

# Long-Term Dynamics of Heavy Metal Concentrations in the Diet and Liver of the Herb Field Mouse (*Sylvaemus uralensis*) During Periods of High and Reduced Emissions from a Copper Smelter

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**Abstract**—The long-term (1990–2023) dynamics of accumulation of essential (Cu, Zn) and toxic (Cd, Pb) heavy metals (HMs) were analyzed in the stomach contents ( $n = 428$ ) and liver ( $n = 561$ ) of individuals of the Herb Field Mouse (*Sylvaemus uralensis*), living at different distances from a large non-ferrous metallurgy plant (Russia, Revda) during periods of high, reduced, and almost cessation of emissions. Testable hypotheses about directed spatiotemporal changes in HM concentrations in the diet and the body of *S. uralensis* were partially confirmed. In the pollution gradient, the concentrations of all elements in the stomach contents, as well as toxic elements in the liver, increased consistently as they approached the plant, reaching maximum values in the immediate vicinity, while the accumulation of essential elements in the liver of animals did not depend on the level of pollution in the area. Multiple (50-fold) reductions in plant emissions during the observation period did not result in an equivalent decrease in element concentrations in the analyzed substrates. In the vicinity of the plant, clear time trends were noted for Pb, the levels of which over 34 years of observations monotonically decreased by 2–3 times both in feed and in the liver of *S. uralensis*. In unpolluted areas, directional changes were noted only for Cd in the liver, the concentration of which decreased by half over the same period.

**Keywords:** industrial pollution, natural restoration, *Sylvaemus uralensis*, copper, zinc, cadmium, lead, diet, liver

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## INTRODUCTION

In recent decades, a reduction in industrial emissions has been recorded throughout Europe and North America, associated with the modernization/repurposing of production facilities or their complete shutdown [1–5]. However in the vicinity of such enterprises, pollutants continue to circulate in the environment for a long time, and without special measures, disturbed areas pose a serious threat to both human health and the ecosystem as a whole [6–9]. For example, near closed ore mines [10–13] and non-ferrous metallurgy enterprises, which significantly reduced emissions [2, 7, 14], the level of potentially toxic elements in the soil after decades still exceeds background values by tens and hundreds of times. Pollutants also accumulate in plant and animal objects, with concentrations often reaching or exceeding toxicity thresholds [3, 11, 15–19].

Today, the processes of post-technogenic restoration of ecosystems are of increased interest to specialists in various fields. At the same time, a significant part of the work is devoted to research within the framework of controlled restoration of ecosystems

(primarily reclamation of disturbed territories), or the study of their successional changes as a result of the cessation of other types of acute/chronic anthropogenic impact: fires, logging, oil spills, salinization, agricultural activities, etc. [20, 21].

The course of natural (without human participation) rehabilitation of territories after their long-term chronic pollution by industrial enterprises has still not been sufficiently studied [5]. Fragmentary data are available only for a limited number of biota objects and they were obtained in the zone of action of individual point sources using different methodological approaches. At the same time, the rates of restoration of individual components of ecosystems are not the same and depend on many external and internal factors [22, 23]. Therefore, to analyze the patterns of natural restoration of ecosystems, information is needed both on the dynamics of pollutants entering the environment and on their content in the components of biota.

Convenient model objects for such assessments are small mammals (SMs): mouse-like rodents and small insectivores. Due to its wide distribution, significant

numbers, high fertility, relative sedentarism, and ease of collection, this group is traditionally used in a variety of field studies [24, 25]. In terrestrial ecosystems SMs play different functional roles: both primary (phytophages) and secondary (zoophages) consumers, influencing the numbers of local populations of prey, higher-order consumers, and detritivores. Their use allows us to characterize the toxic load simultaneously at several levels: the concentrations of elements in the stomach contents provide an integrated assessment of the intake of pollutants into the body with food (plant or animal), and data on accumulation in depot organs serve as a “marker” of exposure at the body level [23, 26, 27]. However, due to their small body size and high metabolic rate, SMs are more susceptible to the effects of pollutants (including HMs) than large mammals [28].

Elements that are conventionally grouped into the “heavy metals” group are normally found in the body of mammals in small quantities (from  $1 \times 10^{-3}$  up to  $1 \times 10^{-6}$ % of mass). These elements are part of enzymes, hormones, and other vital substances, acting as essential, conditionally essential, or toxic [29]. When the environment is chemically polluted, HMs accumulate in living organisms in increased quantities. Depending on the age, sex, physiological state of individuals, their taxonomic and trophic affiliation, as well as the concentration of the elements themselves, and their localization in organs and tissues, SM pollutants entering the body can have the opposite effect on animals [10–13, 18, 19, 29–33]. Thus, many HMs (Cd, Pb, Hg, Ni) can cause a wide range of toxic effects in mammals, which manifest themselves in reproductive disorders, reduced survival of offspring, damage to the digestive, excretory, nervous, and other systems of the body [4, 8, 29, 33]. On the other hand, elements such as Fe, Zn, Cu, Cr are necessary to ensure normal life. However, at high levels they can also cause pathological changes in metabolic processes [4, 21, 29, 31].

Analysis of literary data shows that representatives of the genus *Apodemus* wood mouse (*A. sylvaticus*) and related species more often than other SMs serve as model objects of natural ecotoxicological studies in the territory of Eurasia. Many authors believe that these species are better than other SMs in demonstrating responses to anthropogenic impacts (including industrial pollution) at different levels of organization, from molecular to population [17–19, 28–33].

A comparative analysis of the long-term dynamics of HM accumulation in the diet and body of animals of different trophic levels (shrews and forest voles) living in conditions of industrial pollution allowed us to conclude that there are fundamental differences in the accumulation of toxic elements (Cd and Pb) in the liver of insectivores and rodents [22, 23, 26, 27]. It has been shown [23] that even sympatric species that are close in trophism and taxonomic position can respond

differently to a significant reduction in emissions. Therefore, detailed assessments of spatiotemporal changes in the HM content in the diet and body of representatives of another trophic group, “seed eaters” living in background and polluted areas together with previously studied species, are necessary to understand the processes of natural restoration of disturbed ecosystems.

**Objective**—To analyze the long-term dynamics of concentrations of essential (Cu, Zn) and toxic (Cd, Pb) elements in the diet and liver of the Herb Field Mouse (*Sylvaemus uralensis* Pall, 1811), in the vicinity of a large copper smelter, during periods of high emissions and after significant decrease. The hypothesis was tested that reducing emissions would result in lower HM concentrations in both feed and animal bodies, with greater changes observed at sites located in close proximity to the plant compared to those further away.

## MATERIALS AND METHODS

**Emission source.** The research was carried out in the vicinity of the Middle Ural Copper Smelter (MUCS), the largest enterprise in Russia for the primary smelting of copper and the production of sulfuric acid, located 50 km west of Yekaterinburg. Over a long period of continuous work (since 1940), a contrasting man-made geochemical anomaly was formed in its vicinity, in the soils of which the HM content significantly exceeded background levels. The characteristics of the MUCS as a point source of emission were given earlier [1, 14, 34].

In the 1970s and 1980s, gross emissions, mainly gaseous compounds of S, F and N, as well as dust particles with sorbed Cu, Pb, Zn, Cd, Fe, Hg, and As, reached peak values (225 thousand tons/year), which made the enterprise one of the main sources of industrial pollution in Russia. During the 1990s, the volume of emissions decreased from 148 thousand tons in 1990 to 65 thousand tons in 1999. In the following 10 years, the reduction was even more pronounced, from 63 thousand tons in 2000 to 22 thousand tons in 2009. After the modernization of production (since 2010), gross emissions do not exceed 2.5–5 thousand tons/year. Thus, over the past 30 years, the plant’s gross emissions have been reduced by more than 50 times, with the greatest reduction in SO<sub>2</sub> (80 times), Cu (3000 times), Zn (15 times), and Pb (8.5 times) concentrations [14].

**Study area.** The key plots are 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, differing in the degree of damage to ecosystems: background, buffer, and impact. The severity of degradation processes of forest phytocenoses in different load zones is not the same [34]. The background zone (20–34 km from the

**Table 1.** Volume of analyzed material

Indicator	Zone	Period (years of research)			Total
		I (1990–2000)	II (2001–2010)	III (2011–2023)	
Number of years of observation*	All zones	10	8	13	33
Worked out trap-night**	Background	10,380	9,625	12,825	32,830
	Buffer	12,170	6,015	10,650	28,835
	Impact	16,635	5,975	20,525	43,135
Caught individuals, specimen**	Background	37	37	88	162
	Buffer	30	48	121	199
	Impact	93	19	70	182
Relative abundance of a species, specimens/100 catch-days**	Background	0.36 ± 0.06	0.38 ± 0.06	0.69 ± 0.08	0.49 ± 0.06
	Buffer	0.25 ± 0.05	0.80 ± 0.10	1.14 ± 0.10	0.69 ± 0.06
	Impact	0.56 ± 0.06	0.32 ± 0.06	0.34 ± 0.04	0.42 ± 0.06
Number of years with zero numbers**	Background	3	1	3	7
	Buffer	3	2	1	6
	Impact	2	4	2	8
The content of HM in stomach contents, samples was analyzed	Background	42	33	45	120
	Buffer	30	46	106	182
	Impact	66	13	47	126
The content of HM in the liver of samples was analyzed	Background	36	36	68	140
	Buffer	63	39	156	258
	Impact	74	16	73	163

\* No captures were carried out in 1999 and 2001; \*\*the results of the main captures of animals on stationary research lines are given.

plant) was characterized by a relatively undisturbed state, which due to the action of regional fallout of pollutants. In the buffer zone (moderately polluted areas, 4–10 km), structural changes in ecosystems were caused by the action local pollution are observed. In the impact zone (heavily polluted areas, 1–3 km), the structure of ecosystems was radically different from the background state, extreme variants of technogenic degradation of communities are presented here. A detailed description of the study area, key plots, scheme, and methods of collecting material were published earlier [22].

The studies covered 34 years, which fell during periods of high (1990–2000, period I), reduced (2001–2010, period II), and almost cessation of emissions (2011–2023, period III).

**Collection of material.** The main part of the material was collected at seven key plots located in the impact (1, 1.5, and 2 km from the plant), buffer (4 and 6 km) and background (20 and 30 km) zones. In periods II and III, five additional key plots were also used: impact zone—3 km, buffer zone—5, 7 and 10 km, and background zone—34 km.

The animals were caught annually during the snowless period (from May to September) in all plots

simultaneously to a single scheme. The traps were installed on stationary marked lines (25 pieces every 5–7 m, exposure from 3 to 5 days with a single daily check); 2 to 4 lines were placed in each key plots during trapping round. During the tour, animal captures were carried out simultaneously in all key areas. The use of stationary capture points made it possible to significantly reduce the impact of spatial heterogeneity of conditions, which is especially pronounced in areas near the plant [14, 22, 23, 26, 27, 35–37].

During the entire observation period, the number of *S. uralensis* varied greatly, and in some years the species was absent from captures in all key areas. In this regard, to ensure the “continuity” of the series, samples obtained from animals caught in adjacent areas by other methods were used for chemical analysis, as a result of catching with live traps (liver only) or cones with fences, Barber pitfall traps (both types of samples). The volume of analyzed material is presented in Table 1.

**Object of study.** *S. uralensis* is a typical inhabitant of the forest and forest-steppe zones of Europe and most of Asia. The main habitats of the species are forests of various types, mainly mixed and deciduous, groves, clearings, thickets of ruderal vegetation, and agricul-

tural lands [38]; a typical seed eater. Despite the diversity of the food spectrum, the basis of the species' diet (up to 70%) consists of plant seeds (trees, shrubs, herbaceous plants) and juicy fruits [38–40]. Depending on the season, the diet is supplemented by green and underground parts of plants (especially at the beginning of the growing season), tree bark, berries, mushrooms, and invertebrates (in late winter–early summer). Consequently, HM concentrations in the animals' food can vary greatly throughout the year. The sampling scheme we used (from June to September, simultaneously in all sections of the gradient) allowed us to minimize the contribution of the seasonal factor.

Throughout the observation period, the species were regularly recorded at all key plots. In the background zone, its participation usually did not exceed 5–10% of the total number of SMs, reaching 1/3 in some years. In the vicinity of the plant, *S. uralensis* accounted for an average of 10–20%. During the years of peak abundance of the species in some areas it could exceed 55% of the total SM abundance (in the impact zone), occasionally approaching 100% (in the buffer zone).

It is important to note that the species is characterized by high migratory mobility: in one day the animals can cover a distance of up to 1.5 km, and during the season, up to 10 km [41]. Active movements of animals across areas with mosaic pollution can have a significant impact on the HM content in food and, consequently, in the animals' bodies.

**Chemical analysis.** Collection of samples for chemical analysis throughout the entire study period was carried out according to a single protocol. The liver fragment and stomach contents (individually for each animal) were dried on glass sides at a temperature of 75°C, packed in sealed plastic bags, and stored in a dry, cool place until the start of analytical work. Next, the samples were homogenized, weighed (about 0.1 g) using a KERN-770 analytical balance (with an accuracy of 0.0001 g), and placed in Teflon vessels with 7 mL of 65% HNO<sub>3</sub> (high purity) and 1 mL deionized H<sub>2</sub>O, kept for 30 min and then ashed in a microwave oven MWS-2 (Berghof, Germany). After decomposition, the sample volume was adjusted to 10 mL with deionized H<sub>2</sub>O. The concentration of elements (µg/g dry weight) was determined by the atomic absorption method on AAS6 Vario spectrometer (Analytik Jena, Germany) with flame (Cu, Zn) and electrothermal (Cd, Pb) atomization options.

The quality of the measurements was assessed using the international standard sample CRM 185R (bovine liver). Extraction was, %: Cu—93.2, Zn — 99.8, Cd — 114.2, Pb — 94.4; detection limit, µg/mL: for Cu — 0.013, Zn — 0.005, Cd — 0.001, Pb — 0.013. In cases where the concentration of an element was below the detection limit, a value equal to half the detection limit was used for statistical analysis.

Sample preparation and determination of HM concentrations were carried out in 2015–2017 and 2023 according to standard protocols on the same equipment in the laboratory of ecotoxicology of populations and communities of the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences. A total of 989 samples were analyzed, including 428 stomach contents and 561 liver contents (see Table 1).

**Statistical analysis.** The distribution of element concentrations in most cases was close to lognormal, so logarithmic values (Log<sub>10</sub>). Descriptive statistics (geometric mean, range, coefficient of variation) were calculated for HM concentrations. The statistical unit was considered to be an individual. To assess differences in HM content between contamination zones and study periods, a two-factor ANOVA was used. Multiple comparisons were performed using Tukey's test. Regression analysis was used to analyze spatial (in the pollution gradient) and temporal (by years) changes in HM concentrations in the contents of the stomach and liver samples of *S. uralensis*. In statistical tests, differences were considered significant when  $p < 0.05$ . The calculations were performed in the JMP v.11 package [42].

## RESULTS

**Concentrations of HMs in stomach contents.** The accumulation of all studied elements in the stomach contents depended on the level of contamination (zone); for Cu and Pb, the study period was also statistically significant. The interaction of factors was significant only for Zn, and for Cu and Cd, at the trend level (Table 2).

The minimum levels of HM accumulation were recorded in the background area, and the maximum levels were recorded in the immediate vicinity of the plant. In years of high emissions (period I), the content of Cu, Cd, and Pb in the food of animals from contaminated areas exceeded background values by 2.4–4.2 times, Zn, by 1.5–1.7 times (Table 3). During the period of almost complete cessation of emissions (period III), the differences between background and contaminated areas remained at the same level or decreased (primarily due to impact values). Within all studied zones, a monotonic decrease in levels from period I to III was noted only for Pb, by 2–2.7 times. A clear trend was recorded in the buffer zone: over 34 years of observations, as a result of an annual decrease in Pb concentration by 1.8%, the content of the element in animal feed decreased by 2.3 times (Fig. 1).

Concentrations of the studied elements varied greatly across all sites throughout the study period. The minimum range of values in all zones is recorded for essential elements (Zn and Cu), for toxic elements (Cd and Pb) the *CV* values were 1.5–3 times higher.

**Table 2.** Results of the analysis of variance of differences in HM concentrations in the contents of the stomach (above the line) and liver (below the line) of *S. uralensis* between load zones and periods (F-criterion, in brackets—achieved significance level, *n*—number of samples)

Source of variability	df	Cu	Zn	Cd	Pb
Zone	2	<u>19.1 (&lt;0.0001)</u> 2.4 (0.096)	<u>11.5 (&lt;0.0001)</u> 1.5 (0.219)	<u>12.8 (&lt;0.0001)</u> 26.4 (<0.0001)	<u>9.2 (0.0001)</u> 8.0 (0.0004)
Period	2	<u>9.1 (0.0001)</u> 7.4 (0.007)	<u>0.1 (0.873)</u> 7.7 (0.001)	<u>1.1 (0.349)</u> 4.1 (0.017)	<u>7.7 (0.0005)</u> 14.2 (<0.0001)
Zone × period	4	<u>2.4 (0.051)</u> 1.1 (0.374)	<u>3.3 (0.012)</u> 2.1 (0.082)	<u>2.3 (0.056)</u> 3.1 (0.015)	<u>1.6 (0.183)</u> 1.5 (<0.210)
<i>n</i>		<u>427</u> 559	<u>427</u> 560	<u>425</u> 543	<u>422</u> 541

**Table 3.** Concentration of heavy metals in stomach contents of *S. uralensis* in the studied areas during periods of high (I), reduced (II) and almost cessation of emissions (III), µg/g dry mass

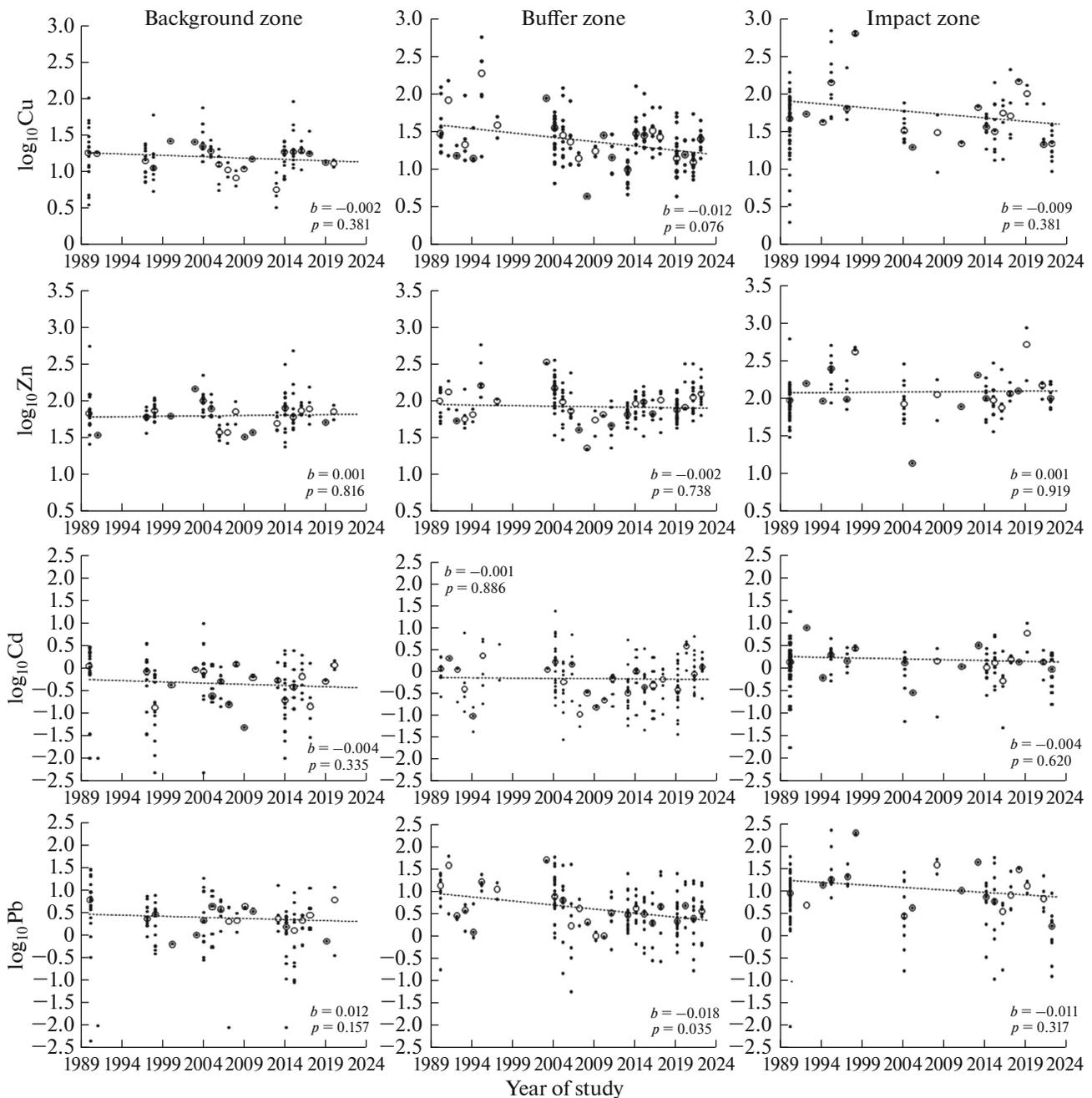
Zone	Period	<i>n</i>	Cu		Zn		Cd		Pb	
Background	I	42	13.89 [94.7] (0.30–103.79)	a	71.38 [94.8] (27.05–560.30)	a	0.33 [101.1] (na–3.68)	a	2.69 [179.0] (na–31.48)	a
	II	33	17.96 [65.7] (5.64–76.65)	a	71.31 [51.6] (27.37–226.38)	a	0.49 [177.3] (na–10.31)	a	2.22 [105.9] (na–20.77)	ab
	III	45	16.43 [71.1] (3.32–92.11)	a	74.69 [86.7] (24.86–492.31)	a	0.34 [104.5] (na–2.60)	a	1.33 [122.3] (na–14.34)	b
Buffer	I	30	40.41 [151.9] (13.36–571.95)	a	104.41 [87.4] (45.24–614.36)	a	0.79 [125.1] (0.05–8.06)	ab	7.01 [104.9] (0.19–63.23)	a
	II	46	26.90 [72.3] (4.49–120.88)	b	108.47 [62.4] (23.96–384.44)	a	0.89 [189.3] (0.03–26.02)	a	5.12 [125.7] (0.06–63.12)	ab
	III	106	18.03 [81.0] (0.60–127.66)	b	89.22 [55.0] (24.58–342.36)	b	0.63 [113.0] (0.04–6.84)	b	3.09 [111.6] (0.18–29.40)	b
Impact	I	66	55.64 [145.3] (2.02–706.50)	a	118.89 [81.8] (32.55–652.21)	a	1.36 [119.1] (0.02–18.52)	a	11.39 [175.8] (0.10–226.42)	a
	II	13	32.23 [51.5] (9.45–76.88)	a	85.03 [72.6] (14.57–303.47)	a	0.75 [73.8] (0.07–2.84)	a	4.64 [124.6] (0.17–52.43)	b
	III	47	36.95 [86.9] (9.58–217.52)	ab	119.19 [89.0] (37.99–904.57)	a	1.13 [100.8] (0.05–10.06)	a	4.18 [129.7] (0.12–57.01)	b

Here and in Table 4 geometric mean values are given in square brackets—coefficient of variation *CV* (%), in parentheses—minimum and maximum values, *n*—number of samples analyzed, na—value below the detection limit. Similar letters indicate the absence of significant differences in element concentration within the zone (Tukey's test).

**Concentration of metals in the liver.** Differences between zones were significant only for toxic elements (Cd and Pb), while the study period was significant for all HMs. The interaction of factors was significant only for Cd (see Table 2). The minimum concentrations of Cd and Pb were observed in the background zone, and the maximum ones were observed in the impact areas (Table 4).

During years of high emissions (period I), in buffer and impact zones, the Pb content in animal livers exceeded background values by 1.6–2.5 times. During

period III, the differences between background and contaminated areas for Pb became less pronounced and did not exceed 5–25%. The main reason for such changes was the decrease in the concentration of the element in the vicinity of the plant. Over 34 years of observations, as a result of an annual decrease in the Pb level by 1.1–1.4%, its content in the liver of animals from buffer and impact sites decreased by 2–2.5 times. Over the same period, Pb concentrations in background areas did not undergo significant changes (Fig. 2).



**Fig. 1.** Long-term dynamics of concentration ( $\text{Log}_{10}$ ,  $\mu\text{g/g}$  dry weight) of HMs in stomach contents of *S. uralensis* in different pollution zones. Here and in Fig. 2: ●—individual values; ○—average value for the year (median); dotted line—linear regression trend;  $b$ —regression coefficient;  $r$ —the achieved level of significance.

For Cd, on the contrary, the differences between background and contaminated areas became more pronounced as a result of the emission reduction. If in the initial period the concentrations of the element in the liver of the background animals were 10–30% lower, then by the end of the observations the differences reached 2–3 times. The increase in differences is due to a decrease in the element level in unpolluted areas (Fig. 2). An annual decrease in Cd concentration

by 1.5% over 34 years of observation resulted in a two-fold decrease in the element level compared to baseline values. In the vicinity of the plant, Cd concentrations in the liver of animals were maintained at the same level throughout the entire time (see Table 4).

Concentrations of essential elements (Cu, Zn) in the liver of *S. uralensis* in the pollution gradient changed insignificantly (not exceeding 10%, in rare cases—20%) during the entire observation interval. In

**Table 4.** Concentration of heavy metals in the liver of *S. uralensis* in the studied areas during periods of high (I), reduced (II), and almost cessation of emissions (III), µg/g dry mass

Zone	Period	<i>n</i>	Cu		Zn		Cd		Pb	
Background	I	36	14.88 [48.5] (6.84–40.03)	a	82.55 [52.9] (39.65–215.11)	a	0.70 [68.9] (0.08–2.30)	a	1.35 [169.1] (na–37.55)	a
	II	36	16.28 [28.8] (7.81–31.34)	a	87.95 [28.2] (54.14–192.77)	a	0.32 [85.7] (na–1.85)	b	1.14 [94.0] (0.09–8.29)	b
	III	68	16.89 [30.4] (6.22–37.42)	a	79.83 [21.8] (40.91–148.22)	a	0.34 [163.6] (na–17.07)	b	1.04 [156.1] (na–25.53)	b
Buffer	I	63	13.98 [43.8] (4.86–40.00)	b	82.99 [48.1] (28.49–215.11)	b	0.79 [151.9] (0.19–13.24)	a	2.20 [129.9] (0.33–17.84)	a
	II	39	16.19 [35.9] (7.34–45.43)	a	94.90 [15.4] (65.08–126.44)	a	1.04 [66.2] (0.19–4.18)	a	1.37 [73.6] (0.07–5.15)	b
	III	156	14.49 [36.9] (5.03–35.34)	ab	82.23 [22.7] (43.35–131.62)	b	0.71 [189.6] (0.01–29.70)	a	1.08 [98.4] (na–9.29)	b
Impact	I	74	13.54 [46.4] (5.47–44.79)	b	95.89 [38.1] (35.53–229.62)	a	1.01 [112.9] (0.05–11.06)	a	3.40 [77.6] (0.14–18.84)	a
	II	16	15.59 [24.8] (11.90–28.89)	ab	95.30 [18.4] (63.94–130.44)	a	1.31 [95.5] (0.36–6.46)	a	2.64 [78.6] (0.33–9.80)	ab
	III	73	16.17 [30.3] (7.13–36.63)	a	78.72 [25.5] (32.71–175.97)	b	1.00 [163.6] (0.01–19.70)	a	1.40 [87.6] (0.01–9.21)	b

the impact zone, the reduction in emissions was accompanied by a monotonic decrease in the Zn level (see Table 4).

As in the diet, HM concentrations in the liver of animals varied greatly throughout the observation period. In all zones, the minimum range was recorded for essential Zn and Cu, and the *CV* values were 2–6 times lower than in feed. For toxic Cd and Pb, the variability of values in the liver was lower than in the stomach contents (up to 3 times), or comparable to them (see Tables 3, 4).

**Changes in HMs concentrations in stomach contents in the pollution gradient.** Since the accumulation of HMs depended on the period of studies, data from only one of them (period III) were used for comparison, but for the maximum number of key plots ( $n = 9$ ), most fully covering the entire pollution gradient. When approaching the plant, the concentrations of both essential elements in the stomach contents increased significantly ( $F = 2.51–2.78$ ,  $p < 0.05$ ), and Cd at the trend level ( $F = 1.92$ ,  $p < 0.10$ ) (Fig. 3). The highest rates of change were recorded for Cu and Cd ( $b = -0.35$ ).

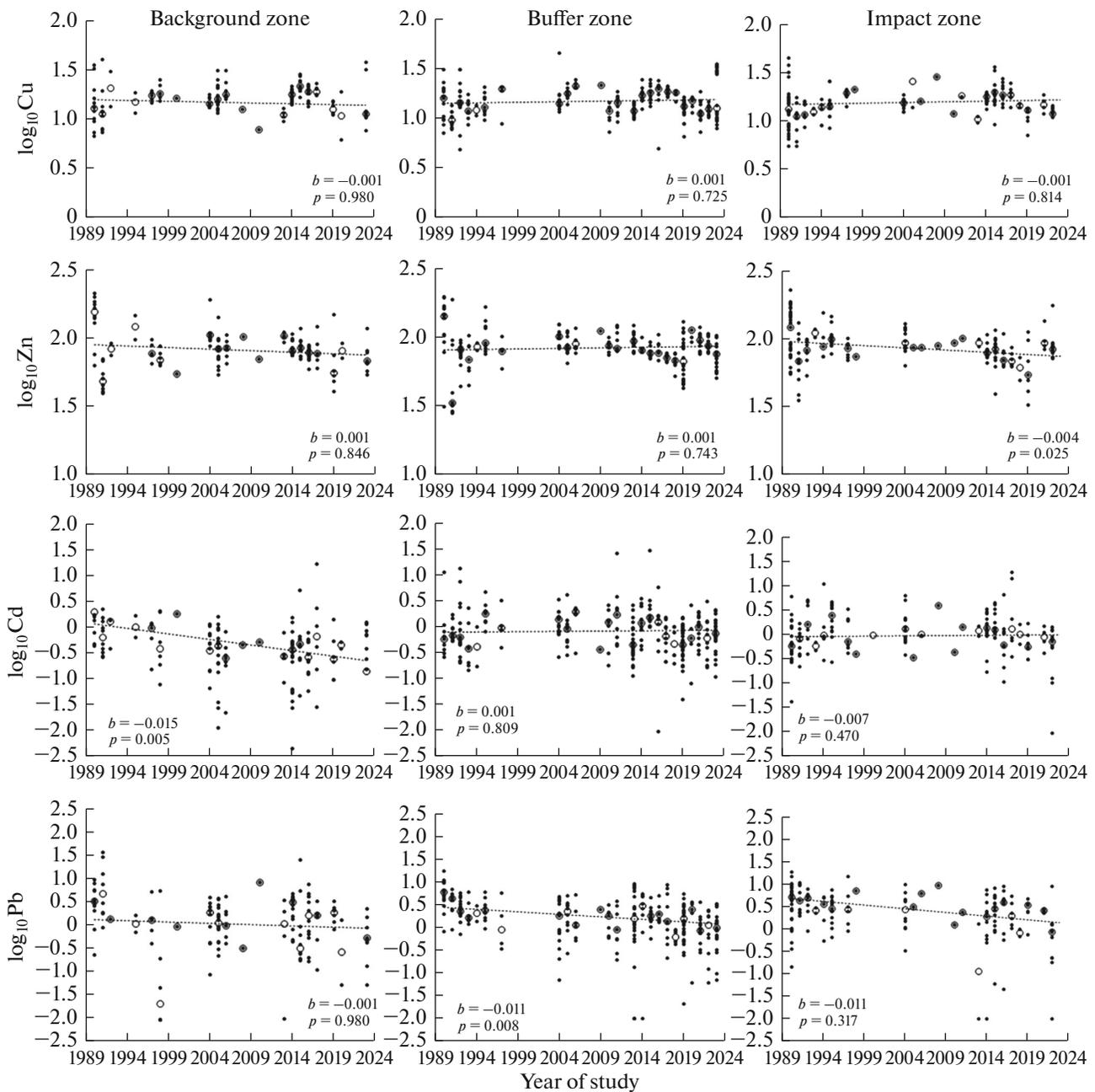
**Changes in HMs concentrations in the liver of animals in the pollution gradient.** For the comparative analysis we used data for period III, obtained at 11 key plots. In addition to those listed above, areas in the vicinity of MUCS (3 and 5 km) have been added. When approaching the plant, the content of Cu, Zn, and Pb did not change significantly, Cd increased monotonically ( $F = 3.41$ ,  $p < 0.008$ ). Moreover, the

rate of changes in Cd were comparable to those in the stomach contents (see Fig. 3).

**Relationship between metal concentrations in feed and liver.** When considering the generalized sample for all sites, the paired correlation coefficients for Cd and Pb in the studied substrates were significant, but the relationship was weak ( $n = 428$ ,  $r = 0.15–0.16$ ,  $p < 0.05$ ). The use of zone-differentiated samples showed that positive correlations were recorded for Cu ( $n = 182$ ,  $r = 0.21$ ,  $p < 0.05$ ), at impact sites and negative for Zn ( $n = 126$ ,  $r = -0.21$ ,  $p < 0.05$ ), but the relationship is weak. No significant interactions were found in the background zone. Note that for Cd and Pb, no interactions were detected within the selected zones.

## DISCUSSION

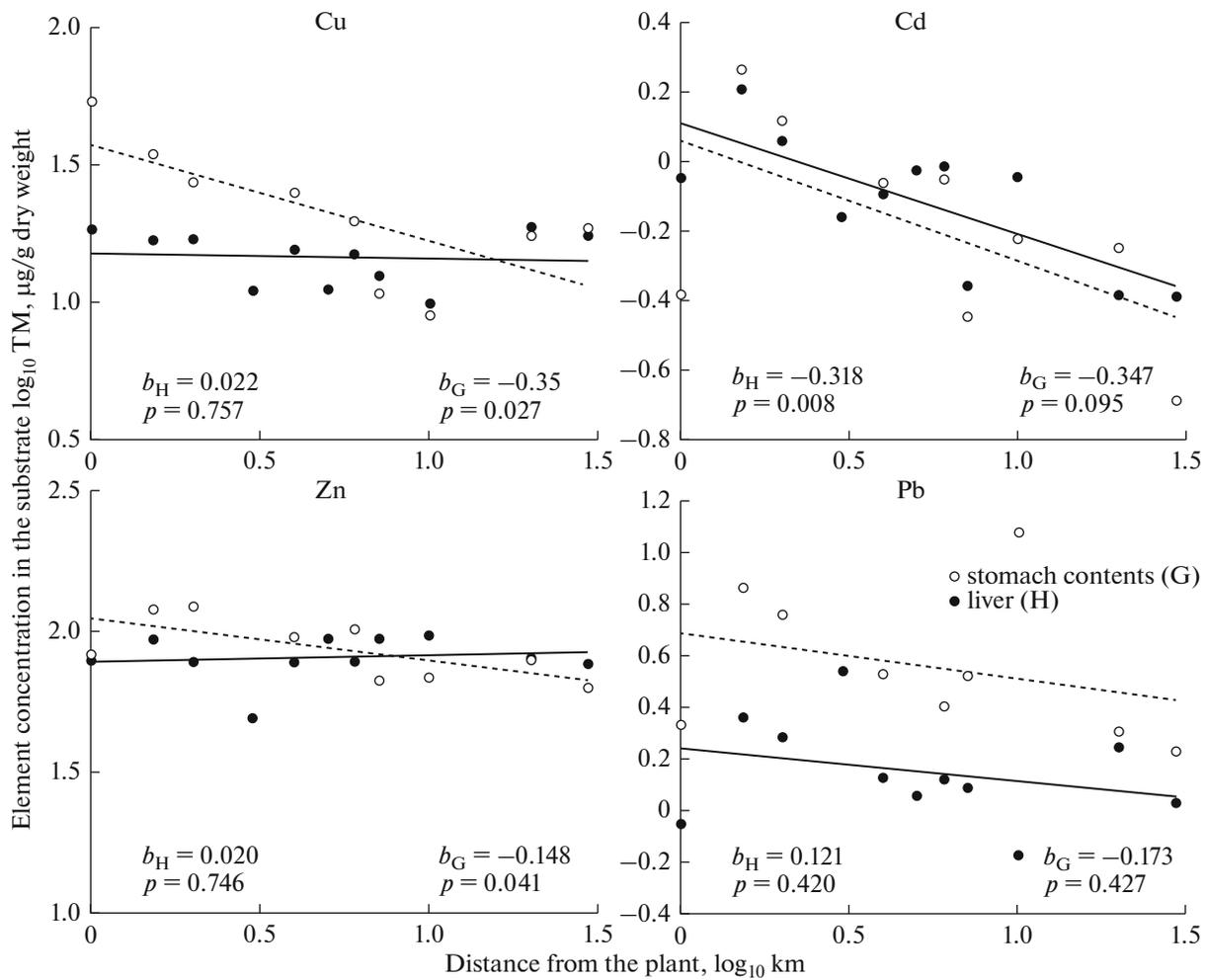
**Changes in the concentration of metals in the gastric contents in the pollution gradient.** Traditionally, to assess the toxic load on the body of mammals, authors use data on the concentrations of elements of interest in environmental objects. However, the varied diet of animals in combination with their active movements in space do not allow for a correct assessment of the intake of HMs into SM organisms based on their content in food objects. To characterize the diet and seasonal and long-term changes in the food spectrum of individual SM species, specialists often examine the contents of their stomachs. We believe that this substrate is the most promising, since it represents an integrated assessment of the current intake of certain elements into the body with food [23, 26, 27, 50].



**Fig. 2.** Long-term dynamics of concentration (Log<sub>10</sub>, µg/g dry weight) of HMs in the liver of *S. uralensis* in different pollution zones.

Unfortunately, information on direct assessments of the elemental composition of diets of mice of the genus *Apodemus*, obtained for individuals from natural populations, are absent from the literature. As noted above, most studies provide calculated data based on the HM content in potential feeds of the studied species [18, 43]. Comparison of HM concentrations in stomach contents of *S. uralensis* and individual components of diets allows us to conclude that such assessments are comparable. For example, in period III, at the most distant key plots of the pollution gradient

from each other, the concentrations of Cd in the stomach contents differed by 7 times, Cu and Pb—by 3 times, Zn — by 2 times (see Fig. 3). In the same gradient, HM concentrations in potential food objects changed in a similar manner [44, 45]. Thus, in the impact zone, the concentrations of Cd (2–7 times) and Pb (2–4 times) were maximally increased in the fruits of wild berries, Cd (2–14 times) and Pb (2–17 times) in mushrooms, and Cu (5–12 times), and Pb (6–16 times) in the vegetative parts of plants.



**Fig. 3.** Change in HM concentrations (µg/g dry mass, Log<sub>10</sub>) in the contents of the stomach (G) and liver (H) *S. uralensis* when moving away from the plant (km, Log<sub>10</sub>) during a period of almost cessation of emissions. Markers are average values (medians) for key plots; lines—linear regression trends;  $b$ —regression coefficient;  $p$ —the achieved level of significance. Light markers, dotted lines—for stomach contents; dark markers, solid lines—for the liver.

It is well known that in natural SM populations, HM accumulation is characterized by trophic specificity. With equal levels of pollutants in the external environment, their unequal content in the body is due to the ecological characteristics of the species and, above all, nutrition [8, 10, 23, 26, 49]. Depending on the distance to the plant, the maximum concentrations of the studied elements were recorded in the stomach contents of shrews (genus *Sorex*) or forest voles (genus *Clethrionomys*) [23, 26, 50]. As expected, in all areas of the gradient throughout the observation period in the series zoophagous > mixophagous > seed-eating *S. uralensis* were characterized by a minimum content of HMs in the feed. These results are in good agreement with the information on the reduced HM content in seeds and fruits compared to other plant parts and invertebrates [46].

Comparison of our results for the Herb Field Mouse with the HMs concentrations in the stomach

contents of two other species of the same genus: the yellow-necked mouse (*A. flavicollis*) and Korean field mouse (*A. peninsulae*), living in background areas and in the zone of operation of non-ferrous metallurgy enterprises from other localities, did not reveal significant interspecies differences [46–48]. It can be assumed that this is due, on the one hand, to the taxonomic proximity of the compared species, their similar position in the trophic chain, and on the other hand, to the comparable type of impact and levels of pollution of the territories. In addition, field collection of material and analytical studies were carried out according to uniform protocols.

**Dynamics of HMs content in the diet.** It is logical to assume that under conditions of multiple (more than 50 times) reduction of emissions, the concentration of pollutants in feed objects will decrease significantly, especially in the vicinity of the plant. However, an equivalent reduction in HM levels in stomach contents

of *S. uralensis*, inhabiting buffer and impact areas, did not occur. Within the selected zones, the concentrations of Zn and Cd changed insignificantly over 34 years of observations, while for Pb (clear trends) and Cu (at the trend level) a decrease in levels was recorded, most pronounced in the buffer zone. This is in good agreement with the information that after the modernization of production, the content of Cu and Pb in the plant's emissions decreased to the maximum [14].

An additional factor in the sharp (3-fold) decrease in the Pb level in the diets of *S. uralensis* in the last two decades was the general reduction in the content of the element in vehicle exhausts as a result of the transition from leaded gasoline to other types of fuel. A significant (2–10 times) decrease in Pb concentrations was recorded in the feed of SMs and other trophic groups: zoophages, green eaters, and mixophages [50].

It is interesting that forest voles of the genus *Clethrionomys* from the vicinity of MUCS, who lived in the same areas and had a similar set of feeds as *S. uralensis* (but with a predominance of vegetative parts of plants), by the end of the observation period, Cd concentrations in stomach contents increased significantly (by 1.5–4 times) [27]. Normalization of soil and litter acidity during the period of almost complete cessation of emissions led to a decrease in Cd mobility and its accumulation in the root layer [14]. This resulted in a more intensive transition of the element into the vegetative parts of plants and an increased intake into the body of voles.

**Changes in the concentration of metals in the liver in the pollution gradient.** The liver is a multifunctional organ that is involved in maintaining the body's homeostasis, bioaccumulation, and detoxification processes, so it is often used to assess the negative impact of pollution on SMs [4]. The content of HMs in the body of different SM species from the vicinity of metallurgical and mining enterprises most often increases when approaching the source of pollution [17, 19, 23, 27–30, 43, 48, 52–54], however, opposite cases have also been described [49].

In our study, the minimum concentrations of toxic elements (Cd and Pb) in the liver were noted in the background zone, and the maximum concentrations were found in the immediate vicinity of the plant (see Table 3 and Fig. 3). The content of essential elements (Cu, Zn) in the liver did not depend on the level of contamination of the territory.

Comparison of our results with data from other authors shows that HM concentrations in the liver of representatives of the genus *Apodemus*, inhabiting background (unpolluted) habitats in the territory of Eurasia, are generally comparable. For each element, the values vary within close ranges (Table 5). As a rule, animals obtained in different regions of Europe had lower average concentrations of toxic elements (Cd and Pb) than individuals of *S. uralensis* from the Ural

populations. It should be noted that the regional background level in the Middle Urals is, as a rule, higher than in the above-mentioned localities [5, 14, 34]. This is due to the intensive development of the mining and metallurgical industry in the region with a long (more than 300-year) history. An exception is *A. flavicollis* from background territories in Slovakia [12], whose liver contained on average 10–15 times more Pb, while Cd excess reached 2–6 times higher concentrations.

Near industrial enterprises, the content of essential elements (average values) in the liver of representatives of the genus *Apodemus* from different localities was similar, while the levels of toxic elements could differ significantly (see Table 5). Pb concentrations can generally be interpreted as comparable. According to literature data (Table 5), near metallurgical plants, average accumulation levels of the element varied in the range from 0.2–2.3 µg/g dry mass in moderately contaminated areas to 0.3–5.4 µg/g in impact zones. According to our data, in the vicinity of MUCS in the buffer zone, Pb concentrations were 1.0–1.3 µg/g, and in the impact zone, 1.4–3.4 µg/g.

According to literature data, the Cd content in the liver of animals varied over a wider range, from 0.1 to 11.8 µg/g dry weight, even in the vicinity of the same pollution sources (see Table 5). For example, according to data from various publications [19, 52, 54], in the liver of wood mice (*A. sylvaticus*) caught several seasons apart in the same areas near a closed Pb and Zn smelting plant in northern France (Metaleurop Nord), the average Cd concentrations differed by 2–3 times, although the range of changes (minimum and maximum values) was the same. According to the results of the present study, the variability of Cd levels in the liver of *S. uralensis* are significantly lower, from 0.7 to 2.2 µg/g, but fully fits into the above-mentioned ranges. The reasons for the observed differences may be both external (composition of dust particles in emissions, acidity of soil horizons, underlying rocks, etc.) and internal (qualitative and quantitative composition of samples, season of capture, features of sample preparation and chemical analysis, etc.) factors. For example, it is well known that the concentrations of elements, especially toxic ones, depend to a large extent on the age and sex of the animals. Unfortunately, such information is absent from most of the literary sources used for comparison. However, the tendency to decrease the concentration of toxic elements with distance from emission sources is the same in all territories.

**Dynamics of metal content in the liver.** Studies of long-term changes in the elemental composition of the body of terrestrial vertebrates under conditions of reduced industrial emissions are still few in number. The dynamics of HM content has been studied in relatively detail using the example of small passerines (pied flycatcher, great tit) living in the area of metal-

**Table 5.** Concentrations of the studied elements in the liver of animals inhabiting contaminated areas in the vicinity of non-ferrous metallurgy enterprises and background areas

Research area	Species	n	Element concentration, µg/g dry weight				Descriptive statistics	Source of pollution	Location-position	Data source
			Cu	Zn	Cd	Pb				
1	2	3	4	5	6	7	8	9	10	11
Background	<i>S_ur</i>	19	12.4 ± 1.7	82.3 ± 12.5	0.72 ± 0.16	0.94 ± 0.13	M ± SEM	RBg CuSm**(2010)	Russia, Middle Ural, Revda	[55]
Impact		65	10.9 ± 0.7	111.6 ± 6.8	0.94 ± 0.13	5.38 ± 0.49				
Background	<i>S_ur</i>	21	14.3 (7.4–29.5)	96.6 (19.3–193.1)	0.06 (na–0.18)	2.25 (na–7.98)	M (min–max)	RBg	Russia, Southern Ural	[56]
Background	<i>S_ur</i>	94	11.6 (5.7–20.3)	93.9 (67.2–157.3)	0.47 (na–1.92)	2.79 (0.22–8.00)	M (min–max)	RBg	Russia, Southern Ural, Karabash	[48]
Buffer		53	13.2 (7.8–70.3)	93.4 (72.8–130.1)	4.00 (0.07–14.41)	2.32 (0.34–23.15)				
Impact		58	11.2 (6.9–23.1)	88.1 (53.8–142.5)	0.69 (0.13–2.28)	4.00 (0.09–12.60)				
Background	<i>A_pen</i>	6	8.0 (5.1–11.2)	57.3 (37.0–78.0)	0.14 (0.07–0.42)	1.08 (0.22–1.66)	M (min–max)	RBg	Russia, Buryatia, Baikalsk	[47]
Background	<i>A_syl</i>	24	16.5	81.0	0.52	0.07	M	RBg	Belgium, G. Antwerpen	[17]
Impact (++++)		21	18.8	106.2	23.21	1.34				
Background		21	13.8 ± 0.9	80.2 ± 9.9	1.75 ± 0.21	0.17 ± 0.03				
Buffer		28	20.9 ± 1.1	117.0 ± 5.5	3.87 ± 0.53	0.21 ± 0.03				
Impact (+)		24	15.3 ± 0.6	89.9 ± 4.0	6.12 ± 1.30	0.30 ± 0.07				
Impact (++)	25	16.9 ± 1.1	97.8 ± 4.9	31.44 ± 6.99	0.61 ± 0.10					
Background	<i>A_syl</i>	23	10.3 (4.5–26.7)	55.8 (40.3–76.0)	2.80 (0.50–12.60)	0.20 (na–3.80)	M (min–max)	RBg	France, Noyelles-Godault, Nord-Pas-de-Calais	[54]
Buffer		23	15.3 (6.0–21.4)	43.5 (36.1–71.1)	3.60 (1.60–15.00)	0.20 (na–0.90)				
Impact (+)		38	16.0 (3.5–72.4)	48.8 (32.0–77.9)	3.40 (0.60–16.90)	1.00 (na–5.70)				
Impact (+++)		25	17.0 (10.3–27.5)	53.5 (30.5–70.6)	5.20 (1.50–18.80)	3.90 (0.30–14.40)				

Table 5. (Contd.)

Research area	Species	n	Element concentration, µg/g dry weight				Descriptive statistics	Source of pollution	Location-position	Data source
			Cu	Zn	Cd	Pb				
1	2	3	4	5	6	7	8	9	10	11
Impact (+)		121	—	—	2.50 (0.14–38.00)	0.72 (0.12–13.00)				
Impact (++)		178	—	—	4.10 (0.01–209.0)	0.95 (0.12–14.00)		PbZnSm*		[52]
Impact (++++)		261	—	—	3.70 (0.01–91.00)	3.40 (0.12–50.00)				
Background	<i>A_syl</i>	17	—	—	1.40 (0.30–4.30)	0.09 (na–0.50)	M (min–max)		France, Noyelles-Godault, Nord-Pas-de-Calais	
Impact (+)		18	—	—	4.80 (1.70–17.00)	0.60 (na–6.30)				
Impact (++)		27	—	—	11.20 (1.60–32.1)	0.80 (na–3.70)		PbZnSm*		[19]
Impact (++++)		22	—	—	5.60 (1.40–18.60)	1.30 (0.20–4.20)				
Background		1	13.6	90.3	0.20	—				
Buffer	<i>A_flv</i>	3	10.5	80.2	0.21	4.58	M		Finland, Harjavalta	Own data
Impact		1	14.5	91.0	0.56	0.73		CuNiSm**		
Background		15	—	68.1 ± 3.3	0.16 ± 0.03	0.11 ± 0.02			Poland, Borecka Forest	
Impact (+)	<i>A_flv</i>	15	—	99.9 ± 3.3	8.66 ± 1.54	7.61 ± 3.30	M ± SEM		Poland, Bukowno	[53]
Impact (++)		15	—	115.2 ± 10.4	4.01 ± 0.84	0.81 ± 0.10		ZnSm***	Poland, Miasteczko Sl.	
Background			—	—	0.01 ± 0.005	0.02 ± 0.004				
Impact	<i>A_flv</i>		—	—	5.80 ± 3.20	1.05 ± 0.45	M ± SEM		Slovenia, Žerjav	[3]
Background		23	5.9	25.5	1.8	15.0	M (min–max)		Slovakia, Banská Stiavnica	[12]
Impact	<i>A_flv</i>	37	9.4–10.8	26.8–34.3	1.00–2.38	20.40–40.08		RBg TCO		

Study areas: background—unpolluted; buffer—moderately polluted; impact—heavily polluted. If several key areas are analyzed within an area, then an increase in the pollution level corresponds to a greater number of “+”. Pollution type: RBg—regional background, CuSm—copper smelting production, CuNiSm—copper-nickel, PbZnSm—lead-zinc; ZnSm—zinc; TCO—waste heaps of polymetallic ore deposits. Enterprise: \*closed, \*\*active after modernization/repurposing, \*\*\*active. In brackets in italics is the date of closure/resumption of work after modernization. Descriptive statistics: M—arithmetic mean; SEM—error of the mean; (min–max)—range of values; na—value below the detection limit; dash—no data. In square brackets is a reference to a literary source. *A\_syl*—*A. sylvatica*, *A\_flv*—*A. flavicollis*, *A\_pen*—*A. peninsulatae*, *S\_ur*—*S. uralensis*.

lurgical enterprises in Fennoscandia [57, 58]. The authors showed that the birds' response to reduced emissions varied even within species and was largely determined by specific conditions, primarily dietary habits.

The most comprehensive studies on mammals were carried out in the vicinity MUCS (Middle Urals, Russia) for two trophic groups: zoophages (shrews of the genus *Sorex*) [23] and mixophages (forest voles of the genus *Clethrionomys*) [27]. It was established that the reduction of industrial emissions led to multidirectional changes in the levels of the studied HMs in the body (liver) of SMs, the nature of which is element-specific and depends on various factors. Despite high concentrations of Cu and Zn in the feed of animals from contaminated sites, their toxic load on the SM organism was insignificant due to the elimination of excess amounts of these elements through the gastrointestinal tract. The Pb content in the liver of animals of both trophic levels decreased gradually, but in zoophages the changes were more pronounced. The nature of long-term changes in Cd concentrations in the liver of animals living near the plant was radically different: in phytophages, the element content increased, while in zoophages it decreased.

Similar trends were demonstrated for individuals of *A. sylvaticus* from the vicinity of Pb and Zn smelting plants in France (Metaleurop Nord) [19, 52, 54] and Slovenia (Žerjav) [3] 3–8 and 25 years (respectively) after the completion of their work. According to such “snapshots” the Pb content in the liver of animals decreased by 2 times, while Cd increased by 1.5–2 times.

The results of our study were generally consistent with the trends noted above: over 34 years of observations in the vicinity of the plant, in the liver of *S. uralensis*, Pb concentrations decreased by 2–2.5 times, Zn, by 30% (only in the impact zone), while no clear trends were identified for Cu and Cd. In background areas, directional changes were noted only for Cd, the concentration of which decreased by half over the same period. Thus, the initial hypothesis about the decrease in HM concentrations in the animals' bodies as a result of multiple reductions in emissions was partially confirmed; targeted temporal changes were observed for Pb (in the vicinity of the plant) and Cd (in background areas).

Interestingly, the Pb levels in the liver of *S. uralensis* declined at a similar gradual rate as in other trophic groups, zoophages and mixophages, inhabiting the areas affected by the MUCS. In contrast, Cd concentrations in the liver exhibited divergent trends: in *S. uralensis* near the plant, they remained stable throughout the observation period, while in other animal groups, they either increased (phytophages) or decreased (zoophages). This once again supports the argument that the key factors determining HM accumulation in SM bodies are the content of elements in

the feed and the system of homeostatic barriers that help protect organisms from metal toxicity [27].

## CONCLUSIONS

There are no known long-term studies on the dynamics of HM content in the diet and body of Muridae family representatives in industrially polluted areas. Based on the analysis of stomach contents and liver samples of *S. uralensis* obtained from annual animal captures (1990–2023), it was found that the minimum concentrations of all studied elements in the diet, as well as toxic ones (Cd, Pb) in the liver, were recorded in unpolluted areas (background zone), while the highest concentrations were observed in close proximity to the plant (impact zone). The content of essential elements (Cu, Zn) in the animals' livers did not depend on the level of environmental pollution.

The initial hypothesis regarding the reduction of HM accumulation in the diet and liver was only partially confirmed: despite a more than 50-fold decrease in plant emissions, there was no equivalent reduction in metal concentrations in food sources or in the bodies of *S. uralensis*. Over the 34-year observation period near the plant, directional changes were observed only for Pb, whose content in the diet and liver decreased by 2–3 times. In background areas, clear temporal trends were noted for Cd in the liver of *S. uralensis*, with concentrations halving over the same period.

The results of our study contribute to the broader understanding of SM responses in the vicinity of a large copper smelter during periods of high emissions and after their significant reduction. Data on the spatiotemporal dynamics of HM concentrations in the diets and bodies of *S. uralensis* are crucial for understanding the patterns of recovery processes in naturally rehabilitating polluted areas. Of particular interest are also assessments of the potential risk of chronic pollution exposure on SMs of different trophic groups during high-emission periods and after their substantial decline; analyses that still need to be conducted.

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#### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The author confirms that all work with animals was carried out in accordance with the requirements established by international and national guidelines for the care and use of animals. All procedures performed in this study complied with the ethical standards of the Institute of Plant and Animal Ecology of the Russian Academy of Sciences (Protocol no. 3 dated December 18, 2014).

#### CONFLICT OF INTEREST

The author of this work declares that she has no conflicts of interest.

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