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Yellow-necked mice (*Apodemus flavicollis*) and bank voles (*Myodes glareolus*) as zoomonitors of environmental contamination at a polluted area in Slovakia

Monika Martiniaková^{1*}, Radoslav Omelka², Birgit Grosskopf³, Alena Jančová¹

Abstract

Background: Free-living wild rodents are often used as zoomonitors of environmental contamination. In the present study, accumulation of cadmium (Cd), copper (Cu), iron (Fe), and zinc (Zn) in critical organs of yellow-necked mice (*Apodemus flavicollis*) and bank voles (*Myodes glareolus*) trapped in a polluted area in Nováky, Slovakia was investigated.

Methods: Yellow-necked mice (n = 8) and bank voles (n = 10) were collected using standard theriological methods for wood ecosystems. All animals were adult males in good physical condition. The concentrations of Cd, Cu, Fe, and Zn in the liver, kidney, and bone were determined by atomic absorption spectrophotometry.

Results: The highest concentrations of Cd and Zn were found in the bone of both species while Cu and Fe accumulated mainly in kidney or liver. Significant higher concentrations of Cd and Cu were detected in the liver of bank voles than in yellow-necked mice. Similar significant higher levels of Cd and Zn were found in the bone of bank voles. In contrast, significant higher concentrations of Cu and Fe were present in the kidney of yellow-necked mice.

Conclusions: In the yellow-necked mouse and bank vole, bone seems to accumulate Cd and Zn following prolonged exposure. On the contrary, kidney and liver store Cu and Fe after a long-term environmental exposure. In the present study, bank voles seemed to be more heavy metal loaded zoomonitors than yellow-necked mice.

Background

The importance of monitoring the exposure and studying the effects of heavy metals on living organisms has increased in the last decades. Studies of small mammals, mainly free-living wild rodents, have demonstrated an ability to accumulate a wide spectrum of pollutants [1,2]. Significant relations have been found between residues of metals in soil and in organs or tissues [1,3]. In addition, the patterns of heavy metal distribution in rodent tissues and their concentrations are similar to those found in humans. Therefore, rodents frequently serve as models for humans in ecotoxicology [4]. Free-living wild rodents are

suitable for monitoring environmental pollution and exposure risk for people living in a contaminated area [5,6].

Mice of the genus *Apodemus* and voles are suitable pollution zoomonitors [7-9]. The yellow-necked mouse (*Apodemus flavicollis*) and bank vole (*Myodes glareolus*; formerly *Clethrionomys glareolus*) belong to the most dominant rodent species in Slovakia. These animals are easily caught and they have a small migration area and a relatively short life span. Compared to larger mammals, their higher metabolic rate may increase their susceptibility to pollutants. Among heavy metals causing environmental contamination, cadmium (Cd) is among the most dangerous metals. This non-essential metal is toxic for humans or animals even in very low concentrations [10]. It primarily damages kidney, lung, and bones, e.g. through altered calcium metabolism leading to osteomalacia [11].

* Correspondence: mmartiniakova@ukf.sk

¹Department of Zoology and Anthropology, Constantine the Philosopher University, Nábřežie mládeže 91, 949 74 Nitra, Slovak Republic
Full list of author information is available at the end of the article

Copper (Cu), iron (Fe), and zinc (Zn) are among the physiologically important metals that although being essential, may induce toxic effects if provided in high concentrations [12]. The aim of the present study was to determine concentrations of Cd, Cu, Fe and Zn in the liver, kidney and bone of yellow-necked mice and bank voles trapped in a polluted area in Nováky, Slovakia.

Methods

Animals

Yellow-necked mice ($n = 8$) and bank voles ($n = 10$) were obtained by means of the standard theriological methods and procedures for wood ecosystems [13] in February 2007. The rodents were trapped in a polluted area in Nováky, Prievidza district, Slovakia, which is considered as a heavily polluted region. Possible sources of pollution in this region are the Nováky chemical plant, the coal power station in Nováky, and Handlová - Cígeľ coal mines (Figure 1). All animals caught were adult males (aged 4-5 months determined by dental wear). They appeared in good physical condition and without gross lesions at necropsy.

Procedures

The animals were euthanized by cervical dislocation shortly after capture and examined for gross lesions. Samples of liver, kidney, and bone (femur) were kept at -18°C until analysis. The concentrations of Cd, Cu, Fe, and Zn were determined by atomic absorption spectrophotometry (Perkin Elmer 4100 ZL) in a graphite furnace [14]. Samples of liver and kidney were weighed and ashed with diluted nitric acid p.a. ($\text{HNO}_3:\text{H}_2\text{O} = 2:1$) at 130°C for 2 h. Undissolved particles were filtered off and the

solution diluted to 25 ml [15]. The bone samples were dried at 105°C until dry mass was obtained. Then all samples were weighed (minimum 2 g) and digested in concentrated nitric acid at 90°C for 10 h. The samples were diluted to 25 ml with distilled water before analysis [16]. Detection limits were as follows Cd = 0.005 ppm, Cu = 0.01 ppm, Fe = 0.02 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 $\text{mg}\cdot\text{kg}^{-1}$.

Statistics

From the final data, basic statistical characteristics were calculated (mean, standard deviation, minimum, maximum, median). Since the distribution of observed levels of heavy metals was normal according to the Shapiro-Wilk test, the parametric Student's t test was used for species comparisons employing the Statistica 7.0 software program.

Results

Concentrations of Cd, Cu, Fe, and Zn in the liver, kidney, and bone of the examined yellow-necked mice and bank voles are listed in Table 1. In both species, the highest concentrations of Cd were found in bone followed by kidney and liver. The hierarchy of Cu and Fe concentrations was kidney > liver > bone in yellow-necked mice. In the bank vole, highest concentration of Cu was detected in the liver followed by bone and kidney while the hierarchy for Fe concentrations was liver > kidney > bone. In both species, the highest concentrations of Zn were found in the bone followed by liver and kidney.

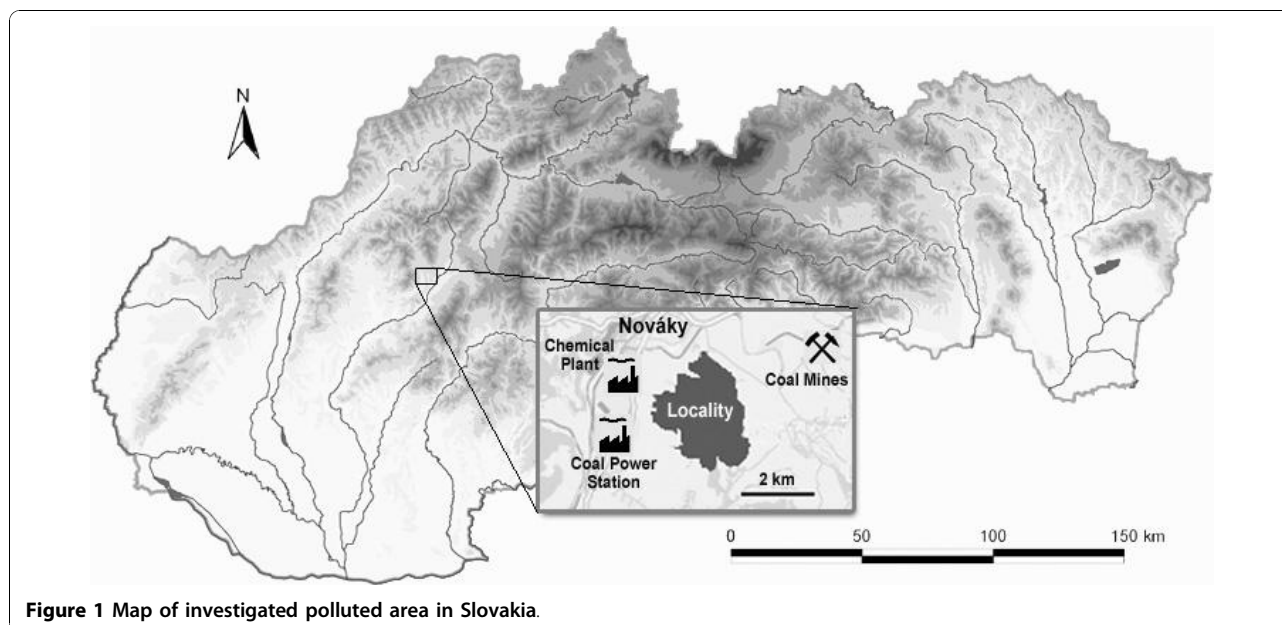


Figure 1 Map of investigated polluted area in Slovakia.

Table 1 Heavy metal concentrations

Tissue	Species		Cd (mg.kg ⁻¹)	Cu (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)
Liver	Yellow-necked mouse	x	0.023	5.13	455.23	101.06
		sd	0.004	1.79	192.39	40.19
		min	0.019	3.24	276.10	55.47
		max	0.027	6.69	658.60	167.60
		med	0.023	5.77	431.00	95.31
	Bank vole	x	0.047*	7.26*	461.77	175.35
		sd	0.027	2.96	77.86	159.25
		min	0.031	3.96	388.20	66.15
		max	0.078	9.65	543.30	358.20
		med	0.032	8.17	453.80	101.70
Kidney	Yellow-necked mouse	x	0.071	5.38*	506.90*	81.64
		sd	0.031	1.20	65.57	53.38
		min	0.035	3.99	434.50	47.43
		max	0.094	6.22	562.30	200.00
		med	0.083	5.92	523.90	62.19
	Bank vole	x	0.075	3.05	394.73	80.28
		sd	0.039	0.98	95.54	36.35
		min	0.031	1.91	287.70	55.38
		max	0.104	3.66	471.40	122.00
		med	0.089	3.57	425.10	63.46
Bone	Yellow-necked mouse	x	2.53	3.60	156.61	126.88
		sd	0.77	0.47	31.64	10.35
		min	1.93	2.89	115.98	110.96
		max	3.95	4.27	204.45	141.35
		med	2.76	3.81	168.57	129.14
	Bank vole	x	4.61*	3.78	138.98	176.49*
		sd	1.13	0.74	10.15	11.20
		min	3.71	3.20	128.19	164.21
		max	5.88	4.61	140.42	186.14
		med	3.76	3.81	138.17	174.14

x – mean, sd – standard deviation, min – minimum, max – maximum, med – median, (*) – $P < 0.05$.

Concentrations of cadmium (Cd), copper (Cu), iron (Fe) and zinc (Zn) in the liver, kidney and bone (femur) of yellow-necked mouse (*Apodemus flavicollis*) (n = 8) and bank vole (*Myodes glareolus*) (n = 10).

When comparing heavy metal levels in the two rodent species, significant higher concentrations of Cd and Cu were detected in the liver of the bank vole than in the yellow-necked mouse ($P < 0.05$). Significant higher levels of Cd and Zn were present in the bone tissue of the bank vole ($P < 0.05$) than in the yellow-necked mouse, while these mice had significant higher kidney concentrations of Cu and Fe ($P < 0.05$).

Discussion

Previous studies have demonstrated that the coal power station in Nováky and the Nováky chemical plant have negative effects on the environment especially by soil pollution [17] and water pollution [18]. One of the most

important sources of environmental contamination with heavy metals is the coal industry [19,20]. The dust emitted from this kind of industries contains Cd, lead (Pb), Cu and Zn, and the associated environmental contamination may increase the heavy metal content of mammals inhabiting the polluted areas.

In general, there is a significant relationship between the amount of heavy metals in the environment and in the organs of free-living wild rodents, first of all in liver and kidneys [13]. However, some metals e.g. Pb, accumulate mainly in bone. Bone tissue has some advantages compared with soft tissues in ecotoxicological studies as metals are subjected to the rather slow bone turnover (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 is a suitable bioindicator of a long-term environmental exposure [16].

We found higher concentrations of Zn in the liver, kidney and bone of bank voles than Milton *et al.* [21], who determined Pb, Zn, and Cd concentrations in selected organs of bank voles trapped at the contaminated abandoned Pb mine at Frongoch in west Wales. The hierarchy of Zn concentrations in their study was bone > liver > kidney > muscle. The same hierarchy was also observed in our study. According to Milton *et al.* [21], the hierarchy of Cd concentrations in the tissues was kidney > bone > liver > muscle. In our study, the highest concentration of Cd was detected in the bone of bank vole followed by liver and kidney. In addition, Cd concentration in the bone was higher than found by Milton *et al.* [21]. These data demonstrate increased accumulation of Zn, Cd in critical organs of bank voles from Nováky and thus provide further evidence of intensive environmental pollution of this area. Since distribution and levels of heavy metals in soft and hard tissues of free-living rodents are similar to those found in humans [5,6], it is believed that the same accumulation of Cd, and Zn occurs also in humans living in studied area of Slovakia. In yellow-necked mice and bank voles, bone accumulates highest levels of Cd and Zn after long-term environmental exposure. On the contrary, Cu and Fe accumulated mainly in kidney or liver of both rodent species.

According to Pokarzhevskij [22], the concentration in the body of a given element is practically directly proportional to its amount in the food. Since the age of the rodents studied was 4-5 months, they foraged on the autumn and winter spectrum of food, including beechnuts and acorns in yellow-necked mouse, and berries, fungi, large amounts of grass leaves in bank vole [23]. Sawicka-Kapusta *et al.* [24] have recorded that Cd, Pb, Cu and Zn concentrations in yellow-necked mice are significantly lower than those in bank voles. The same correlations have been established in the study by Metcheva *et al.* [7] who detected heavy metal concentration in the liver and body of rodent species from different Bulgarian regions. In our study, significant higher concentrations of Cd and Cu were detected in the liver of bank voles than in yellow-necked mice. Also, higher levels of Cd and Zn were found in the bone of this species. In the kidney of yellow-necked mice, significant higher concentrations of Cu and Fe were present, possibly due to lower renal excretion rates for these metals in yellow-necked mice.

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 may be affected by altered feeding patterns, seasonal and flood-related aspects of food availability, habitat suitability and connectivity, and life-stage-related food preference combined with variations in the metal contents in the food items themselves. Finally, exposure time, and therefore age of the animals, might be an explanatory factor [2]. Taking into account all these aspects, we suppose that the bank vole is a more heavy metal loaded zoomonitor than the yellow-necked mouse.

Conclusions

Highest concentrations of Cd and Zn were found in the bone of both yellow-necked mice and bank voles. Cu and Fe accumulated mainly in kidney or liver. Significant higher concentrations of Cd and Cu were detected in the liver of bank vole. In the bone of this species, significant higher levels of Cd and Zn were also found. Significant higher concentrations of Cu and Fe were present in the kidney of yellow-necked mouse. Bank vole is considered as a more pollution loaded zoomonitor in comparison with yellow-necked mouse.

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Author details

¹Department of Zoology and Anthropology, Constantine the Philosopher University, Nábřežie mládeže 91, 949 74 Nitra, Slovak Republic. ²Department of Botany and Genetics, Constantine the Philosopher University, Nábřežie mládeže 91, 949 74 Nitra, Slovak Republic. ³Johann Friedrich Blumenbach Institute of Zoology and Anthropology, Georg-August University, Bürgerstrasse 50, 37 073 Göttingen, Germany.

Authors' contributions

MM was responsible for animal trapping and determination of heavy metals concentrations of bones. RO was responsible for the statistical analyses. BG was responsible for sample preparation for atomic absorption spectrophotometry. AJ was responsible for analyses of liver and kidneys. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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