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Lead Levels in the Bones of Snow Voles *Chionomys nivalis* (Martins, 1842) (Rodentia) from European Mountains: a Comparative Study of Populations from the Tatra (Slovakia), Vitosha and Rila (Bulgaria)

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Abstract: Snow voles (*Chionomys nivalis*) were bait-trapped in the Tatra Mountains, Western Carpathians, Slovakia, and Vitosha and Rila Mountains, Bulgaria. Lead (Pb) content in the tail vertebrae was determined. Adult voles were bait-trapped and sampled in September – October (Slovakia 2009–2010, Bulgaria 2009). The sampling yielded a total of 33 animals from Slovakia and 24 from Bulgaria. Lead concentrations in bones of voles from the Rila and Vitosha Mountains were approximately three-fold lower than in bones of voles from the Tatra Mountains. The concentration of lead in the bones of voles from Rila Mountains was statistically indistinguishable from that of vole bones from Vitosha Mountains. The content of lead in the bones of voles indicates that the Western Carpathians are one of the most polluted with lead alpine regions in Europe. The usefulness of snow voles as a bioindicator of environmental contamination in alpine ecosystems was highly recognised. At regional as well as at global scale, this species is a suitable biological indicator of the environmental hazards of lead pollution.

Key words: Snow vole, atmospheric lead, Vitosha, Rila, Tatra Mountains

Introduction

There is evidence of increased deposition of lead over large mountain areas of Europe. The major source regions are often referred to be in Central and Eastern Europe (UN ECE 1995). For lead deposition in the mountains, the importance of the proximity of the source is known from many studies (Shotyk *et al.* 2000). In alpine habitats the relative importance of more poisonous organolead increases and consideration must be given to altitudes above the planetary boundary layer (ca. 1500 m) which are more influenced by long-range transport processes (Shotyk *et al.* 2002).

Worldwide, environmental pollution may impact animal populations even if acute poisoning effects or levels are not observed (Janiga *et al.* 2012). In comparison to many other alpine mammals, the snow vole *Chionomys nivalis* is exposed to lead mainly by ingestion of contaminated food (Belcheva *et al.* 1998). In our previous study, we showed that the winter diet (mosses, lichens) could constitute a major pathway for the entry of Pb into the food chain of alpine habitats (Janiga *et al.* 2012). Bryophytes collect intensively numerous pollutants due to their physiological features (Zechmeister 1995, Šoltés

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1998). They have no roots, but instead rely exclusively upon atmospheric inputs or nutrient elements. They also receive lead from the air and retain it efficiently. The same applies for lichens but because they grow on a rock substratum, the snow voles may receive additional lead through lithogenic inputs (Shotyk, Le Roux 2005).

The snow vole meets the criteria of good bioindicator of lead contamination in the environment. The species is a small rodent present on the mountain massifs in Europe, Asia Minor and the Middle East (Janeau, Aulagnier 1997). It is very common in high mountains and its presence is not influenced by altitude but by petricolic soils.

We used the snow vole as a model to assess the lead related-induction in terrestrial high mountain environments of the Tatra, Vitosha and Rila Mountains. The main aim was to investigate whether the Western Carpathians are still one of the most polluted mountain ranges in Europe.

Materials and Methods

Sample collection

We studied local populations of snow voles from the Western Carpathians, Slovakia: Brestová Chain (N49°13′29.43″ E19°40′46.07″, 1902 m a.s.l.) and the Valley of Biele Plesá (Belianske Tatra Mts, N49°13′25.87″ E20°13′16.92″, 1673 m a.s.l.), and from Rila Mountain (foothills of Maluk Metchi Vrach, N42°02′00.88″ E23°25′23.85″, 2474 m a.s.l.) and Vitosha Mountain (Vulcha Skala, N42°35′52.73″ E23°17′29.50″, 1769 m a.s.l.) in Bulgaria. We sampled regularly *C. nivalis* from September to October 2009-2010 in Slovakia and 2009 in Bulgaria. The study area included alpine meadows mixed with rocky fields. In the four monitoring fields in Slovakia and Bulgaria, the traps were divided into squares or lines and set up approximately 10 m apart from one another. A hundred Sherman traps baited with fresh apples and commercial seeds for rodents were used to live-trap *C. nivalis*; dry grass was added to the traps as bedding material mainly during cold months. The traps were checked every 12 hours, at dawn and dusk during two or three consecutive days. For the purpose of this study, the global sampling yielded a total of 36 adults from Slovakia and 24 from Bulgaria. Each captured individual was identified; part of the tail was clipped. After the measurement the animals were immediately released at the point of capture. To compare the mean levels of lead in the bones of animals between different mountains, overwintering adults were only selected. The concentration of lead in the bone tissues of voles is highly dependent on the season: significantly higher concentrations of lead were detected in adult voles collected after the winter season than in young animals caught in summer or autumn (Janiga *et al.* 2012).

Detection of lead and statistics

Approximately 0.0020 – 0.0374 g of dried sample was digested with 2 ml of water, 4 ml of concentrated HNO_3 (Merck, Darmstadt, Germany) and 1 ml of H_2O_2 (Slavus, Bratislava, Slovakia) using microwave oven Mars Xpress (CEM Corporation, Matthews, USA). Decomposition temperature was 140 °C, ramp time 15 minutes and hold time 13 minutes. Then the mineralisation solution was diluted to 6 – 10 ml with deionised water. Lead contents were determined by electrothermal atomic absorption spectroscopy (AAS Perkin Elmer 1100B, Norwalk, Connecticut, USA) equipped with deuterium background correction and HGA 700 graphite furnace with automated sampler AS-70 under the conditions: wavelength 283.3 nm; slit 0.7 nm; lamp current 10 mA. Temperature: Drying 1: 70/10/10; Drying 2:150/2/60; Pyrolysis: 800/15/30; Atomization: 1800/0/3; Cleaning: 2500/0/3 (temperature (°C)/ ramp time (s)/ hold time (s)).

To prepare calibration solutions, aliquots were taken from a stock standard solution 1000 $mg.l^{-1}$ of Pb (Merck, Darmstadt, Germany). The calibration range was 5 – 20 $\mu g.l^{-1}$. The matrix modifier $NH_4H_2PO_4$ (0.2 mg) was used by the determination of Pb.

The data were normally distributed; the results were statistically compared using one way ANOVA and Tukey's test at the 95% confidence level ($P < 0.05$). All statistical analyses were performed with Statistica 8 software for Windows.

Results

Lead concentrations in the tail vertebrae of snow voles were clearly higher in the Tatra Mountains than in Vitosha and Rila Mountains (Table 1). Mean total concentrations of lead in the snow vole tail vertebrae from the Eastern – limestone Belianske Tatra Mountains and the Western Tatras are compared in Table 2, and from Vitosha and Rila Mountains in Table 3. In both regional cases, differences between regional samples were not found to be significant.

Discussion

Lead in the mountains

Lead has been used by humans for thousands of years and is a natural constituent found in rocks. Although

Table 1. Mean autumn lead concentrations (μg g^{-1} dry weight) in the tail vertebrae of the snow vole adults from the Tatra, Vitosha and Rila Mountains. Groups with different index in a column are significantly different at P = 0.001. One-way ANOVA

Location	n	Mean	SD
Tatra Mountains	36	9.08a	0.94
Vitosha and Rila Mountains	24	3.29b	1.16

Table 2. Comparison of lead levels (μg g^{-1} dry weight) in the tail vertebrae of *Chionomys nivalis* form the Western Tatra and Eastern Tatra Mountains. The means did not differ significantly (One-way ANOVA at P = 0.05.)

Location	n	Mean	SD
West Tatra Mountains	23	8.65	1.19
Belianske Tatra Mountains	10	11.32	1.81

Table 3. Comparison of lead levels (μg g^{-1} dry weight) in the tail vertebrae of *Chionomys nivalis* form the Vitosha and Rila Mountains. The means did not differ significantly (One-way ANOVA at P = 0.05.)

Location	n	Mean	SD
Rila	14	4.07	1.53
Vitosha	10	2.18	1.82

several adverse health effects of lead have been known for a long time, exposure to lead continues, and has been even increasing in some regions. In contrast to declines in emissions from many sources especially gasoline, the emissions from stationary fossil fuel combustion tend to increase. The main use of Pb today (74%) is in lead-acid batteries, especially for cars (Shotyk, Le Roux 2005). Lead emitted from these sources is released in the air in the form of sub-micron particles, with a median diameter of 0.5 mm. The particles are considered to increase in size rapidly in the atmosphere owing to coagulation with other particles. Due to their longer atmospheric residence time, smaller particles can be carried by winds and deposited over very large areas. (Chamberlain *et al.* 1979). Currently, the major source-polluted mountains in Europe are in the densely populated and heavily industrialised regions of Central and Eastern Europe. Anthropogenous stress to the alpine ecosystems of the Western Carpathians is caused by local emitters and long-range transport. Attention should be paid to the lead depositions. They accumulate in the upper soil layers (Ciriaková *et al.* 2011), increase with altitude (Janiga 2008) and are important because they can influence soil-biological processes (Bengtsson, Tranvik 1989, Janiga 1999, 2001). The stress lead pollutants from the air impose on the vegetation depends on site, exposure, and altitude above the sea level, on the orographic and meteorological conditions, and on the local emission situation (Smidt *et al.* 1996). For example, the cumulative mass of atmospheric anthropogenic Pb was calculated for the Swiss Alps, using peat cores from eight mires (Shotyk *et al.* 2000). The lowest values were found at north -east part of the Swiss Alps, the highest values originated from the south side with direct exposure to the highly industrial region of Northern Italy. The higher levels of lead were also found, for example, in the bog at Bagno in Ukraine, west from the East Carparthian Mountains, and directly exposed to the industrial regions of Eastern Europe, compared to the other bogs from the same country (Shotyk, Le Roux 2005). The occurrence of the highest levels of lead contamination in the Carpathians is consistent with results from an extensive survey of sediments taken from European mountain lakes (Camarero *et al.* 2009). From the sediments and soils, the metal is bioaccumulated by herbivores along the food-chain. Wild vascular plants, mainly mosses and lichens, may influence significantly the uptake of lead (Sivertsen *et al.* 1995, Belcheva *et al.* 1998, Metcheva *et al.* 2008). The amount of lead in the major component of the diet of snow voles, *Poa alpina* and *Nardus stricta*, is at the level from 12 to 15 µg/g in Bulgarian mountains (Belcheva *et al.* 1998). In the Tatra Mountains, the lead level in similar vascular plants is at the average level of 16.1 µg/g (Janiga 2008). The amount of lead in the vascular plants depends on seasons, habitats, soils, local microclimate and therefore, the direct comparative study of intoxication of plants by lead is needed.

Lead concentrations in small mammals are usually substantially higher than lead concentrations from bones of small mammals in many European mountains including the Alps (Madry *et al.* 2015) In this study, we compared the lead concentrations in the bones of voles from Slovak (the Tatras) and Bulgarian mountains (Vitosha and Rila). The comparison showed that the lead concentrations in the Tatra samples were of higher order than those taken from the Bulgarian mountain locations. Rila and Vitosha Mountains in Bulgaria are situated relatively far from heavy industrial activities. Their alpine zones are exposed to long-distance transfer of lead aerosol pollutants; the nearest potential source is the factory for non-ferrous metals (120 km to the East, between Plovdiv and Asenovgrad in the Tracian valley), since 2004 of emission free production. The second possible reason of the relatively low amount of lead in the vole bones is different microclimate (precipitation, wind direction, etc.).

Regional differences: short-term versus long-term bioindicators

Dietary ingestion is often recognised as the primary source of environmental metal intake in small mammals (Hunter *et al.* 1987, Ma 1989). However, the transfer of metals from the environment to terrestrial mammals depends on several abiotic and biotic factors, such as microhabitat and behaviour of the species involved. *Chionomys nivalis* prefers microhabitats characterised by a remarkable scarcity of vegetation (Luque-Larena *et al.* 2002). A physiological argument in support of small mammals as bio-indicators of the short-termed exposure of terrestrial wildlife to environmental pollutants is related to their small body size. Due to a high metabolic rate their intensity of exposure may be expected to be greater than in large mammals which have a slower metabolic rate (Gdula-Argasinska *et al.* 2004). Consequently, the animals may accumulate equal amounts of lead at regional level during one winter season. There were no significant differences in the bone lead concentrations between voles from Rila and Vitosha Mountains, or between voles from the Western (granite) and Eastern limestone Belianske Tatra Mountains. In the alpine areas, small mammals are top bioindicators of short-term and seasonal lead pollution. In contrast, large herbivores such, as chamois, are more sensitive bioindicators of regional differences in lead pollution. The observed drop in the bone lead levels in animals from the Western Tatras to the Eastern Belianske Tatras supports the hypothesis that atmospheric depositions of lead may influence its accumulation in animals in remote areas (Janiga *et al.* 1998). Chamois may serve as usable long-term bioindicator. In the Western Tatras the level of lead in the chamois trophies did not change from 1946 to 1995 as might have been expected. Since the Tatras represent a barrier for the pollutants from the northern part of Central and Eastern Europe (current major source of atmospheric lead in Europe), we suggest that this is the main reason why lead level did not decrease in the bones of chamois for a long time (Janiga *et al.* 1998).

Our results encourage additional research to ascertain the effect of climate change on the environmental cycle of lead (Morueta-Holme *et al.* 2010) Effects of increased temperature and lead deposition are expected to influence distribution and concentration of lead within the bodies of alpine micromammals, their physiology, and probably change and decline of small mammal diversity in the sensitive regions of alpine rocks and meadows (Matz *et al.* 2011).

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In memoriam

Dr Maria Paspaleva-Talpeanu
(1933-2015)

In 2015, Bulgarian and Romanian zoologists have lost one of their brightest representatives. Dr. Maria Georgieva Paspaleva-Talpeanu deceased on 16 May 2015 in Bucharest, Romania.

Maria Paspaleva-Talpeanu was born on 4 July 1933 in Varna, in the family of biologists. At that time, her father, Professor Georgi Paspalev (Corresponding Member of the Bulgarian Academy of Sciences) was the director of the Marine Biological Station in Varna. Her mother, Anastasia Zhelyazkova, was assistant and researcher of Black Sea ciliates. After 1940, the family moved to Sofia. Since the autumn of 1952, Maria became a student in biology at the University of Sofia. Since her first years at the university, she was involved in studies on diversity, biology, ecology and conservation of avifauna, and continued this work during her entire life.

After her graduation, Maria occupied a position as a biologist at the Sofia Zoo. In 1958, she continued her work at the Institute of Zoology, Bulgarian Academy of Sciences, where she completed her doctoral thesis entitled *Studies on the Ornithofauna of the Bulgarian Danube Riverside* (1961). Subsequently, she was appointed as a researcher and became the head of the Bulgarian Ornithological Center. In Bulgaria, she published 25 articles on the avifauna of the country. Simultaneously, she contributed popular articles, mostly in ornithology and nature conservation, and consulted several popular films in the field of ornithology. Together with R. Doychev and S. Dimitrov, she authored the popular science book *Birds* (250 pp.) published by Nauka i Izkustvo Publishers, Sofia.

In 1969, she was married by the outstanding Romanian ornithologist Dr Matey Talpeanu and moved to live and work in Bucharest. In 1970, she occupied a position of a research assistant at the Bucharest Zoo. In 1973, she successfully applied for a research position at the Institute of Biology at the Ministry of Education of Romania and started a new research program on the avifauna of Romania. Substantial parts of her studies in this period were devoted to the diversity and biology of birds from the Danube Delta. Due to the proximity of the two countries and her interests at the avifauna of the Danube Delta and Danubian Riverside, she frequently visited Bulgaria and contributed to the establishment and development of the Natural History Museum at the Srebarna Biosphere Reserve, field studies in Rusenski Lom Natural Park, lectures in ecology and nature protection at school workshops. She also wrote numerous articles for Bulgarian popular magazines. She collaborated with Bulgarian ornithologists from the National Natural History Museum in Sofia and the Regional Museum in Ruse.

Dr. Maria Paspaleva authored more than 60 scientific articles on diversity, biology and ecology of the Bulgarian and Romanian birds, mainly published in scientific journals of the two countries. As a skilled promoter, she also wrote numerous popular articles for Bulgarian and Romanian magazines as well as three popular books: *Babush rafting down the Danube* (1970, 180 p.), *Oiseaux du Delta du Danube* (with M. Talpeanu, 1973, 289 p.) and *Living Delta* (1990, 160 p.). These books inspired many students in biology, ecology and nature conservation.

The life and work of Dr. Maria Paspaleva is an example of passionate love of nature and tireless activities for studying biodiversity, for its preservation for the forthcoming generations in Bulgaria and Romania.

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