

# Concentrations of Selected Heavy Metals in Bones and Femoral Bone Structure of Bank (*Myodes glareolus*) and Common (*Microtus arvalis*) Voles from Different Polluted Biotopes in Slovakia

M. Martiniaková · R. Omelka · A. Jančová ·  
R. Stawarz · G. Formicki

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**Abstract** Concentrations of selected heavy metals in the femora and femoral bone structure of bank (*Myodes glareolus*) and common (*Microtus arvalis*) voles from different polluted biotopes in Slovakia (Kolíňany and Nováky sites) were investigated. Length, weight, and histological structure of vole bones were also analyzed. We observed higher concentrations of lead (Pb), iron (Fe), copper (Cu), and zinc (Zn) in the bones of both species from the Kolíňany site. Significant differences were observed in the concentration of Fe in bank and common voles ( $p < 0.05$ ) and in the concentration of Zn ( $p < 0.05$ ) in common voles. The animals from Nováky had higher concentrations of cadmium (Cd) and nickel (Ni) in their bones; however, the differences were not significant. The measured values for bone length and weight were higher in both species from Nováky ( $p < 0.05$ ). We did not identify differences in qualitative histological characteristics of the femora between the voles (*M. glareolus* and *M. arvalis* separately) between the two biotopes. In addition, no statistically significant differences for any the measured variables of primary osteons' vascular canals were observed. Correlation analysis in *M. glareolus* showed a strong positive relation between Cd and Ni ( $r = 0.52$ ), Pb

and bone weight ( $r = 0.53$ ), Fe and bone weight ( $r = 0.52$ ), and Fe and perimeter size of primary osteons' vascular canals ( $r = 0.55$ ). In common voles, a strong positive relation was found between Fe and Cu ( $r = 0.60$ ) and between Fe and perimeter size of vascular canals of primary osteons ( $r = 0.55$ ). Our results indicate that accumulation of some heavy metals is slightly increased in the femora of both species at Kolíňany.

Many investigations have been performed to determine heavy-metal contamination in various species of mammals. Field studies have shown that high levels of various heavy metals in the environment may appropriately be monitored by the assessment of their concentrations in target organs of free-living small mammals (mainly rodents). The increased levels have usually been considered to relate to environmental pollution, and significant relations have been found between residues of metals in soil and those in body organs or tissues (Shore 1995; Ieradi et al. 2003).

The pattern of heavy-metal distribution and levels of heavy metals in various tissues of rodents are similar to those found in humans. Therefore, in research rodents frequently serve as mammalian surrogates for humans (Shore and Rattner 2001). The bank vole (*Clethrionomys* [= *Myodes glareolus*]) is a small microtine rodent that is ubiquitous throughout Europe and one of the most common woodland rodents in Slovakia. Seeds, fruits, and green vegetation constitute approximately 44% of its food, whereas insects, earthworms and other invertebrates contribute between 9 and 23% depending on the season. In winter, bank voles add tree bark to their food supply (Zemanek 1972; Gdula-Argasinska et al. 2004). This species has been used to monitor environmental pollution from a variety of technogenic sources (Milton and Johnson 1999;

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M. Martiniaková (✉) · A. Jančová  
Department of Zoology and Anthropology, Constantine the  
Philosopher University, 949 74 Nitra, Slovak Republic  
e-mail: mmartiniakova@ukf.sk

R. Omelka  
Department of Botany and Genetics, Constantine the  
Philosopher University, 949 74 Nitra, Slovak Republic

R. Stawarz · G. Formicki  
Institute of Biology, Krakow Pedagogical University,  
31 054 Krakow, Poland

Milton et al. 2003; Topolska et al. 2004). The common vole (*Microtus arvalis*) is the species that has drawn the attention of cytogeneticists for many years, and currently it is one of the most karyologically studied free-living mammals in Europe (Topashka-Ancheva et al. 2003; Moska et al. 2004; Gileva et al. 2005). These voles feed mainly on leaves, stems, and roots of grasses and other plants and seem to preferentially feed on plants with high nitrogen content (Macdonald and Tattersall 2001).

During development, calcified tissues incorporate heavy metals to which they are exposed (Gdula-Argasinska et al. 2004). Bone tissue has some advantages compared with soft tissues, such as liver and kidney, because once incorporated into bone, the metals are subject to bone turnover (remodeling proceeds at approximately 10%/y in adult individuals). Therefore, an accurate historic record of exposure to various elements is retained in the bone, and, consequently, bone tissue would appear to be a suitable long-term bioindicator of environmental exposure.

Among heavy metals, cadmium (Cd) and lead (Pb; nonessential and xenobiotic) often cause acute and chronic environmental contamination. Cd causes damage primarily to kidney, bone, and lung. It also alters calcium (Ca) metabolism, leading to osteomalacia (Kido et al. 1993). Pb accumulates mainly in bone, but the critical organs for Pb intoxication are nervous system, kidney, and bone marrow (Friberg et al. 1986). These nonessential metals are toxic to humans and other animals, even in very low concentrations (Haider et al. 2004). In contrast, copper (Cu), iron (Fe), nickel (Ni), and zinc (Zn) are essential for the correct functioning of living organisms. However, essential metals can also produce toxic effects when the metals intake in high concentrations (Angelova et al. 2004). For example, Fe is essential for the formation of hemoglobin, myoglobin, and many enzymes; it is also involved in the formation of red blood cells and helps fight stress and disease (Leung et al. 1999). In contrast, an excess of Fe causes vomiting, diarrhea, and damage to the intestines in humans (Haider et al. 2004). Deficiency of essential metals can also negatively affect living organisms. A diet that is deficient in a particular essential element can enhance the accumulation and toxicity of some toxic metals. Ca, Fe, and Zn deficiency, for instance, enhance susceptibility to Cd and Pb toxicity. It has also been demonstrated that toxic metals can disrupt essential-element metabolism. Cd toxicity affects Ca metabolism either by direct toxicity to bone or indirectly through renal toxicity (Goyer 1997).

Only a few studies have been focused on the distribution of heavy metals in selected organs of bank voles. They have concentrated on the accumulation of selected toxic elements in kidney, liver, testes, uterus, and teeth (Appleton et al. 2000; Metcheva et al. 2001; Topolska et al. 2004; Gdula-Argasinska et al. 2004; Wijnhoven et al. 2008). The

distribution of selected heavy metals in bones is only rarely found in the literature (Milton and Johnson 1999; Milton et al. 2003).

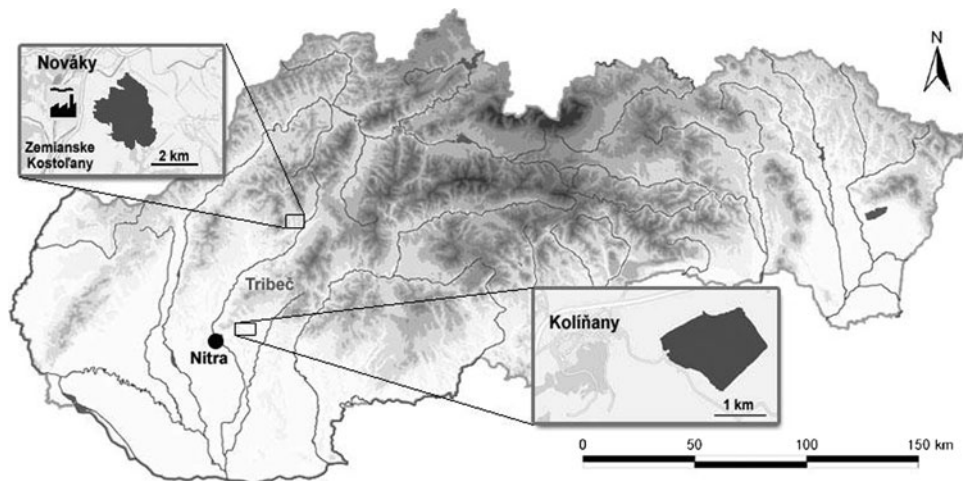
In general, two types of bone can be identified in rodents: compact bone and spongy bone. Compact bone forms the shafts of long bones, the surfaces of their extremities, short bones, and the outer and inner layers (*lamina externa* and *interna*) of the skull vault. Basic constituents of the bones' structural organization are primary and secondary osteons. Primary osteons are not surrounded by a reversal (cement) line, and lamellae around them merge smoothly with the surrounding bone; secondary osteons consist of a central (Haversian) canal, which is surrounded by concentric rings (lamellae) of matrix (Curry 2002; Martiniaková et al. 2007). Spongy bone constitutes the internal part of the long bone extremities (epiphyses) and the middle layer (diploe) of the skull vault in rodents. The bone consists of irregular, sinuous convolutions of lamellae, which create a system of trabeculae and griddles arranged in the direction of biomechanical stress (Rho et al. 1998).

Our study was carried out to contribute to a better evaluation of environmental contamination in two different biotopes in Slovakia. Because bone can serve as a good biomarker of long-term metal accumulation, we analyzed concentrations of selected heavy metals in the femora of two rodent species. Rodents are ideal for monitoring environmental pollution as well as for evaluating the exposure risk for people living in contaminated areas. Therefore, the aim of this study was to analyze concentrations of selected heavy metals (Pb, Cd, Fe, Ni, Cu, and Zn) in the femora of *M. glareolus* and *M. arvalis* trapped at different biotopes of a low hill level in Slovakia (Kolíňany and Nováky). Bone length, bone weight, and histological structure of vole bones were also investigated. Finally, the relations of detected metal concentrations and bone structure were determined.

## Materials and Methods

Individuals of bank (*M. glareolus*,  $n = 24$ ) and (*M. arvalis*,  $n = 15$ ) common voles were obtained by means of standard teriological methods and procedures from wood ecosystems (Jančová et al. 2006) in early spring 2007. The voles were trapped near the coal power station in Nováky (Prievidza district, Slovakia), which is considered to be a strongly polluted region (sources of possible environmental contamination include Nováky chemical plant, coal power station in Nováky, and Handlová–Cígel mines) and near the water pond in Kolíňany (Nitra district, Slovakia), which is located approximately 100 km from the town of Nováky and is considered to be a relatively polluted region (sources

**Fig. 1** Map of investigated biotopes in the Slovak Republic (Central Europe)



of possible environmental contamination include Calmit Žirany stone pit and limestone pit, aluminium production at Žiar nad Hronom, factories near the Nitra region, and intensive agricultural production). The coal power station is located west of Nováky, and the wind blows predominantly northwest and west. These two biotopes are separated by the Tribeč mountains (Fig. 1). All animals (males) used in the experiment were adult (4 months old), in good physical condition, and without anatomic pathologic conditions. We compared 14 bank voles from Koliňany with 10 from Nováky and 8 common voles from Koliňany with 7 from Nováky. Our research was focused on 78 femora taken from the adult voles. We compared bone length, bone weight, and histological structure of the femora between the animals from different types of environment. Values for macroscopical analysis were expressed as mean  $\pm$  SD. Unpaired Student *t* test was used to distinguish possible differences in bone length and bone weight between examined small mammals.

The concentrations of selected heavy metals (Pb, Cd, Ni, Fe, Cu, and Zn) were determined in left femora of investigated mammals ( $n = 39$ ) with the method of atomic absorption spectrophotometry (Perkin Elmer 4100 ZL) in a graphite furnace (Stawarz et al. 2003). The tissue samples were kept at  $-18^{\circ}\text{C}$  until analysis. In the laboratory, the samples were dried at  $105^{\circ}\text{C}$  until dry mass was obtained. Then the bones were weighed (minimum 2 g) and digested in concentrated nitric acid at  $90^{\circ}\text{C}$  for 10 hours. Before analysis, the samples were diluted to 25 ml with distilled water. Detection limits were as follows: Cd = 0.005 ppm, Ni = 0.12 ppm, Fe = 0.02 ppm, Cu = 0.01 ppm, Zn = 0.13 ppm, and Pb = 0.15 ppm. The recovery of the method was 96–98%, and reproducibility was  $>1.0\%$ . All metal concentrations were expressed on a dry-weight basis in  $\text{mg kg}^{-1}$ . From the final data, basic statistical

characteristics were calculated (mean, SD, minimum, maximum, and median). Because the distribution of observed levels of heavy metals was normal according to Shapiro–Wilk test, parametric Student *t* test was used for group comparisons employing the Statistica 7.0 software program.

For histological analysis, each right femur ( $n = 39$ ) was sectioned at the midshaft of its diaphysis. In total, 39 transversal sections of the femur diaphysis were cut. The bone rings were washed in running water for 5 days to remove soft tissues in bone marrow. The bones were then defatted in a mixed solution of chloroform and methanol for 7 days and bleached in 2%  $\text{H}_2\text{O}_2$  solution for 1 day (Martiniaková et al. 2005). After dehydrating through graded alcohols (Martiniaková et al. 2006), the samples were embedded in epoxy resin (Biodur; Günter von Hagens, Germany). Transverse thin sections (70–80  $\mu\text{m}$ ) were prepared with a sawing microtome (Leitz 1600, Germany), mounted on glass slides with Eukitt (Merck, Germany), and examined by light microscope (Jenaval, Germany) at  $225\times$ . The qualitative histological characteristics of the compact bone microstructure were determined according to Enlow and Brown's (1956) and Ricqlés et al.'s (1991) classification systems; the quantitative ones were assessed using the computer software Scion Image (Scion, MD) in anterior, posterior, medial and lateral views of thin section(s). We measured area, perimeter, and the minimum and maximum diameter of primary osteons' vascular canals. The osteons were outlined using the software Scion Image on a microphotograph at a magnification of  $225\times$ . The measured values ( $n = 135$  for *M. glareolus* and  $n = 95$  for *M. arvalis*) were expressed as mean  $\pm$  SD. Unpaired Student *t* test was used to distinguish differences in the quantitative histological characteristics of compact bone between investigated voles. Correlations between

heavy metals and bone structure were determined using the SPSS 8.0 statistical program (SPSS, Chicago, IL).

## Results

Concentrations of selected heavy metals (Pb, Cd, Fe, Ni, Cu, and Zn) in the femora of *M. glareolus* from different types of polluted environments are listed in Table 1. Higher concentrations of Pb, Fe, Cu, and Zn were detected in the bones of bank voles from Koliňany. A significant difference was observed only for Fe ( $p < 0.05$ ). In contrast, concentrations of Cd and Ni were higher in the femora of individuals from the area of the power station in Nováky; however, these differences were not statistically significant.

Similarly, we observed higher concentrations of Pb, Fe, Cu, and Zn in bones of *M. arvalis* from Koliňany (Table 2). Significant differences were detected for Fe and Zn ( $p < 0.05$ ). Concentrations of Cd and Ni were also higher,

although not significantly so, in individuals from the area of the power station in Nováky.

The measured values for bone weight and bone length were higher in bank and common voles from Nováky. The differences were statistically significant (Table 3).

Histological observation of thin sections of a shaft of femora from investigated voles showed an outer and inner nonvascular lamellar layer around a poorly developed reticular layer containing unorganized vascular canals (Figs. 2, 3). We rarely found several secondary osteons in femoral bone microstructure of bank and common voles, showing that the remodeling process of this rodent species is not as intensive compared with nonrodent mammals. In general, we did not identify demonstrable changes in qualitative histological characteristics of the femora in *M. glareolus* and *M. arvalis* (separately) from different biotopes.

With the quantitative histological characteristics, 135 vascular canals of primary osteons and 95 primary osteons' vascular canals were measured in bank and common voles,

**Table 1** Concentrations of selected heavy metals in femora of *M. glareolus* from different polluted biotopes in Slovakia

Locality		Pb (mg kg <sup>-1</sup> )	Cd (mg kg <sup>-1</sup> )	Ni (mg kg <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )	Cu (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )
Koliňany	X	20.46	4.01	9.52	212.99	4.16	188.55
	SD	13.17	1.88	2.80	52.27	2.10	21.61
	Min	9.26	1.08	6.75	140.58	1.87	154.68
	Max	39.74	7.74	13.92	293.09	6.39	219.42
	Med	18.87	3.13	8.46	172.53	4.04	151.46
Nováky	X	20.13	4.61	9.82	138.98*	3.78	176.07
	SD	9.51	1.13	1.89	10.15	0.74	11.20
	Min	14.08	3.71	7.93	128.19	3.20	164.21
	Max	31.09	5.88	11.69	140.42	4.61	186.14
	Med	19.84	3.76	8.17	138.17	3.81	174.14

X mean, min minimum, max maximum, med median

\*  $p < 0.05$

**Table 2** Concentrations of selected heavy metals in femora of *M. arvalis* from Koliňany and Nováky

Locality		Pb (mg kg <sup>-1</sup> )	Cd (mg kg <sup>-1</sup> )	Ni (mg kg <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )	Cu (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )
Koliňany	X	20.41	2.76	6.84	159.27	3.31	138.52
	SD	9.53	0.93	0.94	37.84	1.01	14.21
	Min	13.58	1.98	6.37	103.63	2.24	104.56
	Max	31.17	3.87	7.96	194.82	4.49	170.64
	Med	21.19	3.04	7.05	168.42	3.42	144.98
Nováky	X	20.23	2.84	7.29	113.57*	3.05	129.45*
	SD	8.98	1.11	1.14	26.85	0.87	10.12
	Min	12.88	1.78	6.34	94.56	2.23	113.63
	Max	29.34	4.05	8.69	144.23	4.66	147.37
	Med	20.43	2.96	7.53	121.05	3.12	134.26

X mean, min minimum, max maximum, med median

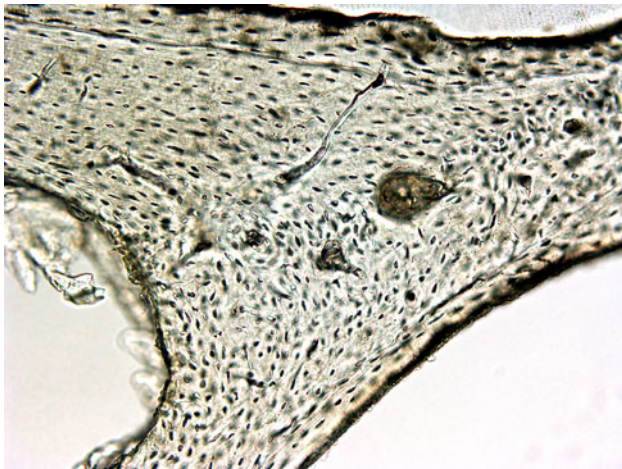
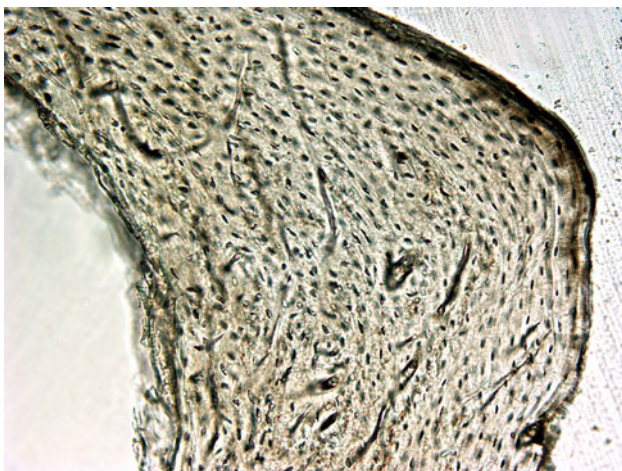
\*  $p < 0.05$



**Table 3** Results of macroscopic analysis of investigated femora from bank and common voles

Animal	Locality	Bone weight (g) ( $X \pm SD$ )	Bone length (cm) ( $X \pm SD$ )
<i>M. glareolus</i>	Kolíňany	0.029 $\pm$ 0.008	1.297 $\pm$ 0.109
	Nováky	0.042 $\pm$ 0.007*	1.637 $\pm$ 0.088*
<i>M. arvalis</i>	Kolíňany	0.026 $\pm$ 0.005	1.261 $\pm$ 0.093
	Nováky	0.037 $\pm$ 0.008*	1.512 $\pm$ 0.071*

X mean

\*  $p < 0.05$ **Fig. 2** Microphotograph of the femur in *M. glareolus***Fig. 3** Microphotograph of the femur in *M. arvalis***Table 4** Measured values of vascular canals of primary osteons' variables in bank and common voles from Kolíňany and Nováky

Animal	Locality	Area ( $\mu\text{m}^2$ ) ( $X \pm SD$ )	Perimeter ( $\mu\text{m}$ ) ( $X \pm SD$ )	Maximum diameter ( $\mu\text{m}$ ) ( $X \pm SD$ )	Minimum diameter ( $\mu\text{m}$ ) ( $X \pm SD$ )
<i>M. glareolus</i>	Kolíňany	100.85 $\pm$ 16.02	27.95 $\pm$ 4.27	12.23 $\pm$ 2.46	4.67 $\pm$ 0.69
	Nováky	102.58 $\pm$ 16.19	28.76 $\pm$ 3.97	12.45 $\pm$ 2.39	4.71 $\pm$ 0.74
<i>M. arvalis</i>	Kolíňany	95.64 $\pm$ 14.21	25.12 $\pm$ 3.56	11.72 $\pm$ 2.24	4.26 $\pm$ 0.58
	Nováky	98.97 $\pm$ 14.89	27.36 $\pm$ 3.78	12.04 $\pm$ 2.47	4.83 $\pm$ 0.69

X mean

respectively. The results are listed in Table 4. We found that all measured values for the vascular canals of primary osteons were higher in both species from Nováky. In contrast, no statistically significant differences were observed.

Correlation analysis in *M. glareolus* showed a strong positive relation between Cd and Ni ( $r = 0.52$ ), Pb and bone weight ( $r = 0.53$ ), Fe and bone weight ( $r = 0.52$ ), and Fe and size of the perimeter of primary osteons' vascular canals ( $r = 0.55$ ). Results of correlation analysis are listed in Table 5. In common voles, a strong positive relation between Fe and Cu ( $r = 0.60$ ) and between Fe and perimeter size of vascular canals of primary osteons ( $r = 0.55$ ) was found (Table 6).

## Discussion

There is a significant relation between the amount of toxic elements in soil, water, food, and mammal organs, including bone. The results obtained by Jančová et al. (2006) suggest that concentrations of Cu, Fe, and Cd in selected organs (kidney, testis, and uterus) of yellow-necked mouse (*Apodemus flavicollis*) from Nováky is significantly higher in all cases compared with those from Mochovce (a part relatively clean region in Nitra), respectively. It demonstrates an increase in pollution (mainly in the soil) due to the coal power station in Nováky resulting from mine work and/or from road traffic. According to Roberts and Johnson (1978) and Ieradi et al. (2003), one of the most important sources of environmental contamination from toxic elements is the coal industry. The dust emitted contains Zn, Cu, Pb, and Cd, and this contamination may increase the content of these elements in

**Table 5** Correlations between heavy metals and bone structure in *M. glareolus*

	Pb	Cd	Ni	Fe	Cu	Zn	Weight	Length	Area	Peri	Max	Min
Pb	1	0.26	0.38	0.09	0.48	0.15	<b>0.53*</b>	0.61	0.21	0.15	0.12	0.17
Cd		1	<b>0.52</b>	0.26	0.14	0.38	−0.09	−0.44	0.16	0.06	−0.16	0.44
Ni			1	0.34	0.42	0.31	0.39	0.36	0.35	0.19	0.25	0.32
Fe				1	−0.08	−0.26	<b>0.52</b>	0.17	0.50	<b>0.55</b>	0.58	−0.09
Cu					1	−0.41	0.33	0.28	0.09	−0.08	0.06	−0.07
Zn						1	−0.30	−0.29	0.11	−0.12	−0.34	0.43

Bolded numbers denoted a strong positive relation

*Peri* perimeter, *max* maximum diameter, *min* minimal diameter

\*  $p < 0.05$

**Table 6** Correlations between heavy metals and bone structure in *M. arvalis*

	Pb	Cd	Ni	Fe	Cu	Zn	Weight	Length	Area	Peri	Max	Min
Pb	1	0.51	−0.10	0.32	0.57	−0.06	0.64	0.59	0.23	0.26	0.16	0.24
Cd		1	0.59	−0.28	0.20	0.49	0.73	0.64	0.18	0.06	−0.17	0.37
Ni			1	0.63	0.44	−0.70	−0.47	−0.36	0.29	0.32	0.34	0.31
Fe				1	<b>0.60*</b>	−0.67	−0.12	−0.08	0.51	<b>0.55*</b>	0.48	−0.07
Cu					1	−0.71	0.17	0.31	0.05	−0.04	0.06	−0.05
Zn						1	0.26	0.24	0.08	−0.20	−0.23	0.42

Bolded numbers denoted a strong positive relation

*X* mean, *min* minimum, *max* maximum, *med* median

\*  $p < 0.05$

the tissues of mammals inhabiting polluted areas. In addition, it is known that concentrations of selected toxic elements (e.g., Cd, Pb, Cu, and Zn) in soft tissues and whole-body of *M. glareolus* are higher compared with *Apodemus flavicollis* (Sawicka-Kapusta et al. 1987; Metcheva et al. 2001). Therefore, we supposed that significantly higher concentrations of these elements would also be detected in the femora of *M. glareolus* from Nováky. Surprisingly, higher concentrations of Pb, Fe, Cu, and Zn were detected in bones of bank voles from Kolíňany, for which Fe concentration differences were significant. Similarly, we observed higher concentrations of Pb, Fe, Cu, and Zn in the femora of common voles trapped at the same biotope, with both Fe and Zn differences being significant. These results can be explained by intensive agricultural production and subsequent contamination of soil, water, and food by road traffic pollution as well as by various factories in industrial zones in Nitra. Intensive agricultural production and the use of chemicals are characteristic for the whole region of Nitra. It is generally known that an application of agricultural chemicals can lead to a higher accumulation of specific elements, including Fe and Zn, into the soil. In addition, there is heavy road traffic near the capture locality, which is also considered to be a significant source of heavy metals that have a potential ability to be

transported by air flow over large distances. There is also a possibility of falling dust being transported in the air from large industrial regions, such as Bratislava, Vienna, Budapest, or factories near Nitra. This hypothesis may be supported by studies indicating the possibility of long-range transportation of heavy metals (Coggins et al. 2006).

Animals of both vole species trapped at Nováky had higher concentrations of Cd and Ni in their bones. However, the differences were not significant. Our results demonstrate a slightly increased accumulation of other heavy metals in the bones of *M. glareolus* and *M. arvalis* from Kolíňany and thus provide evidence of environmental contamination.

We observed higher concentrations of Cd and Zn in the femora of *M. glareolus* (from both Nováky and Kolíňany) compared with the data reported by Milton et al. (2003). These investigators analyzed Pb ( $203 \pm 13.1 \mu\text{g g}^{-1}$  dry weight), Zn ( $173 \pm 5.1 \mu\text{g g}^{-1}$  dry weight) and Cd ( $0.6 \pm 13.1 \mu\text{g g}^{-1}$  dry weight) concentrations in the femora of bank voles trapped at the contaminated, unused Pb mine at Frongoch in west Wales. Accordingly, the Pb concentration was lower in our study for both biotopes. In contrast, our values (Pb concentrations in the femora of bank voles from Kolíňany and Nováky) were higher than the reference value mentioned by Milton et al. (2003) (Pb concentration

$3.2 \pm 0.8 \mu\text{g g}^{-1}$  dry weight). Concentrations of Cd and Pb were also higher in our study (from both Kolíňany and Nováky) compared with the value reported Milton and Johnson (1999). These investigators analyzed concentrations of Pb ( $0.99 \pm 0.14 \mu\text{g g}^{-1}$  dry weight), Zn ( $261.1 \pm 7.4 \mu\text{g g}^{-1}$  dry weight), and Cd ( $0.15 \pm 0.04 \mu\text{g g}^{-1}$  dry weight) in femora of laboratory-bred bank voles exposed to increased levels of dietary Zn. The Zn concentrations in the femora of bank voles from Kolíňany and Nováky were lower in our study. In contrast, it was not possible to compare our results of heavy-metal concentration in the bones of *M. arvalis* with published data because of their absence in the literature.

We also found significant changes related to femoral length and weight between *M. glareolus* and *M. arvalis* from different types of polluted biotopes. The voles trapped at Nováky had higher values for both of these variables. Because all individuals were adult and of the same sex, and came from similar biotopes, we speculate this fact could indicate a growth-stimulating effect of some heavy metals. Some investigations based on the experimental exposures of individuals to risk elements have shown evidence of associations between body (bone) length and body (bone) weight and increased intake of Pb, Zn, or Cd. According to Osweiler et al. (1985), Pb affects the metabolically active growth centers of long bones in young animals, having a positive effect on bone growth. Supplementation with Zn results in stimulation of skeletal growth and maturation (Moser-Veillon 1995). In addition, it has been demonstrated that long-term accumulation of Cd leads to decreased body weight and weight of some digestive organs in rats and rabbits (Toman et al. 1999, 2002). Our results could be explained by the above-noted findings only partially because femoral length and weight were higher in *M. glareolus* and *M. arvalis* with a higher concentration of Cd but not those of Pb and Zn. However, it is known that low levels of the elements taken up from the natural environment may have a somewhat different effect on bone structure than exposure occurring under artificial conditions. These differences may be related to the differences in food quality and availability, the level of toxic elements in the ground, or differences in locomotive activity. According to the study by Velickovic (2007), black-striped mice (*Apodemus agrarius*, rodents) trapped at a contaminated site in Serbia had decreased body size and poorer body condition compared with mice caught in an unpolluted reference site. Decreased body size means that an individual also has decreased bone size. We observed lower values for bone length and weight in bank and common voles from Kolíňany along with higher contents of Pb, Fe, Cu, and Zn in the femora.

Our results from the qualitative histological analysis correspond with the data published by Enlow and Brown

(1958) and by Martiniaková et al. (2005) for compact bone microstructure of rodents. We rarely found several secondary osteons in our bone samples. It is generally known that aged rats and mice (rodents) lack true Haversian cortical bone remodeling but not cancellous bone remodeling activity (Erben 1996). Therefore, some secondary osteons can be observed in their long bones (near the endosteal border). The presence of several secondary osteons in our voles might also be related to biomechanics, body size, or phylogenetic origin of rodent species. In general, we did not identify demonstrable changes in qualitative histological characteristics between bank and common voles from different polluted biotopes. In addition, histomorphometry showed no statistically significant differences for measured variables of primary osteons' vascular canals between the animals. Therefore, we suppose that environmental factors (including heavy metals in the environment) influence the macroscopic structure of the bones in *M. glareolus* and *M. arvalis* trapped at different types of polluted environments in Slovakia. The voles from Nováky had significantly higher femoral length and weight compared with those from Kolíňany in our study. In contrast, heavy metals did not affect compact bone morphology. According to Havill (2003), bone microstructural morphology is also genetically mediated and provides the direction for further exploration into this phenomenon. Beamer et al. (2001) mentioned that environmental factors are important contributors to variability in bone microstructure, but genetics still play a substantial role, and estimates of heritability range from 40 to 93%.

It has been reported that essential and nonessential metals may interact with each other, thus affecting uptake, bioaccumulation and toxicity. The results of such interactions are highly variable and range from antagonism to synergism depending on the metal, its external concentration and exposure scenario, length of exposure, species, and specific organs (Norwood et al. 2003; Komjarova and Blust 2008). We observed a strong positive relation between Cd and Ni in the femora of bank voles. It is known that Cd as well as Ni and Zn interact actively with Ca channels (Paquin et al. 2002), which is typical for bone cells. According to Komjarova and Blust (2008), Cd, Ni, and Zn uptake rates are closely related to each other. A high correlation coefficient of 0.62 between Ni and Cd has also been observed in bones of people working in steel mills (Brodziak-Dopierala et al. 2009). In our study, a strong positive interaction between Fe and Cu in the femora of common voles was identified. Cu forms a synergetic system with Fe (Cu–Fe), which advantageously influences the course of enzymatic processes. This interaction ( $r = 0.83$ ) has been noted in trabecular bone of humans (Brodziak-Dopierala et al. 2009). In addition, research by Hisanaga et al. (1988) performed on bone samples taken

from human ribs showed interactions between Fe and Cu that were characterized by a high correlation coefficient ( $r = 0.52$ ). In both vole species, a strong positive relation between Fe and the perimeter size of primary osteons' vascular canals was found. This indicates that a higher content of Fe in bones significantly influences some variables of vascular canals of primary osteons in bank and common voles.

In conclusion, our contribution is a pilot study about the relation between femoral bone structure and accumulation of some heavy metals in the bones of bank and common voles from different types of polluted environments in Slovakia. Further research in this direction must extend the number of analyzed skeletal elements and verify the results that were obtained from our skeletal samples, especially in other species of small mammals.

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## References

- Angelova V, Ivanova R, Delibaltova V, Ivanov K (2004) Bio-accumulation and distribution of heavy metals in fiber crops (flax, cotton, and hemp). *Ind Crops Prod* 19:197–205
- Appleton J, Lee KM, Sawicka-Kapusta K, Damek M, Cooke M (2000) The heavy metal content of the teeth of the bank vole (*Clethrionomys glareolus*) as an exposure marker of environmental pollution in Poland. *Environ Pollut* 110:441–449
- Beamer WG, Shultz KL, Donahue LR, Churchill GA, Sen S, Wergedal JR et al (2001) Quantitative trait loci for femoral and lumbar vertebral bone mineral density in C57BL/6J and C3H/HeJ inbred strains of mice. *J Bone Miner Res* 16:1195–1206
- Brodziak-Dopierala B, Kwapulinski J, Kusz D, Gajda Z, Sobczyk K (2009) Interactions between concentrations of chemical elements in human femoral heads. *Arch Environ Contam Toxicol* 57:203–210
- Coggins AM, Jennings SG, Ebinghaus R (2006) Accumulation rates of the heavy metals lead, mercury and cadmium in ombrotrophic peatlands in the west of Ireland. *Atmos Environ* 40:260–278
- Currey JD (2002) *Bones: structure and mechanics*. Princeton University Press, Princeton, NJ
- de Ricqlés AJ, Meunier FJ, Castanet J, Francillon-Vieillot H (1991) Comparative microstructure of bone. In: Hall BK (ed) *Bone 3: bone matrix and bone specific products*, vol 1. CRC Press, Boca Raton, FL, pp 1–78
- Enlow DH, Brown SO (1956) A comparative histological study of fossil and recent bone tissues. Part I *Texas J Sci* 8:405–412
- Enlow DH, Brown SO (1958) A comparative histological study of fossil and recent bone tissues. Part III *Texas J Sci* 10:187–230
- Erben RG (1996) Trabecular and endocortical bone surfaces in the rat: modeling or remodeling? *Anat Rec* 246:39–46
- Friberg L, Nordberg GF, Vouk VB, Kessler E (1986) *Handbook on the toxicology of metals*. Elsevier, Amsterdam
- Gdula-Argasinska J, Appleton J, Sawicka-Kapusta K, Spence B (2004) Further investigation of the heavy metal content of the teeth of the bank vole as an exposure indicator of environmental pollution in Poland. *Environ Pollut* 131:71–79
- Gileva EA, Yalkovskaya LE, Polyavina OV, Bořshakov VN (2005) Common voles of the *Microtus arvalis* group in the Urals: genome instability and chromosomal polymorphism. *Dokl Biol Sci* 405:458–460
- Goyer RA (1997) Toxic and essential metal interactions. *Annu Rev Nutr* 17:37–50
- Haider S, Naithani V, Barthwal J, Kakkar P (2004) Heavy metal content in some therapeutically important medicinal plants. *Bull Environ Contam Toxicol* 72:119–127
- Havill LM (2003) Osteon remodeling dynamics in *Macaca mulatta*: normal variation with regard to age, sex and skeletal maturity. *Calcif Tissue Int* 74:95–102
- Hisanaga A, Hirata M, Tanaka A, Ishinishi N, Eguchi Y (1988) Variation of trace metals in ancient and contemporary Japanese bones. *Biol Trace Elem Res* 22:221–231
- Ieradi LA, Zima J, Allegra F, Kotlánová E, Campanella L, Grossi R et al (2003) Evaluation of genotoxic damage in wild rodents from a polluted area in the Czech Republic. *Folia Zool* 52:57–66
- Jančová A, Massányi P, Naď P, Koréneková B, Skalická M, Drábeková J et al (2006) Accumulation of heavy metals in selected organs of yellow necked mouse (*Apodemus flavicollis*). *Ekol Bratislava* 25:19–26
- Kido T, Nogawa K, Hoshi Y, Hayano M, Honda R, Tsuritani I et al (1993) The renal handling of calcium and phosphorus in environmental cadmium-exposed subjects with renal dysfunction. *J Appl Toxicol* 13:43–47
- Komjarova I, Blust R (2008) Multi-metal interactions between Cd, Cu, Ni, Pb and Zn in water flea *Daphnia magna*: a stable isotope experiment. *Aquat Toxicol* 90:138–144
- Leung PL, Huang HM, Sun DZ, Zhu MG (1999) Hair concentrations of calcium, iron, and zinc in pregnant women and effects of supplementation. *Biol Trace Elem Res* 69:269–282
- Macdonald DW, Tattersall FH (2001) *Britain's mammals: the challenge for conservation*. People's Trust for Endangered Species, London, UK
- Martiniaková M, Grosskopf B, Vondráková M, Omelka R, Fabiš M (2005) Observation of the microstructure of rat cortical bone tissue. *Scripta Medica* 78:45–50
- Martiniaková M, Grosskopf B, Omelka R, Vondráková M, Bauerová M (2006) Differences among species in compact bone tissue microstructure of mammalian skeleton: use of a discriminant function analysis for species identification. *J Forens Sci* 51:1235–1239
- Martiniaková M, Grosskopf B, Omelka R, Dammers K, Vondráková M, Bauerová M (2007) Histological study of compact bone tissue in some mammals: a method for species determination. *Int J Osteoarch* 17:82–90
- Metcheva R, Teodorova S, Topashka-Ancheva M (2001) A comparative analysis of the heavy metals and toxic elements loading indicated by small mammals in different Bulgarian regions. *Acta Zool Bulg* 53:61–80
- Milton A, Johnson M (1999) Biomonitoring of contaminated mine tailings through age accumulation of trace metals in the bank vole (*Clethrionomys glareolus*). *J Environ Monit* 1:219–225
- Milton A, Cooke JA, Johnson MS (2003) Accumulation of lead, zinc, and cadmium in a wild population of *Clethrionomys glareolus* from an abandoned lead mine. *Arch Environ Contam Toxicol* 44:405–411
- Moser-Veillon RB (1995) Zinc needs and homeostasis during lactation. *Analyst* 120:895–897



- Moska M, Kosowska B, Brzezinska K (2004) Karyotype of the common vole (*Microtus arvalis*) from the Sowie mountains. *EJPAU* 7:#08
- Norwood WP, Borgmann U, Dixon DG, Wallace A (2003) Effects of metal mixtures on aquatic biota: a review of observations and methods. *Hum Ecol Risk Assess* 9:795–811
- Osweller GD, Carson TL, Buck WB, Van Gelder GA (1985) Clinical and diagnostic veterinary toxicology. Kendall/Hunt Publishing Company, Dubuque, IA
- Paquin PR, Gorsuch JW, Apte S, Batley GE, Bowles KS, Campbell PGC et al (2002) The biotic ligand model: a historical overview. *Comp Biochem Physiol C* 133:3–35
- Rho JZ, Kukn-Spearing L, Zioupos P (1998) Mechanical properties and the hierarchical structure of bone. *Med Eng Phys* 20:92–102
- Roberts RD, Johnson MS (1978) Dispersal of heavy metals from abandoned mine transference through terrestrial food chains. *Environ Pollut* 16:293–310
- Sawicka-Kapusta K, Górecki A, Lange R (1987) Heavy metals in rodents from polluted forests in southern Poland. *Ekologia Polska* 35:345–354
- Shore RF (1995) Predicting cadmium, lead and fluoride levels in small mammals from soil residues and by species-species extrapolations. *Environ Pollut* 88:333–340
- Shore RF, Rattner BA (2001) *Ecotoxicology of wild mammals*. Wiley, London
- Stawarz R, Zakrzewski M, Marenčík A, Hraška Š (2003) Heavy metal concentration in the toad *Bufo Bufo* from a region of Mochovce, Slovakia. *Ekol Bratislava* 22:292–297
- Toman R, Massányi P, Kováčik J (1999) Growth and reproductive ability of male rabbits after cadmium intake from diet. *Folia Vet* 43:182–185
- Toman R, Massányi P, Čupka P, Lukáč N, Ducsay L, Kolenkáš M, et al. (2002) Changes in weight of some organs and weight of rats under the influence of cadmium receiving in the diet from wean to sexual maturity [in Slovak]. In: Risk factors of the food chain: proceeding book, pp 154–157. Nitra, Slovakia
- Topashka-Ancheva M, Metcheva R, Teodorova S (2003) A comparative analysis of the heavy metal loading of small mammals in different regions of Bulgaria II: chromosomal aberrations and blood pathology. *Ecotoxicol Environ Saf* 54:188–193
- Topolska K, Sawicka-Kapusta K, Cieslik E (2004) The effect of contamination of the Krakow region on heavy metals content in the organs of bank voles (*Clethrionomys glareolus*, Schreber, 1780). *Pol J Environ Stud* 13:103–109
- Velickovic M (2007) Measures of the developmental stability, body size and body condition in the black-striped mouse (*Apodemus agrarius*) as indicators of a disturbed environment in northern Serbia. *Belg J Zool* 137:147–156
- Wijnhoven S, Leuven RSEW, van der Velde G, Eijsackers HJP (2008) Toxicological risk for small mammals in a diffusely and moderately polluted floodplain. *Sci Total Environ* 406:401–406
- Zemanek M (1972) Food and feeding habits of rodents in a deciduous forest. *Acta Theriol* 23:315–325