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Long-Term Dynamics of Heavy Metal Concentrations in the Food and Liver of Shrews (g. *Sorex*) during High and Reduced Emissions Periods from the Copper Smelter

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Abstract—The long-term changes in the contents of heavy metals (Cu, Zn, Cd, Pb) in the food and liver of four shrew species of the g. *Sorex*, in the vicinity of the the Middle Ural Copper Smelter (MUCS) during periods of its high (1990–1997), reduced (1998–2009), and almost ceased (2010–2019) emissions. The minimum concentrations of all elements in the animal organism were noted in unpolluted areas (background zone), the maximum concentrations of Cu, Zn, and Cd were in the immediate vicinity of the plant (impact zone), Cd—at moderate pollution (buffer zone). The species specificity of the accumulation of the considered elements in the liver was determined by the composition of diets and was expressed in increased accumulation of Cu and Cd in the liver of *S. araneus* and increased accumulation of Pb in the liver of *S. caecutiens*. A multiple reduction in industrial emissions did not lead to an equivalent decrease in the content of heavy metals (HMs) either in the feed or in the body of shrews. Over 30 years, directed changes in HM concentrations in the liver were noted only in *S. caecutiens*: in the impact zone (1–3 km from the plant), the content of Zn did not change, the concentrations of Cd and Pb decreased by 1.2–1.5 times, while the concentration of Cu, on the contrary, increased by 1.5 times. In the buffer zone (4–10 km) Cu, Zn, and Pb concentrations remain unchanged, while the concentrations of Cd decreased by 1.5 times; in the background area (20–34 km), the content of essential elements (Cu, Zn) was maintained at the same level, while the concentration of toxic elements (Cd and Pb) decreased by 4–5 times.

Keywords: industrial pollution, natural ecosystem restoration, copper, zinc, cadmium, lead, liver, food spectrum, *Sorex araneus*, *S. caecutiens*, *S. minutus*, *S. isodon*

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INTRODUCTION

In recent decades, in most countries, for various reasons, emissions from industrial enterprises have been declining [1, 2]. However, various pollutants, including heavy metals (HMs), continue to circulate in the environment for a long time, exerting adverse effect on the biota [3]. The rate of recovery of different components of terrestrial ecosystems after emission reduction is not the same and depends on many factors, including the decreasing of toxic load [4–12]. Therefore, information on the dynamics of pollutants entering the environment and their content in biota components is required for the study of the patterns of natural restoration of ecosystems [2].

Small mammals (mouse-like rodents and small insectivores) are traditionally considered as model objects of ecotoxicological studies due to their widespread occurrence, significant abundance, high fecundity, resident mode of life, and ease of collection [13, 14]. In the terrestrial ecosystems small mammals play different functional roles as both primary (phytophages) and secondary (zoophages) consumers. Their use allows characterizing the toxic load simultaneously at two levels: the concentrations of elements in the stomach content provides an integral assessment of the intake of pollutants with feed, and the data on accumulation in depot organs serve as its "marker" of the level of pollutants in the body [15].

Small insectivores, including shrews of the g. *Sorex* are of potential interest for long-term research. Small body size, high metabolic rate, and a food spectrum based on invertebrates that accumulate significant amounts of HMs make them more vulnerable to xenobiotics than other mammals [16–18]. Anthropogenic pollution leads to significant changes in shrews at different levels of organization, from coenotic and population to organismic and cellular levels [8, 16, 19–23]. Experimental [24, 25] and natural studies [16, 20, 26] established that shrews are capable of accumulating HMs in amounts significantly exceeding the levels that cause irreversible damage in the body of mouse-like rodents, up to lethal ones. However, actual data on the dynamics of HM content in the feed and body of shrews under conditions of chronic industrial pollution, as well as after a significant reduction in emissions from enterprises, are not known to us.

For the past 30 years, we have performed annual surveys of the population of small mammals in areas exposed to emissions from the Middle Ural Copper Smelter (MUCS). Previously [15], the results of the analysis of the long-term dynamics of the content of HMs (Cu, Zn, Cd, Pb) in the food spectrum and body of *Myodes (Clethrionomys) glareolus* were obtained. This widespread, ecologically plastic species is a typical phytophage. A multiple (by 50 times over 25 years) reduction in industrial emissions of MUCS did not lead to an equivalent decrease in HM concentrations either in the food or in the body of voles: in the zone of severe pollution, the Cd content doubled; in the background zone Pb concentrations have decreased by 1.7–2.5 times; in other cases, no directed changes were detected. This led to the conclusion about the key role of diets and the system of element-specific homeostatic barriers in the accumulation of HMs in the body of phytophages. It seems interesting to study such changes in representatives of another trophic level, zoophages, and try to answer the question whether the same patterns would be observed.

The purpose of this study was to analyze the long-term dynamics of HM (Cu, Zn, Cd, Pb) concentrations in the food and liver of shrews of the g. *Sorex* in the vicinity of a large copper smelter during periods of high emissions and after significant reduction. We tested two hypotheses: (1) the accumulation of HMs in the body of cohabiting shrew species is not the same and is determined by the specifics of their food spectrum; (2) reduction of emissions leads to a decrease in HM concentrations in the body of shrews.

MATERIALS AND METHODS

Source of emissions. The studies were carried out in the zone of influence of atmospheric emissions from the Middle Ural Copper Smelter (MUCS), located 50 km west of Yekaterinburg. The enterprise has been operating since 1940 and it is Russia's largest works producing crude copper and sulfuric acid. In the 1980s total emissions of MUCS (mainly gaseous compounds S, F and N, as well as dust particles with sorbed Cu, Pb, Zn, Cd, Fe, Hg, As) reached $150–225 \times 10^3$ t, which made the enterprise one of the main sources of industrial pollution in Russia. During the 1990s the volume of emissions decreased by 2 times, from 148×10^3 t in 1990 to 65×10^3 t in 1999; over the next 10 years, the decline was even more pronounced, from 63×10^3 t in 2000 to 22×10^3 t in 2009. Since 2010, after the reconstruction of the plant, gross emissions do not exceed $3–5 \times 10^3$ t/year. Over the past 30 years, the gross emissions of the plant have been reduced by more than 50 times, with SO_2 concentration decreasing the most (80 times), Cu (3000 times), Zn (15 times), and Pb (8.5 times) [27].

Study area. Key plots were located in spruce-fir forests at different distances (from 1 to 34 km) to the west of the MUCS (against the prevailing wind direction). They are grouped into three zones that differ in the degree of damage to ecosystems: background, buffer, and impact. The severity of the processes of degradation of forest phytocenoses in different load zones was not the same: the background zone (20–34 km from the MUCS) was characterized by a relatively undisturbed state, due to the action of only regional pollutant fallout; in the buffer zone (4–10 km) structural changes in ecosystems were caused by the action of local pollution; in the impact zone (1–3 km), the structure of ecosystems was fundamentally different from the background state; extreme variants of technogenic digression of communities were presented here [28, 29]. A detailed description of the study area, key plots, and methods for collecting material were published earlier [8].

The studies were performed over 30 years, which covered periods of high (1990–1997, period I), reduced (1998–2009, period II) and almost ceased (2010–2019, period III) emissions. Most of the material was collected at seven key plots located in the impact (heavy pollution; 1, 1.5, and 2 km from the MUCS), buffer (moderate pollution, 4 and 6 km), and background (pollution at the regional background level, 20 and 30 km) zones. During period III, the population of small mammals was additionally studied at 4 more key plots in the impact (3 km), buffer (7 and 10 km), and background (34 km) zones.

Collection of material. Animals were caught annually (in May, July, and September) in all plots simultaneously using wooden snap traps arranged in lines at stationary positions [30]. Wooden snap-traps with a hook were installed on stationary marked lines (25 traps every 5–7 m for 3–5 days with a single daily check); two to four lines were placed at each plot during each trapping round. When analyzing the long-term dynamics of HM accumulation in the food and body of shrews, the use of stationary trapping points allows significantly reducing the effect of spatial heterogeneity of conditions, especially expressed in contaminated areas (including the mosaic nature of pollution fields, the composition and abundance of food resources, and the quality of microhabitats for different species) [8, 27, 28]. Taking into account the limitations of this method for counting shrews, additional trapping of animals was regularly carried out in adjacent areas using other schemes and means (live traps, cones with fences, and Barber pitfall traps). This allowed us to obtain more complete data on the species composition of shrews of the g. *Sorex* in different zones of pollution and collect more representative material. During the research, more than 100000 trap–days were conducted, 952 individuals were included in the analysis. The species identification of shrews was carried out based on exterior features (body weight, length of the body, tail, and hindfoot), condylobasal length of the skull, and features of the dentition [31, 32]. Latin names and sequence of species in Table 1 correspond to the summary "Mammals Species of the World" [33].

Research objects. Shrews of the g. *Sorex* are present in all study areas [8]. The share of the group in the population of small mammals in the background and buffer zones averaged 12–13%, and the share in the impact zone was about 17%. Only in some years, in some areas, the contribution of shrews reached 40–60% of the total number of small mammals [34]. Four species were represented in all zones: *Sorex araneus* Linnaeus, 1758, *S. caecutiens* Laxmann, 1788, *S. minutus* Linnaeus, 1766, and *S. isodon* Turov, 1924. The first two species are common in all zones and are regularly recorded in catches. Although *S. minutus* is present in all zones, due to its small size it is rarely caught with snap- and live traps, and the peculiarities of other methods of accounting did not allow the use of samples for chemical analysis. *S. isodon* is the most numerous in the background zone, and occurs sporadically in the contaminated areas.

For shrews of the g. *Sorex,* characterized by high metabolic activity and a significant need for food, the daily dietary intake in most species is from 100 to 250% of body weight [35–39]. The food spectrum is wide and consists mainly of insects, arachnids and earthworms (in large species) [40, 41]. The specificity of the food spectrum of cohabiting species is manifested in the predominant consumption of different groups of invertebrates, although the lists of the main victims almost completely overlap. [42]. This is achieved by separating habitat and foraging horizons: for *S. araneus* and *S.isodon*—soil and (partially) ground layers, for *S. caecutiens* and *S. minutus*—ground-litter layer [43, 44]. An important factor determining the choice of food objects is the correspondence between the size of prey and the features of the jaw apparatus [42]. The basis of the food spectrum of *S. araneus* and *S.isodon* are earthworms (more than 60%) and Diptera larvae, and the main food spectrum of *S. caecutiens* and *S. minutus* consists of arachnids, ground beetles (Carabidae, Elateridae, Staphylinidae), Diptera larvae, and bugs [40–42]. At the same time, trophic relationships with soil invertebrates became weaker in the series *S. isodon* > *S. araneus* > *S. caecutiens* > *S. minutus* [43].

Chemical analysis of samples. During the entire observation period, samples for chemical analysis were collected according to a single protocol, which was created at the beginning of the study and then steadily followed. The liver and stomach content (for each individual) were dried on glass slides at a temperature of 75°C, packed in sealed plastic bags, and stored in a cool dry place until the start of analytical investigation. The samples were weighed using a KERN-770 analytical balance (accuracy 0.0001 g), placed in Teflon vessels with 7 mL of 65% HNO_3 (ultra high purity) and 1 mL deionized H_2O, incubated for 30 min, and then incinerated in MWS-2 microwave oven (Berghof, Germany). After ashing, the sample volume was adjusted to 10 mL of deionized H_2O. The concentrations of elements (μg/g dry weight) were measured by atomic absorption using an AAS6 Vario spectrometer (Analitik Jena, Germany) with flame (Cu, Zn) and electrothermal (Cd, Pb) atomization options.

The quality of measurements was evaluated according to the international standard sample CRM 185R (bovine liver). The extraction was, %: Cu, 93.2; Zn, 99.8; Cd, 114.2; Pb, 94.4; limit of detection, μg/mL: for Cu, 0.013; for Zn, 0.005; for Cd, 0.001; and for Pb, 0.013. In the case where the concentration of the element was below the detection limit, a value equal to half the detection limit was used for statistical analysis. 939 liver samples, 91 samples of stomach content were analyzed.

Sample preparation and determination of HM concentrations were carried out during 2015–2017 and 2019 according to standard protocols on the same equipment in the Laboratory of Ecotoxicology of Populations and Communities, Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences. The exception was a small series (about 90 samples of *Sorex araneus* liver, collected in 1990–1995) analyzed in 1995 using similar equipment (AAS-3). Blind retesting of samples was not performed due to small volumes of starting material.

Statistical analysis. The distribution of element concentrations in most cases was close to lognormal. Descriptive statistics were calculated for HM concentrations (geometric mean, range, and coefficient of variation). An individual was considered a statistical unit. For the determination of differences in HM concentrations between key plots and periods, as well as between species, a two-way ANOVA was used (the values were preliminarily logarithmic, Log_{10}). Multiple comparisons were performed using Tukey's test. Linear regression was used to analyze the dynamics of HM concentrations, including the mixed effects variant. In statistical tests, differences were considered significant for $p < 0.05$. The calculations were performed using the JMP v.11 package [45], analysis of models with mixed effects was done in the R v.4.1 environment [46] using the lme4 v. 1.1 package [47]. The source data and code are hosted on the Zenodo repository (https://doi.org/10.5281/zenodo.6560130).

RESULTS

HM concentration in the body and food spectrum of shrews. Data on the concentrations of HMs in the liver and stomach content of four species of the g. *Sorex*, summarized for the entire period of observations

Table 1. The concentration of HMs in the stomach content and liver of shrews of the g. *Sorex,* μg/g dry weight

Zone	Stomach content					Liver				
	n	Cu	Zn	Cd	Pb	*n*	Cu	Zn	Cd	Pb
Sorex Araneus										
Background	30	16.7 [80.4] (2.9–86.6)	160.2 [42.0] (60.4–359.8)	7.6 [63.9] (0.6–24.8)	4.7 [91.5] (na–36.2)	185	19.3 [33.0] (6.6–46.5)	92.3 [36.2] (34.2–343.6)	15.2 [65.7] (0.03–65.3)	1.5 [97.3] (na–14.0)
Buffer	7	39.8 [86.9] (12.8–136.9)	310.1 [20.0] (220.5–393.9)	24.3 [52.8] (9.8–49.4)	28.8 [91.6] (14.5–97.6)	206	27.9 [44.8] (8.7–80.3)	100.1 [26.8] (27.0–210.8)	37.5 [66.3] (4.7–165.8)	3.7 [152.0] (na–94.2)
Impact	5	54.3 [65.2] (19.0–131.5)	142.1 [29.9] (88.3–209.4)	3.2 [85.7] (1.5–10.2)	15.2 [88.4] (4.7–50.2)	23	28.0 [39.1] (7.8–47.5)	105.0 [43.9] (32.9–237.4)	26.0 [46.5] (2.7–59.4)	5.2 [71.2] (0.4–19.2)
Sorex caecutiens										
Background	10	19.2 [60.7] (8.2–45.4)	148.3 [66.1] (48.7–472.4)	1.7 [100.8] (0.2–9.9)	5.5 [101.5] (1.2–29.5)	126	18.4 [23.0] (11.2–36.9)	97.8 [29.2] (49.9–272.1)	6.6 [89.4] (0.8–44.8)	2.8 [141.3] (na–68.8)
Buffer	10	36.0 [60.8] (19.4–98.2)	157.4 [19.3] (104.6–211.7)	3.3 [57.7] (0.4–7.7)	17.9 [96.0] (4.0–87.7)	149	19.0 [35.2] (10.4–74.7)	94.6 [24.8] (24.2–229.6)	11.9 [85.8] (0.1–97.1)	4.9 [116.0] (na–81.1)
Impact	20	46.0 [44.3] (17.2–107.5)	158.9 [37.3] (94.6–330.8)	3.1 [92.7] (0.2–19.8)	11.9 [125.5] (1.3–92.8)	155	22.5 [30.0] (7.8–55.4)	97.2 [39.1] (32.9–336.2)	11.2 [72.5] (1.3–61.0)	6.8 [116.9] (0.3–103.0)
Sorex isodon										
Background	7	14.7 [72.2] (9.7–44.5)	215.4 [51.7] (116.6–492.3)	6.5 [53.9] (1.0–14.8)	6.0 [73.4] (1.2–29.5)	67	20.4 [33.3] (11.8–45.4)	97.4 [30.2] (42.0–216.0)	16.2 [80.0] (1.70–77.3)	1.8 [197.8] (0.04–56.0)
Buffer	0	–	–	–	–	10	27.1 [35.8] (16.1–46.9)	95.4 [20.6] (74.1–131.2)	26.4 [76.3] (6.2–84.2)	3.2 [113.8] (0.6–21.0)
Impact	0	–	–	–	–	1	20.5	91.6	37.3	0.4
Sorex minutes										
Background	1	34.8	60.2	1.8	2.9	7	20.2 [30.4] (13.6–32.4)	119.2 [29.8] (67.6–168.2)	8.7 [71.6] (3.2–25.7)	5.4 [59.4] (0.6–13.2)
Buffer		–	–	–	–	4	20.6 [10.2] (19.4–23.8)	113.1 [18.0] (83.7–127.2)	5.9 [69.8] (3.0–12.8)	3.4 [62.9] (1.4–7.5)
Impact	1	41.1	195.6	6.4	9.2	6	18.7 [32.6] (11.8–29.6)	84.7 [16.2] (61.7–100.9)	8.5 [71.5] (3.1–20.9)	5.6 [60.1] (1.5–12.2)

The geometric mean value is shown, the coefficient of variation (%) is shown in square brackets, the minimum and maximum values are shown in in round brackets, dash—no data, na—concentration of the element below the detection limit, accountable item was individual.

Table 2. Results of ANOVA analysis of differences in HM concentrations in the liver of *S. araneus* and *S. caecutiens* between load zones and periods (*F*-criterion, the achieved level of significance is shown in parentheses, *n* is the number of samples)

Source of variability	df	Cu	Zn	Cd	Pb
Sorex araneus					
Zone	2	41.7 (<0.0001)	12.4 (<0.0001)	61.9 (<0.0001)	40.5 (<0.0001)
Period	2	10.3 (<0.0001)	0.7 (0.484)	0.1 (0.881)	8.9 (0.0002)
Zone×period	4	3.5 (0.008)	2.6 (0.038)	2.3 (0.056)	3.5 (0.009)
n		410	410	410	341
Sorex caecutiens					
Zone	2	10.6 (<0.0001)	0.5 (0.615)	8.8 (0.0002)	18.5 (<0.0001)
Period	2	39.5 (<0.0001)	7.2 (0.001)	11.4 (<0.0001)	24.1 (<0.0001)
Zone×period	4	2.4 (0.048)	1.6 (0.167)	3.2 (0.014)	1.6 (0.184)
n		430	430	430	405

(1990–2019), are shown in Table 1. Within the zone, the elemental composition of the liver and stomach content was the closest in pairs of species characterized by a similar food spectrum and foraging layer: *S. araneus*—*S. isodon* and *S. caecutiens*—*S. minutus*. Due to lack of data on *S. isodon* and *S. minutus* for further comparison, the results for only *S. araneus* and *S. caecutiens* were used, suggesting that they can also characterize another species from “their” pair. Differences between these species were statistically significant for all HMs ($F = 4.6–144.5$, $p = 0.032–0.0001$), therefore in the further study the species were considered separately.

HM concentration in the liver of *S. araneus*. The accumulation of HMs in the liver was dependent on the level of pollution; for Cu and Pb, the period and interaction of factors were also statistically significant (Table 2). The minimum levels of HM accumulation were recorded in the background territory, the maximum concentrations of Cu, Zn, and Pb were in the impact zone, and the maximum concentration of Cd was revealed in the buffer zone (see Table 1).

In the years of high emissions (period I), the content of Pb in the contaminated sites exceeded the background values by 2–3.2 times, Cd, by 2.7 times, Cu, by 1.6–2.8 times, and Zn, by 1.2–1.5 times (Table 3). After the almost complete cessation of emissions (period III), the differences between the background and polluted areas remained at the same level for Cu and Zn, while for Cd and Pb they increased by 4 and 5–6.5 times. The increase in differences in Cd was due to a twofold increase in the concentration in the liver of animals in the buffer zone at constant background values. For Pb, on the contrary, such changes were associated with a threefold decrease in the concentration in the background zone with a less significant decrease (up to 30–70%) in the vicinity of the MUCS (Fig. 1).

Throughout the observation period, HM concentrations in the liver were characterized by high variability: in all zones, the minimum variation was recorded for essential elements (Cu, Zn), while for toxic elements (Cd, Pb) the coefficients of variation were 2–4 times higher (see Table 3).

HM concentration in the liver of *S. caecutiens*. The accumulation of HMs (with the exception of Zn) depended on the level of pollution and the period of observation; the interaction of these factors for Cu and Cd was also significant (see Table 2). The minimum HM levels were recorded in the background zone, the maximum concentrations of Cu and Pb were recorded in the impact zone, and the maximum concentration of Cd was observed in the buffer zone (see Table 1).

During period I, the Pb content in the impact zone exceeded the background values by 1.5 times, Cu and Zn contents were higher than the background values by 10–15%, while the Cd concentrations, on the contrary, were 1.2–1.4 times lower than the background values. During period III, the differences between the background and contaminated areas for Cu, Cd, and Pb became more pronounced, while for Zn they remained at the same level (Table 4). Strengthening differences in the content of elements was due to the presence of clear trends. In the impact zone, the Cu content in the animal liver increased at a rate of 1% per year, which over 30 years of observations led to a 30% increase compared to the initial values (Fig. 2). The increase in differences in Cd (by a factor of 2.3–2.7) was associated primarily with directional changes in the background zone, where, as a result of a gradual decrease (by 3% per year), the concentration of the element decreased by a factor of 4. In contaminated areas, the result of a directed decrease in Cd level (by 1.2–1.3% per year) over the same time interval was a general decrease in the concentration of the element by about 1.5 times. For Pb, such changes were even more pronounced: in the background zone, a decrease

Table 3. The concentration of heavy metals in the liver of *Sorex araneus* in the gradient of environmental pollution during periods of high (I), reduced (II) and almost ceased emissions (III), μg/g dry weight

Zone	Period	*n*	Cu		Zn		Cd		Pb	
Back-ground	I	65	17.6 [37.4] (6.6–45.1)	b	79.4 [34.2] (34.2–154.6)	b	12.5 [66.2] (0.03–47.2)	a	1.7 [71.3] (0.06–7.9)	a
	II	88	19.8 [32.2] (7.2–46.5)	ab	101.5 [21.4] (42.6–166.4)	a	18.0 [66.1] (1.3–65.3)	a	2.1 [89.8] (0.3–12.3)	a
	III	32	21.8 [24.1] (13.8–35.8)	a	87.2 [14.0] (72.5–122.4)	b	14.4 [42.4] (1.2–31.4)	a	0.5 [117.5] (0.01–6.3)	b
Buffer	I	146	27.4 [41.4] (8.7–78.2)	b	95.5 [28.3] (27.0–210.8)	b	33.7 [59.8] (8.8–104.9)	b	3.5 [54.5] (0.4–11.8)	a
	II	35	23.2 [20.7] (16.2–38.5)	b	114.2 [15.9] (72.7–164.4)	a	42.9 [48.7] (9.1–101.6)	ab	4.5 [75.4] (0.7–20.7)	a
	III	25	39.7 [44.3] (15.6–80.3)	a	109.4 [27.9] (70.1–193.8)	ab	58.4 [67.4] (4.7–165.8)	a	2.7 [97.8] (0.1–21.7)	a
Impact	I	10	49.7 [25.1] (7.8–44.1)	a	117.6 [46.8] (32.9–237.0)	a	33.8 [31.2] (16.9–51.9)	a	5.5 [12.2] (4.9–6.2)	a
	II	6	26.3 [32.2] (14.2–39.3)	a	94.2 [16.8] (65.7–111.9)	a	22.8 [46.2] (11.0–40.0)	a	9.1 [58.2] (3.8–19.1)	a
	III	7	34.8 [28.2] (17.6–28.2)	a	98.0 [43.5] (65.8–169.1)	a	20.2 [68.0] (2.7–59.4)	a	3.2 [80.1] (0.4–10.9)	a

The geometric mean is shown here and in Table 4, minimum and maximum values are shown in parentheses, coefficient of variation (%) is shown in square brackets, *n* is the number of analyzed samples. The same letters mean the absence of significant differences within the zone for each element (according to Tukey's test).

Table 4. The concentration of heavy metals in the liver of *Sorex caecutiens* in the gradient of environmental pollution during periods of high (I), reduced (II) and almost ceased emissions (III), μg/g dry weight

Zone	Period	*n*	Cu		Zn		Cd		Pb	
Back-ground	I	10	17.1 [18.7] (13.1–25.2)	b	76.3 [25.1] (49.9–111.2)	b	16.4 [50.5] (4.3–27.8)	a	4.4 [72.3] (1.3–12.6)	a
	II	96	17.9 [21.9] (11.2–36.9)	b	98.3 [22.0] (62.4–159.2)	a	6.6 [86.8] (0.8–33.2)	b	3.3 [102.2] (0.2–31.0)	a
	III	20	21.6 [22.1] (12.6–29.8)	a	99.7 [34.6] (53.8–202.3)	a	3.9 [56.0] (1.3–10.6)	c	0.9 [125.1] (0.05–8.85)	b
Buffer	I	34	18.3 [27.0] (10.4–36.9)	b	89.4 [17.4] (52.9–139.8)	a	13.5 [58.4] (5.0–39.1)	a	3.3 [114.6] (0.9–29.4)	b
	II	96	18.3 [21.9] (12.2–45.1)	b	95.8 [24.2] (24.2–177.0)	a	11.3 [81.8] (0.1–80.0)	a	5.9 [77.7] (0.1–32.4)	a
	III	19	22.9 [23.3] (12.3–32.6)	a	92.8 [27.2] (70.3–159.4)	a	8.9 [65.8] (2.3–26.8)	b	2.6 [140.5] (0.05–37.4)	b
Impact	I	37	18.7 [27.1] (7.8–36.1)	b	88.5 [20.4] (32.9–137.0)	a	12.0 [64.6] (3.1–36.9)	a	6.5 [47.9] (1.1–20.0)	a
	II	54	19.7 [21.3] (12.7–40.2)	b	102.3 [30.3] (65.2–199.2)	a	11.6 [68.9] (1.3–45.3)	a	10.3 [70.9] (1.2–50.1)	a
	III	64	27.6 [19.3] (16.8–37.6)	a	93.5 [41.3] (63.6–222.9)	a	10.4 [63.4] (1.3–36.2)	b	4.4 [118.6] (0.3–46.0)	a

in concentration at a rate of 4% per year led to a fivefold drop compared to the initial values, while in the impact zone, the decrease was less significant (by 1% per year) and amounted to 1.5 times (see Table 4).

HM concentrations in the liver varied significantly throughout the entire observation period and, as a rule, exceeded the coefficient of variation values for *S. araneus*. In all zones, the minimum variation was

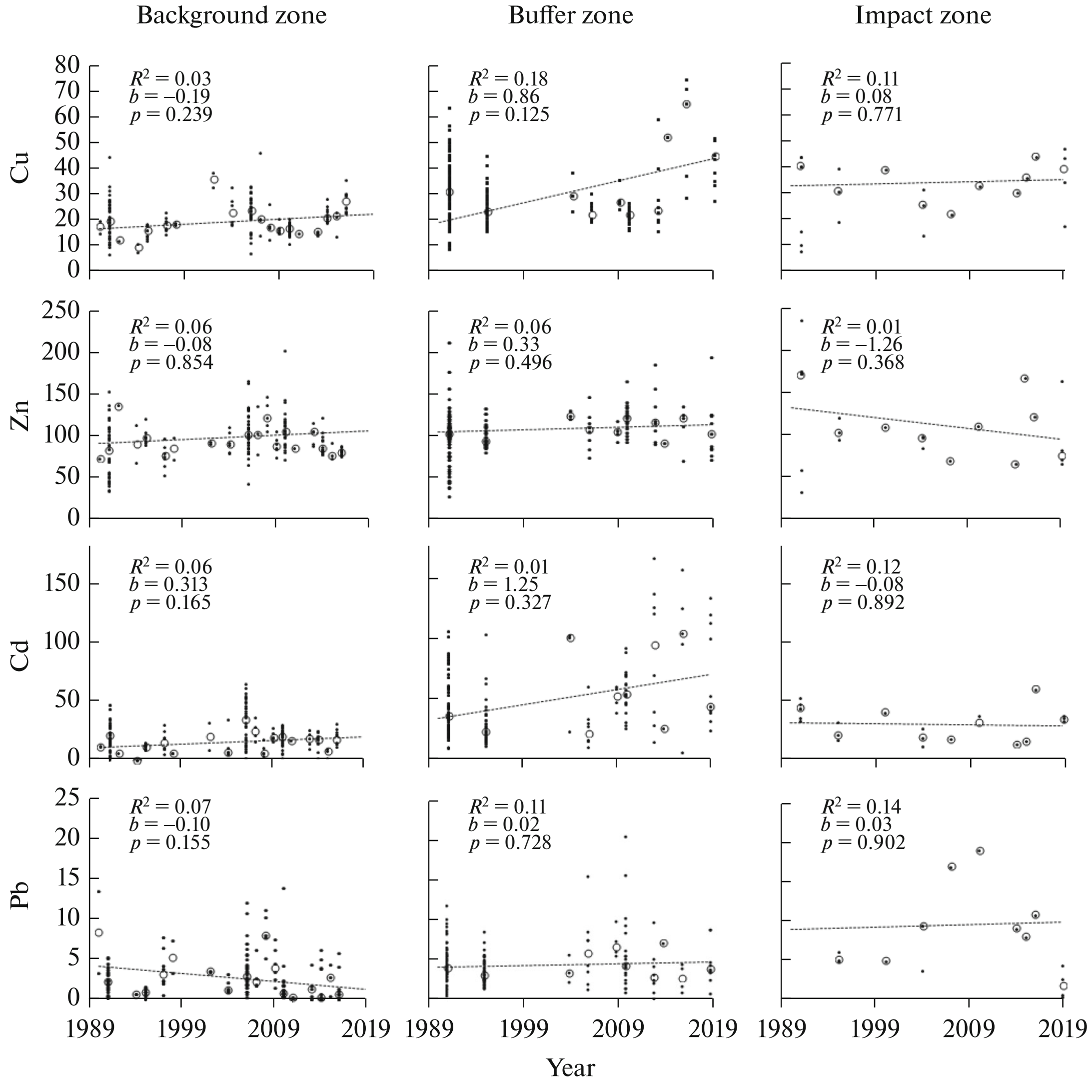

Fig. 1. Long-term dynamics of HM concentration (μg/g dry weight) in the liver of *Sorex araneus* in different zones of pollution. The results of linear regression were calculated based on average annual values (medians): here and in Fig. 2 points are individual values; circles—mean value for the year; R^2_{adj}—coefficient of determination; *b*—regression coefficient; *p*—achieved level of significance; lines are linear regression trends.

recorded for essential elements (Cu, Zn), while for toxic elements (Cd, Pb), the coefficient of variation values were 2–6 times higher (see Table 4).

Changes in HM concentrations in the liver of shrews in the pollution gradient. Since the accumulation of HMs in the liver depended on the period of observation, we used data from only one of them (period III), but for the maximum number of key plots (n = 11), which most fully cover the entire pollution gradient (from 1 to 34 km). When approaching the MUCS, a monotonous increase in the concentrations of all HMs was observed in the liver of both shrew species (except for Zn in *S. caecutiens*). At the same time, Pb concentrations were higher in *S. caecutiens*, while Cu and Cd, on the contrary, accumulated more intensively in *S. araneus,* in some areas, interspecies differences reached 8 times (Fig. 3).

Changes in HM concentrations in the stomach content of shrews in the pollution gradient. The data of only one period (III) obtained at 11 key plots were analyzed. When approaching the MUCS, the concentrations of Cu in both species increased monotonously, and the rates of changes were higher than in the liver: for *S. araneus* by 2.6 times, for *S. caecutiens* by 3.4 times (see Fig. 3). Cd concentration in feed of *S. caecutiens* monotonously increased when approaching the plant, while Cd concentration in feed of *S. araneus* changed non-linearly with a maximum in moderately polluted areas (6–10 km from the plant) at similar background and impact values (see Fig. 3). A comparison of the two models showed that the non-linear model better

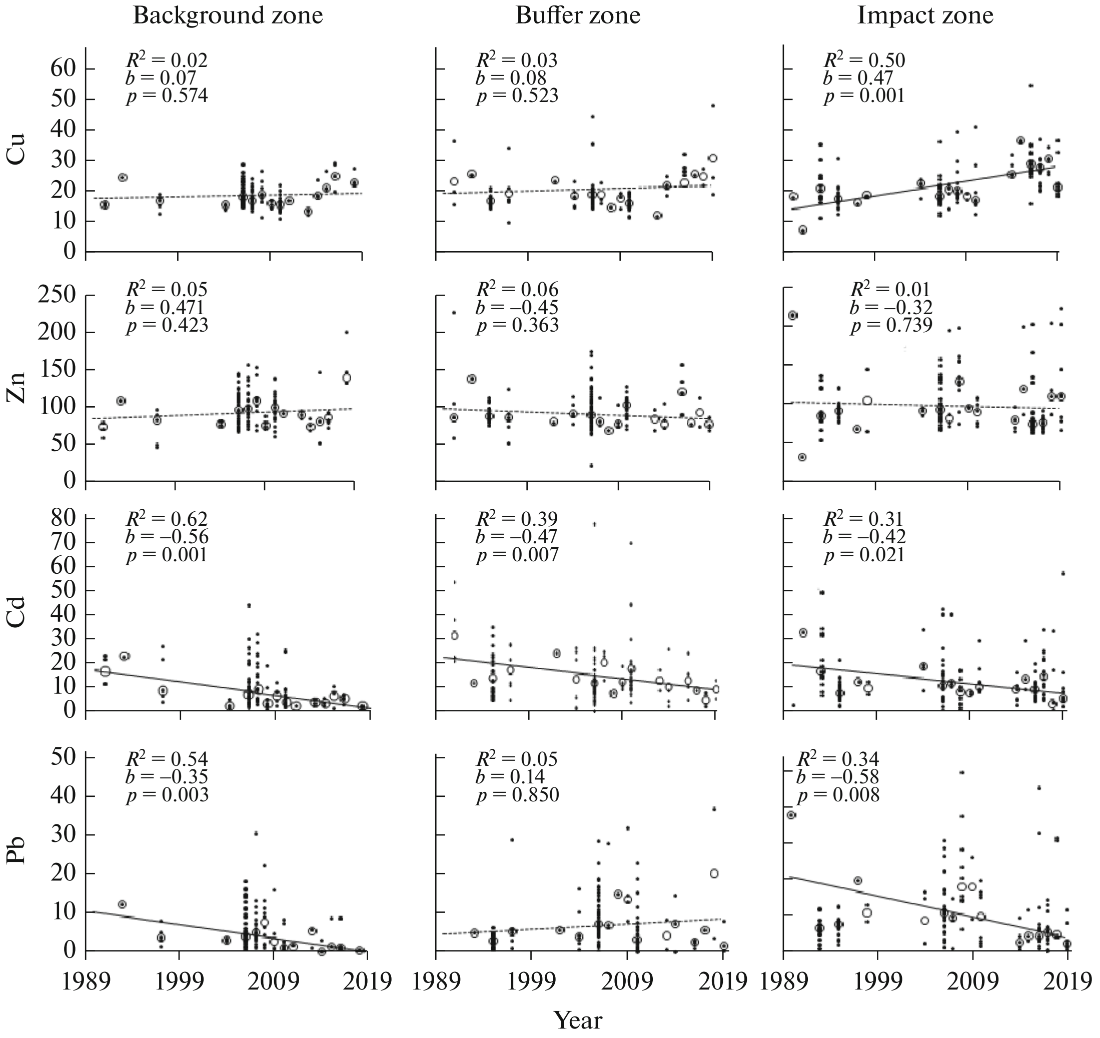

Fig. 2. Long-term dynamics of HM concentration (μg/g dry weight) in the liver of *Sorex caecutiens* in different zones of pollution. Designations see in Fig. 1.

described changes in Cd accumulation levels in feed of *S. araneus*: the coefficient of determination was higher ($R^2 = 0.24$ versus $R^2 = 0.03$ for the linear model), while AIC was lower (50.28 versus 67.99, respectively). There were no directional changes for other elements. Accumulation levels of Cu, Zn, and Cd in the stomach content of *S. araneus* were generally higher than in the stomach content of *S. caecutiens* (see Fig. 3).

Relationship between HM concentrations in feed and liver. The content of HMs in the feed and liver of animals correlated for all elements, except for Zn (Fig. 4): for a generalized sample for all plots ($n = 79$) paired correlation coefficients were significant ($r = 0.31$–0.49, $p < 0.01$). The use of samples for individual species showed that in *S. araneus* the relationship was significant for Cu, Cd, and Pb ($r = 0.37$–0.57, $n = 35$, $p < 0.05$), in *S. caecutiens* the relationship was significant only for Cu and Cd ($r = 0.29$–0.37, $n = 44$, $p < 0.05$).

The simultaneous influence of the factors described above on the accumulation of HMs in the feed and body of shrews was additionally analyzed using general linear models with mixed effects. Data on two species *S. araneus* and *S. caecutiens* were used for the assessment. The area and period of the study were considered as fixed effects, and the species with embedded factors “element” and “sample type” (liver/stomach content) were considered as random effects (AIC = 3029, $R^2 = 0.666$). The use of more detailed quantitative predictors (year, key plots) instead of categorical predictors (period, zone) did not improve the quality of the model (AIC = 3059, $R^2 = 0.663$). In the pollution gradient, the increase in HM concentrations was more significant from the background key plots to the buffer areas; in the vicinity of the MUCS, the accession rates decreased (Table 5). The long-term dynamics of HM concentrations was

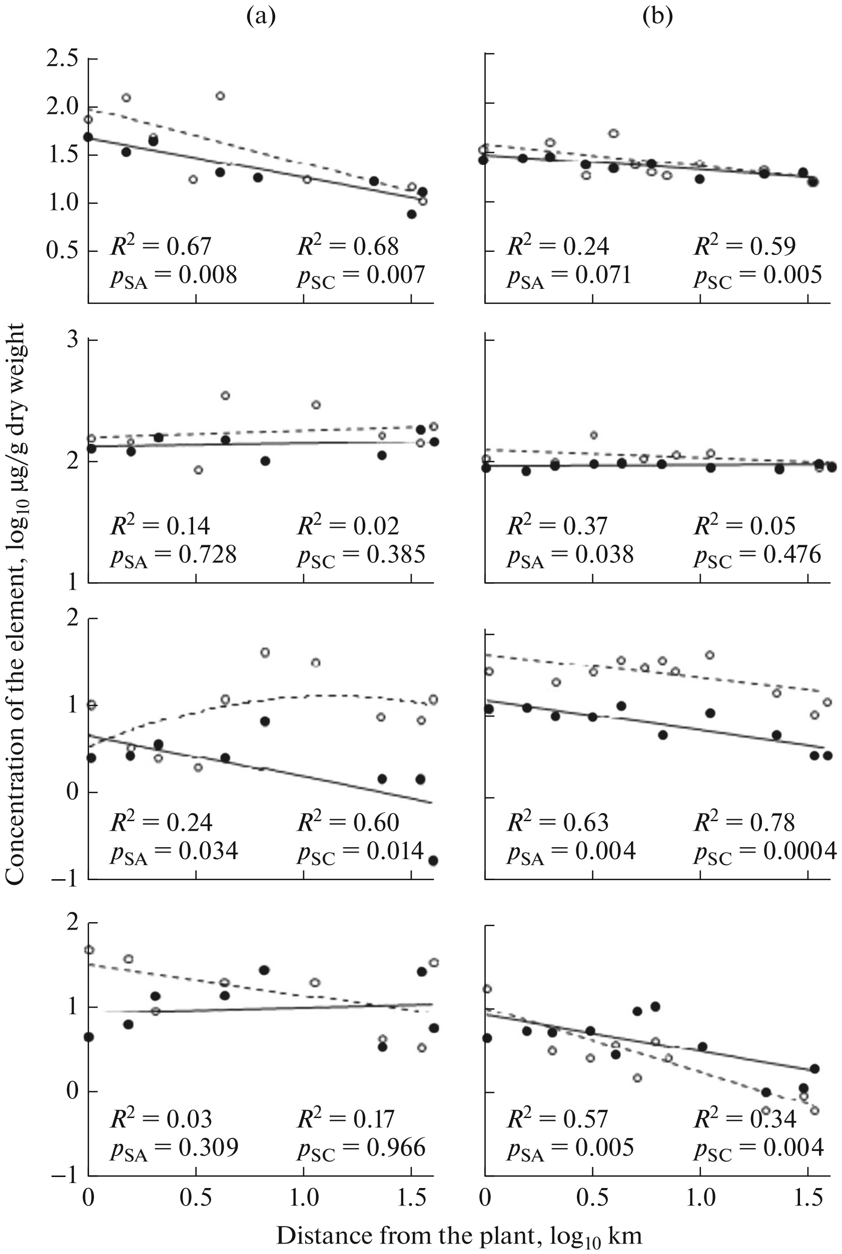

Fig. 3. Change in HM concentrations (μg/g dry weight, $\log_{10}$) in the stomach content (a) and liver (b) of *S. araneus* (SA) and *S. caecutiens* (SC) at distance from the MUCS (km, $\log_{10}$). The results of linear regression were calculated based on the average values for the key plot (medians). Markers are plot averages, lines—linear regression trends, R^2_{adj}—coefficient of determination; *b*—the regression coefficient, *p*—achieved level of significance. Light markers, dotted lines indicate *S. araneus;* dark markers, solid lines indicate for *S. caecutiens*.

characterized by a gradual (but weak) decrease in the body of shrews. Analysis of random effects showed that the food spectrum of both shrew species contained more HMs than the liver, while differences in the accumulation levels between samples within the species were more pronounced in *S. araneus*. In addition, the concentrations of HMs in the liver of *S. araneus*, usually exceeded the corresponding values for *S. caecutiens*.

DISCUSSION

Species specificity of HM accumulation. The main data on the accumulation of HMs in the body of small insectivores under conditions of anthropogenic pollution refer to typical European species—*S. araneus* and *S. minutus* [16, 20, 21, 47]. Information on other (Asian) species—*Sorex caecutiens* and *Sorex isodon* was fragmented. Since habitat horizons and spectra of food objects of *S. minutus* and *S. caecutiens* are close

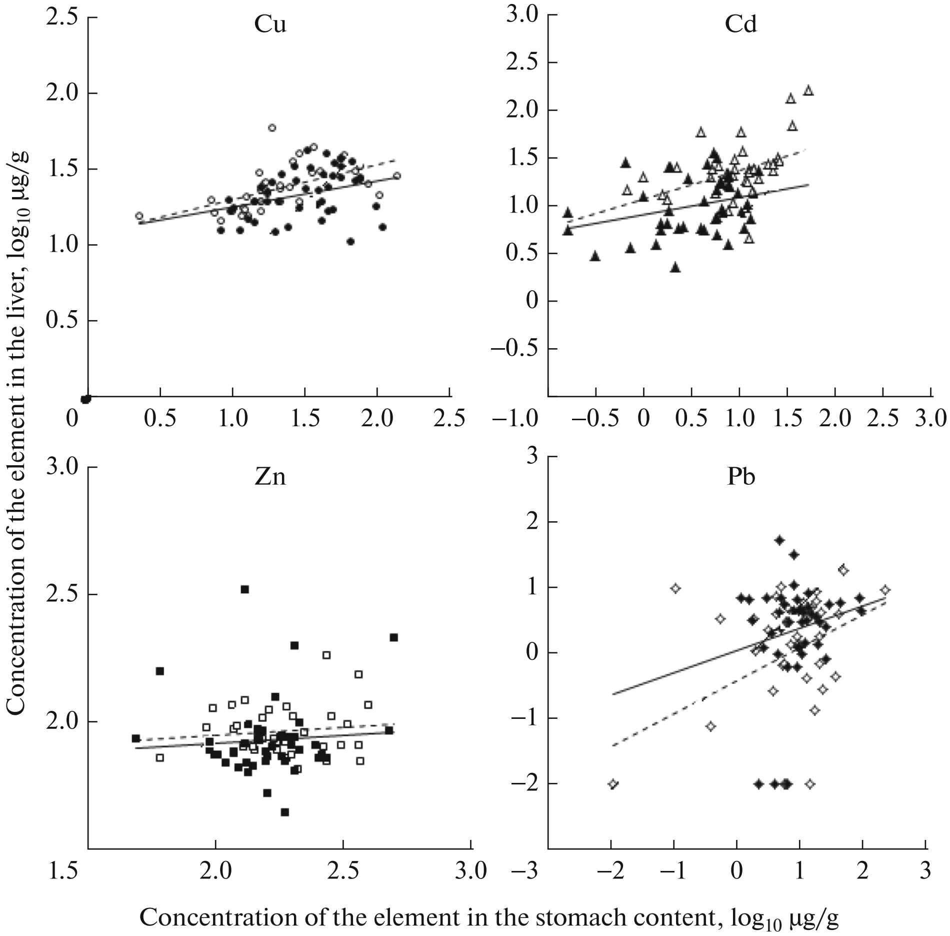

Fig. 4. Dependence of HM accumulation in the liver (µg/g, $\log_{10}$) of shrews of the g. *Sorex* on their concentrations in the stomach content (µg/g, $\log_{10}$). Markers are individual values; lines are linear regression trends. Light markers, dotted lines indicate for *S. araneus*, dark markers, solid lines indicate *S. caecutiens.*

[42, 44], it is possible to use for comparison the data obtained by other researchers for *S. minutus*.

Usually, interspecific differences in the content of HMs in the body of shrews of the g. *Sorex* are insignificant. For example, the concentrations of Cu, Zn, Cd, Pb, and Cr in the liver of *S. araneus* and *S. minutus* from the vicinity of the copper-nickel plant (Harjavalta, Finland) did not differ significantly, although a higher amount of Zn accumulated in the kidneys of *S. araneus* [16]. In the area of action of the lead-zinc plant (England), there were no interspecies differences in the accumulation of HMs in the liver, while the kidneys of *S. araneus* contained more Cd [47]. In anthropogenic biotopes with a high regional level of pollution (Northern Bohemia, Czech Republic), Cd concentrations in the liver of *S. araneus* was twice higher than the levels of *S. minutus*, whereas Pb accumulated 7 times more intensively in the liver of *S. minutus* [20]. Other authors have recorded elevated levels of Cd, Pb, and Zn in the liver of *S. araneus* compared with *S. minutus* [21]. Interspecies differences in HM accumulation were usually associated by the cited authors with different nutritional strategies: food spectrum of *S. araneus* consists of insects actively moving on the soil surface, as well as earthworms, the digestive tract of which contains particles of contaminated soil, while *S. minutus* feeds on litter-dwelling invertebrates, but not worms.

The results of our study indicate the presence of specificity in the accumulation of HMs in the organisms of different species. Throughout the pollution gradient, Cu, Zn, and Cd accumulated more intensively in the liver of *S. araneus,* while Pb accumulated more intensively in *S. caecutiens* (see Fig. 3). For the other two species *S.isodon* and *S. minutus* the data were limited to small samples, but even limited data provided an idea of the levels of accumulation of these elements (see Table 1). As expected, similar concentrations of HMs in the food spectrum and liver were noted for pairs of species that were most similar in terms of food spectra and foraging horizons (*S. isodon*–*S. araneus* and *S. minutus*–*S. caecutiens*).

Similarly with that discussed above, we believe that the interspecies differences were mainly due to the

Table 5. Results of analysis of differences in HM concentrations in the liver and stomach content of *S. araneus* and *S. caecutiens* according to the analysis of general linear models with mixed effects

Source of variability	Estimate	Std. error[1]/Std.dev.[2]	*t*-value
Fixed effects			
Background zone	0.197	0.014[1]	13.928
Impact zone	0.215	0.017[1]	12.361
Period	–0.016	0.009[1]	–1.831
Random effects			
Element	0.341	0.584[2]	
LgCu	0.074		
LgZn	0.735		
LgCd	–0.129		
LgPb	–0.680		
Sample type based on species	0.003	0.059[2]	
Stomach content of *S. araneus*	0.064		
Stomach content of *S. caecutiens*	0.118		
Liver of *S. araneus*	–0.033		
Liver of *S. caecutiens*	–0.042		
Species specificity	0.005	0.068	
S. araneus	0.040		
S. caecutiens	–0.040		
Residual		0.133[2]	0.364

feeding habits of the compared species. In the background zone, soil invertebrates are diverse and abundant [6], interspecies differences in the feeding spectra of shrews are insignificant and are determined mainly by the foraging layer and the size of prey. Therefore, the concentrations of Cu, Zn and Pb in the feed of *S. araneus* and *S. caecutiens* were similar, and the content of Cd increased in the food spectrum of *S. araneus*, which included a significant number of earthworms.

In the zone of moderate pollution, the food composition characteristic of each species insignificantly differed from the background plots, while the levels of soil pollution [27], and, consequently, soil invertebrates, were higher here. This explains the sharp (2–6 times) increase in the concentrations of the majority of HMs in the feed of shrews compared to the background values (see Table 1). The results are in good agreement with direct estimates of HM accumulation in the body of earthworms and the contents of the mole stomach from the buffer zone (7 km from the MUCS), which showed an increase in concentrations of Cd and Pb by 4–7 times in the comparison with background values [48].

In the impact areas, the range of food objects of shrews was depleted as a result of cardinal rearrangements in the composition of soil invertebrates: some groups (Lumbricidae, Enchytraeidae, Diplopoda, Mollusca) disappeared, a number of others (Chilopoda, Carabidae, Staphylinidae, Arachnidae, Diptera larvae) decreased sharply, while the proportion of others (Elateridae larvae), on the contrary, increased significantly [5, 6]. Coleoptera (Elateridae, Staphylinidae, Carabidae) formed the basis of the food spectrum of both shrew species in the impact zone, characterized by a reduced accumulation of HMs [48]. As a result of a partial change in feed, some "purification" of the food spectrum of shrews in the impact zone occurred, which was expressed in a decrease in the concentrations of toxic elements (Cd and Pb) by 1.5–8 times compared with the buffer values. At the same time, the levels of accumulation of essential elements were maintained at the same level (Zn) or increased (Cu) (see Table 1). A similar effect of reduced (compared to the background zone) accumulation of toxic elements in the feed of shrews inhabiting areas in the immediate vicinity of the MUCS was noted earlier for *S. caecutiens* [19]. Thus, the species specificity of HM accumulation in the food spectrum of shrews inhabiting together in areas with different levels of pollution was probably associated with differences in the composition of diets.

The close relationship between the elemental composition of the liver and food objects was shown by the results of experiments on feeding invertebrates to different types of shrews [24, 25, 50]. For example, when eating earthworms with an increased content of Pb (by 2 times), Zn (by 3 times), and Cd (by 4 times), the concentrations of Pb and Cd in the liver of shrews increased by 1.6 and 2.4 times, respectively [51].

Our results show that, despite the high concentrations of Cu, Zn, and Pb in the feed of animals from contaminated areas, the toxic load of these elements on the body was insignificant due to the effective excretion of their excessive amounts through the gastrointestinal tract (see Fig. 4). For Cd, such a barrier is not so effective; therefore, its increased intake led to a significant increase in the concentration of the element in the liver of shrews (especially in *S. araneus* and *S.isodon*). Previously, similar features of HM accumulation in the liver were noted for small mammals of another trophic level, herbivorous bank voles inhabiting the same areas in the vicinity of the MUCS [15].

Thus, the initial hypothesis about the unequal accumulation of HMs in the body of cohabiting shrew species was confirmed. The food spectrum of animals played a key role in the accumulation of HMs in the liver of shrews.

Changes in HM concentrations in the pollution gradient. Most often the HM content in the body of shrews of *Sorex* and *Crocidura* genera, inhabiting the vicinity of metallurgical and mining enterprises, increased when approaching the pollution source [20, 22, 48], however, opposite cases have also been described. Thus, E. Pankakoski et al. [16], noted higher concentrations of Pb in the liver and kidneys of *S. araneus* from the background areas compared to the sites near iron works (Koverhar, Finland), although the upper soil horizons in the vicinity of the plant were heavily contaminated with HMs (especially Pb and Zn). The authors explained this discrepancy by the possible effect of soil acidity on the bioavailability of individual elements: the alkalization of sites in the vicinity of the plant (pH 7.6) compared to the background areas (pH 4.6) was associated with the influx of significant amounts of calcium-containing dust.

The results of our study showed that the minimum concentrations of all HMs in the liver and feed of four shrew species were noted in the background zone, while the maximum concentrations were observed in the impact areas (Cu, Zn, and Pb) or in the zone of moderate pollution (Cd). When approaching the plant (from 34 km to 1 km), the HM content changed in a similar way for *S. araneus* and *S. caecutiens*: concentrations of Cu, Cd, and Pb (in both species) and Zn (in *S. araneus*) increased monotonically. At the same time, the levels of HM accumulation in *S. araneus* were generally higher than in *S. caecutiens*.

Thus, the concentrations of HMs in the feed and liver of four species of shrews from the vicinity of the plant were significantly higher than in uncontaminated areas, which is in good agreement with the conclusions of other authors.

Long-term dynamics of the content of metals in the liver. Long-term studies of the dynamics of the elemental composition of the body of terrestrial vertebrates under conditions of reduced industrial emissions are still limited [2]. The main studies were carried out on small passerines in the vicinity of a nonferrous smelter in Finland [12] and Sweden [10, 11]. It was shown that even within the same species (*Ficedula hypoleuca*), the response to a significant (from 60 to 95%) reduction in emissions was ambiguous and depended on specific conditions: in some cases, the content of HMs in the body of nestlings rapidly decreased [12], in others, there was almost no decrease [11]. The authors associated such differences with the peculiarities of nutrition (a different range of food objects).

Such information on small mammals is fragmentary [52, 53]. The only detailed study of the long-term dynamics of the HM content in the food spectrum and body of the bank vole (*Myodes glareolus*) was performed in the area of the MUCS [15]. It turned out that a significant reduction of the emission of Cu, Zn, and Pb in the environment did not affect their content in the liver, which was associated with the presence of effective barriers in the body at the level of the gastrointestinal tract. Since Cd overcomes this barrier, its increased dietary intake resulted in an equivalent increase in the concentration of the element in the liver of impact animals even after reduction of emissions [15]. In addition, over 25 years of observations, the content of Pb in the feed of the bank vole in the background zone decreased significantly (by 3 times); as a result, the concentration of the element in the liver decreased twice [15].

The results of our study indicate that a multiple reduction in emissions did not lead to time-directed changes in the concentrations of HMs in the liver of *S. araneus*, while in *S. caecutiens* clear trends were recorded for Cu, Cd, and Pb. Over 30 years of observations, the concentrations of toxic elements in the liver have significantly decreased: Cd, in the impact zone (by 1.2 times), buffer zone (by 1.5 times), and background zone (by 4 times), Pb, in the impact zone (by 1.5 times) and background zone (by 5 times). Over the same period, the content of Cu in the liver of *S. caecutiens* from the impact areas increased by 1.5 times. The observed changes in the HM levels in the feed and organisms of small insectivores radically differ from the results of similar studies performed on the bank vole, which represents a different trophic level. A multiple reduction in MUCS emissions during 25–30 years of observations caused multidirectional changes in Cd concentrations in the liver of animals living in contaminated areas: they increased in phytophages, while they decreased in zoophages. The content of Pb gradually decreased in the liver of animals of both trophic levels, but the changes were more pronounced in zoophages.

Thus, the hypothesis of a decrease in HM concentrations in the body of shrews of the g. *Sorex* as a result of multiple reductions in emissions was only partially confirmed: clear temporal trends were identified only for *S. caecutiens*, in the liver of which the concentrations of Cd and Pb decreased over 30 years of observation.

Based on the data presented above, it can be expected that the recovery rates of natural populations of small mammals of different trophic levels inhabiting the territories in the vicinity of the MUCS will be different. Positive shifts in the population of small insectivores will be more pronounced, which in the near future will lead to an anticipatory increase in the abundance of shrews in the immediate vicinity of the plant compared to mouse-like rodents. First of all, this will be facilitated by changes in the communities of soil and terrestrial invertebrates in the impact and buffer zones. These assumptions are confirmed by the results of direct observations of the population of small mammals: over the past 20 years, the total abundance of shrews in the impact zone increased by 3 times, while the number of bank voles remained practically unchanged.

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CONFLICT OF INTEREST

Conflict of interest. The author declares that she has 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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