Original Research

Accumulation of Lead, Cadmium, Nickel, Iron, Copper, and Zinc in Bones of Small Mammals from Polluted Areas in Slovakia

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Abstract

The accumulation of lead (Pb), cadmium (Cd), nickel (Ni), iron (Fe), copper (Cu), and zinc (Zn) in the femora of four rodent species (*Apodemus flavicollis*, *Apodemus sylvaticus*, *Myodes glareolus*, and *Microtus arvalis*) trapped at different polluted areas in Slovakia (Kolíňany and Nováky) was investigated in the present study. We detected the highest concentration of Pb in bones of *A. sylvaticus* from both polluted areas; however, no significant changes between species were observed. In the femora of *M. glareolus*, the highest concentrations of Cd, Ni, and Zn were detected at both areas. Significant differences were observed between *A. flavicollis* and *M. glareolus*, *M. glareolus* and *M. arvalis* (for Cd concentration); and between *M. glareolus* and remaining species (for Zn concentration). The highest concentration of Fe was found in bones of *A. flavicollis* and *M. glareolus* from Nováky and Kolíňany areas, respectively. Significant differences (for Ni, Fe, and Cu concentrations) were examined between *A. flavicollis* and *A. sylvaticus*, *A. flavicollis* and *M. arvalis*. Although the highest concentration of Cu was found in bones of *M. glareolus* and *A. flavicollis* from Nováky and Kolíňany areas, respectively, no significant differences between species were observed. Our results demonstrate increased accumulation of some heavy metals (mainly Cd, Fe, Pb, and Zn) in the femora of small mammals from both polluted areas in Slovakia in comparison with other polluted biotopes in Europe and thus provide evidence of intensive environmental contamination. *M. glareolus* may be considered the most loaded zoomonitor with heavy metal contamination as compared to other species.

Keywords: heavy metals, bone, small mammals, Slovakia

Introduction

Small mammals (mainly rodents) are sensitive and accurate monitors of exposure to environmental pollution by heavy metals. Moreover, the pattern of heavy metal distribution and levels of heavy metals in their tissues are similar to those found in humans [1]. Therefore, small mammals frequently serve as mammalian surrogate for humans in research [2].

Mice from the genus *Apodemus* and voles have been shown to be relevant pollution zoomonitors [3-6]. The yellow-necked mouse (*Apodemus flavicollis*), wood mouse (*Apodemus sylvaticus*), bank vole (*Myodes glareolus*) (formerly *Clethrionomys glareolus*), and common vole (*Microtus arvalis*) belong to the most dominant rodent species in Slovakia. They are small, easy to catch, have a territory of limited range, a fairly short life span, and they are closely adjusted to their environment [7].

During development, calcified tissues incorporate heavy metals to which they are exposed [8]. Bone tissue has

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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% per year in adult individuals). Therefore, an accurate historical 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, lead (Pb) and cadmium (Cd; nonessential and xenobiotic) often cause acute and chronic environmental contamination. Pb accumulates mainly in bone but the critical organs for Pb intoxication are the nervous system, kidneys, and bone marrow [9]. Cd causes damage primarily to kidney, bone, and lungs. It also alters the calcium (Ca) metabolism that leads to osteomalacia [10]. These nonessential metals are toxic to humans and other animals, even in very low concentrations [11]. In contrast, nickel (Ni), iron (Fe), copper (Cu), and zinc (Zn) are essential for the correct functioning of living organisms. However, essential metals can also produce toxic effects when metals intake reaches high concentrations [12].

Since bone can serve as a good biomarker of long-term metal accumulation, we analyzed concentrations of selected heavy metals (Pb, Cd, Ni, Fe, Cu, and Zn) in the femora of four rodent species (*A. flavicollis*, *A. sylvaticus*, *M. glareolus*, and *M. arvalis*) trapped at different polluted areas in Slovakia (Kolíňany and Nováky) to evaluate the exposure risk for people living in these areas.

Materials and Methods

Individual yellow-necked mice (*Apodemus flavicollis*, n=17), wood mice (*Apodemus sylvaticus*, n=15), bank voles (*Myodes glareolus*, n=24) and common voles (*Microtus arvalis*, n=15) were obtained by means of the standard teriological methods and procedures from wood ecosystems [13] in early spring 2007. The mice and voles were trapped near a 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, the coal power station in Nováky, and Handlová-Cígeľ mines) and near a water pond in Kolíňany (Nitra district, Slovakia), located approximately 100 km from the town of Nováky and considered to be a relatively polluted region (sources 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 pathological conditions. We analyzed 9 yellow-necked mice from Kolíňany and 8 from Nováky, 8 wood mice from Kolíňany and 7 from Nováky, 14 bank voles from Kolíňany and 10 from Nováky, and 8 common voles from Kolíňany and 7 from Nováky.

The concentrations of selected heavy metals (Pb, Cd, Ni, Fe, Cu, and Zn) were determined in the left femora of investigated mammals (n=71), using atomic absorption spectrophotometry (Perkin Elmer 4100 ZL) in a graphite furnace [14]. The tissue samples were kept at -18°C until analysis. In the laboratory, the samples were dried at 105ºC until dry mass was obtained. Then, the bones were weighed (minimum 2 g) and digested in concentrated nitric acid at 90ºC for 10 hours. Before analysis, the samples were diluted to 25 ml with distilled water. Detection limits were as follows: Pb=0.15 ppm, Cd=0.005 ppm, Fe=0.02 ppm, Ni=0.12 ppm, Cu=0.01 ppm, and Zn=0.13 ppm. The recovery of the method was 96-98% and reproducibility was better than 1.0%. All metal concentrations were expressed on a dry weight basis in mg·kg^{-1}. From the final data, basic statistical characteristics were calculated (mean, standard deviation, minimum, maximum, median) for each species. Analysis of variance and Tukey's test were used to identify the species-specific differences in heavy metal accumulation with SPSS 8.0. software.

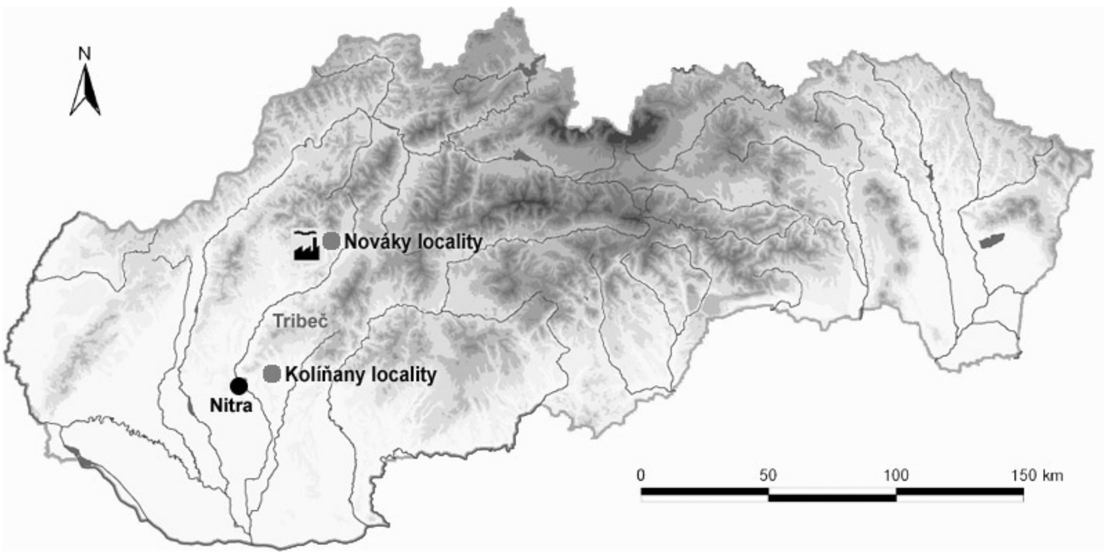

Fig. 1. Map of investigated areas in Slovakia.

Table 1. The concentrations of select heavy metals in the femora of small mammals from the Nováky area.

Species		Pb	Cd	Ni	Fe	Cu	Zn
		(mg·kg⁻¹)					
Apodemus flavicollis (1)	mean	20.18	2.53	7.95	156.61	3.60	126.88
	standard deviation	3.87	0.77	1.94	31.64	0.47	10.35
	minimum	15.28	1.93	6.29	115.98	2.89	110.96
	maximum	26.68	3.95	11.34	204.45	4.27	141.35
	median	18.84	2.76	7.07	168.57	3.81	129.14
Apodemus sylvaticus (2)	mean	22.48	3.12	7.26	103.35	3.01	119.99
	standard deviation	1.85	0.75	0.90	31.24	0.89	14.49
	minimum	19.28	1.84	6.13	85.14	2.18	109.38
	maximum	24.54	3.95	9.34	143.38	4.86	142.59
	median	22.02	2.81	7.17	101.57	3.04	120.98
Myodes glareolus (3)	mean	20.13	4.61	9.82	138.98	3.78	176.07
	standard deviation	9.51	1.13	1.89	10.15	0.74	11.20
	minimum	14.08	3.71	7.93	128.19	3.20	164.21
	maximum	31.09	5.88	11.69	140.42	4.61	186.14
	median	19.84	3.76	8.17	138.17	3.81	174.14
Microtus arvalis (4)	mean	20.23	2.84	7.29	113.57	3.05	129.45
	standard deviation	8.98	1.11	1.14	26.85	0.87	10.12
	minimum	12.88	1.78	6.34	94.56	2.23	113.63
	maximum	29.34	4.05	8.69	144.23	4.66	147.37
	median	20.43	2.96	7.53	121.05	3.12	134.26
Tukey's test			1:3 +		1:2 +		1:3 +
			3:4 +		1:4 +		2:3 +
							3:4 +

(+) – $P<0.05$

Results and Discussion

Concentrations of selected heavy metals (Pb, Cd, Fe, Ni, Cu, and Zn) in the femora of four rodent species (*A. flavicollis*, *A. sylvaticus*, *M. glareolus*, *M. arvalis*) from different polluted areas in Slovakia are listed in Tables 1 and 2. We detected the highest concentration of Pb in bones of *A. sylvaticus* from both polluted areas; however, no significant differences between *A. sylvaticus* and other species were observed. The highest concentrations of Cd, Ni, and Zn were detected in the femora of *M. glareolus* trapped in both areas. Significant differences were observed between *A. flavicollis* and *M. glareolus*, *M. glareolus* and *M. arvalis* (for Cd concentration); and between *M. glareolus* and remaining species (for Zn concentration). The highest concentration of Fe was found in bones of *A. flavicollis* and *M. glareolus* from Nováky and Kolíňany areas, respectively. Significant differences were examined between *A. flavicollis* and *A. sylvaticus*, *A. flavicollis* and *M. arvalis*. Although the highest concentration of Cu was found in bones of *M. glareolus* and *A. flavicollis* from Nováky and Kolíňany areas, respectively, no significant differences between species were observed.

In general, it is known that the coal power station Nováky has a negative effect on environmental (mainly soil) pollution resulting from mine work and/or road traffic. According to Ieradi et al. [6] and Roberts and Johnson [15], one of the most important sources of environmental contamination with heavy metals is the coal industry. The dust emitted contains Cd, Pb, Cu, and Zn, and this contamination may increase the content of the elements in the tissues of mammals inhabiting polluted areas. Therefore, we supposed that significantly higher concentrations of these elements would also be detected in the femora of small mammals from Nováky. Surprisingly, higher concentrations of investigated elements were detected in bones of *A. flavicollis*, *A. sylvaticus*, *M. glareolus*, and *M. arvalis* from Kolíňany in most cases. These results can be explained by

Table 2. Concentrations of selected heavy metals in the femora of small mammals from the Kolíňany area.

Species		Pb	Cd	Ni	Fe	Cu	Zn
		(mg·kg^{-1})					
Apodemus flavicollis (1)	mean	19.82	2.84	9.16	163.27	4.43	143.84
	standard deviation	5.44	0.35	1.89	73.91	1.19	16.52
	minimum	14.11	2.24	7.06	97.64	3.01	117.73
	maximum	32.05	3.28	12.04	358.40	6.40	163.82
	median	19.87	2.91	8.88	155.74	4.47	151.46
Apodemus sylvaticus (2)	mean	22.30	3.03	7.80	109.10	3.33	147.55
	standard deviation	1.27	0.73	0.84	35.61	1.06	13.35
	minimum	20.83	2.48	6.94	86.22	2.37	132.78
	maximum	22.10	3.86	8.63	150.13	4.47	158.76
	median	22.96	2.74	7.83	90.95	3.15	151.13
Myodes glareolus (3)	mean	20.46	4.01	9.52	212.99	4.16	188.55
	standard deviation	13.17	1.88	2.80	52.27	2.10	21.61
	minimum	9.26	1.08	6.75	140.58	1.87	154.68
	maximum	39.74	7.74	13.92	293.09	6.39	219.42
	median	18.87	3.13	8.46	172.53	4.04	171.46
Microtus arvalis (4)	mean	20.41	2.76	6.84	159.27	3.31	138.52
	standard deviation	9.53	0.93	0.94	37.84	1.01	14.21
	minimum	13.58	1.98	6.37	103.63	2.24	104.56
	maximum	31.17	3.87	7.96	194.82	4.49	170.64
	median	21.19	3.04	7.05	168.42	3.42	144.98
Tukey's test			1:3 +		1:2 +		1:3 +
			3:4 +		1:4 +		2:3 +
							3:4 +

(+) – $P<0.05$

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 into the soil. In addition, there is heavy road traffic near the capture location, which is also considered to be a significant source of heavy metals that have a potential ability to be transported by the air flow over long 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 the long-range transportation of heavy metals [16].

We observed higher concentrations of Cd and Fe in the femora of *A. flavicollis* (for both Kolíňany and Nováky areas) in comparison with the data observed by Damek-Poprawa and Sawicka-Kapusta [1]. These authors analyzed Pb (172.36±14.16 μg·g^{-1}), Cd (0.43±0.12 μg·g^{-1}), Zn (166.3±7.6 μg·g^{-1}), and Fe (153.0±9.9 μg·g^{-1}) concentrations in the *femur* of yellow-necked mice from zinc smelters in Bukowno (Poland) that are considered to be extremely polluted. On the other hand, Pb and Zn concentrations were lower in our study for both areas.

Pb concentrations in the femora of *A. sylvaticus* were lower in our study (for both areas) as compared to the value of Milton et al. [4]. These investigators analyzed concentrations of Pb (27.0±8.0 mg·kg^{-1}) in ecosystems developed on tailings from lead/zinc mines in Wales and Ireland. Our values were indeed higher as compared to the reference value (2.2±0.3 mg·kg^{-1}). On the other hand, Cd concentrations in the femora of wood mice from Kolíňany and Nováky were higher than those observed by Milton et al. [5]. The animals were again caught at the same ecosystems as in a previous study (polluted biotopes in Wales and Ireland, Cd concentration 0.27±0.07 mg·kg^{-1}).

We observed higher concentrations of Cd (approximately 7 times) and Zn (approximately 1.2 times) in the femora of *M. glareolus* (for both Kolíňany and Nováky) compared with the data reported by Milton et al. [17]. These authors analyzed Pb (203±13.1 $\mu g \cdot g^{-1}$), Zn (173±5.1 $\mu g \cdot g^{-1}$), and Cd (0.6±13.1 $\mu g \cdot g^{-1}$) concentrations in the *femur* of bank voles trapped in the contaminated, unused Pb mine at Frongoch in west Wales. Accordingly, the Pb concentration was lower in our study for both areas. In contrast, our values for Pb concentration were higher (approximately 6 times) than the reference value mentioned by Milton et al. [17] (3.2±0.8 $\mu g \cdot g^{-1}$). Concentrations of Cd and Pb were also higher in our study (for both areas) compared with the values of Milton and Johnson [18]. These investigators analyzed concentrations of Pb (0.99±0.14 $\mu g \cdot g^{-1}$), Zn (261.1±7.4 $\mu g \cdot g^{-1}$), and Cd (0.15±0.04 $\mu g \cdot g^{-1}$) in femora of laboratory-bred bank voles exposed to increased levels of dietary Zn. For this aim, Zn concentration in the femora of bank voles was lower in our study. On the other hand, it was not possible to compare our results of heavy metal concentrations in the bones of *M. arvalis* with published data because of their absence in the literature.

Anyway, our results demonstrate increased accumulation of some heavy metals (mainly Cd, Fe, Pb, and Zn) in the femora of small mammals from both polluted areas in Slovakia in comparison with other polluted biotopes in Europe and thus provide evidence of intensive environmental contamination. Since distribution and levels of heavy metals in soft and hard tissues of rodents are similar to those found in humans, it is believed that increased accumulation of Cd, Fe, Pb, and Zn also occurs in humans living in these areas.

According to Pokarzhevskij [19], the concentration of a given element in animal organisms is practically directly proportional to its contents in the food. The *Apodemus* food could be determined like the "purest" in comparison with all other zoomonitors. These 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 also supplement their diet with invertebrates – animal food forming up to 20% of all food consumed. According to Metcheva et al. [3], heavy metal concentration in the liver of *Apodemus* is in last place. However, the body's heavy metal loading is relatively high, which may be due to lower excretion by the kidneys. It is possible that this factor results in greater sensitivity of *Apodemus* to heavy metal pollution. On the other hand, the voles are characterized by good excretion and, therefore, lower contaminant retention in the organism. However, voles feed mainly on the green parts of the plants. They are polluted in the greatest degree like more accessible to the precipitations, atmosphere, dust, etc. Also, seeds and roots are present in the food of voles.

It is interesting to note the observations of Sawicka-Kapusta et al. [20], who investigated heavy metal content in free-living rodents populating industrial polluted forests in southern Poland. The authors recorded that Cd, Pb, Cu, and Zn concentrations in *A. flavicollis* are significantly lower than those in *M. glareolus*. The same correlations were established in the study by Metcheva et al. [3], who detected heavy metal concentration in the liver and body of various rodent species from different Bulgarian regions. In our study, the highest concentrations of Cd, Ni, and Zn were found in bones of *M. glareolus* trapped at both polluted areas in comparison with other species. Significant differences were observed for concentrations of Cd and Zn between yellow-necked mice and bank voles. In contrast, the highest concentrations of Fe and Cu were found in the femora of *A. flavicollis* from Nováky and Kolíňany, respectively. But significant differences between yellow-necked mice and bank voles were not detected.

We also observed significant differences for Fe concentrations between *Apodemus* species (*A. flavicollis* and *A. sylvaticus*) and also for Cd and Zn concentrations between vole species (*M. glareolus* and *M. arvalis*). These facts can be explained by specific food differences of all rodents studied. Diets of both *Apodemus* species are similar, with *A. flavicollis* tending to eat more animal food and less green plant matter [21]. The herbivorous *M. arvalis* feeds on spatio-temporally predictable food resources (e.g. roots, tubers, and shoots of plant tubers), the omnivorous *M. glareolus* additionally subsists on temporally unpredictable food resources (e.g. insects and seeds) [22].

In general, it is known that differences in average metal concentrations between species can be the result of differences in population structure between the species. In addition, the metal concentrations in free-living rodents could also be affected by shifts in feeding patterns, seasonal and flood-related aspects of food availability, habitat suitability and connectivity, and life-stage-related food preference, combined with the same variations in the metal contents in the food items themselves. Finally, exposure time, and therefore age of the animals, might be an explanatory factor [23]. Taking into account all these aspects, we suppose that *M. glareolus* may be considered as the most loaded zoomonitor with heavy metal contamination (mainly regarding Cd, Zn, and Ni) in comparison with other species in our study.

In conclusion, observed animals inhabiting the studied areas in Slovakia show higher heavy metal bioaccumulation compared to small mammals in other polluted biotopes in Europe. It is believed that similar bioaccumulation is also present in humans living in these areas. Therefore, it is necessary to improve the quality of environment in Nováky and Nitra, including dust emission reduction, improvement of the technologies in agriculture and industry, and road traffic reorganization. These steps may bring significant benefits for human health and life in these regions of Slovakia.

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