Element Composition of Diet and Tissues of Small Mammals of Different Trophic Levels as a Bioindicator of the Chemical Pollution of Environment

V. S. BEZEL[1], K. P. KJOUTSENOGII[2], S. V. MUKHACHEVA[1], T. I. SAVCHENKO[2] and O. V. CHANKINA[2]

[1]*Institute of Ecology of Plants and Animals, Ural Branch of the Russian Academy of Sciences, Ul. 8 Marta 202, Yekaterinburg 620144 (Russia)*

E-mail: bezel@ipae.uran.ru

[2]*Institute of Chemical Kinetics and Combustion, Siberian Branch of the Russian Academy of Sciences, Ul. Institutskaya 3, Novosibirsk 630090 (Russia)*

E-mail: koutsen@ns.kinetics.nsc.ru

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Abstract

Comparative analysis of the element composition of diet and tissues (liver) of the individuals of bank voles (*Clethrionomys glareolus*) and common shrews together inhabiting chemically polluted (impact) and non-disturbed (background) territories of the Middle Urals was carried out. The obtained data allow one to estimate the role of ecological factors in the formation of biogenic cycles of chemical elements participated by the mammals belonging to different links of trophic chains. The group of phytophages (voles) occupies a special place in the translocation of chemical elements over the trophic levels of mammals. The specific features of feeding for these species and the existence of the gastrointestinal barrier limit the accumulation of elements in animal organisms. Under the same conditions, carnivores (common shrew) play the part of concentrators of a number of chemical elements (Pb, Cd, Cr, As *etc.*) in the mammal community. The concentrations of these elements increase in the animal organisms in comparison with their concentrations in their food. The toxic load on an animal organism (liver) does not depend on the trophic specialization.

INTRODUCTION

Chemical elements distributed in environmental objects comprise a very important natural phenomenon depicting the intensity of global and regional biogeochemical cycles. It is known that natural systems and living organisms consist of 10–15 major chemical elements by more than 99 %. Other elements, defined by V. I. Vernadskiy [1] as scattered elements, in spite of low concentrations exhibit high geochemical activity; the scales of their exchange in environment are huge. According to the biospheric function of the living matter, one should speak of several levels of biogenic cycles of chemical elements. These cycles are formed by the living organisms of different trophic groups. A specific geochemical selection of microelements takes place between these levels of natural ecosystems [2]. This selection is determined by different biological availability of the chemical elements and by the forms of their compounds in soil, by the specific features of zonal types of vegetation, by the selectivity of their assimilation and deposition by the organism of different trophic groups [3–5]. In view of this, we deal with a complicated multilevel geochemical portrait of the integral biocenosis and its possible deformation as a result of anthropogenic activities.

At present, numerous facts on the accumulation of such chemical elements as radionuclides [4, 6], macro- and microelements including heavy metals [7–9] in separate components of natural ecosystems has been obtained also for the conditions of the chemical pollution of environment [10–14]. However, much smaller number of works deal with the migration of heavy metals over the food chains in ecosystems [15, 16]. In addition, the majority of authors limit themselves to the investigations of only a small number of chemical elements, as a rule, those playing the role of toxicants. Meanwhile, the necessity to obtain the data for the microelement composition of the constituents of the biota for a wide range of elements is evident. Only in this case a detailed analysis of the biogeochemical cycles of specific natural and industry-related ecosystems and their transformation in the system of trophic levels becomes possible.

The urgent character of the investigation of the microelement composition of populations and communities of different groups of the biota is caused by the necessity to explore the mechanisms of formation of biogenic cycles and to develop, on the basis these mechanisms, the methods of regulation of the environmental impact of industrial enterprises. To develop adequate notions in these aspects, it is necessary to carry out extensive multi-aspect ecological and analytical investigations including the analysis of the microelement composition of model objects at different levels of the organization of biological systems: from primary producers (the components of phytocenoses that are food items for phytophage animals) to consumers (food items for carnivore species) of the higher trophic levels.

The goal of the present investigation was to estimate the geochemical selection of elements by small mammals of different taxonomic and trophic groups together inhabiting chemically polluted and undisturbed territories. In order to achieve this goal, we made an attempt to carry out a comparative analysis of the concentrations of 18 chemical elements belonging to the macro- and microelements, in diet and in the tissues (liver, skeleton) of the individuals of voles and common shrews.

OBJECTS AND METHODS OF INVESTIGATION

The investigation is based on the data obtained in the studies of the population of small mammals in the vicinity of a large copper-smelting plant and in the undisturbed (background) territories (the Middle Urals). The plant works for more than 65 years; the affected zones around the plant are clearly exhibited. The range of heavy metals entering the atmosphere in the form of aerosol and polluting the environment is broad. We chose the elements present in emissions in the highest concentrations as the priority pollutants. For instance, the mass concentration of Cu is 44.9, Zn 31.5, Pb 10.1 % [17]. A detailed description of the investigated ground was presented by us previously [18]. The data obtained in July 2004 in course of irretrievable withdrawal of small mammals using Gero traps according to the standard procedure [19] were used in the investigation. Trapping was carried out simultaneously in two regions: in the vicinity of the source of industrial emission (1–2 km, impact zone) and at a distance of 30 km, in the region chosen as the background one where the concentrations of chemical elements in soil did not exceed the clarke level.

The trapped animals were subjected to the standard zoological examination determining their species, sex, functional age state, reproductive status of an individual, and measuring the major morphophysiological indices.

Two small mammal species belonging to different taxonomic and trophic groups: mainly herbivorous European vole (*Clethrionomys glareolus* Shreber, 1780) and insectivorous common shrew (*Sorex caecutiens* Laxmann, 1788) were chosen as the main objects of investigation. The choice of the objects was also determined by the dominant position of the species in the communities of murine rodents and small insectivorous animals inhabiting the disturbed and background territories.

In our opinion, in view of the complicated composition and plasticity of food items of small mammals, the most correct method is to determine the concentrations of elements in the contents of the animal stomachs [12]. For this purpose, stomach dissection of the trapped animals was carried out; a dry clean spatula was used to sample the contents for chemical analysis. Special investigations show [20] that the concentrations of major pollutants in food items of the animals depend on the season of capturing. Because of this, in the present work we used animal samples maximally close in capturing dates and in the functional age status.

The concentrations of 18 chemical elements (K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Br, Rb, Sr, Y, Pb, Cd) were compared in diet ($n = 20$) and in liver ($n = 20$) of the animals. The sampled material was dried in a drying chamber at a temperature of 70 °C to the absolutely dry mass. Further preparation of the samples for analysis was described in detail elsewhere [21]. The samples of liver and stomach contents were analysed in the form of tablets 1 cm in diameter and a mass of 30 mg. Energy dispersion X-ray fluorescence element analysis of biosubstrates was carried out at the Station for element analysis of the Centre for SR VEPP-3 of the Budker Institute of Nuclear Physics, SB RAS (Novosibirsk) [22]. The samples were recorded with the excitation energy of 21 keV. The obtained the quantitative data on the elemental composition, the external standard was used. The Russian standard of cereal mixed grass crop SORM 1 GSO 8242–2003 was used for this purpose because this standard is most close to the samples under analysis. The concentration of cadmium in liver and in stomach contents was determined by means of atomic absorption with ASS-6 spectrometer (Carl Zeiss) in the Laboratory of Population Ecotoxicology of the Institute of Plant and Animal Ecology, UrB RAS (Yekaterinburg). The samples were preliminarily ashed by means of humid mineralization in concentrated nitric acid using microwave decomposition.

The data obtained were processed with the help of Microsoft Excel software. The quantitative intergroup differences in the concentrations of elements for the individuals of the groups under comparison at different territories were estimated using Student's t-criterion ($p < 0.05$).

RESULTS AND DISCUSSION

Composition of diet of the small mammals of different trophic groups

It is logical to assume that the differences in the microelement composition of food items of the animals can be due either to differences in the composition of food objects or to different concentrations of chemical elements in food. The data on the elemental composition of stomach contents, obtained with XFA SR, allow us to conclude that the level of accumulation of the majority of elements in food items of both species is within the limits from 0.10 to 1000 μg/g; the ranges of concentration measurement are close to each other (Table 1). Since our data are adequately described by the lognormal distribution, the reliability of intergroup differences were estimated using Student's t-criterion for the geometric mean concentrations of the elements.

If the concentration of each element in the food items of voles inhabiting the background regions is taken as unity, the levels of accumulation of these elements in the food items of the animals of other groups (voles from the polluted region, and shrews from both regions) expressed in relative units will provide evidence how much this parameter varies in comparison with its value for the voles of the background territory.

A comparative analysis of the diet of the animals of two trophic groups inhabiting the background regions allows us to conclude that the food items of shrews in comparison with those of voles are enriched with Co, as well as with some of physiologically significant elements (Fe, Zn) (Fig. 1). No reliable differences were detected for other elements.

It is natural to expect that under the conditions of chemical pollution of the environment the food items of animals (first of all phytophages) can contain toxic elements. Indeed, for the voles inhabiting the regions situated in the zone of intense chemical pollution, the admission of Ca, Fe, Co, Cu, Zn, As, Cd, Pb into the gastrointestinal tract increased. The concentrations of some elements exceed the background values by a factor of eight and more (Fig. 2, *a*).

The mineral composition of the diet of shrews at the polluted territory changed, too. However, the concentrations of the majority of chemical elements (12 of 18 investigated ones) decreased in comparison with the background regions; significant differences were recorded for Rb and Y (see Fig. 2, *b*). An increase was observed only for the pollutants of priority for this territory: Pb and Cu. Partial "purification" of the diet of this group of an-

TABLE 1

Elemental composition (average values and the ranges of variation of element concentrations, μg/g of dry mass) and chemically polluted (B) territories

Element	Vole			
	Stomach contents		Liver	
	A (1)	B (2)	A (3)	B (4)
K	26 800	17 580	12 270	9990
	12 741–31 197	10 413–25 427	9920–15 670	8565–10844
Ca	5950	2290	703	230
	4090–7749	1057–3658	205–2711	168–341
Ti	30	104	4.6	5.6
	21–39	18–250	2.7–11.7	3.8–8.2
V	04	08	0.02	–
	0.27–0.43	0.05–1.83	0.01–0.05	–
Cr	175	1110	7	11
	72–393	36–3061	5.5–9.9	4.3–17.2
Mn	263	334	16	15
	198–359	99–615	13–20	9–24
Fe	470	2170	500	1300
	329–644	785–3947	234–742	707–2041
Co	0.16	07	02	04
	0.14–0.21	0.27–1.24	0.11–0.22	0.29–0.65
Ni	21	26	0.6	0.6
	17.6–29.0	7.6–39.3	0.48–0.91	0.44–0.82
Cu	20	117	13	13.5
	17–21	63–214	11–16	11–17
Zn	110	316	103	100
	100–132	132–424	96–110	85–117
As	04	2.8	02	2
	0.02–1.22	2.71–3.11	0.17–0.29	0.81–5.02
Br	10	85	9	39
	8–13	19–307	6–13	10–63
Rb	37	15	30	15
	15–52	9–22	21–37	10–20
Sr	27	14	04	03
	20.24–45.25	1.36–23.33	0.19–0.71	0.22–0.52
Y	2	22	1.7	0.9
	1.01–2.79	1.35–3.64	1.11–2.09	0.58–1.22
Pb	24	307	0.9	3.6
	14–38	155–481	0.38–1.17	0.88–5.67
Cd	1.7	63	1.4	11
	0–14.43	0.59–16.82	0–8.97	0–16.10

Notes. 1. Figures in brackets show numbers of groups for the comparison on the basis of Student's t-criterion. d – 7–8, e – 1–5, f – 2–6, g – 3–7, h – 4–8. 3. n/f – not found.

in diet and in liver of vole and shrew individuals inhabiting the background (A)

Shrew				p
Stomach contents		Liver		
A (5)	B (6)	A (7)	B (8)	
14 300	7750	10 250	11265.67	
8075–24786	5347–12499	9269–12362	10105–1222	
7315	2900	230	269.60	a
2215–20436	1254–4796	179–279	218–329	
375	48	5	9.94	d
40–576	20–80	3.6–9.0	7.8–12.9	
2	0.3	n/f	n/f	
0.43–6.17	0.23–0.39			
383	507	11	28	
27–939	73–1137	7.2–16.0	10.0–54.4	
312	131	33	31	g, h
101–592	110–160	28–38	20–37	
2933	1233	1050	1380	a, b, e
666–6720	883–1641	564–2099	796–1918	
0.7	0.4	0.4	0.4	a, b, e
0.22–1.42	0.32–0.47	0.23–0.63	0.30–0.53	
12	6	0.4	0.5	f
4.10–19.00	4.8–8.1	0.02–0.58	0.25–0.71	
29	84	15	18	a, c
17–58	65–98	14–16	15–21	
268	350	82	85	a, b, f, g
177–426	287–464	74–88	82–89	
1.2	0.8	2	0.8	b, h
0.31–2.35	0.26–1.25	0.90–3.22	0.28–1.22	
17	16	7	10	b, h
12–23	11–21	6–9	9–12	
16	4	15	6	b, c, d, f, g, h
8–29	3–6	12–19	4–8	
24	33	0.3	2	
7.37–62.69	7.02–82.74	0.11–0.52	0.06–9.39	
2	1.3	0.9	0.4	b, c, d, f, g, h
0.85–3.84	0.58–2.52	0.63–0.88	0.33–0.53	
100	169	0.8	13	a, b, c, d, f
23–192	116–217	0.24–1.39	3.45–25.46	
7.5	7.3	18	54	a, e
1.54–19.78	0.89–16.08			

2. The letters in the last column designated essential differences between groups ($p < 0.05$): a – 1–2, b – 3–4, c – 5–6,

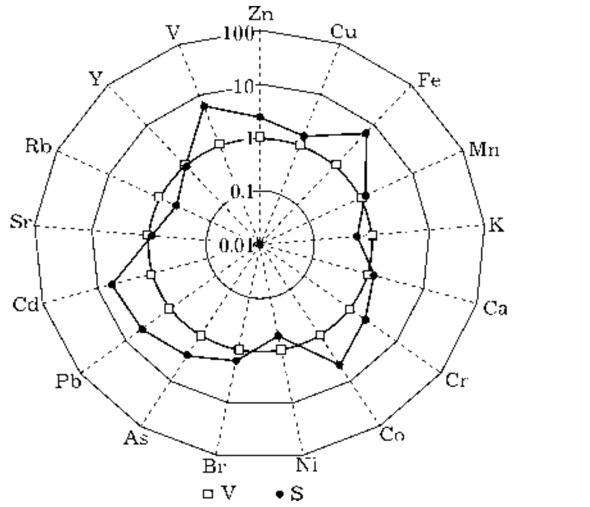

Fig. 1. Microelement composition of diet of voles (V) and shrews (S) at the background territory (the Middle Urals, 2004). The data are represented in relative units; the concentrations of the elements in diet of voles at the background territory are accepted to be unity.

imals can be connected with the changes in the species composition of food objects and therefore with decreased concentrations of chemical elements in food.

Concentrations of elements in the tissues of the animals of different trophic groups

We considered liver as the depositing substrate, since liver takes an active part in the mineral exchange. In spite of differences in the trophic specialization, the animal species under investigation belong to the class of mammals, so one could not expect significant differences in the mechanisms of absorption and deposition of the elements under investigation. The detected differences in the levels of elements in liver are most likely due to the differ-

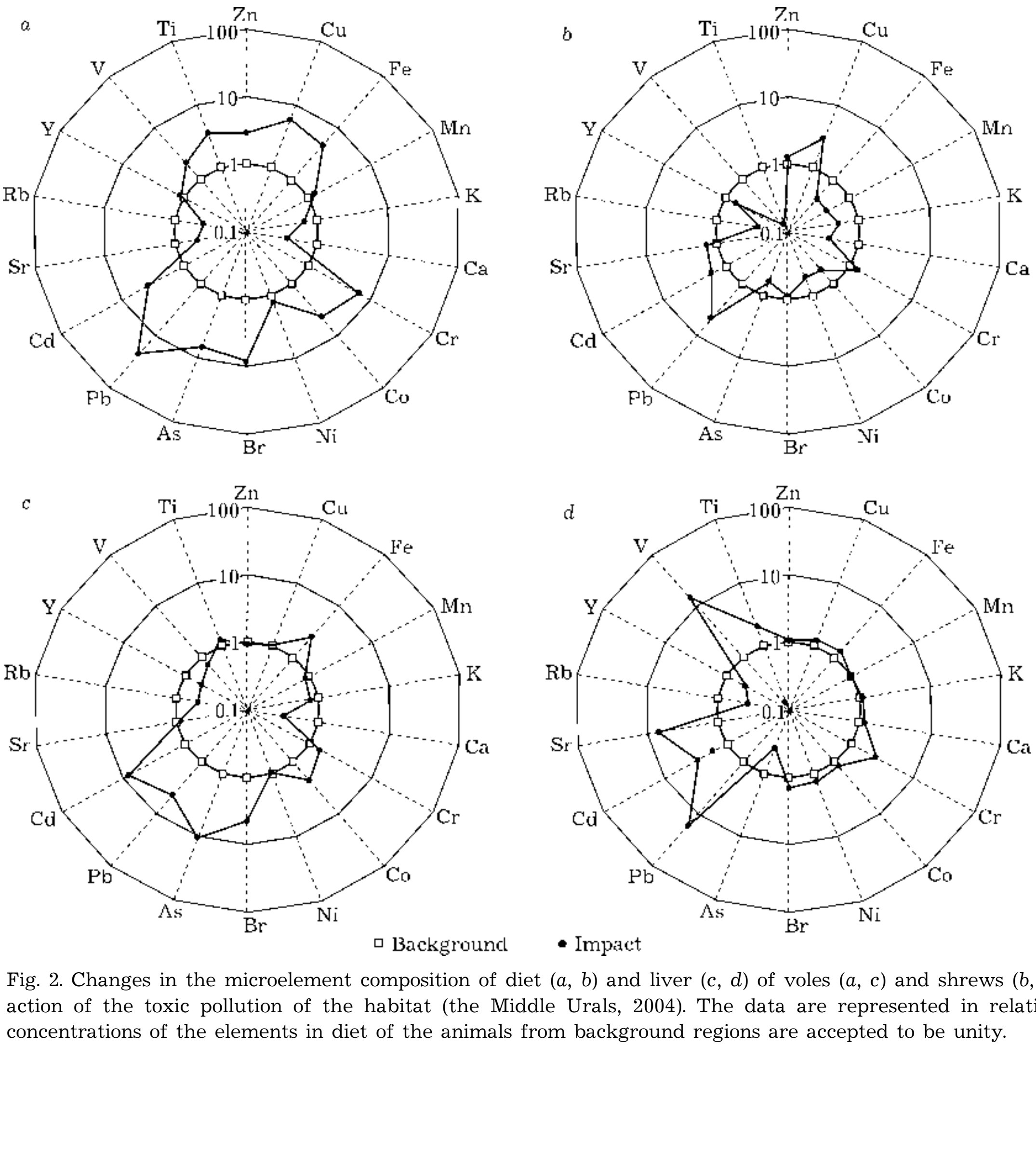

Fig. 2. Changes in the microelement composition of diet (*a*, *b*) and liver (*c*, *d*) of voles (*a*, *c*) and shrews (*b*, *d*) under the action of the toxic pollution of the habitat (the Middle Urals, 2004). The data are represented in relative units; the concentrations of the elements in diet of the animals from background regions are accepted to be unity.

ences in the microelement composition of food items. The levels of element accumulation in liver were compared using the scheme proposed above for the diet; the concentrations of elements in the liver of voles from background regions was taken as unity.

Analysis of the elemental composition of vole liver showed (see Table 1) that the presence of such elements as Co, As, Cd, Pb in increased concentrations at the polluted territories determines the increase of the concentrations of these elements in the depositing organ. At the same time, an increase in the concentrations of Fe, Cu, Zn in diet does not cause a substantial increase in their concentrations in liver (see Fig. 2, *c*). This phenomenon may be a manifestation of some barrier regulating the admission of physiologically necessary elements into the internal environment. A similar comparative analysis of the elemental composition of diet and liver of shrews at the polluted territories showed that such typical pollutants as Pd and Cd are able to get accumulated in liver in substantial amounts (see Fig. 2, *d*).

The intensity of biogenic cycles of chemical elements in natural ecosystems is controlled by a system of biological barriers. At the level of an organism (ontogenetic), ones speaks of the presence of the root system in plants or the walls of the intestinal tract in animals; these formations either promote intense inclusion of an element into the biological circulation or limit these processes. This barrier function preventing the admission of chemical elements into mammal organisms has been rather thoroughly studied within the framework of experimental toxicology. For the majority of heavy metals, the level of their absorption in mammals is within 3–10 % of the amount entering the intestinal tract [23].

In addition to the mentioned processes of the ontogenetic level, the intensity of biogenic cycles of chemical elements in natural ecosystems is also controlled by a system of ecological barriers including the composition of food items and level of their pollution, the abundance of the organisms of a definite trophic level. Under chemical pollution and related environmental degradation, one may expect changes in the species composition and number of both producers and consumers.

As we have already stressed above, the shrews from the polluted region exhibited partial "purification" of diet. First of all, this is connected with the changes of food objects. It is known that the main diet of shrews are *Arachnida*, ground coleopterous insects (representatives of Carabidae, Elateridae, Staphylinidae families), larvae of dipteran insects (dipteran *Larvaeinsect*), hemipterans, earthworms. The main zone of food search is the surface litter (mulch) layer [24]. Cardinal changes in the population of soil mesofauna occur in the impact zone: some groups (Lumbricidae, Enchytraeidae, Diploda, Mollusca) get completely eliminated; others (Carabidae, Staphylinidae, Arachnidae, Diptera) substantially decrease in number. A characteristic feature of the population of impact regions is an increase in the fraction of Elateridae larvae from 0.7–4.0 (background region, 30 km) to 35–50 %. At these regions one also observes the trend of the displacement of soil invertebrates into the litter layer. For instance, at the background territory 10–30 % of the total number of organisms is concentrated in the mulch layer, while at a distance of 1–2.5 km from the emission plume this index increases to 50–80 % [17].

Analysis of the accumulation of the main pollutants in the representatives of different groups of invertebrates in the gradient of toxic load showed that the concentrations of metals and the ability to their bioaccumulation in the invertebrate organisms differ susbtantially [15]. At the background territory, the minimal concentrations of Cu are observed in ants, Pb in lepidopterans, Cd and Zn in phytophagous hemipterans; the maximal concentrations of Cu were observed in hemipterans, Pb in blood-sucking dipterans, Cd and Zn in ants. In the impact zone, the minimal levels of Cu accumulation were recorded for butterflies, other priority pollutants in bugs. The maximal concentrations of Cu at the polluted territory were observed in predacious coleopterous insects, Cd and Zn in blood-sucking dipteran insects, Pb in ants.

So, it may be stated that due to the industry-caused transformation of the population of invertebrates, the diet of the shrews in the impact zone change substantially in comparison with the background territories. An important part in food items of the small animals in polluted regions is played by the representatives of *Coleoptera* order (including Elateridae, Staphylinidae, Carabidae) characterized by reduced bioaccumulation of elements.

The data obtained allow us to estimate the role of ecological factors in the formation of biogenic cycles of chemical elements involving the participation of mammals belonging to different links of trophic chains. The ratios of the concentrations of chemical elements for impacted regions to those for background ones are plotted in Fig. 3: in diet along the abscissa and in liver along the ordinate axis, for voles and for shrews. If an increased concentration of an element in the polluted territory causes a proportional increase in its concentration in the depositing medium (which is liver in the case under consideration), we observe a direct (without limitations) translocation of an element to the next trophic level. If an element is located below the bisector, its transition to the next trophic level is limited by a barrier at the level of the gastrointestinal tract.

For voles, the majority of chemical elements including toxic ones (V, Cr, Mn, Ni, Pb), as well as a number of physiologically significant elements are discriminated by this barrier, so the transition from the level of producers (plant food) to the level of primary consumers is limited (see Fig. 3, *a*). Increased (in comparison with food items) concentrations of the elements are

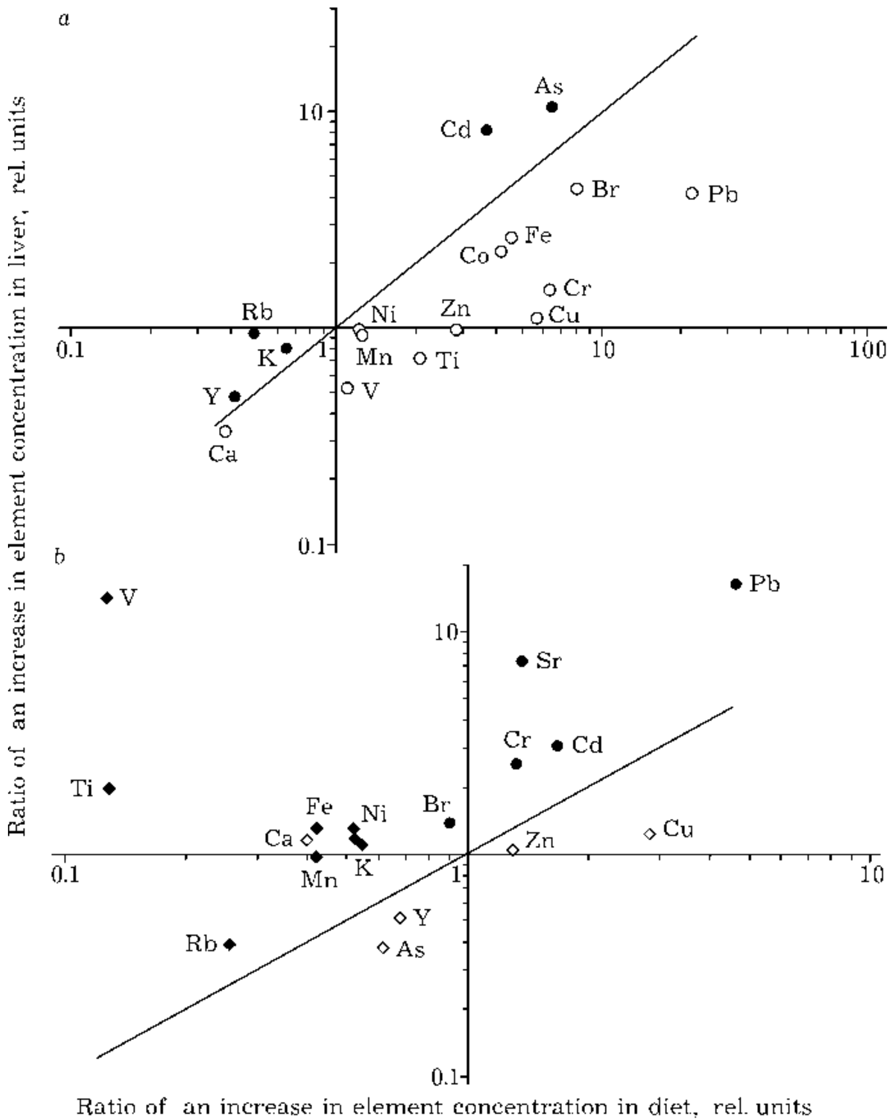

Fig. 3. Changes in the microelement composition of the liver of voles (*a*) and shrews (*b*) depending on the concentrations of the elements in their diet (the Middle Urals, 2004).

observed in voles for typical toxicants (As and Cd). For carnivores (shrews) a limited inclusion of an element to the level of secondary consumers was observed only for Cu, Zn, As, Y (see Fig. 3, *b*). The positions of the majority of elements at the plane above the bisector mean that an excess (in comparison with food items) accumulation of these elements occurs in shrew organisms.

From the viewpoint of ecological toxicology, the determining factor is not the concentrations of chemical elements in food items or in organisms of animals but the toxic load originating from the presence of the elements. The level of toxic load is most frequently accepted to be the overall excess of element concentrations over the background values calculated according to equation

$$S_n = (1/n)\Sigma C_i/C_f$$

where C_i and C_f are the concentrations of elements that can be considered as toxic at the polluted and background regions, respectively; n is the number of elements taken into account. The load can be calculated for the conditions of element arrival with food, and also for their accumulation in tissues.

The results of calculation of total toxic load are shown in Fig. 4. If the background load is accepted to be equal to one relative unit, all the considered versions for impacted territories exhibit excess of the toxic action over the background values. The maximal load is that formed by chemical elements in the diet of phytophages. This high toxicity of vole diet is formed by a wide range of elements: Ti, V, Cr, Co, Cu, Zn, As, Cd, Pb (see Fig. 2, *a*). The food load for shrews is about two times lower; it is formed mainly by Cu, Cd and Pb (see Fig. 2, *b*). In spite of the mentioned differences in the chemical composition of diet and in the levels of food load, liver of both species in the polluted territory is subjected to about the same toxic action, which is likely to be connected with the above-mentioned increased absorption of chemical elements in the gastrointestinal tract of insectivorous animals (see Fig. 3, *b*).

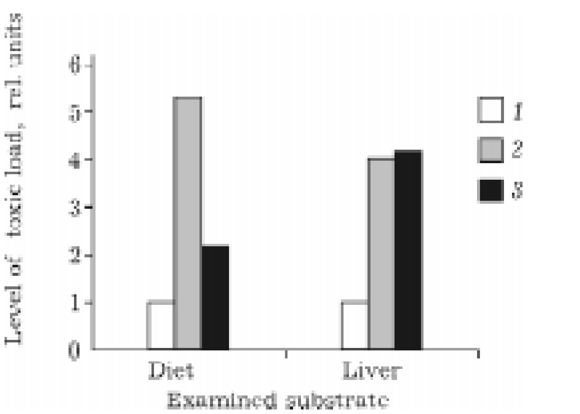

Fig. 4. Toxic load on small mammals inhabiting the impacted territories, calculated on the basis of the concentrations of chemical elements in diet and in liver of the animals: *1* – background value equal to 1 rel. unit; *2* – voles; *3* – shrews.

SUMMARY

1. The special place in the translocation of chemical elements over the trophic levels of mammals is occupied by a group of phytophages (voles). The specific features of diet of these species and the existence of the gastrointestinal barrier limit the accumulation of the elements in animal organisms.

2. Under the same conditions, in the mammal community, the carnivores (shrews) play the part of concentrators of a number of chemical elements (Pb, Cd, Cr, As *etc.*); the concentrations of these elements in animal organisms increase in comparison with their levels in diet.

3. The toxic load on animal organisms (liver) does not depend on heir trophic specialization.

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