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Effect of Chemical Pollution on the Fertility of Male Rodents from Natural Populations: Comparing the Response of Sperm Morphology, Motility, and Concentration

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Abstract—The results of studies of epididymal spermatozoa of three species of rodents (bank vole *Clethrionomys glareolus*, northern red-backed vole *Cl. rutilus*, and herb field mouse *Sylvaemus uralensis*) living under long-term exposure to atmospheric emissions from two large copper smelters in the Middle Urals are summarized (Middle Ural and Kirovgrad copper smelters). The impact of pollution (including at the individual level on the accumulation of Cu, Zn, Pb, and Cd in the liver) was assessed for indicators characterizing the quality of sperm from different aspects: morphology (proportion of cells with head and tail defects), motility (proportion of motile cells, velocity, and straightness of movement) and concentration. Sperm motility responds to pollution: in impact zones, the proportion of motile cells and their velocity were lower than in background zones. The occurrence of abnormal cells and sperm concentration were not statistically significantly different between impact and background zones. The reaction of sperm to chemical pollution is species-specific: voles react more strongly than the herb field mouse. The consistency of changes in sperm parameters (in the direction of their deterioration) in response to increased pollution was found only in the bank vole. Effect sizes for sperm parameters are much smaller compared to those for liver Cd accumulation and animal abundance. In general, the reaction of sperm to pollution turned out to be weak, none of the studied indicators can be a reliable marker of industrial pollution.

Keywords: sperm quality, reproductive success, voles, mice, copper smelter, heavy metals

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INTRODUCTION

The resistance of mammalian populations to toxic load is largely determined by the efficiency of reproduction, which depends on the reproductive characteristics of individuals. This is why the response of various reproductive parameters to pollution has always attracted the attention of ecotoxicologists [1–9]. The impact of point sources of intense pollutant emissions (for example, metallurgical plants with primary smelting of non-ferrous metals) is often chosen as model situations.

When studying the contribution of males to population reproduction, all parts of male reproduction are considered, from the morphology and physiology of tissues and organs to behavioral patterns [5, 10, 11]. The most important element is sperm quality, which can be considered as a proxy assessment of potential fertility. For humans, the need for such an assessment is regulated by the guidelines of the World Health Organization [12] and the International Organization for Standardization [13].

Fertility diagnosis is based on sperm assessment: morphology, motility, and concentration of cells, which together characterize their fertilizing ability. In practice, all three parameters are most often studied in humans, laboratory, or commercial animals [14, 15]. Other species are involved in these studies to a lesser extent. Nevertheless, spermatozoa have been studied to varying degrees of completeness in mammals from natural populations: marsupials [16], artiodactyls [17, 18], predatory [19], large rodents (*Castor fiber*) [20], and mouse-like rodents [21, 22]. Most studies on rodents from natural populations have been performed using epididymal sperm, which are equivalent in fertilizing ability to ejaculated sperm [23–25].

Ecotoxicological aspects of the functioning of the reproductive system of male rodents have been studied fragmentarily, and its resistance to the action of toxicants (including potentially toxic metals) is still a subject of debate. Most researchers consider male reproductive cells to be sensitive to pollution of the environment [26, 27]. On the other hand, the toxic effects of metals are not always detected in organisms: For example, in animals living in contaminated zones, no decrease in sperm motility or concentration or increase in the proportion of abnormal cells was

recorded [1, 28, 29]. There are also few studies in which reproductive parameters (usually only the structure of the testis) and metal concentrations in the body were assessed on the same individuals. [5, 6]. To our knowledge, the influence of individual toxic load on sperm quality has not been previously examined. There are also very few studies that simultaneously assess the impact of pollution on all three groups of parameters: morphology, motility, and sperm concentration. This does not allow us to give a comparative description of the information content of different parameters. Note that in works outside the problem of pollution, a comprehensive analysis of sperm parameters is traditional [22, 30].

Objective—To compare the response of parameters of epididymal sperm of different species of rodents to pollution of the territory by emissions from copper smelters. To do this, we compared the morphology (occurrence of abnormal cells), motility (velocity indicators and proportion of motile cells), and sperm concentration in animals inhabiting background and impact zones with contrasting levels of chemical pollution. The concentrations of priority pollutants (Cu, Zn, Cd, Pb) in the body were also determined, which made it possible to characterize the relationship between sperm quality and individual toxic load. At the same time, we assumed that, (1) the sensitivity of different parameters of spermatozoa to chemical pollution is not the same and (2) the reaction of spermatozoa to pollution is species-specific.

These assumptions are based in part on our previous studies. So, of the two forest vole species (bank and northern red-backed voles) living in areas of copper smelters, only in one species (bank vole) the velocity and proportion of motile cells were lower in impact zones compared to background [31]. The results of the analysis of the occurrence of abnormal sperm in bank voles from another sample were unexpected: there were fewer of them in contaminated zones than in background ones [32].

Unlike our previous studies, in which individual sperm parameters were characterized for different samples, in this work, their entire complex was examined in the same individuals. The basis for this analysis was samples of bank and northern red-backed voles, in which motility indicators were determined [31], but the morphology and concentration of germ cells have not been studied. In addition, in addition to the closely related bank and northern red-backed voles (family Cricetidae), this work also included the herb field mouse (family Muridae), a species that differs from voles in morphology and lifestyle (type of food, preferred habitats, mobility). Taken together, this made it possible to assess the species specificity and consistency of changes in the parameters under consideration.

The effect of pollution on spermatozoa was analyzed taking into account the variant of ontogenesis of rodents, since it is believed that it can determine differences in reproductive characteristics between underyearlings and overwintered individuals [33, 34].

MATERIALS AND METHODS

Study Area

Areas were selected near the two largest copper smelters in the Middle Urals, MUCS (Middle Ural copper smelter, Revda) and KCS (Kirovgrad copper smelter, Kirovgrad). The enterprises have several similar features, which makes it possible to correctly compare the effects of pollution. Both plants are located in the southern taiga subzone (with a predominance of dark coniferous forests) of the western macroslope of the Urals and at the time of the study had comparable volume and composition of emissions (sulfur dioxide, metals, and metalloids).

In the vicinity of the enterprises, technogenic geochemical anomalies with increased (10–100 times compared to the background) content of heavy metals and other elements in the soil have been formed [35–38]. Despite the reorganization of production, which resulted in a significant reduction in emissions (Fig. S1), the impact on ecosystems remains high [39, 40]. In the immediate vicinity of pollution sources, the death of the tree stand continues, and there are no restoration processes in the herb-shrub layer [41, 42]. The depressed state of forest ecosystems is associated with the preservation of a thick layer of weakly decomposed forest litter due to the low rate of destruction of plant litter [38] and extremely slow purification of the upper soil horizons from metals. The latter is associated with an increase in pH, which in turn reduces the transit of metals into underlying soil horizons [37].

Animal capture sites were located to the west of the plants (opposite to the prevailing wind direction) in two zones contrasting in terms of pollution levels, background (Bg) and impact (Im). Background zones (30–40 km from factories) are characterized by a relatively undisturbed state of ecosystems, which is caused only by regional fallout of pollutants. In impact zones (2–6 km from plants), structural changes in ecosystems are observed, caused by the effects of local pollution, up to extreme variants of technogenic digression of communities, technogenic wastelands [36, 38, 41, 42].

Sampling, Keeping, and Examining Animals

Three species of rodents (Rodentia) from two families were studied: bank vole (*Clethrionomys glareolus* (Schreber, 1780)) and northern red-backed vole (*Clethrionomys rutilus* (Pallas, 1779)) of the subfamily Arvicolinae of the family Cricetidae [43] and herb field mouse (*Sylvaemus uralensis* (Pallas, 1811)) subfamily Murinae of the family Muridae [44]. These

Table 1. Sample size and structure

Area	Pollution zone	Average relative abundance of rodents (ind./100 trap-day) in 2018–2020	*Cl. glareolus*, $n = 71$		*Cl. rutilus*, $n = 52$		*S. uralensis*, $n = 18$	
			m	ow	m	ow	m	ow
MUCS	Background	12.8	0	24	1	1	2	2
	Impact	8.5	8	11	16	9	4	2
KCS	Background	19.3	7	12	6	6	1	3
	Impact	9.7	5	4	6	7	2	2

Intrapopulation groups: m—mature young of the year (mature), ow—overwintered individuals (overwintered).

species are common in the Middle Urals and often dominate local taxocenes.

Captures in the MUCS and KCS areas were carried out in 2018–2020 from May to August. At each site, two to three lines of wooden live traps (20–30 each) were placed at a distance of 5–10 m from each other [45]. The traps were exposed for 3–5 days and checked daily in the morning and evening. To increase the sample size, a "maximum catch" of animals was used: after triggering (capturing an animal), the trap was again installed in working condition, in contrast to the classical scheme, in which the trap can only be triggered once during the day. Thus, abundance estimates turned out to be somewhat biased (increased).

After capture, the animals were brought to the laboratory for measurement, weighing, determination of the mass of internal organs, and collection of biological samples. The rodents were kept for 1–3 days in laboratory conditions under natural light and room temperature, trying to house the animals as quickly as possible (1–3 individuals in plastic cages with a mesh lid). Overexposure of animals helped reduce the impact of stress from transportation and the new environment. The animals were fed (*ad libitum*) oats, carrots, cucumbers, and apples; sawdust and hay were used for bedding. Animals were killed by dislocation of the cervical vertebrae.

Males were differentiated into three intrapopulation groups based on a set of characteristics (body weight and size, the presence of a thymus, the state of the gonads): immature underyearlings (immature, im), sexually mature young of the year (mature, m) and overwintered individuals (overwintered, ow). The analysis of sperm parameters included only sexually mature males without visible signs of involution of the testes and accessory glands, dividing them into two groups, sexually mature young of the year (I variant of ontogenesis, breed in the year of their birth) and overwintered individuals (variant II, breed after wintering). The sexual maturity of males was determined by the degree of development (weight and size) of the testes and accessory glands and the presence of sperm in the epididymis. To verify the definition of the group, the absolute age of all captured rodents was determined based on age-related changes in teeth: for voles, with an accuracy of 10–45 days [34], for the herb field mouse, up to 10–142 days [46].

Sample Size and Structure

The samples partially reflected the status of the small mammal population in the vicinity of MUCS and KCS during the study period. The relative abundance of rodents in the background zones was 1.5–2.0 times higher compared to the impact zones (Table 1). Interannual differences in the relative abundance made it possible to identify years with low (2018, 4.6 (1–8) individuals/100 traps-day), medium (2019, 14.3 (6–22) individuals/100 traps-day) and high (2020, 17.5 (9–35) individuals/100 trap-day) numbers and classify them as phases of "depression," "growth," and "peak" of the population cycle [47, 48]. The small number of years of observations and the lack of repetitions within the cycle phase did not allow us to assess the interannual variability of reproductive parameters, so they were considered in total for all three years.

Analysis of Sperm Morphology

Smear preparations were prepared from the contents of the caudal part of the right epididymis in 2–3 replicates. A suspension of unstained cells was applied to a glass slide and a smear was made, which was then fixed in 95% alcohol. Cells were photographed using a Leica DM1000 LED microscope and a Leica DFC 295 digital camera (Leica Microsystems, Germany) at ×400 (for voles) or ×200 (for the wood mouse).

Three groups of sperm were distinguished: normal (without deformations of the structural elements of the cells), with a head defect (including acrosome deformation), and with a tail defect (various types of loops and hairpins) [32]. In total, 200 spermatozoa were examined in each animal: the proportion of normal and abnormal cells was determined in 15–30 random fields of view.

Analysis of Sperm Motility and Concentration

After autopsy, epididymis were removed from males, a thin puncture was made in their tail part, then using a micropipette dispenser (Proline, Sartorius AG, Finland, 0.1–2.5 μL), 0.5 μL of epididymal fluid was taken and placed in a nutrient medium with a volume of 2 mL for 10–15 minutes. Manipulations with the epididymis were performed under an MS-2 stereomicroscope (Biomed, Russia). A mixture of solutions of DMEM (Dulbecco's Modified Eagle's Medium, with L-glutamine and glucose 4.5 g/L) and BSA (bovine serum albumin, 2% in the final solution) was used as a nutrient medium. Both solutions are produced by BioloT (Russia). A mixture of DMEM and BSA is a modification of culture media used to analyze sperm motility in laboratory mice (Crlj:CD1 (ICR) mice) [49] and bank voles from a laboratory colony [50]. The finished mixture was poured into sterile microtubes and placed on a thermotable (Microstat, Tekhnom, Russia) at a temperature of 37°C.

The prepared suspension was filled into both chambers (10 μL of suspension per chamber) of glass slides (2X-CEL chamber, Hamilton Thorne, United States; chamber depth 80 μm) and mounted on a slide heater (MiniTherm, Hamilton Thorne, United States) under the microscope lens. To analyze sperm motility, the right epididymis was used, except for two cases of pronounced asymmetry of the epididymis (the right organ was 2 times or more smaller than the left) and two cases of a technical error during sampling.

Sperm motility was measured using the CEROS CASA system (Computer-assisted sperm analysis): specialized program MouseTraxx v. 12.3 (Hamilton Thorne, United States), microscope (Olympus CX41, Japan) and video camera (Sony XC-ST50, Japan). The movement of spermatozoa was filmed with a ×4 lens magnification for 30 consecutive frames at a speed of 60 fps in three or more fields of view. Cells that appeared in only part of the captured frames were not included in the analysis. An average of 450 (240–1782) sperm movement tracks per individual were analyzed. The following indicators were assessed: Motile—proportion of motile cells; VCL (μm/s)—velocity measured over the actual point-to-point track followed by the sperm cell; VSL (μm/s)—velocity of cell movement in a straight line from the beginning to the end of the track; VAP (μm/s)—velocity of cell movement along its average pathway; ALH (μm)—twice the maximum displacement of a sperm head from its spatial average path; BCF (Hz)—frequency of sperm head movement across the middle plane of its averaged path; STR (%)—straightness of the average path (VSL/VAP) × 100; LIN (%)—linearity of the curve of the captured track (VSL/VCL) × 100 (Appendix, Table S1, Fig. S2).

Concentration values of sperm in the samples were obtained using CASA simultaneously with their motility indicators. To control the hardware determination and check the reproducibility of the results, an MMC-SR counting camera (MMCSoft, Russia) was used. For this purpose, an additional sample of epididymal fluid was taken and diluted in physiological solution (NaCl, 0.9%). A drop of the finished suspension was placed into the counting chamber using a pipette. Cells were photographed and counted using a Leica DM1000 LED microscope and a Leica DFC 295 digital camera (Leica Microsystems, Germany) at a total magnification of ×200 in ten camera grid cells.

To convert the sperm concentration from a diluted suspension into values for the epididymal fluid (epididymate) for manual and automatic determination, the following formula was used: $C = L \times (Vl/Ve)$, where C is the cell concentration in epididymal fluid (cells/mL); L is the measured cell concentration in solution (DMEM or saline) (cells/mL); Vl is the volume of nutrient solution (DMEM or saline) (mL); and Ve is the volume of collected epididymal fluid (mL).

Measuring Metal Concentrations in the Body

Concentrations of priority (based on content in emissions) metals (Cu, Zn, Cd, Pb) were determined in the liver. The choice of organ is due to the known fact of the predominant deposition of toxicants in it [51, 52].

Liver samples from each animal were dried at 75°C until dry. Then, the samples were crushed and weighed on a KERN-770 analytical balance (Germany) with an accuracy of 0.01 mg. A sample of ~100 mg was placed in a Teflon vessel, 7 mL of 65% HNO_3 (OSH) and 1 mL deionized H_2O were added, kept for 30 min, and decomposed in a MWS-2 microwave oven (MWS-2 Berghof, Germany) according to the manufacturer's instructions. The volume was then adjusted to 10 mL of deionized H_2O. Metal concentrations (μg/g dry mass) were measured on a ContrAA 700 vario atomic absorption spectrometer (Analytik Jena, Germany) using flame (for Cu and Zn) and electrothermal (for Cd and Pb) atomization.

The quality of measurements was controlled using the international standard sample BCR-185R (bovine liver). The recovery was 81.5% for Cu, 83.6% for Zn, 94.0% for Cd, and 95.0% for Pb. The detection limit in the flame version of atomization was 0.03 μg/mL for Cu and 0.015 μg/mL for Zn; in the electrothermal version, it was 0.0008 μg/mL for Cd and 0.0025 μg/mL for Pb. If the concentration of an element was below the detection limit, a value equal to half of it was used for further analysis. The analysis (including sample preparation) was carried out in the laboratory of ecotoxicology of populations and communities of the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences.

Statistical Analysis

Statistical analysis was performed using STATISTICA 8 software [53] and the R programming environment (R-project, v. 4.3.2, IDE RStudio v. 2023.09.1+494) [54, 55], as well as the ggplot2 package for visualization [56]. In all cases, the statistical unit was the individual.

Frequency of abnormal sperm. The relationship between the frequencies of various anomalies was assessed using the Spearman rank correlation coefficient (R). The search for possible predictors of the development of sperm anomalies (region, pollution zone, species, intrapopulation group) was carried out using generalized linear models (GLM) for dichotomous characteristics (multiple additive logit regression). Odds ratios (OR) and their 95% confidence intervals (CI) are given after potentiation of logit regression coefficients, logarithms of odds ratios (exp(lnOR) or 1/exp(lnOR)). Mature underyearlings from the background zone in the KMS region were selected as a reference group.

Sperm motility and concentration. The arithmetic mean for the individual was used, since most sperm motility parameters were normally distributed (according to the Shapiro–Wilk test). The structure of correlations between motility indicators was analyzed by the method of principal components (varimax raw rotation was used). Since the initial data was an unbalanced complex, the influence of factors (region, pollution zone, intrapopulation group) on sperm motility and concentration was analyzed using Student's t-test and Fisher's test (F) to test equality of variances. General linear models (LM) were used to analyze the proportions of explained variance in R^2, and one-way analysis of variance was used to assess interspecific differences. Multiple comparisons were performed using Tukey's test. The relationship between instrumental and manual concentrations was assessed using Pearson's linear correlation coefficient (r) (values were pre-logarithmized).

Relationship between sperm parameters and individual toxic load. Since the distribution of metal concentrations had pronounced asymmetry (Shapiro–Wilk test), data preliminarily logarithmized (natural logarithm). To assess the influence of factors (region, pollution zone, species, intrapopulation group) we used multivariate analysis of variance. Multiple comparisons were performed using Tukey's test. The relationship between sperm parameters and individual levels of heavy metal accumulation was assessed using the Pearson linear correlation coefficient (r). To control the false discovery rate (FDR) during multiple tests of statistical hypotheses, the Benjamini–Yekutili correction was used.

Species specificity of changes in indicators. The contribution of different groups of sperm parameters to differences between species was assessed using canonical variates analysis.

The structure of correlations between different sperm parameters were estimated using the principal component method. The values of indicators measured on a multiplicative scale (proportion of abnormal and motile cells, straightness (STR)) were previously converted to an additive scale, logarithm of odds (Log Odds (LO) or logit).

Effect size, i.e., an assessment of the strength of the influence of pollution on the parameter was calculated using the response ratio (log Response Ratio) as the natural logarithm of the ratio of the value in the impact zone to the value in the background zone. The confidence interval was estimated using the LRR function of the SingleCaseES v. 0.7.2 package [57].

RESULTS

Frequencies of Abnormal Sperm

The frequencies of occurrence of sperm head and tail defects were not related to each other (for the bank vole—$R = 0.02$, $p = 0.88$, northern red-backed vole—$R = -0.27$, $p = 0.06$, herb field mouse—$R = 0.07$, $p = 0.80$), so they were further analyzed independently of each other.

The "species" factor significantly influenced the occurrence of abnormal cells: for head defects—$\chi^2(1)_{\text{Wald}} = 21.6$, for tail defects – $\chi^2(1)_{\text{Wald}} = 56.2$ ($p < 0.001$). The odds of detecting head defects in the bank vole were 1.3 (95% CI: 1.2–1.4) times, compared to the herb field mouse – 1.7 (1.3–2.0) times. The chances of detecting tail defects, on the contrary, were 1.2 (1.1–1.2) times lower in the bank vole compared to the northern red-backed vole, and 1.4 (1.3–1.6) times lower than in the herb field mouse. Since the incidence of abnormal sperm varied significantly between species, the influence of factors was considered separately for each species.

All factors (district, pollution zone, group) had different effects on the frequency of abnormal cells. In the MUCS region, head and tail defects were more common in northern red-backed voles, and in KMS region—sperm tail defects in the bank vole (Table S2). In impact zones, the proportion of all defects in *Cl. glareolus* and the proportion of tail defects in *S. uralensis* was higher than in the background zones. In *Cl. rutilus* the frequency of head defects was higher in background zones (Fig. 1, Table S2).

The frequency of abnormal cells in some cases varied among rodents of different intrapopulation groups. In sexually mature bank vole underyearlings, the incidence of tail defects was lower than in overwintered animals. In *Cl. rutilus* head defects were more common in mature yearlings, and tail defects were more common in overwintered individuals. In *S. uralensis* all types of sperm pathologies were more common in mature underyearlings. In *Cl. glareolus* most of the effects found were weak since the odds ratio was close to unity (see Table S2). In other words, the likelihood

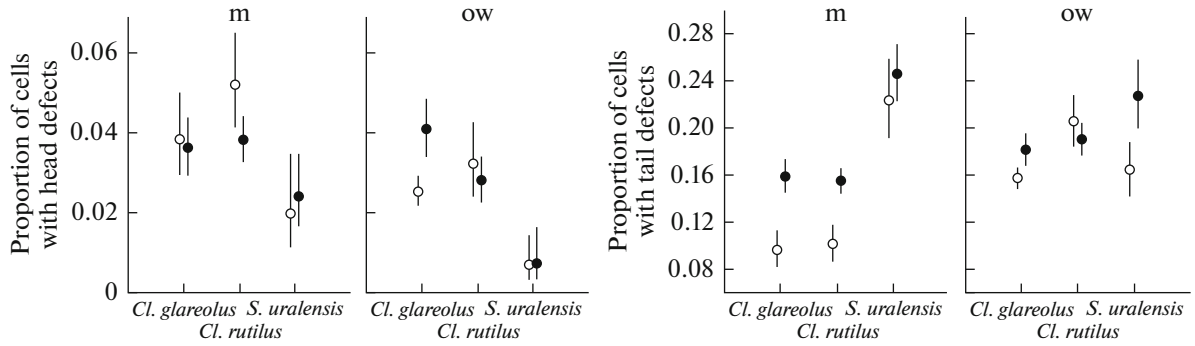

Fig. 1. Frequencies of abnormal spermatozoa (mean, 95% CI) in rodents from background (white marker) and impact (black marker) zones. Intrapopulation groups: m—mature young of the year, ow—overwintered individuals.

of detecting abnormal sperm due to one factor or another is low.

In the northern red-backed vole and the herb field mouse, some effects associated with head defects were more pronounced. For example, the odds of detecting head anomalies were 2–3 times higher depending on the "area" or "group." But even with the maximum chance (in *S. uralensis* the probability of detecting head defects was 3.3 times higher in underyearlings than in overwintered individuals); the frequency of occurrence of head defects in this species did not exceed 3.5% (see Fig. 1).

Sperm Motility

Due to the discovered species-specificity of sperm motility indicators (Application, Table 3) they were analyzed separately for each species. In *Cl. glareolus* the first two main components (PC 1 and PC 2) of the variability in sperm motility indicators accounted for 84.2% of the total variance; *Cl. rutilus*—84.4%, in *S. uralensis*—92.1% (Appendix, Table S4). In all three species, the main contribution to the first principal component was made by indicators characterizing the velocity of spermatozoa, and the second, by the direction of movement. For further analysis, we chose two well-interpreted indicators with high factor loadings that do not correlate with each other: VCL (reliably contributes to PC 1) and STR (reliably contributes to PC 2). The proportion of motile cells (Motile) was also analyzed.

Belonging to one or another intrapopulation group did not affect sperm motility indicators: for *Cl. glareolus*—$|t| = 0.82–1.65$, $p = 0.103–0.417$; for *Cl. rutilus*—$|t| = 0.47–1.30$, $p = 0.200–0.637$; For *S. uralensis*—$|t| = 0.13–1.70$, $p = 0.108–0.900$. The "area" factor also did not affect the indicators of sperm motility in rodents: for *Cl. glareolus*—$t| = 0.31–1.27$, $p = 0.209–0.756$; for *Cl. rutilus*—$|t| = 0.32–0.56$, $p = 0.575–0.752$; for *S. uralensis*—$|t| = 0.61–1.58$, $p = 0.133–0.548$. Since the influence of these factors turned out to be insignificant, animals from different groups and from different areas were combined for further analysis.

The effect of the pollution zone on Motile and VCL in the bank vole turned out to be significant: in the impacted zones the proportion of motile cells and their velocity were lower than in the background ($|t|_{Motile} = 2.60$, $p = 0.011$; $|t|_{VCL} = 2.43$, $p = 0.018$) (Fig. 2). However, the factor "pollution zone" explained only 9% of the total variance for Motile and 8% for VCL. In the northern red-backed vole and the herb field mouse, both indicators did not differ between zones: for *Cl. rutilus* – $|t|_{Motile} = 0.21$, $p = 0.837$; $|t|_{VCL} = 1.87$, $p = 0.067$); For *S. uralensis* – $|t|_{Motile} = 0.29$, $p = 0.772$; $|t|_{VCL} = 0.92$, $p = 0.373$.

The directionality index (STR) of sperm in all species did not differ between zones: for *Cl. glareolus*—$|t| = 0.60$, $p = 0.548$; for *Cl. rutilus*—$|t| = 0.76$, $p = 0.451$; for *S. uralensis* – $|t| = 1.21$, $p = 0.242$. Also, the bank vole in the impact zones had higher variability in the Motile index ($CV_{Bg} = 14.85$; $CV_{Im} = 25.18$; $F_{Motile}(27, 42) = 2.26$, $p = 0.017$), only here were individuals found with extremely low sperm motility rates (see Fig. 2). In the herb field mouse, on the contrary, the variability of the Motile index was higher in animals in the background zones: $CV_{Bg} = 63.81$; $CV_{Im} = 32.81$; $F_{Motile}(9, 7) = 4.34$, $p = 0.045$.

Sperm Concentration

The concentration values for manual and automatic measurements correlated with each other ($r = 0.63$, $p < 0.05$). Sperm concentrations in DMEM and epididymal fluid *Cl. glareolus* and *Cl. rutilus* did not differ, while in both species of voles the concentration of cells was almost 5 times higher than in *S. uralensis*. The total number of sperm in the epididymis varied in different species (Appendix, Table S5).

Indicators of the concentration of epididymal spermatozoa in rodents (as well as indicators of motility) did not depend on belonging to a particular group and region: for *Cl. glareolus* – $|t|_{group} = 0.56$, $p = 0.579$, $|t|_{area} =$

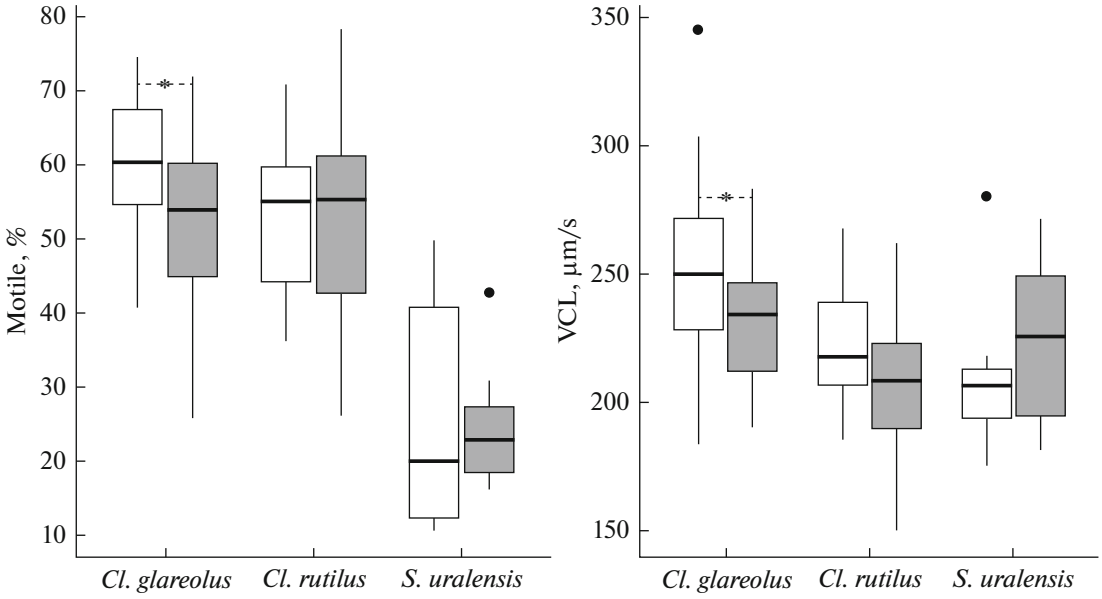

Fig. 2. The proportion of motile sperm (Motile) and the curvilinear line velocity (VCL) in rodents from background (without filling) and impact (gray filling) zones: horizontal line—median, box boundaries—interquartile range, whiskers—minimum and maximum values, not exceeding 1.5 interquartile range, point—outlier; *—differences are statistically significant ($p < 0.05$).

0.25, $p = 0.803$; For *Cl. rutilus*—$|t|_{group} = 0.40$, $p = 0.694$, $|t|_{area} = 1.17$, $p = 0.246$; for *S. uralensis*—$|t|_{group} = 0.55$, $p = 0.592$, $|t|_{area} = 1.24$, $p = 0.232$.

Since the differences between area and groups turned out to be insignificant, the samples were then combined. For all three species, the influence of the pollution zone also turned out to be insignificant: for *Cl. glareolus*—$|t| = 0.37$, $p = 0.711$; For *Cl. rutilus*—$|t| = 0.68$, $p = 0.497$; For *S. uralensis*—$|t| = 0.48$, $p = 0.638$. Although no statistically significant effects associated with the pollution zone were detected; in both species of voles, the lowest sperm concentrations were found in impact zones. In addition, in the northern red-backed vole, the variability of concentration indicators in impact zones was higher than in background zones: $CV_{Bg} = 30.03$, $CV_{Im} = 49.83$; $F(37, 13) = 3.37$, $p = 0.022$ (Fig. 3).

Relationship between Sperm Parameters and Individual Toxic Load

The studied factors had different effects on the accumulation of metals in rodents (Fig. 4, Appendix, Table S6). The influence of the intrapopulation group on the accumulation of metals turned out to be insignificant (Appendix, Table S7), therefore, to analyze other sources of variability, underyearlings and overwintered individuals were combined. The "region" factor influenced the accumulation of all elements except Cu: rodents accumulated more Cd near MUCS, and Zn and Pb near KCS (Table 2). The pollution zone affected only the accumulation of Cd: in animals in the impacted zones, the concentrations of this element were higher than in the background ones. Species differences were also found in the accumulation of Cd (*Cl. rutilus* > *Cl. glareolus* > *S. uralensis*) and Zn (*Cl. rutilus* > *S. uralensis*): voles accumulated more metals than the herb field mouse.

In none of the species were sperm parameters associated with individual toxic load (Appendix, Table S8). The detected weak negative relationships between

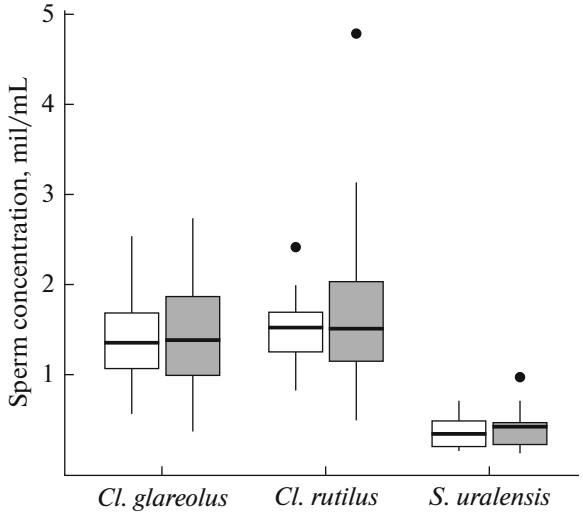

Fig. 3. Concentration of epididymal spermatozoa in rodents from background (without filling) and impact (gray filling) zones. For other designations, see Fig. 2.

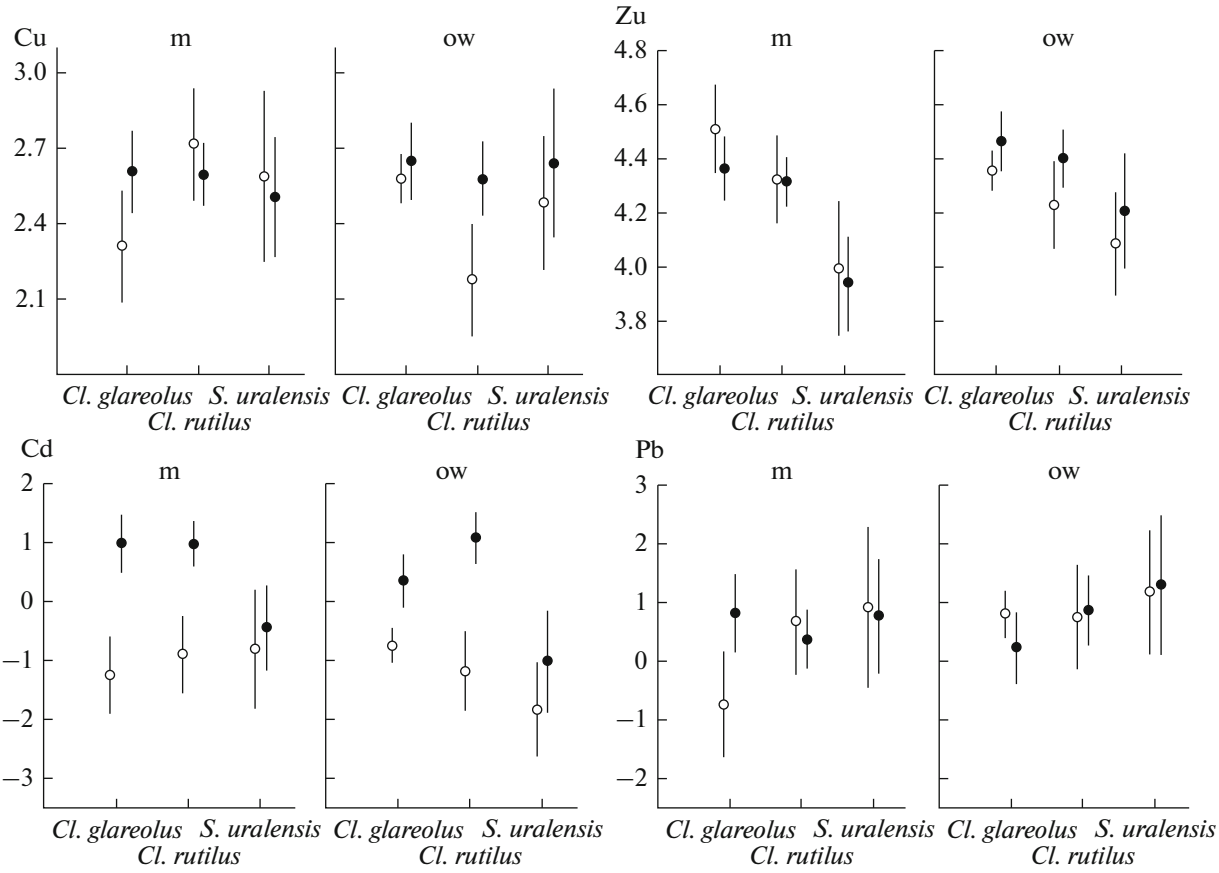

Fig. 4. Concentrations (μg/g) of Cu, Zn, Cd and Pb in the liver (mean, 95% CI, logarithm values) of rodents from background (white marker) and impact (black marker) zones; intrapopulation groups: m—mature yearlings, ow—overwintered individuals.

some parameters and metal concentrations were leveled out by correction for multiple testing of statistical hypotheses.

Species Specificity of Sperm Parameters

The species differed significantly in terms of motility (velocity and straightness of movement, the proportion of motile cells) and sperm concentration, but the proportion of cells with head and tail defects turned out to be insignificant for their differentiation (Appendix, Table S9).

In the first canonical variate axis (CV 1, explains 91.6% of the variance), the greatest contribution was made by concentration, straightness of movement (STR) and the proportion of motile cells (Motile), in the second (CV 2, explains 8.6% of the variance), the velocity of sperm movement (VCL). The differences in spermatozoa, as expected, were maximum between voles and the herb field mouse, and minimal between vole species (Fig. 5, Appendix, Table S10).

Structure of Correlations between Sperm Parameters

In detail, the structure of correlations between sperm parameters in each species differed, however, in all rodents, the indicators of motility and cell concentration turned out to be the most interconnected (see Table 3): for example, in *Cl. glareolus* basic contribution to variability was made by the concentration and straightness of cell movement (PC 1); in *Cl. rutilus*, in addition to these indicators (which were also included in PC 1), the maximum contribution was made by the proportion of motile cells (PC 2); in *S. uralensis*, the main contribution to variability was made by cell movement indicators (VCL, STR; PC 1) and the proportion of cells with tail defects (PC 2).

Some sperm parameters were similarly associated across species, but others were not. In both species of voles, the proportion of motile spermatozoa (Motile) was positively correlated with movement speed (VCL, $r = 0.3–0.4$), and their concentration is negative with straightness of movement (STR, $r = -0.8$ to -0.4) (Fig. 6).

Although no strong connection was found between the proportion of motile cells and the proportion of cells with tail defects, these two indicators turned out

Table 2. ANOVA results of metal accumulation in the liver of rodents

Element	Source of Variability	MS	*df*	*F*	$p \leq$
Cu	Area	0.04	1	0.51	0.4752
	Pollution zone	0.12	1	1.40	0.2388
	Species	0.02	2	0.29	0.7478
Zn	Area	0.34	1	7.83	**0.0059**
	Pollution zone	0.04	1	0.96	0.3299
	Species	0.81	2	18.72	**0.0001**[b, c]
Cd	Area	9.18	1	12.18	**0.0007**
	Pollution zone	28.91	1	38.36	**0.0001**
	Species	8.85	2	11.74	**0.0001**[a, b, c]
Pb	Area	9.20	1	6.49	**0.0120**
	Pollution zone	0.30	1	0.21	0.6441
	Species	1.63	2	1.15	0.3192

Capital letters indicate differences (Tukey test, $p < 0.05$; $df = 109$) between: (a) *Cl. glareolus* and *Cl. rutilus*, (b) *Cl. glareolus* and *S. uralensis*, and (c) *Cl. rutilus* and *S. uralensis*; values of $p < 0.05$ are in bold font.

Table 3. Structure of correlations between rodent sperm parameters (results of principal component analysis)

Index	*Cl. glareolus*, $n = 71$		*Cl. rutilus*, $n = 52$		*S. uralensis*, $n = 18$	
	PC 1	PC 2	PC 1	PC 2	PC 1	PC 2
Proportion of cells with head defects	−0.19	−0.40	0.04	−0.65	−0.62	0.37
Proportion of cells with tail defects	−0.46	−0.25	−0.24	0.16	−0.15	**0.83**
Curvilinear line velocity (VCL)	0.57	0.55	0.42	−0.49	**−0.78**	0.30
Straightness (STR)	**−0.88**	0.35	**−0.89**	−0.23	**−0.72**	−0.42
Proportion of motile cells (Motile)	0.57	0.30	0.16	**−0.72**	−0.61	−0.25
Cell concentration	**0.72**	−0.62	**0.90**	0.20	0.31	0.41

Maximum (>70%) factor loadings on the principal components (PC 1, PC 2) are highlighted in bold.

to be in different directions: in all rodent species, as the proportion of abnormal cells increased, the proportion of motile cells decreased. Only in one species *Cl. glareolus*, a consistency in the response to pollution was found between all sperm parameters: a simultaneous increase in the proportion of abnormal cells, a decrease in the proportion of motile cells, and their velocity in impact zones (see Appendix, Fig. S3). However, despite the statistical significance, all detected effects were weak and explained only 2.9–9.0% of the total variance.

Effect Sizes

For only one species, the bank vole, confidence intervals for effect sizes (differences between logarithms of values from impact and background sites) do not include zero, i.e., they can be considered statistically significant. Of the sperm measures, effect sizes are statistically significant only for movement velocity (VCL) and proportion of motile cells (Motile), but they are much smaller compared to the effect sizes for Cd accumulation and relative abundance (Fig. 7). Confidence intervals for the remaining sperm parameters include zero, although the effect sizes themselves take on nonzero values. All the considered parameters in the bank vole change in a consistent manner: with an increase in the toxic load (Cd concentration in the liver of animals), the relative abundance, the proportion of motile sperm and their velocity decrease, but the number of abnormal cells increases.

DISCUSSION OF THE RESULTS

The Influence of Chemical Pollution on the Occurrence of Abnormal Spermatozoa

A study of sperm morphology, performed on new samples, confirmed earlier conclusions about the difference in the frequency of occurrence of cells with head and tail defects [32]. While tail defects can always

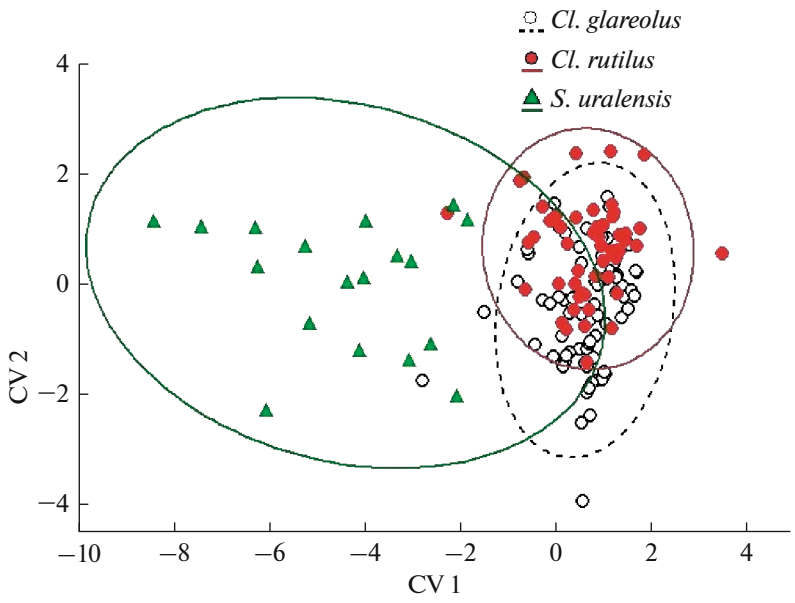

Fig. 5. Distribution of individuals of three rodent species according to sperm parameters (proportion of cells with head and tail defects, STR, Motile, VCL, cell concentration) in the space of two canonical variate axes (CV 1, CV 2); ellipses—95% CI.

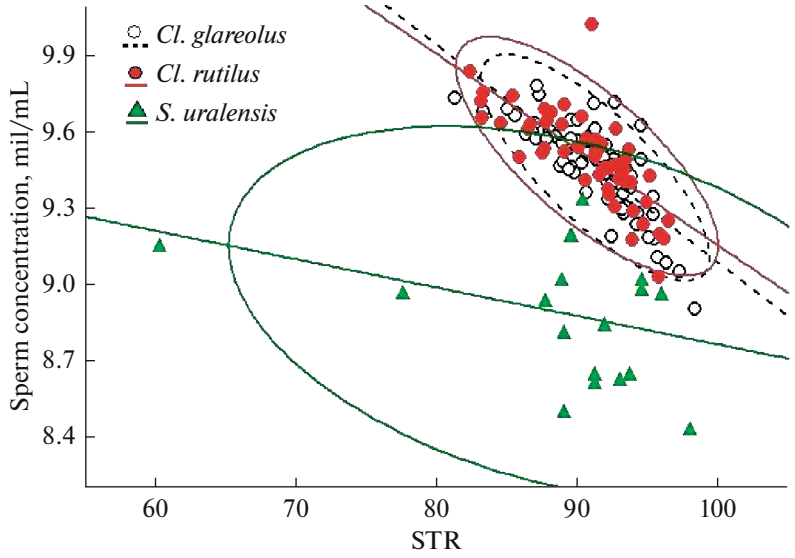

Fig. 6. Relationship between sperm straightness (STR) and sperm concentration; ellipse—95% CI.

be found among epididymal spermatozoa of rodents, head defects are less common or may be absent. However, they are not related to each other, and the differing frequencies of occurrence and different reactions to the factors under study support the opinion of different reasons for their occurrence [58].

Disturbances in the structure of sperm indicate a decrease in their fertilizing ability, therefore it is no coincidence that the influence of environmental factors is more often studied in relation to extreme and therefore clearly distinguishable variants of morphological variability of sperm anomalies [29, 59, 60]. There are several classifications of abnormal forms of sperm, including for rodents [50, 61–63]. We used a rough version of the classification (without detailed defects) and considered only two groups of anomalies (head and tail defects), which, if necessary, allows us to compare our results with the materials of other authors.

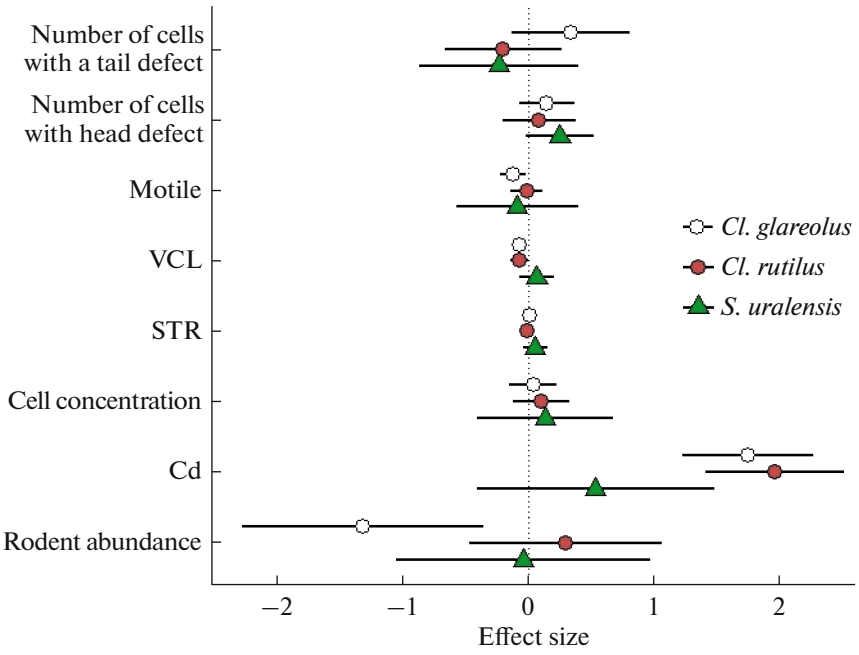

Fig. 7. Effect sizes (95% CI) for sperm parameters, liver Cd concentrations, and relative rodent abundance.

Previously, we examined in detail the controversial issues about the importance of morphological indicators for assessing potential fertility, as well as the effect of chemical pollution on structural indicators [32]. Despite skepticism regarding the prognostic value of morphological characteristics of sperm (in reproductive medicine, it is reflected in a decrease in the reference value of the proportion of normal sperm [64]), the study of the structure of germ cells remains an important part of assessing their quality. In addition, there is inconsistency and scarcity of data on the effect of chemical pollution on the morphology of sperm of "wild" rodents [6, 26, 27] determine the need to continue full-scale and experimental studies of this group of animals.

The results on the variability of frequencies of abnormal cells are consistent with data for other samples of rodents from background and impact zones of both regions [32, 65–67]. Although the direction of the effects did not always coincide (for the polluted zones and the intrapopulation group), the strength of the effects was always at a very low level: odds ratio estimates were close to unity in all cases. Thus, slight differences and incomplete agreement in the frequencies of abnormal sperm in different samples (i.e., different species and intrapopulation groups) may indicate a natural level of variability in this indicator, and not the influence of chemical pollution.

The Effect of Chemical Pollution on Sperm Motility

Motility is considered the most informative parameter of sperm [68], since it is what determines their functional properties. We have previously reviewed in detail methods for measuring sperm motility (including using automatic analysis systems), determining reference parameters in voles, and comparing the obtained values with data from other authors [31].

Motility analysis performed for three species also showed the species-specific characteristics of spermatozoa: most of them in the herb field mouse are significantly lower than in voles. Differences may be due to characteristics of spermatozoa in representatives of the group/section *Apodemus* (including *Sylvaemus*). Apart from the obvious differences related to cell size (acrosome size, head area, and tail length, *S. uralensis* more than in voles), these features include the ability of cells to form clusters (the so-called sperm-train). This ability may provide a more reliable path to the egg compared to individual cells. However, the relationship between cell size, sperm-train formation, and motility remains controversial [22, 69, 70].

Sperm motility indicators did not depend on the region or population group. In two species (*Cl. rutilus* and *S. uralensis*), motility (Motile, VCL, STR) did not differ between populations living in background and polluted zones. However, for *Cl. glareolus* in heavily polluted zones, the proportion of motile cells (Motile) and sperm velocity (VCL) were lower compared to background, but pollution explained only 9 and 8% of their variance, respectively. In other words, pollution affected sperm motility, but very weakly.

Effect of Chemical Pollution on Sperm Concentration

Determination of sperm concentration in humans and commercial animals is a routine procedure regulated by WHO and various national and international guidelines. [13, 71, 72]. For laboratory animals, including rodents, regulations have also been developed that involve excision and homogenization of the epididymis [49, 73]. However, the search for new, optimal methods for determining cell concentration and related methods for selecting ejaculated or epididymal sperm is still ongoing.

A variety of methods are used to measure the concentration (or absolute number) of sperm in rodents, causing results to differ from one study to another, which makes it difficult or impossible to compare [21, 26–28, 50, 74–76]. The method we propose allows us to simultaneously study indicators of sperm motility and concentration in several replicates, and samples from the caudal part of the epididymis can be taken multiple times.

We did not find a significant effect of the pollution zone on sperm concentration. This is contrary to the results of some toxicology experiments in which the classical dose relationship between toxicant levels and sperm concentration has been demonstrated. So, in the experimental group of *Cl. glareolus* from a laboratory colony seeded with copper sulfate and aluminum chloride decreased the concentration of epididymal sperm due to a decrease in the activity of the spermatogenic epithelium of the testis (according to the representation of different cell types) [26, 27]. The authors cited suggested that the effect is due to an increase in the amount of testosterone (so-called testosterone overload), since this hormone in large quantities suppresses sperm production and inhibits the secretion of hypothalamic hormones involved in steroidogenesis [77].

In contrast, L.V. Tannenbaum et al. [28, 29, 74, 78], did not find differences in the concentration of spermatozoa of rodents living in background and polluted zones: the values never reached "threshold" levels (a decrease in concentration by 60–80%). In our study, this indicator was also insensitive to recorded pollution levels.

Relationship between Individual Toxic Load and Sperm Parameters

Data on individual levels of accumulation of heavy metals in animal organs and tissues serve as an indicator of the toxic load on the body [5, 79–81]. For these purposes, as a rule, we examine the organs of primary deposition of heavy metals: liver, kidneys, and bones of the postcranial skeleton. We examined the concentrations of biophilic (Cu and Zn) and toxic (Cd and Pb) elements in the liver, since it is considered one of the main target organs for metals [51, 52].

Many researchers [80–89] believe that animals in polluted zones accumulate more toxicants than in background ones, and the concentrations of toxicants are higher in adult animals than in young ones. In addition, different species (supraspecific taxa) accumulate toxicants differently: lower concentrations of elements in mice compared to voles are explained by the peculiarities of their biology, predominant feeding on seeds, which are maximally protected from the intake of excess metals, greater motility, etc. [90].

Our data did not fully confirm the known patterns of metal accumulation by small mammals. With the exception of a single element (Cd), no relationship was found between the concentrations of elements in the body with the zone of pollution and the age of the animals. In contrast to what was expected, there was no dependence of sperm parameters on metal concentrations in the body.

The reasons for the absence or weakness of individual toxic effects have been repeatedly discussed. These include different behavior of essential and toxic elements in the body (including their synergistic or antagonistic interactions), high individual variability and specificity of accumulation of elements by different intrapopulation groups, and insufficiently high levels of recorded pollution and/or its mosaic nature [5, 28, 29, 91–95].

The discovered differences between the regions (in the MUCS region rodents accumulate more Cd, in the KCS region—Zn and Pb) well illustrate the polyetiological nature of the accumulation of heavy metals in the body. These differences can be explained by the geographic location of pollution sources, production cycles, emission treatment systems, ore composition, etc. For example, the KCS region is distinguished from MUCS by a more complex orography (determines a highly mosaic distribution of pollutants) and a less sharp decrease in gross emissions after the reconstruction of the enterprise (see Appendix, Fig. S1).

Species-Specific Response of Sperm to Pollution

Most sperm parameters turned out to be species specific, even when considering closely related *Cl. glareolus* and *Cl. rutilus*. This appears to reflect different reproductive strategies of the species. The results of the analysis of the structure of relationships between predictors and dependent variables (see Table S7) confirm significant interspecies differences in rodent spermatozoa, discovered when testing separate groups of indicators (morphology, motility, concentration).

The reaction of sperm to pollution also turned out to be species-specific: voles reacted more strongly compared to the herb field mouse. The severity of the detected effects (for sperm motility) decreased in the series *Cl. glareolus* > *Cl. rutilus* > *S. uralensis*. Unfortunately, it cannot be ruled out that less pronounced

effects (or lack thereof) in *Cl. rutilus* and *S. uralensis* may be due to small sample sizes for these species. However, extremely low concentrations of metals in the liver of *S. uralensis* in impact zones correspond to the lack of influence of intoxication of the body with heavy metals on sperm parameters.

Structure of Correlations between Different Sperm Parameters and Effect Sizes

One of the key issues of our work is the analysis of the consistency of changes between groups of sperm parameters. Many authors have studied several sperm parameters simultaneously [26–29, 60, 96], however, general patterns regarding the direction of changes in indicators under the influence of various factors have not yet been identified. Our analysis of the structure of correlations between sperm parameters showed that motility and cell concentration were the most related to each other. Although the variability of morphological parameters was statistically insignificant, the proportion of abnormal cells and motility indicators changed in the impact zones in the same direction, towards a deterioration in sperm quality. This consistency of response to chemical pollution can lead to the summation of many weak effects, making the resulting population effect significant.

It is important to emphasize that studying the quality of sperm is necessary to assess the potential fertility of males, but is not sufficient to determine their actual fertilizing ability [97, 98]. It is impossible to accurately determine the optimal quality of sperm for pregnancy, the number of fetuses, or the survival rate of offspring, since after the formation of a zygote is a complex of factors, associated not only with the quality of sperm.

The use of effect sizes made it possible to clearly compare entities of different sizes, expressing them on a single scale. The effect sizes for sperm parameters were significantly smaller compared to the relative rodent abundance, the resulting estimate of population reproduction (see Fig. 7). On the other hand, the unidirectionality of these effects (in impact zones the relative rodent abundance and motility of sperm are lower) can be considered as indirect evidence of the existence of a connection between the quality of sperm and the final population size. To a certain extent, this allows us to get closer to estimating the realized fertility of males.

In the discussion about the sensitivity of sperm parameters to toxic load, our results can be regarded as contradictory: on the one hand, germ cells react to chemical pollution, but on the other hand, the reaction to it is weak (see Fig. 7). The weakness of the effects of pollution can be explained by the presence of evolutionarily developed homeostatic barriers, primarily hematotesticular, which reliably protect the reproductive function of animals [99, 100]. The reasons for the weakness of the effects can also include the spatial mosaic nature of the toxic load and the motility of rodents, which allows them to avoid unfavorable habitats. In addition, it cannot be ruled out that the level of pollution studied was insufficient to cause a significant shift in reproductive rates. This necessitates the verification of our findings for the effects of other sources of industrial pollution. In any case, our results add arguments to those researchers who consider sperm to be "invulnerable" to toxic effects [1, 28, 29], and not declaring the high sensitivity of germ cells [26, 27].

CONCLUSIONS

In this work, we for the first time compared for several species of rodents (*Cl. glareolus*, *Cl. rutilus*, *S. uralensis*) living in zones with contrasting levels of chemical pollution from industrial emissions, the information content of various indicators of epididymal sperm. It turned out that pollution worsens the quality of sperm in rodents from natural populations, but the reaction to pollution is weak. Therefore, none of the sperm parameters studied can be a reliable marker of industrial pollution, at least for the levels of metal pollution studied.

Insignificant differences between the MUCS and KCS areas in most tests may indicate similarities in the impact of the two pollution sources and the identification of patterns that are not related to the specific emissions of a particular enterprise. The indicators of morphology, motility, and concentration of epididymal sperm in the studied rodents also do not depend on the variant of ontogenesis and reach definitive values in mature underyearlings and overwintered individuals; therefore, the potential contribution of males of these groups to the reproduction of the population can be considered equivalent.

Our assumptions about the different sensitivity of sperm parameters and the species-specificity of their response to pollution were confirmed. Sperm motility turned out to be more sensitive to pollution compared to morphology, which is consistent with the idea that cellular structures are conserved. At the same time, the detected response, although weak, of sperm motility to pollution allows for the future study of other important functional characteristics (for example, the level of apoptosis, the content of extra- and intracellular nucleic acids).

The reaction of sperm to pollution turned out to be species-specific: voles reacted more strongly compared to the herb field mouse. This result highlights the risk of extrapolating results from one species to another and the importance of including different species in ecotoxicological studies.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at https://doi.org/10.1134/S106741362470005X.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures performed when working with animals complied with the ethical standards of the Institute of Plant and Animal Ecology of the Russian Academy of Sciences (Protocol no. 4 of January 26, 2021).

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

REFERENCES

1. Llobet, J.M., Colomina, M.T., Sirvent, J.J., et al., Reproductive toxicology of aluminum in male mice, *Fundam. Appl. Toxicol.*, 1995, vol. 25, no. 1, pp. 45–51.
2. Mukhacheva, S.V., Reproduction of the bank vole population *Clethrionomys glareolus* (Rodentia, Cricetidae) along gradient of industrial environmental pollution, *Zool. Zh.*, 2001, vol. 80, no. 12, pp. 1509–1517.
3. Mukhacheva, S.V., Long-term dynamics of bank vole reproduction rates during the period of reduction of industrial emissions, *Prints. Ekol.*, 2016, no. 3, pp. 103.
4. Damek-Poprawa, M. and Sawicka-Kapusta, K., Damage to the liver, kidney, and testis with reference to burden of heavy metals in yellow-necked mice from areas around steelworks and zinc smelters in Poland, *Toxicology*, 2003, vol. 186, nos. 1–2, pp. 1–10.
5. Damek-Poprawa, M. and Sawicka-Kapusta, K., Histopathological changes in the liver, kidneys, and testes of bank voles environmentally exposed to heavy metal emissions from the steelworks and zinc smelter in Poland, *Environ. Res.*, 2004, vol. 96, no. 1, pp. 72–78.
6. Ieradi, L.A., Zima, J., Allegra, F., et al., Evaluation of genotoxic damage in wild rodens from a polluted area in the Czech Republic, *Folia Zoologica*, 2003, vol. 52, no. 1, pp. 57–66.
7. Bezel', V.S., *Ekologicheskaya toksikologiya: populyatsionnyi i biotsenoticheskii aspekty* (Ecological Toxicology: population and Biocoenotic Aspects), Ekaterinburg: Goshchitskii, 2006.
8. Ivanter, E.V. and Medvedev, N.V., *Ekologicheskaya toksikologiya prirodnykh populyatsii ptits i mlekopitayushchikh Severa* (Environmental Toxicology of Natural Populations of Birds and Mammals in the North), Moscow: Nauka, 2007.
9. Amuno, S., Niyogi, S., Amuno, M., and Attitaq, J., Heavy metal bioaccumulation and histopathological alterations in wild Arctic hares (*Lepus arcticus*) inhabiting a former lead-zinc mine in the Canadian high Arctic: A preliminary study, *Sci. Total Environ.*, 2016, vol. 556, pp. 252–263.
10. Ambaryan, A.V., Mal'tsev, A.N., and Kotenkova, E.V., Relationship between characteristics of sexual behavior and male sperm competitive ability in taxa of superspecies complex *Mus musculus sensu lato, Zh. Obshch. Biol.*, 2015, vol. 76, no. 3, pp. 212–224.
11. Potapova, O.F., Potapov, M.A., Kondratyuk, E.Yu., and Evsikov, V.I., Dynamics of testosterone concentration in male steppe lemmings (*Lagurus lagurus*) in the reproductive cycle reflects the species-specific mating system, *Dokl. Ross. Akad. Nauk*, 2016, vol. 468, no. 6, pp. 716–718.
12. *WHO Laboratory Manual for the Examination and Processing of Human Semen*, Geneva: World Health Organization, 2021.
13. International Organization for Standardization ISO 23162: 2021 Basic semen examination – Specification and test methods. https://www.iso.org/ru/standard/74800.html. Cited December 1, 2023.
14. Wadi, S.A. and Ahmad, G., Effects of lead on the male reproductive system in mice, *J. Toxicol. Environ. Health, Part A*, 1999, vol. 56, no. 7, pp. 513–521.
15. Pichardo-Matamoros, D., Sevilla, F., Elizondo-Salazar, J., et al., Exploration of semen quality analyzed by casa-mot systems of brahman bulls infected with BLV and BHV-1, *Sci. Rep.*, 2023, vol. 13, no. 1, p. 18659.
16. Hughes, R.L., Comparative morphology of spermatozoa from five marsupial families, *Aust. J. Zool.*, 1965, vol. 13, no. 4, pp. 533–544.
17. Malo, A.F., Gomendio, M., Garde, J., et al., Sperm design and sperm function, *Biol. Lett.*, 2006, vol. 2, no. 2, pp. 246–249.
18. Gizejewski, Z., Szafranska, B., Steplewski, Z., et al., Cottonseed feeding delivers sufficient quantities of gossypol as a male deer contraceptive, *Eur. J. Wildl. Res.*, 2008, vol. 54, no. 3, pp. 469–477.
19. Wolf, K.N., Wildt, D.E., Vargas, A., et al., Age-dependent changes in sperm production, semen quality, and testicular volume in the black-footed ferret (*Mustela nigripes*), *Biol. Reprod.*, 2000, vol. 63, no. 1, pp. 179–187.
20. Bierla, J.B., Gizejewski, Z., Leigh, C.M., et al., Sperm morphology of the Eurasian beaver, *Castor fiber*: An example of a species of rodent with highly derived and pleiomorphic sperm populations, *J. Morphol.*, 2007, vol. 268, no. 8, pp. 683–689.
21. Gómez Montoto, L., Magaña, C., Tourmente, M., et al., Sperm competition, sperm numbers and sperm quality in muroid rodents, *PLoS One*, 2011, vol. 6, no. 3, p. 1-10/e18173.

22. Tourmente, M., Zarka-Trigo, D., and Roldan, E.R.S., Is the hook of muroid rodent's sperm related to sperm train formation? *J. Evol. Biol.*, 2016, vol. 29, no. 6, pp. 1168–1177.

23. Lakoski, K.A., Carron, C.P., Cabot, C.L., and Saling, P.M., Epididymal maturation and the acrosome reaction in mouse sperm: Response to zona pellucida develops coincident with modification of M42 antigen, *Biol. Reprod.*, 1988, vol. 38, no. 1, pp. 221–233.

24. Cooper, T.G., Sperm maturation in the epididymis: A new look at an old problem, *Asian J. Androl.*, 2007, vol. 9, no. 4, pp. 533–539.

25. Monteiro, G.A., Papa, F.O., Zahn, F.S., et al., Cryopreservation and fertility of ejaculated and epididymal stallion sperm, *Anim. Reprod. Sci.*, 2011, vol. 127, nos. 3–4, pp. 197–201.

26. Miska-Schramm, A., Kruczek, M., and Kapusta, J., Effect of copper exposure on reproductive ability in the bank vole (*Myodes glareolus*), *Ecotoxicology*, 2014, vol. 23, no. 8, pp. 1546–1554.

27. Miska-Schramm, A., Kapusta, J., and Kruczek, M., The effect of aluminum exposure on reproductive ability in the bank vole (*Myodes glareolus*), *Biol. Trace Elem. Res.*, 2017, vol. 177, no. 1, pp. 97–106.

28. Tannenbaum, L.V., Bazar, M., Hawkins, M.S., et al., Rodent sperm analysis in field-based ecological risk assessment: Pilot study at Ravenna army ammunition plant, Ravenna, Ohio, *Environ. Pollut.*, 2003, vol. 123, no. 1, pp. 21–29.

29. Tannenbaum, L.V., Thran, B.H., and Williams, K.J., Demonstrating ecological receptor health at contaminated sites with wild rodent sperm parameters, *Arch. Environ. Contam. Toxicol.*, 2007, vol. 53, no. 3, pp. 459–465.

30. Roldan, E.R.S., Sperm competition and the evolution of sperm form and function in mammals, *Reprod. Domest. Anim.*, 2019, vol. 54, pp. 14–21.

31. Smirnov, G.Yu., Sperm motility in bank (*Clethrionomys glareolus*) and northern red-backed voles (*Cl. rutilus*) exposed to industrial pollution, *Russ. J. Ecol.*, 2022, vol. 53, no. 1, pp. 48–57.

32. Smirnov, G.Yu. and Davydova, Yu.A., Effect of industrial pollution of the environment on the frequency of abnormal spermatozoa in the bank vole, *Myodes glareolus, Russ. J. Ecol.*, 2018, vol. 49, no. 5, pp. 459–463.

33. Olenev, G.V., Alternative types of ontogeny in cyclomorphic rodents and their role in population dynamics: An ecological analysis, *Russ. J. Ecol.*, 2002, vol. 33, no. 5, pp. 321–330.

34. Olenev, G.V., Determining the age of cyclomorphic rodents: Functional-ontogenetic determination, ecological aspects, *Russ. J. Ecol.*, 2009, vol. 40, no. 2, pp. 93–104.

35. Vorobeichik, E.L., Davydova, Yu.A., Kaigorodova, S.Yu., and Mukhacheva, S.V., Study of small mammals of the Visimsky Nature Reserve: contribution to population ecotoxicology? in *Rezul'taty izucheniya prirody Visimskogo biosfernogo zapovednika* (Results of Nature Studies in the Visim Biosphere Reserve), Ekaterinburg, 2006, pp. 108–129.

36. Vorobeichik, E.L. and Pishchulin, P.G., Industrial pollution reduces the effect of trees on forming the patterns of heavy metal concentration fields in forest litter, *Russ. J. Ecol.*, 2016, vol. 47, no. 5, pp. 431–441.

37. Vorobeichik, E.L. and Kaigorodova, S.Yu., Long-term dynamics of heavy metals in the upper horizons of soils in the region of a copper smelter impacts during the period of reduced emission, *Eurasian Soil Sci.*, 2017, vol. 50, no. 8, pp. 977–990.

38. Korkina, I.N. and Vorobeichik, E.L., Humus Index as an indicator of the topsoil response to the impacts of industrial pollution, *Appl. Soil Ecol.*, 2018, vol. 123, pp. 455–463.

39. Kozlov, M., Zvereva, E., and Zverev, V., *Impacts of Point Polluters on Terrestrial Biota: Comparative Analysis of 18 Contaminated Areas*, Dordrecht: Springer Science & Business Media, 2009, vol. 15.

40. Mukhacheva, S.V., Long-term dynamics of small mammal communities in the period of reduction of copper smelter emissions. 1. Composition, abundance, and diversity, *Russ. J. Ecol.*, 2021, vol. 52, no. 1, pp. 84–93.

41. Vorobeichik, E.L., Trubina, M.R., Khantemirova, E.V., and Bergman, I.E., Long-term dynamic of forest vegetation after reduction of copper smelter emissions, *Russ. J. Ecol.*, 2014, vol. 45, no. 6, pp. 498–507.

42. Trubina, M.R., Vorobeichik, E.L., Khantemirova, E.V., et al., Dynamics of forest vegetation after the reduction of industrial emissions: Fast recovery or continued degradation? *Dokl. Biol. Sci.*, 2014, vol. 458, no. 1, pp. 302–305.

43. Kryštufek, B., Tesakov, A.S., Lebedev, V.S., et al., Back to the future: the proper name for red-backed voles is *Clethrionomys* Tilesius and not *Myodes* Pallas, *Mammalia*, 2020, vol. 84, no. 2, pp. 214–217.

44. Pavlinov, I.Ya. and Khlyap, L.A., Order Rodentia, in *Mlekopitayushchie Rossii: sistematiko-geograficheskii spravochnik* (Mammals of Russia: Systematic and Geographical Reference Book), Moscow: KMK, 2012, vol. 52, pp. 142–312.

45. Karaseva, E.V. and Telitsina, A.Yu., *Metody izucheniya gryzunov v polevykh usloviyakh* (Methods of Study of Rodents in Natural Habitats), Moscow: Nauka, 1996.

46. Kolcheva, N.E., Dynamics of the ecological structure of wood mouse populations in the Southern Urals, *Candidate Sci. (Biol.) Dissertation*, Ekaterinburg, 1992.

47. Kshnyasev, I.A. and Davydova, Yu.A., Population cycles and the Chitty syndrome, *Russ. J. Ecol.*, 2021, vol. 52, no. 1, pp. 70–75.

48. Mukhacheva, S.V. and Sozontov, A.N., Long-term dynamics of small mammal communities in the period of reduction of copper smelter emissions: 2. β-Diversity, *Russ. J. Ecol.*, 2021, vol. 52, no. 6, pp. 533–542.

49. Tayama, K., Fujita, H., Takahashi, H., et al., Measuring mouse sperm parameters using a particle counter and sperm quality analyzer: A simple and inexpensive method, *Reprod. Toxicol.*, 2006, vol. 22, no. 1, pp. 92–101.

50. Kruczek, M., Styrna, J., and Kapusta, J., Reproductive capacity of male bank voles (*Myodes glareolus* Schreber, 1780)-age-dependent changes in functional

activity of epididymal sperm, *Belg. J. Zool.*, 2013, vol. 143, no. 2, pp. 131–141.

51. Hébert, C.D., Elwell, M.R., Travlos, G.S., et al., Subchronic toxicity of cupric sulfate administered in drinking water and feed to rats and mice, *Fundam. Appl. Toxicol.*, 1993, vol. 21, no. 4, pp. 461–475.
52. Aburto, E.M., Cribb, A., Fuentealba, I.C., et al., The failure of selenium supplementation to prevent copper-induced liver damage in Fischer 344 rats, *Can. J. Vet. Res.*, 2001, vol. 65, no. 2, pp. 104–110.
53. StatSoft Inc. STATISTICA (data analysis software system), version 8.0. http://statsoft.ru. Cited November 23, 2023.
54. Posit team. RStudio: Integrated Development Environment for R. PBC, Boston, MA: Posit Software. http://www.posit.co/.2023.
55. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/.2023.
56. Wickham, H., *Ggplot2: Elegant Graphics for Data Analysis*, New York: Springer-Verlag, 2016.
57. Pustejovsky, J.E., Chen, M., and Swan, D.M., SingleCaseES: A calculator for Single-Case Effect Sizes. R package version 0.7.2. 2023.
58. Pesch, S. and Bergmann, M., Structure of mammalian spermatozoa in respect to viability, fertility and cryopreservation, *Micron*, 2006, vol. 37, no. 7, pp. 597–612.
59. Mamina, V.P., Morphofunctional analysis of testes and sperm in the assessment of male reproductive success in the bank vole (*Clethrionomys glareolus*), *Izv. Ross. Akad. Nauk, Ser. Biol.*, 2012, no. 5, pp. 554–562.
60. Kotula-Balak, M., Grzmil, P., Chojnacka, K., et al., Do photoperiod and endocrine disruptor 4-tert-octylphenol effect on spermatozoa of bank vole (*Clethrionomys glareolus*)?, *Gen. Comp. Endocrinol.*, 2014, vol. 201, pp. 21–29.
61. Wyrobek, A.J., Gordon, L.A., Burkhart, J.G., et al., An evaluation of the mouse sperm morphology test and other sperm tests in nonhuman mammals: A report of the US environmental protection agency genetox program, *Mutat. Res.*, 1983, vol. 115, no. 1, pp. 1–72.
62. Styrna, J., Kilarski, W., and Krzanowska, H., Influence of the CBA genetic background on sperm morphology and fertilization efficiency in mice with a partial Y chromosome deletion, *Reproduction*, 2003, vol. 126, no. 5, pp. 579–588.
63. Osadchuk, L.V. and Osadchuk, A.V., Genetic variability of spermatozoon production and morphology in laboratory mice, *Bull. Exp. Biol. Med.*, 2010, vol. 149, pp. 739–742.
64. Menkveld, R., Clinical significance of the low normal sperm morphology value as proposed in the fifth edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen, *Asian J. Androl.*, 2010, vol. 12, no. 1, pp. 47–58.
65. Smirnov, G.Yu., Qualitative assessment of the morphology of spermatozoa of the bank vole *Myodes glareolus* Schreber under conditions of industrial pollution, in *Mater. Konf. molodykh uchenykh "Ekologiya: fakty, gipotezy, modeli"* (Proc. Conf. Yong Sci. "Ecology: Facts, Hypotheses, Models"), Ekaterinburg: Lisitsa, 2017.
66. Smirnov, G.Yu., Occurrence of abnormal spermatozoa in rodents in the areas of two copper smelters, *Morfologiya*, 2020, vol. 157, no. 2.
67. Smirnov, G.Yu. and Davydova, Yu.A., Ontogenetic changes in bank vole (*Clethrionomys glareolus*) sperm morphology, *Russ. J. Ecol.*, 2020, vol. 51, no. 2, pp. 195–198.
68. Amann, R.P. and Waberski, D., Computer-assisted sperm analysis (CASA): Capabilities and potential developments, *Theriogenology*, 2014, vol. 81, no. 1, pp. 5–17.
69. Dvorakova, K. and Stopka, P., Sperm trains and mating behaviour in wood mice of the genus Apodemus, *Reprod., Fertil. Dev.*, 2004, vol. 16, p. 260.
70. Gómez Montoto, L., Sánchez, M.V., Tourmente, M., et al., Sperm competition differentially affects swimming velocity and size of spermatozoa from closely related muroid rodents: Head first, *Reproduction*, 2011, vol. 142, no. 6, pp. 819–830.
71. Björndahl, L., Mortimer, D., Barratt, C.L.R., et al., *A Practical Guide to Basic Laboratory Andrology*, Cambridge: Cambridge Univ. Press, 2010.
72. Chenoweth, P.J. and Lorton, S., *Animal Andrology: Theories and Applications*, Wallingford: Cabi, 2014.
73. Behringer, R., Gertsenstein, M., Nagy, K.V., and Nagy, A., *Manipulating the Mouse Embryo: A Laboratory Manual*, New York: Cold Spring Harbor Lab. Press, 2014.
74. Tannenbaum, L.V., Thran, B.H., and Willams K.J., Testing the limits of rodent sperm analysis: azoospermia in an otherwise healthy wild rodent population, *Arch. Environ. Contam. Toxicol.*, 2009, vol. 56, no. 1, pp. 157–164.
75. Tourmente, M., Villar-Moya, P., Varea-Sánchez, M., et al., Performance of rodent spermatozoa over time is enhanced by increased ATP concentrations: the role of sperm competition, *Biol. Reprod.*, 2015, vol. 93, no. 3, pp. 64, 1–13.
76. Chen, X., Hou, X., Feng, T., et al., Anti-fertility effect of levonorgestrel and/or quinestrol on striped field mouse (*Apodemus agrarius*): evidence from both laboratory and field experiments, *Integr. Zool.*, 2021, vol. 17, no. 6, pp. 1041–1052.
77. Matsumoto, A.M., Karpas, A.E., and Bremner, W.J., Chronic human chorionic gonadotropin admininstration in normal men: evidence that follicle-stimulating hormone is necessary for the maintenance of quatitatively normal spermatogenesis in man, *J. Clin. Endocrinol. Metab.*, 1986, vol. 62, no. 6, pp. 1184–1192.
78. Tannenbaum, L.V. and Beasley, J.C., Validating mammalian resistance to stressor-mediated reproductive impact using rodent sperm analysis, *Ecotoxicology*, 2016, vol. 25, no. 3, pp. 584–593.
79. Sánchez-Chardi, A., Peñarroja-Matutano, C., Borrás, M., and Nadal, J., Bioaccumulation of metals and effects of a landfill in small mammals Part III: Structural alterations, *Environ. Res.*, 2009, vol. 109, no. 8, pp. 960–967.
80. Sánchez-Chardi, A., Ribeiro, C.A.O., and Nadal, J., Metals in liver and kidneys and the effects of chronic

exposure to pyrite mine pollution in the shrew *Crocidura russula* inhabiting the protected wetland of Doñana, *Chemosphere*, 2009, vol. 76, no. 3, pp. 387–394.

81. Tête, N., Durfort, M., Rieffel, D., et al., Histopathology related to cadmium and lead bioaccumulation in chronically exposed wood mice, *Apodemus sylvaticus*, around a former smelter, *Sci. Total Environ.*, 2014, vol. 481, pp. 167–177.
82. Talmage, S.S. and Walton, B.T., Small mammals as monitors of environmental contaminants, *Rev. Environ. Contam. Toxicol.*, 1991, no. 119, pp. 47–145.
83. Pankakoski, E., Koivisto, I., Hyvärinen, H., and Terhivuo, J., Shrews as indicators of heavy metal pollution, in *Advances in the Biology of Shrews*, Pittsburgh, Pennsylvania: Carnegie Museum of Natural History, 1994, vol. 18, pp. 136–147.
84. Pereira, R., Pereira, M.L., Ribeiro, R., and Gonçalves, F., Tissues and hair residues and histopathology in wild rats (*Rattus rattus* L.) and Algerian mice (*Mus spretus* Lataste) from an abandoned mine area (Southeast Portugal), *Environ. Pollut.*, 2006, vol. 139, no. 3, pp. 561–575.
85. Beernaert, J., Scheirs, J., Leirs, H., et al., Non-destructive pollution exposure assessment by means of wood mice hair, *Environ. Pollut.*, 2007, vol. 145, no. 2, pp. 443–451.
86. Rogival, D., Scheirs, J., and Blust, R., Transfer and accumulation of metals in a soil–diet–wood mouse food chain along a metal pollution gradient, *Environ. Pollut.*, 2007, vol. 145, no. 2, pp. 516–528.
87. Wijnhoven, S., Leuven, R.S.E.W., van der Velde, G., et al., Heavy-metal concentrations in small mammals from a diffusely polluted floodplain: importance of species- and location-specific characteristics, *Arch. Environ. Contam. Toxicol.*, 2007, vol. 52, no. 4, pp. 603–613.
88. Levengood, J.M. and Heske, E.J., Heavy metal exposure, reproductive activity, and demographic patterns in white-footed mice (*Peromyscus leucopus*) inhabiting a contaminated floodplain wetland, *Sci. Total Environ.*, 2008, vol. 389, nos. 2–3, pp. 320–328.
89. Bezel', V.S. and Mukhacheva, S.V., Geochemical ecology of small mammals at industrially polluted areas: Is there any effect of reduction in the emissions? *Geochem. Int.*, 2020, vol. 58, pp. 959–967.
90. *Concentrations of Selected Elements, Physical Condition, Reproductive Activity, and Demographic Patterns in Small Mammalas Inhabiting DePue Wildlife Management Area*, Heske, E.J., Levengood, J.M., and Caldwell, K.D., Eds., Champaign: Illinois Waste Management and Research Center, 2003.
91. Hunter, B.A., Johnson, M.S., and Thompson, D.J., Ecotoxicology of copper and cadmium in a contaminated grassland ecosystem. III. Small mammals, *J. Appl. Ecol.*, 1987, vol. 24, no. 2, pp. 601–614.
92. Au, D.W.T., The application of histo-cytopathological biomarkers in marine pollution monitoring: A review, *Mar. Pollut. Bull.*, 2004, vol. 48, nos. 9–10, pp. 817–834.
93. Schipper, A.M., Loos, M., Ragas, A.M.J., et al., Modeling the influence of environmental heterogeneity on heavy metal exposure concentrations for terrestrial vertebrates in river floodplains, *Environ. Toxicol. Chem.*, 2008, vol. 27, no. 4, pp. 919–932.
94. Brumbaugh, W.G., Mora, M.A., May, T.W., and Phalen, D.N., Metal exposure and effects in voles and small birds near a mining haul road in Cape Krusenstern National Monument, Alaska, *Environ. Monit. Assess.*, 2010, vol. 170, no. 1, pp. 73–86.
95. Mukhacheva, S.V., Long-term dynamics of heavy metal concentrations in the food and liver of bank voles (*Myodes glareolus*) in the period of reduction of emissions from a copper smelter, *Russ. J. Ecol.*, 2017, vol. 48, no. 6, pp. 559–568.
96. Osadchuk, L.V. and Kleshchev, M.A., Interstrain differences in the parameters of spermatogenesis in inbred mice, *Morfologiya*, 2016, vol. 149, no. 2, pp. 54–57.
97. Muller, C.H., Rationale, interpretation, validation, and uses of sperm function tests, *J. Androl.*, 2000, vol. 21, no. 1, pp. 10–30.
98. Amann, R.P. and Hammerstedt, R.H., Detection of differences in fertility, *J. Androl.*, 2002, vol. 23, no. 3, pp. 317–325.
99. Marchlewicz, M., Effectiveness of blood-testis and blood-epididymis barriers for lead, *Ann. Acad. Med. Stetin.*, 1994, vol. 40, pp. 37–51.
100. Mruk, D.D. and Cheng, C.Y., The mammalian blood-testis barrier: its biology and regulation, *Endocr. Rev.*, 2015, vol. 36, no. 5, pp. 564–591.