters of follicles in the ovaries of the pregnant bank voles, we also used similar three-factor model of dispersion analysis: 'Geochemical conditions'. The sizes of the follicles in the pregnant females from the background and anomalies do not differ (F = 1.17, p = 0.31). But triple interaction of the factors 'Geochemical factor', 'Phase of population cycle' and 'Type of follicle' (F = 0.87, p = 0.42 Fig. 2, b) suggests that, in the background, an increase in their sizes during the population 'growth' phase and the decrease during the 'peak' phase occurs. An increase in the maximum diameters of the compact follicles in the pregnant females from the background during the population 'growth' phase compared to the 'peak' phase was found (p = 0.01, LSD test, Fig. 2, b).

In analysis of variability factors for a generative function of the ovaries in the immature bank vole females, we used a two-factor model of dispersion analysis. The model included the factors of geochemical conditions and type of follicle. The factor 'phase of cycle' was not considered because during the population 'growth' phase, only solitary immature Bank Vole females were caught.

Estimation of the number of follicles in the immature Bank Vole females during the population 'growth' phase found an increase in the number of secondary and tertiary follicles in animals residing in natural geochemical anomalies (Location 1 and 2) compared with those from the background (Table 2).

Estimation of the follicle sizes in immature females with the use of similar two-factor dispersion analysis revealed higher values of the maximum diameters for the compact and cavity follicles in the ovaries of the immature females residing in natural geochemical anomalies compared with those from the background (Table 2). Thus, the increase both in the size and in number of follicles suggests intensification of the folliculogenesis process in the ovaries of the immature females from Locations 1 and 2.

IV. DISCUSSION

Geochemical anomalies that provoke no endemic diseases can though result in the organism growth, development, reproduction disturbance and modify the course of widely distributed diseases [21]. At the present time, the issue of impacts from the non-endemic geochemical anomalies on the biota has not been much highlighted. The studied anomalies with a surplus of nickel, chrome and cobalt cause no endemic diseases but, as our research showed, trigger certain adaptive responses from the animal organisms. The geochemical anomalous environment accounts for low abundance of small mammals, changes in the interior weight (morpho-physiological) characteristics of animals, alterations in the adrenal cortex structure and modification of the reproductive function.

Morpho-physiological parameters of animals are widely addressed in the scientific literature because variability in the body mass and interior characteristics reflects certain shifts in the metabolism of animals under the pressure of various environmental factors [22]. It is known that the natural geochemical factor along with the other factors can provoke changes in the morpho-physiological characteristics of animals. Our research found that the relative and absolute mass of the bank voles adrenal increased in natural geochemical anomalies associated with ultrabasic rock distribution, which was determined largely by an increase of the organ blood filling and indicated activation of this organ function. In addition, increased kidney relative mass and lowered index of animal fatness and lowered hepato suprarenal coefficient were recorded in the anomalies. The changed interior parameters identified, taken both separately and collectively, suggest intensification of metabolism in the mice from the anomalies and, as a consequence, lowered their energy potential. One of the primary causes for increased metabolism intensity and high metabolic costs of organisms in the anomalies is likely to be the energy deficiency in the animal tissues because the heavy metals affect the tissue respiration and energy metabolism of the cells [23]. In anomalies, we also recorded an increase in the testis relative mass, which indicates accelerated maturation of young animals and increased reproductive potency of the mature males. Increased number and size of the ovarian follicles in the immature vole ovaries evidence the female accelerated maturation in the anomalies because the number of ripening follicles and their diameters correlate with hormonal activity of the ovaries.

Acceleration of puberty of animals was recorded previously in technogenic contamination areas [24-26], in radioactive contamination areas [27] and in artificially dispersed populated areas [28] and was associated with disturbance of the population social environment. In our case, accelerated maturation is facilitated with high postnatal mortality of animals in the anomalies based on the density-related mechanism principle. Our assumption about excessive mortality rates in the anomalies is made based on the female potential and actual fecundity data and presence of the voles generally in small numbers (Table III). In anomalies, chromium salts can also induce acceleration of the maturation process in animals because this element in certain concentrations stimulates gonadotroph function of the hypophysis. Numerous studies showed that diverse changes in the environment conditions result in activation of the adrenal cortex with the hormones involved in a broad range of adaptive responses.

TABLE III THE NUMBER BANK VOLE ON THE TERRITOTIES OF NATURAL GEOCHEMICAL ANOMALIES (LOCATION 1 AND 2) AND THE BACKGROUND

Territory / Year	**Relative Abundance, Individuals/100 Trap Days**					
	2001 ‘peak’	2002 ‘depression’	2003 ‘growth’	2004 ‘peak’	2005 ‘depression’	2006 ‘growth’
Background	40.0	-	5	22	0	13.5
location 1	12.5	0.5	3	11.5	-	-
location 2	-	-	-	5.5	0.5	2.3

Note: The crossed out section means absence of the data.

Structural and functional characteristics of the adrenal cortex depend on the nature, intensity and duration of the stimuli. Many authors record the changes in the mammal adrenal cortex in extreme conditions. Along with the main characteristics of the general adaptation syndrome, Selye paid attention to the adrenal hypertrophy in mammals under the stress [29]. Changes in the morpho-functional state of the adrenal cortex in animals were found in the course of laboratory introduction of xenobiotics, under higher radiation conditions in areas with the natural radioactive background and radionuclide contamination [30], in the course of exogenous introduction of gonadotropic and sex hormones [31] in the case of density-related mechanism effects [32]. In our case, weight adrenal hypertrophy under extreme geochemical conditions, as described above, is followed by an increase in the area of the adrenal cortex fasciculate zone, its cells and nuclei. In our opinion, an increase in the fascicular cells metrics is explained by intensified production of the glucocorticoids that participate in a broad range of adaptive responses and ensure increased non-specific resistance of animals under the action of excess heavy metals contained in the environment. Animal adaptation to conditions of a natural biogeochemical province is likely to occur through reduction of the energetic reserves as a result of the glucocorticoid catabolic effect.

The reproductive system is one of the systems that provide most active response to the changing environment [33]. An increase in the fecundity parameters was recorded in technogenic contamination and higher radiation conditions [34]. Changes in the morpho-functional state of the ovaries were found in the course of laboratory introduction of chromium VI, under combined action of the lead and radiation, in areas with high radiation levels and in the case of animal density-related mechanism effects [35]. Our study revealed certain characteristics of the female reproductive system in natural geochemical anomalies. Increased potential and actual fecundity, pre-implantation mortality decline were recorded. The high values of potential and actual fecundity in anomalies agree well with literature data on increase in these parameters in extreme habitat conditions. Increased potential and actual fecundity, pre-implantation mortality decline are thought to be population adaptive response to impacts from the natural geochemical anomalies to offset a higher-level animal pre-implantation mortality in these areas.

In natural geochemical anomalies, this study found synergizing effect that occurred under pressure of other factors upon the bank vole organism. Estimation of factor interaction effects (multifactor dispersion analysis) showed that extreme geochemical conditions aggravate functional tension in the geosystems studied under the impact of other factors. In anomaly areas, widening sex differences in the adrenal cortex morphology and differences related to the population density changes occur. Intensity of folliculogenesis depends on the phase of population cycle in natural mouse rodent populations. The maximum follicles are recorded at the population 'growth' phase and the minimum at the 'peak' because density-related functional tension in the adrenal cortex increases. Our data on folliculogenesis in the females from the background reflect this regularity: an increase in the number of secondary follicles and sizes of compact follicles at the population 'growth' phase was recorded. Increased functional activity of the adrenal cortex in geochemical anomalies results in no changes in the folliculogenesis intensity in the pregnant females depending on the population cycle phase ('growth', 'peak'). This implies the maximum reproductive function operation in the females in anomalies.

V. CONCLUSION

Thus, natural geochemical anomalies serve as the factor of the additional functional load upon animal reproductive and endocrine systems. In such areas, we should thereby expect lowered animal resistance to an adverse environment especially in those situations in which the organism requires significant metabolic costs.

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