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Bioaccumulation of Cadmium and Lead in Rodent Species from the Region of Lead-Zinc Smelting Factory – Plovdiv (South Bulgaria)

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Abstract. The levels of the toxic metals, Cd and Pb, were measured in liver of yellow-necked mouse (*Apodemus flavicollis* Melchior, 1834), Mediterranean mouse (*Mus macedonicus* Petrov & Ružić, 1983) and common vole (*Microtus arvalis* Pallas, 1778) from the vicinity of Plovdiv (South Bulgaria), where the lead-zinc smelting factory is the main source of pollution. The study was carried out at three sites located along a pollution gradient. An unpolluted region, the Strandzha Natural Park was used as a background region. MANOVA analysis revealed significant differences by species ($F=9.61$, $p=0.003$), site ($F=24.12$, $p=0.0001$) and exposure ($F=3.79$, $P=0.013$) effects. Significant increase of Pb and Cd bioaccumulation was found along the pollution gradient. Cd and Pb mean concentrations were highest at the site closest to the smelter and decreased with increasing the distance from them. The bioaccumulation of Pb was significant highest in the individuals of the yellow-necked mouse, followed by Mediterranean mice and common voles, whereas the common voles accumulated more Cd in comparison with the yellow-necked-, and Mediterranean mice. However, there is little evidence of adverse cadmium-mediated effects in yellow-necked- and Mediterranean mice and this species may be tolerant to Cd exposure. High Cd concentrations in body organs may simply reflect an ability to store the metal in a nontoxic, metallothionein-bound state. Liver Pb and Cd concentration did not differ significantly among sexes.

Key words: Bioaccumulation, lead, cadmium, *Apodemus flavicollis*, *Mus macedonicus*, *Microtus arvalis*

Introduction

Industrial pollution has become a new environmental factor that has essentially influenced the normal functioning of ecosystems. Therefore, it is necessary to analyze, in detail, the entry of various substances as a result of human activity into the environment and their interaction with living organisms at different levels. An essential stage in the overall ecological risk assessment is the establishment of residue content and distribution of specific pollutants of anthropogenic origin such as heavy metals in animal organisms that are especially sensitive to the change of the quantitative content of xenobiotics in the environment (SÁNCHEZ-CHARDI *et al.*, 2007).

Over the last decades the production of lead (Pb) and cadmium (Cd) in the industrial areas increased 2- and 15-fold,

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respectively, and the subsequent release of these metals into the environment is of some concern (Nriagu, 1988). The ecosystems seem to offer an effective filter by retaining contaminants in soil profiles, transferring them into aquatic (Négrel & Roy, 2002) and/or terrestrial systems, and thereby increasing the bioavailability and poisoning risk both to humans and the environment (Al Sayegh Petkovšek *et al.*, 2015). Lead and cadmium, two non-essential elements that are widely distributed, are well known for their highly toxic effects on biological systems (Wolfe *et al.*, 1998; Lewis *et al.*, 2001).

The pollution status at the area of the Plovdiv (Bulgaria) lead-zinc smelting factory is well documented, particularly for certain heavy metals present in freshwaters and soils. In this region the industrial polymetallic dust emission of lead, cadmium and zinc microaggregates from the smelting factory remain the primary sources of *in situ* metal pollution. In the first quarter of 2014 the concentration of polymetallic dust in the air pool over the town Plovdiv increased from 0.75 to 1.0 mg/m^3 or was 1.5 to 2 times higher than the TLV (Threshold Limit Value) (source PRIEWs, 2014).

The intake and bioaccumulation of pollutants by mammals is known to occur (Talmage & Walton, 1991; Shore, 1995; Komarnicki, 2000; Sánchez-Chardi *et al.*, 2007; Al Sayegh Petkovšek *et al.*, 2015). Several studies have shown that rodents and voles are mammals suitable for eco-toxicological research, especially due to their widely distribution, large number, r-type reproductive strategy, relatively small home range, high trophic chain position and metabolic rate (Ma & Talmage, 2001). Small mammals respond to stress effects in the environment, such as the intensity of changes in their organism correlates with the intensity of stress factors (Mitkovska *et al.*, 2012a). The fact that concentrations of heavy metals found in natural rodent populations regularly correlates with environmental pollution makes it possible to regularly use small mammals in eco-monitoring studies (Metcheva *et al.*, 2001; Ieradi *et al.*, 2003; Markov, 2012; Mitkovska *et al.*, 2012a, b). Moreover, the pattern of heavy metals distribution and the levels of heavy metals found in their tissues are similar to those found in humans (Damek-Poprava & Savicka-Kapusta, 2003). Furthermore, consumers at higher trophic levels in terrestrial ecosystems may be useful in predicting risks to human health (Komarnicki, 2000). In several studies rodent species have shown to be relevant zoomonitors (Abramson-Zetterberg *et al.*, 1997; Metcheva *et al.*, 2001; Ieradi *et al.*, 2003; Topashka-Ancheva *et al.*, 2003; Velickovic, 2004; Topashka & Yordanova, 2008; Al Sayegh Petkovšek *et al.*, 2015).

The main goal of this study was to quantify the bioaccumulation of non-essential metals (Pb and Cd) in rodent species along the pollution gradient in the area of the Plovdiv lead-zinc smelting factory and to evaluate the exposure-, species-, and gender- related effects.

Material and Methods

Study area. The area of study covers two regions determined by "National Biomonitoring Program of Bulgaria" (Peev & Gerassimov, 1999) as impact (polluted – the area of the lead-zinc smelting factory near Plovdiv) and background (unpolluted – Strandzha Natural Park) (Fig. 1). The lead-zinc smelting factory is located in the Thracian valley, at 230 m asl. The natural forest vegetation has been completely destroyed, only fractions of scattered mosaic mixed deciduous forests, bushy and grassy components are still preserved. The areas around the smelting factory are agricultural ecosystems. The industrial pollution is presented by SO_2, NO_2, Pb, Cd, Zn and other toxic substances. Microaggregates of Pb, Cd and Zn are emitted in the atmosphere by aerosols in the torch of pollution. They accumulate in the ground, spreading over vegetation and aquatic areas. Larger Zn aggregates (from 250 to 370 microns) fall in the vicinity of the factory, while the heavier particles of Pb, which have a minimum size of 30 to 70 microns, are blown away along the direction of the prevailing wind. Extensive investigations indicate that the polymetal pollution appears in soil samples. The heavy metals spread in the shallow plow layer of the soil and penetrate in downward direction to a limited extent (SENGALEVICH, 1998). By the end of 2010 the content of heavy metals and metalloids in soil samples from the factory area was: 4077.40 mg/kg Zn (by THV of 360 mg/kg); 3414.10 mg/kg Pb (by THV of 80 mg/kg) and 68.32 mg/kg Cd (by THV of 3.0 mg/kg). The average annual concentrations of Pb aerosols in the atmosphere remain below the corresponding average rate of 0.5 $\mu g/m^3$ over the last few years, while the concentrations of Cd aerosols increase (source PRIEWs, 2010).

The sites of study inside the area of the smelting factory cover locations along a pollution gradient. Pollution is highest on the eastern side. Three study sites were selected: site 1, adjacent to the smelter, where a green belt of vegetation exists; site 2, located 2 km east of the smelter and site 3, located 4 km east, respectively (Fig. 1). The Strandzha Mountain is located in the southeastern part of Bulgaria (Fig. 1a). This clean and uninhabited area also suffers from global air pollution caused by industrial emissions in this part of South-East Europe. However, the yearly average concentrations of pollutants are considerably lower and there are no important local sources of industrial pollutions and the animals are not directly exposed to environmental pollution (PEEV & GERASSIMOV, 1999).

Material. In total, 84 specimens of 3 rodent species were collected (Table 1). The common voles and Mediterranean mice were not catch in enough numbers in the sampling area of the Strandzha Natural Park to perform statistical analysis. To avoid intraspecific differences related to age, only adult specimens were examined. The age was determined according to criteria of molar root development and growth (FELTEN, 1952; GUSTAVSSON *et al.*, 1982).

a b

Fig. 1. Topographic location of the investigated regions and sites: a) location of the impact (lead-zinc smelting factory) and background (NP "Strandzha") regions and b) location of the investigated sites (1, 2 and 3) along the pollution gradient in the impact region.

Table 1. Number of investigated species in the studied areas.

Species	Lead-zinc smelting factory - Plovdiv			NP "Strandzha"	Total
	Site 1	Site 2	Site 3		
Apodemus flavicollis	8	10	7	16	41
Mus macedonicus	6	8	11	-	25
Microtus arvalis	6	7	5	-	18
				Total number:	**84**

Sampling. Trapping sessions were carried out from the second half of September 2012 until the end of October 2013. Sherman live traps were placed at dusk, left active overnight, and collected the next morning. The rodents were brought to the laboratory, where they were sexed and weighed. The animals were sacrificed by cervical dislocation after deep anesthesia. The liver was dissected for heavy-metal analyses. The liver tissue was stored at -20° C until further analysis.

The liver samples were dried for 24 h at 60°C until dry mass was obtained and then weighed. Afterward, a mixture of 5 ml of HNO_3 (70%) and 250 µl of H_2O_2 (30%), both ultrapure grade, was added to the dried samples to start digestion. Pb and Cd concentrations were determined using atomic absorption spectrophotometry (Perkin-ElmerISP-7000). The determination of lead was carried out in a graphite furnace. All metal concentrations were expressed on a dry weight basis in mg/kg.

The data were checked for both normal distribution (D'Agostino and Pearson omnibus normality test) and homogeneity of variance (Levene, F-test). Initially, an overall measure of the effect of sex, species and site effects was obtained by a three-way analysis of variances (MANOVA). Site and species divergences in the concentrations of heavy metals were performed using one-way analyses of variance (ANOVA), followed by Tuky's multiple comparison post-test. Intraspecies differences between both sexes and regions (impact and background) were calculated by Student's t-test. The differences in metal concentration distributions were statistically tested at P<0.05. All calculations were performed with the software program PRISM, version 4.02.

Results

Cd and Pb concentrations in the liver of the animals from the impact region were above the detection threshold (in mg/kg, Pb: 0.05; Cd: 0.05). MANOVA revealed significant differences by species (F=9.61, P=0.003), by different sites (F=24.12, P=0.0001) and exposure (F=3.79, P=0.013). Therefore, basic descriptive statistic (sample size, arithmetic mean, standard error, and range) of heavy metal concentrations are shown in Table 2.

Gender effect. The one-way analysis of variance (ANOVA) showed a lack of statistically significant sex differences in the accumulation of Pb and Cd in the liver of the studied species (F=0.51, P=0.76). Although, the average values of Pb were higher in females of the yellow-necked mice (Table 2), no statistically significant differences were found (t=0.8923; df=13; P=0.3885). Both males and females voles have approximately the same amount of Pb, while the values for the females are slightly higher (Table 2). No statistically significant differences were observed between both sexes of common voles (t=0.3554; df=2; P=0.7562) and Mediterranean mouse, either (t=0.7908; df=78; P=0.4550).

The average liver concentrations of Cd were higher in females in all studied species. In voles this trend is particularly emphasized as females show a substantially higher concentration of Cd compared to that of males, although no statistically significant differences between both sexes were observed (t=1.434, df=2, P= 0.2881). The samples result of the Mediterranean mice were more or less the same (t=1.198, df=7, P=0.2698).

The liver concentrations of Pb and Cd showed no gender effect and this enable data for both sexes to be combined in general samples in order to evaluate the species and exposure effects.

Exposure effect. The differences in mean concentrations of Pb and Cd between the impact and background regions were tested only by yellow-necked mice samples, because they inhabit both regions. The common voles and Mediterranean mice were catch in enough numbers to perform statistical analysis only in the region of lead-zinc smelting factory. In general, yellow-necked mice from the polluted region showed a significant increase in Pb (t=2.193, df=18, P=0.0417) and Cd concentrations (t=2.764, df=19, P=0.0123) than in the individuals from the background region (Fig. 2).

Table 2. Sample size (N), mean concentration in mg/kg (X), standard deviation (SD), minimal (Min) and maximal (Max) values of Pb and Cd by region and species

Region	Metal	Species	Male					Female				
			N	X	SD	Min	Max	N	X	SD	Min	Max
Lead-zinc smelting factory	Pb	*Apodemus flavicollis*	14	15.5	19.4	3.63	53.3	11	24.2	24.0	4.8	77.6
		Mus macedonicus	13	17.1	10.8	3.39	31.4	12	11.6	6.50	4.43	17.0
		Microtus arvalis	10	7.53	2.95	5.44	9.62	8	8.59	2.97	6.48	10.7
NP "Strandzha"		*Apodemus flaicollis*	8	0.47	0.35	0.22	0.71	8	0.79	0.66	0.39	1.77
Lead-zinc smelting factory	Cd	*Apodemus flavicollis*	14	12.5	10.3	4.46	27.6	11	16.7	15.1	2.39	49.7
		Mus macedonicus	13	11.8	9.69	2.53	30.0	12	21.6	15.1	9.14	38.4
		Microtus arvalis	10	12.2	4.27	9.15	15.2	8	34.6	21.7	19.3	49.9
NP "Strandzha"		*Apodemus flavicollis*	8	0.05	0.03	0.03	0.07	8	0.11	0.05	0.04	0.15

In the impact region, liver concentration of Pb differed among the sites. All rodents inhabiting site 1 accumulated significant more Pb (F=8.61, P=0.006) in comparison with these from sites 2 and 3 (Fig. 3). The mean liver concentration of Pb in the yellow-necked mice from site 1 was significant higher (F=20.46, P=0.0001) than that in individuals from sites 2 and 3 (Fig. 3).

In Mediterranean mice the mean amount of Pb did not differ significantly among sites (F=0.53, P=0.75) along the pollution gradient. Lack of statistically significant differences were also observed between the common voles from the different sites (F=2.53, P=0.07).

Significant differences in the mean values of cadmium accumulated in livers of the species from the three different sampling sites were observed (Fig. 4). The yellow-necked mice accumulated significantly more cadmium in the liver in the green belt area, less in the site 2 and at least in site 3 (F=13.92, P=0.0007). The average liver concentration of Cd in Mediterranean mice also differed significantly between individuals from the different sampling sites (F=6.48, P=0.009).

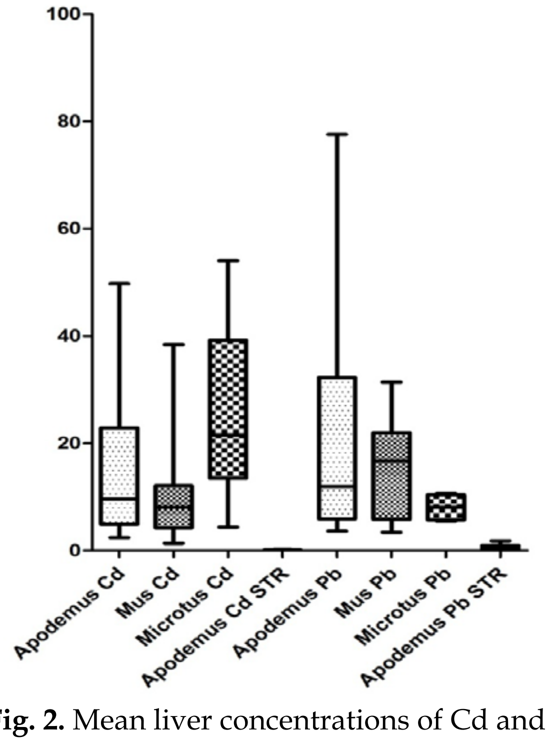

Fig. 2. Mean liver concentrations of Cd and Pb (mg/kg) in the investigated rodent species from the impact (lead-zinc smelting factory) and background regions (STR – Strandzha Natural Park). Bottom and top of the box represent 25 and 75% percentile values, respectively, with median values within the box. Error bars indicate minimum and maximum values.

More cadmium was accumulated in individuals from site 1. Such pattern of Cd accumulation was observed also in the common voles. Individuals of common vole accumulate significantly more cadmium in the green belt area (F=14.28, P=0.0003).

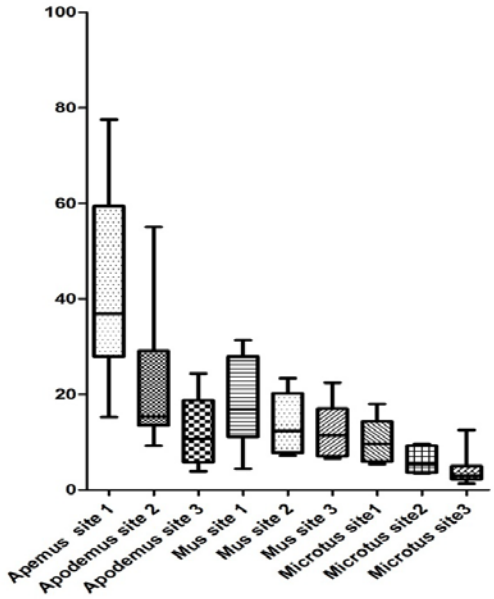

Fig. 3. Mean liver concentration of Pb (mg/kg) in the investigated rodent species along a pollution gradient in the impact region (lead-zinc smelting factory near Plovdiv). Bottom and top of the box represent 25 and 75% percentile values, respectively, with median values within the box. Error bars indicate minimum and maximum values.

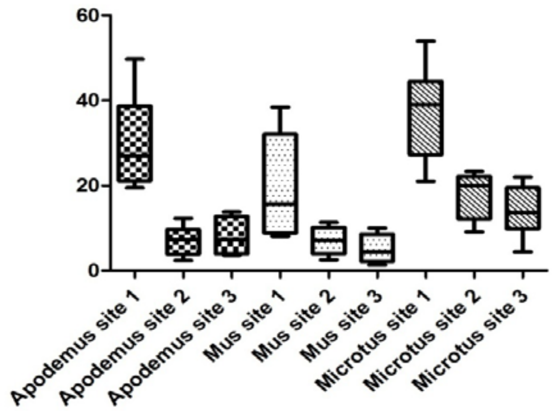

Fig 4. Mean liver concentration of Cd (mg/kg) in the investigated rodent species along a pollution gradient in the impact region (lead-zinc smelting factory near Plovdiv). Bottom and top of the box represent 25 and 75% percentile values, respectively, with median values within the box. Error bars indicate minimum and maximum values.

Species effect

In the impact region the mean concentration of Pb was highest in yellow-necked mice (20.48±21.77), followed by Mediterranean mice (15.26±9.54), and lowest in common voles (8.06±2.5) (Fig. 2). Outlined differences were not statistically significant (F=2.53, P=0.077).

The average concentrations of Cd in the livers of the investigated rodents differ statistically significant between the investigated species (F=5.82, P=0.006). The mean concentration of Cd was higher in voles (25.22±14.49), followed by yellow-necked mice (14.75±12, 79), and lowest concentrations were observed in the Mediterranean mice (10.55± 9.64) (Fig. 2).

Discussion

The obtained results revealed that the accumulation of Pb and Cd in the analyzed rodents varied according to site, species and exposure gradient, arranged in descending order of importance, whereas the sex remains statistically invariably for all three species.

Exposure related effect in metal bioaccumulation

The high levels of Pb and Cd found in the rodents from the polluted region can be easily explained as a result of intense industrial activities carried out in this area. According to data from the Plovdiv regional inspectorate of environment and waters (PRIEWs, 2012) the concentrations of Pb and Cd in the soil were 3108.18 mg/kg by TLV (Threshold Limit Value) of 80 mg/kg and 63.99 mg/kg by TLV of 3.0 mg/kg, respectively. Such anthropogenic impact had led to an increase of bioavailability of non-essential heavy metals by biota, and consequently to a chronic exposure for small mammals inhabiting the area of the smelter. Liver Cd concentrations in rodents from the polluted region were comparable to levels observed in other studies of rodents from polluted sites. The highest liver Cd concentrations observed until now in rodents were reported by HUNTER *et al.* (1989) for *Microtus agrestis* (mean, 22.7 mg/g) living near a copper-cadmium refinery. This liver concentration is comparable to those observed in the present study. High values for yellow-necked mice also were observed in the latter study (mean, 18.2 mg/g) and in a study of yellow-necked mice living on a mining waste site (range, 10.3–39.7 mg/g) (JOHNSON *et al.*, 1978). The obtained in our study liver levels of Pb were far below the extremes reported from other polluted sites (JOHNSON *et al.*, 1978; ROBERTS *et al.*, 1978). The concentrations of metals in yellow-necked mice from the background region were consistent with those reported for the same species and other rodents inhabiting non-polluted sites (TALMAGE & WALTON, 1991; SHEFFIELD *et al.*, 2001; BEERNAERT *et al.*, 2007; ROGIVAL *et al.*, 2007). As can be deduced from the obtained data, the range of variation in metal concentration is generally wider in polluted areas than in uncontaminated sites (Table 1). This circumstance may indicate an individual response due to a different metal exposure and/or to particular ecological, genetic, and physiological factors in chronically exposed animals (TALMAGE & WALTON, 1991). Additionally, the tissular turnover that occurs in liver causes changes in metal concentration, particularly in lead, over the life of an animal, which may also partially explain the great variation observed at the population level. In contrast, in non-polluted sites, the animals do not need detoxification mechanisms to control the intake of metals and to prevent toxic effects to their organs.

The decrease in Pb and Cd levels with increasing distance from the smelter indicates that heavy-metal exposure decreased with increasing distance from the smelter, which agrees with the findings of previous studies (BERCKMOES *et al.*, 2005).

Gender related effects in metal bioaccumulation

Data on the effect of biotic factors, such as sex, on metal bioaccumulation in the investigated species are scarce. Those available do not show clear patterns as the effect of this factor vary greatly between populations. Although several authors have reported sex-dependent variation of metal concentrations in wood mice (e.g. LOPES *et al.*, 2002; SCHEIRS *et al.*, 2006; BEERNAERT *et al.*, 2007), we did not detect these differences in yellow-necked mice. This discordant result may be attributed mainly to inter-population variation caused by differences in exposure and uptake of elements.

Species related effect in metal bioaccumulation

The long-term pollutant activities from smelting factories may disturb or destroy ecosystems, thereby making them less suitable for wildlife. In fact, these polluted sites may produce an increase in the levels and bioavailability of toxic compounds, such as heavy metals, which stress populations during multiple generations as a result of extended bioavailability. Therefore, continuous biomonitoring of pollutants by means of selected species is required. In our study, *A. flavicollis* did indeed show the highest lead concentrations of the three small mammal species in the green belt site of the lead-zinc smelting factory. In this context, our results corroborate other studies that reported the mice from *Apodemus* genus to an effective bioindicator of non-essential metals and the effects of environmental pollution (TALMAGE & WALTON, 1991; BARGAGLI *et al.*, 1997; SHEFFIELD *et al.*, 2001; GONZÁLEZ *et al.*, 2006; SCHEIRS *et al.*, 2006; WIJNHOVEN et al., 2007; ROGIVAL *et al.*, 2007; AL SAYEGH PETKOVŠEK *et al.*, 2015). The fact that a particular small-mammal species accumulates larger amounts of heavy metals does not necessarily mean that this is the species most at risk of toxic effects from pollutants. Some species could be more sensitive to heavy metals than others, and storage and transformation of heavy metals to less harmful products in organs, such as the liver, could be a good mechanism to cope with toxicants (SHORE & DOUBEN, 1994).

The significant difference between Cd concentration in mice and voles might be particularly linked to their food preferences. The species share similar levels in the trophic chain (primary consumers), but are specialized to take different plant foods – mice are weevils and voles – phyllophagous. According to POKARZHEVSKIJ (1985) the concentration of a given element in animal organisms is practically directly proportional to its content in the food. The yellow-necked mice feed mainly on seeds and fruits. The green parts of the plants, which are more dusted, are present in lower degree in their feeding. Mice supplement their diet with invertebrates – up to 20% of all food consumed (MARTINIAKOVA *et al.*, 2012). However, voles feed mainly on the green parts of the plants. They are polluted in the greatest degree and more accessible to precipitation and atmospheric dust. Particularly, with respect to Cd exposure, it was established that Cd is more readily taken up by plants than other metals, such as Pb (GOYER, 1996). This could be the reason for the leading position of *M. arvalis* in mean liver Cd bioaccumulation. According to the Pb concentration, voles were less loaded. They are characterized by good excretion and therefore, lower contaminant retention in the organism.

Biomonitoring of pollution through wild animals is crucial for the assessment of environmental quality and to improve our understanding of the response capacity of natural populations to pollution. The requirement for this systematic control is greater in protected areas inhabited by endangered species.

Conclusions

Based on the obtained results the following conclusions could be made:

1. The established bioaccumulation and the increased concentrations of Cd and Pb in the liver of the studied small rodents prove the existence of industrial pollution in the area of lead-zinc smelting factory - Plovdiv and testify to the presence of environmental risk.

2. The bioaccumulation of Cd and Pb in the liver of the studied small rodent species showed no gender effect.

3. Different degrees of bioaccumulation of Cd and Pb in the liver of the studied species were established:

- The investigated species of the Muridae family accumulate more Pb in the liver compared to the voles from the Arvicolinae subfamily;
- The investigated species of the Arvicolinae subfamily accumulate more Cd in the liver compared to species of the Muridae family.

4. Different levels of bioaccumulation in relation to the exposure of industrial pollution were registered. The highest concentrations of Cd and Pb were detected in species of green belt in the area of lead-zinc smelting factory, i.e. accumulation of heavy metals decreases with increasing distance from the smelter.

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