Oecologia Montana 2017,
26, 12-18

Bio-indication of environmental pollution in alpine environments using X-ray analysis in snow vole (*Chionomys nivalis*) population

V. FTÁČNIKOVÁ[1] and M. NÉMETHY[1]

[1]*Institute of High Mountain Biology, Žilina University, Tatranská Javorina 7, SK-059 56, Slovak Republic; e-mail: ftacnikova.veronika@gmail.com*

Abstract. In this study we focused on bio-indication of pollution in alpine environments the using snow vole as a bio-indicator species. The research took place in the Dolina Bielych Plies valley, Belianske Tatras, Slovakia. The samples of tails from live-trapped snow voles, were analyzed using energy dispersive X-ray fluorescence. From a total of 26 detectable elements we detected 13 elements. Statistical analysis was performed to examine mutal relationships between the accumulated of elements between sexes and age categories. Measured elements in most cases shared lower values except in the case of molybdenum. We found significantly higher accumulation of chrome, zinc and manganese in females and similar but less significant amounts of lead and barium. There were no significant differences between the age categories. Adverse detected elements could be attributed to pollution from the mining industry and traffic.

Key words: snow vole, *Chionomys nivalis,* ED-XRF, heavy metals, pollution

Introduction

Despite the fact that chemical analyses of soils, atmosphere and water provide information on concentrations of specific compounds present in the environment, they cannot provide sufficient information on assessing their toxicity and concentrations within the life cycle. To aid in further investigation of this issue, the best indicators of environmental quality are living organisms naturally inhabiting affected areas of interest (Talmage and Walton 1991). We refer to these organisms as bio-indicators. Generally, their tolerance toward contamination is limited and their current status (presence, absence, fitness) may inform us about the quality of environmental conditions (Gadzala-Kopciuch *et al.* 2004). Small mammals are often a suitable bio-indicators. They are small in size and relatively easy to trap within their territory. During their short life span they are able to accumulate relevant pollutants in various body structures, organs and tissues where these compounds may be detected and quantified. The intensity of accumulation varies between the species, sexes, seasons and means of absorption. Suitable bio-indicators are mostly found in three families of small mammals: Soricidae, Cricetidae and Muridae. (Martiniaková *et al.* 2010; Martiniaková *et al.* 2012).

It is generally known that the mountains represent a barrier for atmospheric flow. This results in increased precipitation and subsequent deposition of atmospheric pollutants in the montane and alpine vegetation zone.

The aim of this thesis was to further investigate this effect and assess possible environmental pollution by using energy dispersive X-ray flourescence analysis to measure the accumulation of polluting elements such as heavy metals, in the tails of snow voles. Our hypothesis presumed the presence of at least some polluting elements which are detectable by the analyzer. A secondary goal was to gain deeper insight into the matter of the accumulation of polluting elements within a population structure - age, class, and sex- as well as to record other patterns in accumulation.

Material and Method

The sample site was located in the upper part of the Dolina Bielych Plies (DBP) valley. Geographically it is located in the eastern part of the High Tatras (the Western Carpathians, Central Europe). This valley covers approximately 1.8 km. The DBP valley has proven to be fruitful in terms of of successful capture of samples in past research. The next considerable advantage of this site was its proximity to the Institute of high mountain biology, where the samples could be analyzed and stored within a few hours of initial capture. Sample species included snow vole and other species from the family Cricetidae, as well as bank vole (*Myodes glareolus*).

Animals were trapped between summer (July) 2016 and late autumn 2016 (November) using H.B. Sherman traps. In the summer the traps were lined with grass vegetation picked up directly at the sample site to support thermal regulation of trapped animals. Later in the season, or when surrounding grass was too wet, hay was brought in to be used.

Trapping occurred over the course of 1-3 nights in intervals 2-3 weeks. The traps were set up at dusk and control was performed early in the morning from 5:00 to 6:00am and then again in the afternoon between 16:00 and 18:00.

We used approximately 90 traps at each sampling event. The number of traps varied as some were damaged in the course of the trapping season. The traps were set up 5-10 meters apart from each other covering all types of present habitat: under rocks, near to dwarf pine, close to grassy patches and throughout the middle parts of moraine.

After the control, traps with captured animals were brought together to one flat place where we could perform measurements and collection of samples comfortably.

Live animals were weighted with spring weights. Basic morphometric parameters were taken, including: length of a body, length of tail, length of a hind foot, and length of earlobe.

Furthermore, we noted overall condition including sexual activity and scoped the fur, ear lobes and eyes for the presence of external parasites. We identified the sex of each animal based on the distance between anus and papilla. These morphological parameters provided information on age, and we recorded two age categories: first year (juveniles and subadults), and second year (adults). After the measurements and sample collection individuals were restored to the place of their initial trapping.

We measured the concentrations of 26 elements within the tails of snow voles with ED-XRF spectrometer handheld Delta from Olympus Innov-X. Pieces of tails were placed into a small plastic vial with plastic foil at the bottom. The diameter of the vial was 2 cm and tails were placed exactly at the center of the vial. The vial was then placed on the top of the measuring window into the shielding of the X-Ray.

Each sample was measured 3 times in a non-consecutive manner and measured values were averaged. Blind controls were performed at the beginning of analyses and randomly to reject false positives or contamination.

Data from all measured variables were combined into a large matrix in MS Excel. Statistics were performed using Statistica version 12 (www.statsoft.cz 2004) starting with descriptive statistics, followed by an analysis of variance (ANOVA), which compared the variance of concentrations of elements in different categories. The first of these was the comparison of accumulated elements between the sexes, followed by comparison of accumulations between the age classes.

Advanced statistics included principal component analysis (PCA). The PCA allows for the investigation of the inner structure of the data and simplifies the description of mutually correlated traits. The basic function of PCA is the reduction of a number of variables in analysis without major loss of information (Meloun 2012). The component scores were then compared to categorical variables - sex and age classes - using two way factorial ANOVA.

Results

The ED-XRF analysis detected and provided data for thirteen out of twenty-six detectable elements. For these elements: P, Ti, Co, Ni, Cu, As, Se, Zr, Cd, Hg, Ag, the concentration was lower than the detection limit. For the elements Sb and Sn, concentrations were only partially detected. Elements where analysis did not provided sufficient data were excluded from further analyses. Table 1 displays descriptive statistics of the data on concentrations of remaining detected elements.

One way ANOVA was used to separately examine the relationship of sex and concentrations of accumulated elements. The overall results are presented in table 2. Significant variance between sex categories was observed in manganese, zinc and chromium. Nearly significant differences between males and females were observed in concentrations of barium and lead. Figures 1-6 graphically display significant differences in variance. Similarly, one-way ANOVA was used to test the variance of element concentration between age classes: 0-1 Year and 1+

Element	N	Mean (ppm)	Mininum (ppm)	Maximum (ppm)	St. deviation
S	37	4,571.14	540	16,536	3,819.61
Cl	36	975.42	413	2,768	514.86
K	37	1,264.73	254	6,438	1,206.75
Ca	37	1,535.03	606	5,716	1,172.47
Cr	37	27.95	11	62	12.04
Mn	37	59	23	223	34.39
Fe	34	212.65	55	592	126.47
Zn	37	72.49	37	156	24.76
Rb	36	16.12	10	30	3.99
Sr	23	6.19	2	19	4.15
Mo	37	7.26	4.30	9.70	1.10
Ba	37	65.97	33	138	23.32
Pb	37	15.46	10	25	3.45

Table 1. Data from ED-XRF analysis. N- sample size; ppm- parts per million.

Element	**F**	**p**	**Element**	**F**	**p**
S	1.58	0.22	Zn	4.96	0.03
Cl	1.15	0.29	Rb	2.56	0.12
K	2.21	0.15	Sr	2.22	0.15
Ca	2.42	0.13	Mo	0.10	0.75
Cr	**7.62**	**0.01**	**Ba**	**3.63**	**0.07**
Mn	**6.30**	**0.02**	**Pb**	**3.67**	**0.07**
Fe	1.54	0.22			

Table 2. Analysis of variance, difference in accumulation of elements between the sexes, results of interest are in bold.

Element	**F**	**p**	**Element**	**F**	**p**
S	0.6	0.45	Zn	0	0.95
Cl	1.49	0.23	Rb	0.11	0.74
K	0.91	0.35	Sr	0.7	0.42
Ca	0.05	0.82	Mo	0	1
Cr	0.02	0.89	Ba	0.37	0.55
Mn	0.35	0.56	Pb	0.39	0.54
Fe	0.06	0.82			

Table 3. One-way ANOVA, analysis of variance. Difference in accumulation of elements between two age classes: under 1 year, 1 year and older.

Element	**Factor 1**	**Factor 2**	**Factor 3**	**Factor 4**	**Factor 5**
S	**-0.85**	0.25	-0.28	0.19	-0.14
Cl	**-0.77**	-0.48	-0.19	0.24	-0.16
K	**-0.89**	-0.33	-0.04	0.13	-0.14
Ca	**-0.78**	**0.53**	0.07	0.07	0.1
Cr	**-0.83**	0.32	0.21	-0.29	0
Mn	**-0.89**	-0.07	-0.17	0.06	0.19
Zn	**-0.94**	-0.03	-0.13	-0.02	-0.1
Rb	**-0.69**	-0.36	0.29	**-0.46**	-0.24
Mo	-0.32	0.03	**0.88**	**0.34**	-0.01
Ba	**-0.7**	**0.63**	-0.08	-0.07	0.06
Pb	**-0.66**	**-0.5**	0.04	-0.08	**0.52**
% Variance	59.81	14.36	9.79	4.96	4.13

Table 4. Principal component analysis. % Variance - denotes the percentage of data variation represented by each factor.

Year. ANOVA (Table 3) did not reveal significant differences in any of the measured elements.

Following the statistical method was PCA. Based on the correlation matrix the analysis calculated eleven factors. Most of the variation in data was explained by the first three factors. The results are presented in table 4 together with information on how much variation in data each factor represents. The first three factors can be described and analyzed in more detail:

Factor 1 was a unipolar vector, suggesting that if values in measured variables are low they tend to be low in all variables. This effect was studied further by comparing variance in factor scores between the sexes and different age classes. Results of this analysis are presented in Fig. 4.

The comparison suggested higher accumulation of measured elements in females than in males, F=5.5 (p=0.03). This effect is present in both age classes, as there is no significant difference in metal concentrations between the age classes F=0.261 (p=0.6). There was no significant interaction between these two categorical variables F=0.026 (p=0.87).

Factor 2 proposed an inverse relationship of Pb with Ca and Ba. This indicates a higher accumulation of Ca and Ba at the expense of Pb or vice versa. The result of two-way ANOVA (Fig. 5), where the variation of factor scores 2 was compared within and between categorical variables of sex and age. This effect tended to be higher in the first year voles F=1.13 (p=0.3). However, these results were not significant. There was also no significant diffe-

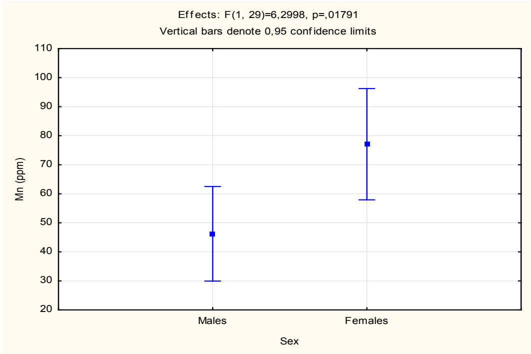

Fig. 1. One-way ANOVA. Manganese. Difference in concentrations of manganese between the sexes. ppm - parts per million.

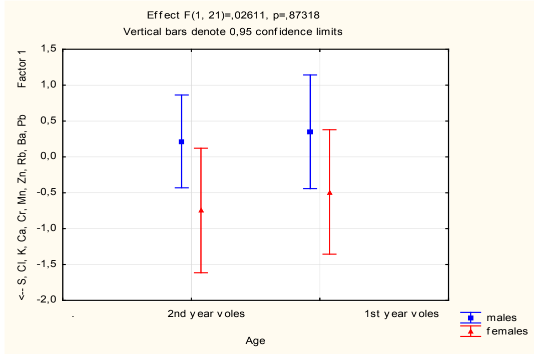

Fig. 4. Two-way ANOVA. Unipolar vector of S, Cl, K, Cr, Mn, Zn, Rb, Ba, Pb. Difference in variance of factor scores 1 in males and females and two age classes.

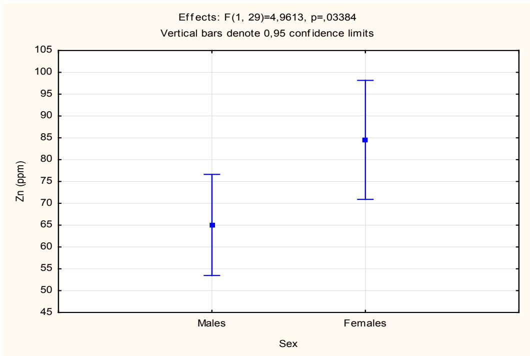

Fig. 2. One-way ANOVA. Zinc. Difference in concentrations of zinc between the sexes. ppm - parts per million.

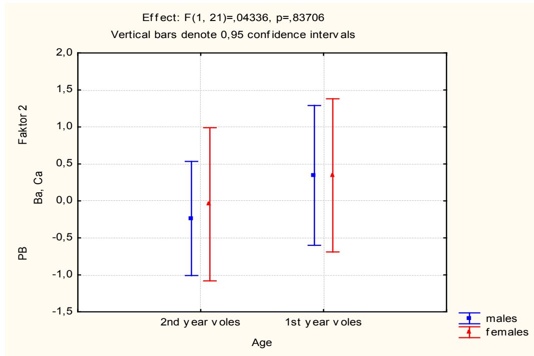

Fig. 5. Two-way ANOVA. Inverse relationship of Pb with Ca and Ba. Variances of factor scores 2 in males and females and two age classes.

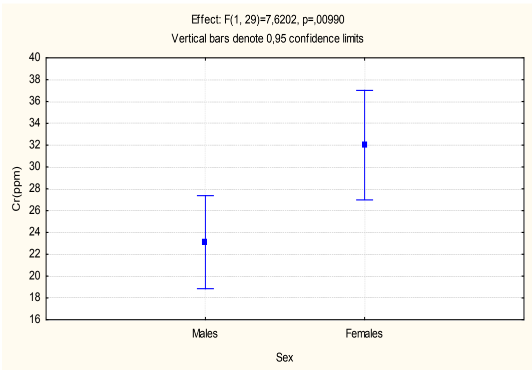

Fig. 3. One-way ANOVA - Chromium. Difference in concentrations of chromium between the sexes. ppm - parts per million.

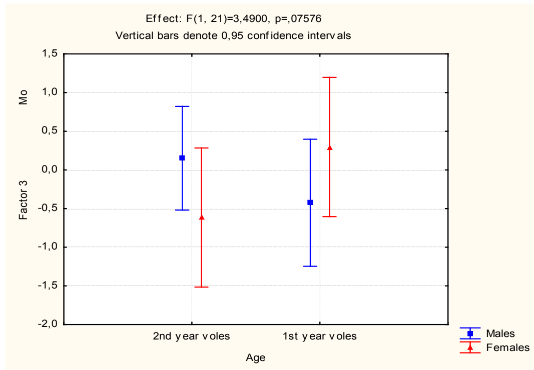

Fig. 6. Two-way ANOVA. Molybdene. Difference in variance of factor score 3 in males and females and two age classes, suggesting different accumulation of Mo in between the sexes and age classes.

rence in variation of factor 2 between sexes F=0.04 (p=0.83) and between two selected categories F=0.04 (p=0.84).

The third factor presented variability of Mo concentrations in tails. Two way ANOVA did not reveal a significant relationship in the variation of Mo with age F= 0.18 (p=0.68) or sex (F=0.03; p=0.95). More interestingly, the comparison of shared variation between age classes and sexes suggests that the Mo concentrations are decreasing in adult females and increasing in adult males compared to juveniles F=3.49 (p=0.08) (Fig. 6) but the results were not statistically significant.

Discussion

In this work we have monitored the bio-indication of environmental pollution in alpine environments using X-ray analysis of tail vertebrae from the snow vole (*Chionomys nivalis*) population. This species has been successfully used as a bio-indicator in many other environmental studies throughout Europe, including in Italy, Bulgaria, Romania and Spain (Teodorova 2001; Metcheva *et al.* 2002; Topashka-Ancheva *et al.* 2003; Metcheva *et al.* 2008). Altogether we trapped 80 animals. And took 80 tail samples. From these, 42 were tails from snow voles and 38 from bank voles (*Myodes glareolus*).

Most of the detected elements were bio-gene elements which form a fundamental part of the body and are naturally present within the body structures and tissues in larger concentrations. Such elements included K, Ca, S and Cl. Other bio-gene elements are present only in subtle concentrations and are called trace elements. They are required for certain biochemical and physiological functions (Nordberg, Fowler and Nordberg 2014), and for our study included Cr, Mo, Mn, Zn and Fe.

Some of the detected elements represent hazardous materials and their presence within the samples is adverse and undesirable. These elements do not naturally occur within the body and originate from anthropogenic activities. Such detected elements were rubidium (Rb), strontium (Sr), barium (Ba) and lead (Pb). These were also detected in the study by Belcheva et al. (1998), who investigated pollution in the area of Rila Mountain, precisely the peak of Moussala and also in the study by Metcheva, Teodorova and Topashka-Ancheva (2002).

In this study we found significant difference in the accumulation of Zn, Cr and Mn, where significantly lower values were measured in males. Similar but less significant effect was observed for Pb and Ba. The age classes did not suggest differentiation in the content of measured elements. The relationship between zinc and lead content in the liver and body of *Chionomys nivalis* versus their content in food is well documented in work of Metcheva *et al.* (2003). However, as investigated in work of Walshe *et al.* (1994), the concentration of zinc does not build up with continued exposure, because the amount of accumulated zinc is affected by homeostatic mechanisms.

Martiniaková *et al.* (2012) studied accumulation of zinc and lead among other heavy metals in the femurs of four distinct rodent species (*Apodemus flavicolis*, *Apodemus silvaticus*, *Myodes glareolus* and *Microtus arvalis*) in two polluted areas located in Slovakia (Nováky- coal mine and powerplant, Nitra-intensive agriculture). The mean values were similar in all species, ranging from 19.82 to 22.30ppm in Pb and 119.99 - 188.55ppm for Zn. In our study the mean amount of Zn was considerably higher (72.49 ppm ± 24.76), while the mean lead concentration was substantially lower (15.46ppm ± 3.45).

The study by Damek-Poprawa and Sawicka-Kapusta (2003) found nearly double the level of Zn (*Apodemus flavicollis*) (mean 166 ppm ± 7.6), and higher mean concentrations of Pb (172.36 ppm ± 14.16) in the yellow-necked mouse. However, their study area was in close proximity to zinc smelters in Bukowno (Poland), and this must be considered. This smelter is 127 km to the northwest of our study area, thus it is possible that increase amounts of Zn detected at our locality was due to aerial transport from southern Poland which lies headwind (Prevailing winds western, north-western) from our locality.

In the study by Dimitrov *et al.* (2016) the concentrations of Pb in the livers of three rodent species (*Microtus arvalis, Apodemus flavicollis, Mus macedonicus*) were studied at three sites located along the pollution gradient. The source of pollution was was a Pb and Zn smelter impacting the Strandzha national park (Bulgaria). The concentrations of Pb for species in the polluted areas were 7.53 ppm, 17.1 ppm and 15.5 ppm respectively. Dimitrov argued that the low retention of Pb in voles is an effect of good excretion compared to other rodents. Furthermore, their study not only revealed similar values of Pb, but also the tendency of higher accumulation of Pb in females (however, statistically insignificant). Considering the similar amount of Pb at our locality compared to the locality in Dimitrov's study (which was in close proximity to the source of pollution), we can infer that our study area is largely affected by airborn Pb.

Additional differences in concentrations between the sexes occured in Cr, Mn and Ba. As mentioned above, Cr (in oxidative state CrIII) occurs naturally within the bodies of mammals in trace concentrations. Similar to zinc, it is responsible for maintenance of homeostasis and optimal physiological functioning of organisms. Its content is thus dependent on physiological processes in which gender may be decisive. Cr play a substantial role in the metabolism of basic nutrients like saccharides, lipids and proteins. Cr is also needed for correct function of the brain and leucocytes. Most of the Cr accumulates within the liver, kidneys, muscles, lipids, and skin (Koréneková 2010; Tchounwou *et. al.* 2012). Therefore it is possible that X-Ray analysis measured traces of Cr from the skin at the end of tails. The source of the Cr in the bodies of snow voles is likely *Juncus trifidus* which constitutes a significant part of their diet (Pryor 1985). Concentrations of Cr have been recorded in parallel research conducted by Krendželák (2017). However, X-ray methodology poses a limitation in that that it cannot distinguish the compound in which the Cr was present in the body. Thus, we are unable to differentiate between bio-gene Cr (III) and the presence of other forms of Cr (different oxidation state Cr (VI)). There is no proof of bio-accumulation within the terrestrial food chain (soil-plants-animals) (Clay 1982, ATSDR 1992)

Another element subjected to deeper investigation based on the results of ANOVA with sex as the categorical variable is Mn. Here the most probable explanation of higher content in females is as a result of iron deficiency due to pregnancy. This deficiency may affect dysregulation of other metals including Mn as explained by Fitsanakis *et al.* (2009) who tested this effect on rats with different diets. Mn plays a role in the activation of various enzymes, and is necessary for the production of sex hormones, transformation of lipids, saccharides and proteins. It also affect the development of cartilage.

Finally, Ba was found in higher concentrations in females than in males. Barium represents approximatly 0.05 % of Earth crust, and its mechanism of entry into the environment is both natural and manmade. Naturally, it enters the environment through the weathering of minerals and rocks. Anthropogenic Ba is released into the air during the mining and processing of ore, through manufacturing, and burning of coal and fossil fuels (Nordberg *et. al.* 2014). Normally Ba occurs in the environment in subtle concentrations: 0.0015 ppb in clean air, 0.33 ppb in polluted areas and only 0.38 ppm in ground water (ATSDR 1992). The intake of Ba in animals is most prominently dietary, and its absorption after ingestion is dependent on the ability of Ba salt to dissolve, age, starvation and presence of sulfates in the diet. As our study did not recorded significant differences in age, young females could have higher absorption rates as a result of dietary stress caused by gestation periods.

A study from the United States (NTP 1994) investigated the accumulation of Ba in femur bones of rats through dietary ingestion. Here the control group had mean values of 3 ppm, while exposed to water including 2,500 ppm of Ba over the course of 15 months had mean values of accumulation around 1,200 ppm. They found that Ba competes with Ca in the process of ossification (Nordberg *et al.* 2014). As such, it is possible that more Ba was accumulated in females due to higher demand on calcium needed for prenatal development of their younglings.

The PCA investigated mutual relationships between analyzed elements. The most important principal component (Factor 1) explained almost 60 percent of variability the data. It suggests that in individuals with a lower concentration of elements, the concentration tended to be lower in all measured elements except Mo. Possibly these were the individuals with the lowest contamination rate. This effect was stronger in males than in females and did not differ in between the age classes.

Factor 2 explained an inverse relationship of PB with Ba and Ca. This factor was not dependent on age or gender categories. As mentioned previously, the Ba and Ca competes in the process of ossification, and in rats the Ba accumulates in the following ratio to Ca: 1.4:1 (NTP 1994). The effect of accumulation of Pb in bones when Ca levels are low is well known (Rowland 1991). As such the most plausible explanation of factor 2 is lower accumulation of Pb when there is enough Ca and Ba in the nutrition to outcompete Pb.

Factor 3 represented almost 10% of variability in the data (stands for about four samples), and thus must be discussed carefully. It proposed the rise of Mo concentrations independent from the rise of concentrations of other elements. The phenomenon described in factor 1 was prevalent in all analyzed elements but lowest in Mo. The tendency of this dataset therefore suggests that the concentration of Mo follows its own variability irrespective of other elements. In small rodents Mo is absorbed in the stomach and small intestine (Nielsen 1996). In other studies it has not been found to correlate with other elements apart from Cu and sulfates, as they share the same metabolic pathway (NRC 2005).

Acknowledgements

First of all, we would like to thank to Mgr. Zuzana Hrehová for her valuable advices and assistance during the fieldwork. We would also like to extend our gratitude to all students of IHMB who gladly helped with sample collection: Natália Kubjatková, Lenka Zábojníková, Marek Bielesh, Daniela Dúhová, Bibiana Martinková and Simona Grešíková. The research was supported by projects ITMS (Grant No. 26210120006 and Grant No. 26210120016).

References

ATSDR (Agency for toxic substances and disease registry) 1992: Toxicological Profile for Chromium. U.S. Department of Health & Human Services, Washington, D.C

Belcheva, M., Metcheva, R., Artinian, A. and Nicolova, E. 1998: Assessment of toxic elements in the snow vole (*Chionomys nivalis*) and its food from Rila mountains. *Observatoire de Montagen de Moussala OM2,* **7**: 276-280.

Clay, R.E. 1992: Multimedia Environmental Distribution of Gaseous, Dissolved, and Particle-Bound Pollutant. Master of Science Thesis, Chemical Engineering Department, University California, Los Angeles.

Damek-Poprawa, M. and Sawicka-Kapusta, K. 2003: Damage to the liver, kidney, and testis with reference to burden of heavy metals in yellow-necked mice from areas around steelworks and zinc smelters in Poland. *Toxicology*, **186**: 1–10.

Dimitrov, H.A, Mitkovska, V.I., Tzekov, V.D. and Chassovnikarova, T.G. 2016: Bioaccumulation of Cadmium and Lead in Rodent Species from the Region of Lead-Zinc Smelting Factory – Plovdiv (South Bulgaria). *Ecologia Balcanica*, **8**: 9-18.

Fitsanakis, V.A., Thompson, K.N., Deery, S.E., Milatovic, D., Shihabi, Z.K., Erikson, K.M., Brown, R.W. and Aschner, M. 2009: A chronic iron-deficient/high-manganese diet in rodents results in increased brain oxidative stress and behavioral deficits in the morris water maze. *Neurotoxicity Research*, **15**: 167-178.

Gadzała-Kopciuch, R., Berecka, B., Bartoszewicz, J. and Buszewski, B. 2004: Some considerations about bioindicators in environmental monitoring. *Polish Journal of Environmental Studies*, **13**: 453-462.

Koréneková, B. 2010: Chróm – Prečo ho potrebujeme? Online: https://www.zzz.sk/clanok/9054-chrom-preco-ho-potrebujeme (retrieved 30.3.2017).

Krendželák, P. 2017: Ecotoxicological assessment of *Juncus trfidus* in the Dolina Bielej vody valley, High Tatras. Bachelor thesis. IHMB, University of Žilina,Tatranská Javorina.

Martiniaková, M., Omelka, R., Jančová, A., Stawarz, R. and Formicki, G. 2010: Heavy metal content in the femora of yellow-necked mouse (*Apodemus flavicollis*) and wood mouse (*Apodemus sylvaticus*) from different types of polluted environment in Slovakia. *Environmental monitoring and assessment*, **171**: 651-660.

Martiniaková, M., Omelka, R., Stawarz, R. and Formicki, G. 2012: Accumulation of lead, cadmium, nickel, iron, copper, and zinc in bones of small mammals from polluted areas in Slovakia. *The Polish Journal of Environmental Studies*, **21**: 153-158.

Metcheva, R., Teodorova, S. and Topashka-Ancheva, M. 2002: A comparative analysis of the heavy metal loading of small mammals in different regions of Bulgaria. In *Monitoring points and bioaccumulation features. Ecotoxicology and Environmental Safety*, **54**:176-187.

Metcheva, R., Beltcheva, M. and Chassovnikarova, T. 2008: The snow vole (*Chionomys nivalis*) as an appropriate environmental bioindicator in alpine ecosystems. *Science of the total Environment*, **391**: 278-283.

Metcheva, R. and Topashka-Ancheva, M. 2003: Assesment of environmental contaminants in small mammals. *Observatoire de Montagne de Moussala OM2. Sofia*, **9**: 225- 230.

Meloun M. 2012: Interactive statistical analysis of data, Charles University, Prague

National Toxicology Program 1994: Toxicology and carcinogenesis studies of barium chloride dihydrate, *National Toxicology Program technical report series*, **432**: 1-285.

Nielsen, F.H. 1996: Other trace elements. In *Present knowledge in nutrition* (eds. J.W.Erdman, I.A Mcdonald and S.H. Zeisel), pp 353-377. International Life Sciences Institute, Washington, D.C.

Nordberg, F.G., Fowler, B.A. and Nordberg, M. 2014: Handbook on the Toxicology of Metals, 4th edition, Academic Press, Cambridge.

NRC (National Research Council) 2005: Mineral Tolerance of Animals: Second Revised Edition. Division on Earth and life studies, Board on agriculture and natural resources, Committee on minerals and toxic substances in diets and water for animals, National Academic Press, Washington, D.C.

Pryor, P.J. 1985: The effects of disturbance on open *Juncus trifidus* health in the Cairngorm Mountains, Scotland. In *The ecological impacts of outdoor recreation on mountain areas in Europe and North America*. RERG Report, *Reation Ecology Research Group*, **9**: 53-62.

Rowland, I.R. 1991: Nutrition, Toxicity and Cancer, CRC Press, Boston.

Talmage, S.S. and Walton, B.T. 1991: Small mammals as monitors of environmental contaminants. In *Reviews of Environmental Contamination and Toxicology*, pp. 47-145. Springer, New York.

Teodorova, S., Metcheva, R. and Topashka-Ancheva, M. 2001: Bioaccumulation and damaging action of polymetal industrial dust on laboratory mice *Mus musculus alba*. Analysis of Zn, Cu, Pb, and Cd disposition and mathematical model for Zn and Cd bioaccumulations. *Environmental Resources*, **39**:717-27.

Topashka-Ancheva, M., Metcheva, R., and Teodorova, S. 2003: Bioaccumulation and damaging action of polymetal industrial dust on laboratory mice *Mus musculus alba* II. Genetic, cell, and metabolic disturbances. *Environmental research*, **92**: 152-160.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A. K., and Sutton, D. J. 2012: Heavy Metals Toxicity and the Environment. *EXS*, **101**: 133–164.

Walshe, C.T., Sanddstead, H.H. and Prasad, A.S. 1994: Zinc: health effects and research priorities for the 1990s. *Environmental Health Perspectives*, **102**: 5–46.

www.statsoft.cz 2004: Statistica - komplexní analytický nástoj pro správu a spracovaní dat ve všech oblastech lidské činnosti. Online: http://www.statsoft.cz/ (retrieved 15.4. 2017).

Received 5 August 2017; accepted 10 November 2017.