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Heavy metals and some other elements in the teeth of the Tatra marmot (*Marmota marmota latirostris*)

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Abstract. In this study we examined element composition and accumulation in the teeth and skull bones of the Tatra marmot. In total, 88 bones of the Tatra marmot were examined. We found differences in the levels of Pb, K, Ca. Zn, Sr, Cl and S between teeth and skull bones of marmots. The accumulation of some elements depended on the age of the animals. Elements including Zn, Ca, Sr, K, Mn, Pb, Cl and S accumulated to a greater extent in molars and skull bones than in incisors. The toxic elements, mercury and cadmium were not found in the bones by XRF spectrometry. Lead was detected in 34 percent of samples**.**

Key words: Tatra marmot, XRF spectrometry, heavy metals, teeth, skeletal bone

Introduction

Heavy metals can cause harm to humans and other biotic segments of an ecosystem at specific concentrations. Levels of toxicity depend on the type of metal, the biological function of the metal, and the organism that is exposed to these effects. Toxic metals endure in an environment for years or even centuries. The most harmful toxic metals are lead, cadmium, arsenic and mercury. Marginally less harmful metals include plutonium, beryllium and chromium (Frankovská *et al.* 2010). It is well-known fact that wild animals serve as excellent bio-indicators of environmental pollution. Indicator species used in terrestrial biomonitoring studies are usually small mammals or rodents (Sheffield *et al.* 2001).

Rodents are suitable indicators in eco-toxicological studies mainly because of their short life spans, generalized diets and the preference of habitats that increase exposure through ingestion and inhalation of contaminated soils (Talmage and Walton 1991). In addition, they are geographically widespread, easily collected, and prevalent in both contaminated and uncontaminated sites (Jenkins 1981).

Teeth serve as very good markers of exposure to pollution in environment. It has been proven that elements are integrated into the mineral phase of dental tissues (Curzon and Cutress 1983). Lee *et al.* (1999) show that elements including heavy metals are integrated into the dental tissues forming at the time of their exposure. Teeth accumulate and retain lead over time and so can be reliable and sensitive indicators of the biological impact and accumulation of lead pollution (Appleton *et al.* 1999). Teeth, therefore, provide a suitable cumulative and steady record of environmental exposure, unlike bone, in which the mineral phase is subject to turnover (Budd *et al.* 1998; Sargentini-Maier *et al.* 1988, Frank *et al.* 1989).

Shore (1995), demonstrated a significant correlation between cadmium and lead residues in soil and in the liver and kidneys of some small mammals. It was concluded that soil residue data could be used to predict mean cadmium and lead concentrations in the soft tissues of these mammals. From this study it has been seen that levels of cadmium in the teeth also reflect the differing levels of cadmium in the environment.

The Tatra marmot (*Marmota marmota latirostris*), an endemic subspecies of the alpine marmot, inhabits open areas in the subalpine and alpine zones. Marmots are primarily herbivorous organisms (Frase and Armitage 1989, Mann *et al.* 1993) but their diet appears to change seasonally (Mann, Macchi and Janeau 1993). Tatra marmots mainly forage in tall-stem grasslands and tall-herb plant communities such as *Calamagrostion villosae*, or *Trisetion fusci* (Ballová and Šibík 2015). Karč (2006) has noted these marmots feeding on 21 plant species, as well as larvae of an insect from the *Carabidae* family. This investigation focuses on element composition and toxic element occurrence in hard tissues, teeth and skeletal bones of the Tatra marmot.

Material and Methods

We have chosen the Tatra marmot´ s teeth and skull bones as our research sample for heavy metal and other element accumulation. These samples were borrowed from Ing. Ján Korňan – director of Protected Landscape Area Kysuce. The origin of these samples is denoted by the name of a valley in the Western Tatra Mountains. Sample locations were recorded in relation to golden eagle (*Aquila chrysateos*) nests. Teeth were found in the nests of golden eagles and in close surroundings. Additional teeth and skull bones come from the collection at the Institute of High Mountain Biology. All the samples were collected in the valleys of the Western, High, and Belianske Tatra Mountains between 1992 and 2014. Bones, leftovers and undigested remains from golden eagle nests were sampled in the second half of June and early part of July, after young eagles had left the nest. Nests were approached through the use of climbing equipment and techniques. Monitoring of nests continued later in summer and also during autumn (Fig. 1).

After sampling, remains were sorted according to animal species, with bones subsequently separated from flesh using highly caustic sodium hydroxide. Afterward, clean bone samples were stored in plastic bags. For analysing teeth and skull bones of marmots we used a hand-held XRF spectrometer - DELTA CLASSIC (USA). XRF spectrometry identifies and quantifies elements easily and quickly over a wide concentration range. It does not destroy the sample and requires little specimen preparation (Innov-X Systems, 2005). Fig. 2 represents the principle of XRF Spectrometry.

We set the spectrometer to alloy analysis mode, as we were analysing solid material. The spectrometer was calibrated before each analysis. X-ray safety was abided by keeping distance from spectrometer while analysing. Samples were not homogenized, only lightly grinded on the measured area. We inserted the XRF spectrometer into a homemade wooden holder, and then we placed the bone in the modelling clay and inclined it toward the measurement window, so that instrument´ s nose was firmly placed on the flattest surface of the bone and would not move during analysis. Skeletal bones were measured in the frontal and parietal area of the skull, mandible, molars and incisors. We set the mode to a total of three repetitions-two with a duration of thirty seconds, and one with a duration of forty-five seconds. Average values were calculated from measurements over three data sets.

Fig. 1. Remains of food including bones of marmots in the nest of golden eagle (Korňan 2014).

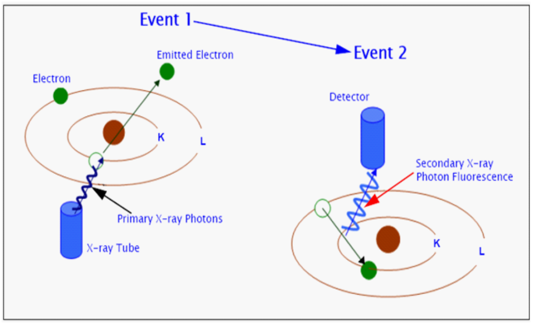

Fig. 2. The principle of XRF spectrometry (Innov-X Systems 2005).

After measuring, we populated tables with raw data. We added additional data to these tables such as the age of animals and bone type, and worked with average values only. After adjustments were made to the tables, a completed matrix was used for statistical analysis using the graphic system STATISTICA, ver. 12 for Windows. The multivariate technique of principal component analysis was used for the study of mutual accumulation of some elements in the bones. One-way ANOVA was used to test the differences among different groups. Statistical methods were applied to the following elements: P, S, Cl, K, Ca, Ti, Mn, Fe, Ni, Zn, Sr, Pb.

Results

We measured elements from samples of incisors, molars and skull bones. Using XRF spectrometry, the following elements were not detected in any of the samples: Hg, Sb, Mo, Ag, Cd, Se, Rb and Zr. Elements detected in a few samples out of 88 were: As, Cu, Cr, Sn, and Ba. Arsenic was detected in three samples ranging from six to eight ppm. All the detected samples were molars from Žiarska and Tichá Valley, from both young and old marmots.

Copper was detected in 4/88 samples ranging from 21 to 73 ppm. These concentrations were detected in molars of one old and three young marmots found in Žiarska Valley.

Chromium was detected in one sample of skeletal bone of an old marmot from Tichá Valley. Concentration of chromium in this bone sample was 15.6 ppm. There was no chromium detected in teeth.

Tin was found in five specimens ranging from 30.3 to 40 ppm. In four of five samples, tin was repeatedly found primarily in molars, and only detected once in skeletal bone. Tin was not found in incisors at all. All the specimens were from Žiarska Valley and included young and old animals.

Barium was detected in 15 samples of young and old marmots ranging from 41 to 164 ppm and found in skeletal bones, incisors, and molars from localities of in the Western, High and Belianske Tatra Mountains. In most cases, barium was detected in skeletal bone; however the highest concentration was measured in the molar of an old marmot from Veľká Studená Valley.

Calcium accumulated to a greater extent in molars in comparison to incisors. The animals did not differ in the accumulation of these elements according to age groups (Fig. 3).

While calcium accumulated mainly in molars, zinc was more prevalent in the skull bone, and older animals contained more zinc in their bones than young ones (Fig. 4).

Skull bones also contained the highest levels of strontium, but the quantity of strontium did not increase or decrease based on age group (Fig. 5).

Incisors contained significantly less potassium and manganese compared to molars (Fig. 6, 7). Molars also contained more chlorine (Fig. 8) and sulphur (Fig. 9).

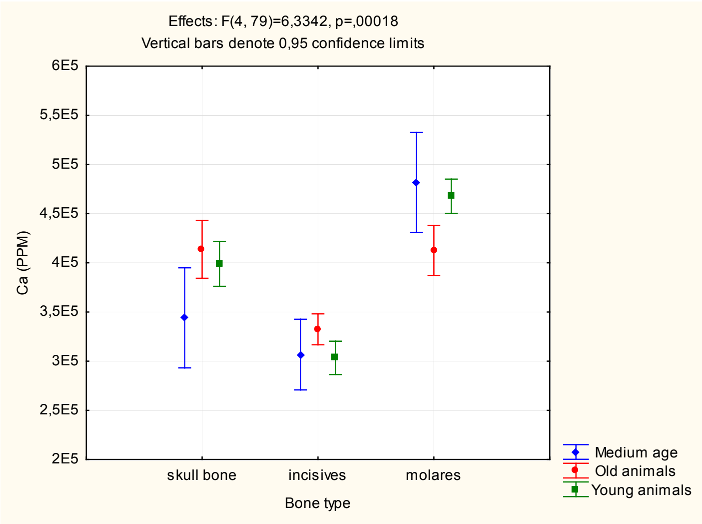

Fig. 3. Molars contained more calcium than incisors or skull bones (F=66.9, p=0.00, N=88), molars of medium aged animals contained more Ca than old or young marmots, and it was vice versa in skull bones. In general (see significant difference in the effects of interactions of the two factors in the picture), the amount of calcium in bones or teeth did not differ based on the age of the sample (F=0.39, p=0.68, N=88).

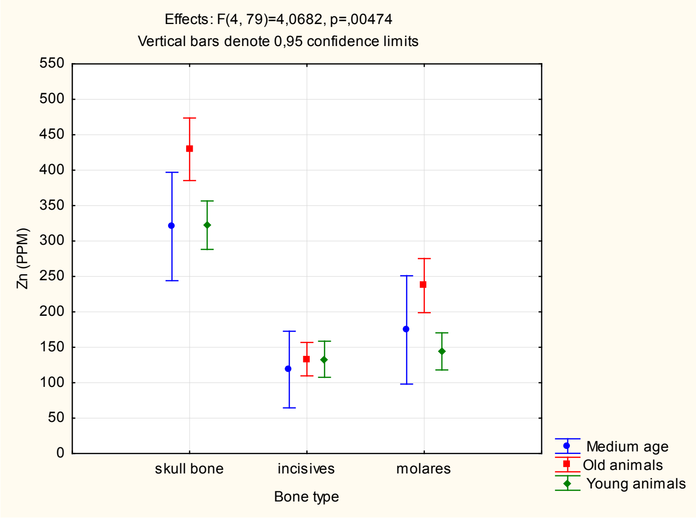

Fig. 4. The amount of zinc was higher in the skull bones than in the teeth of marmots (F= 71.9, p=0.00), old animals contained the highest levels of zinc (F=12.7, p=0.01), and old animals contained more zinc in skull bones than they did in incisors (effects of interaction are denoted in figure) N=88. Note that the effects of interaction between two measured factors - age and bone type - were not significan at p = 0.05

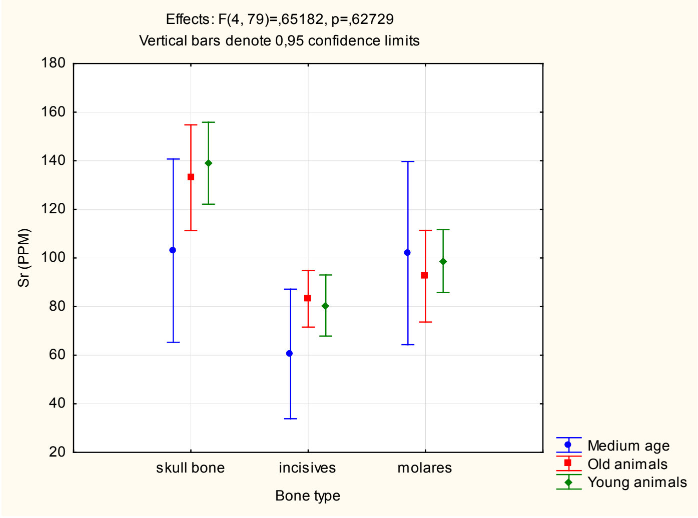

Fig. 5. Skull bones contained the highest amount of Sr, molars and incisors significantly less. (F=14.5, P=0.00). Animals did not differ in age groups. (F=1.32, P=0.27) N=88. Note that the effects of interaction between two measured factors - age and bone type - were not significan at p = 0.05.

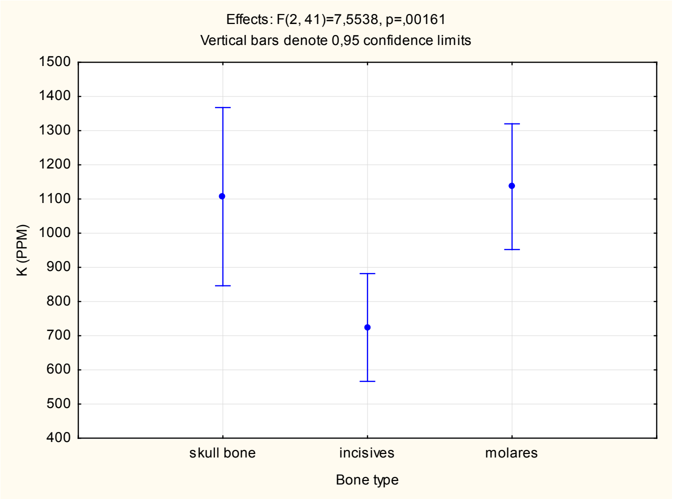

Fig. 6. The amount of potassium differed in bone types (F=7.6, P=0.02). The highest concentration was measured in molars; the lowest amount was in incisors. Animals did not differ in age groups (F=2.5, P=0.1) N=46.

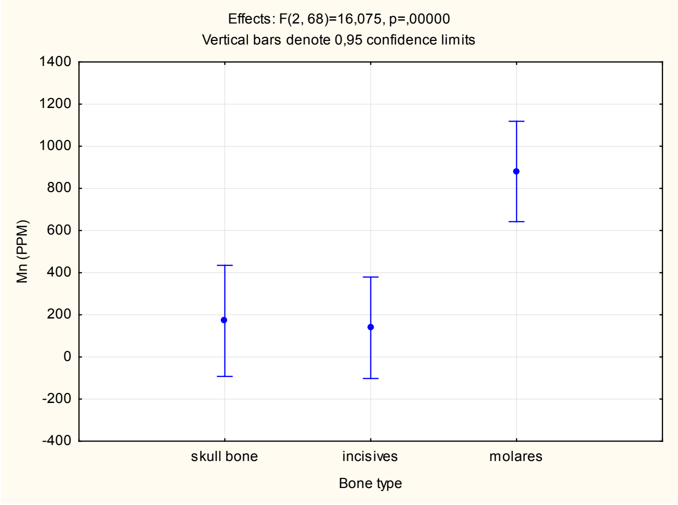

Fig. 7. Amount of manganese did not differ in age groups. Highest amount of manganese was detected in molars. (F=0.9, P=0.4) N=73.

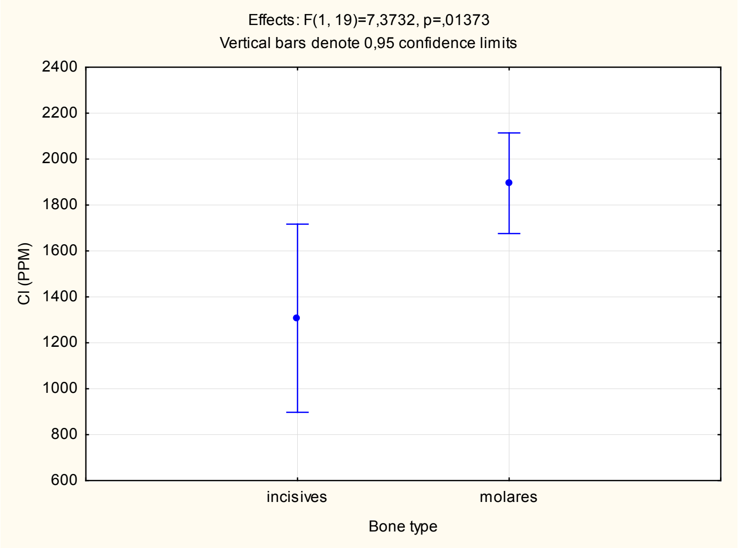

Fig. 8. The amount of chlorine differed across bone types; molars contained more chlorine than incisors. Chlorine did not vary according to age (F=2.2, P=0.1) N=23.

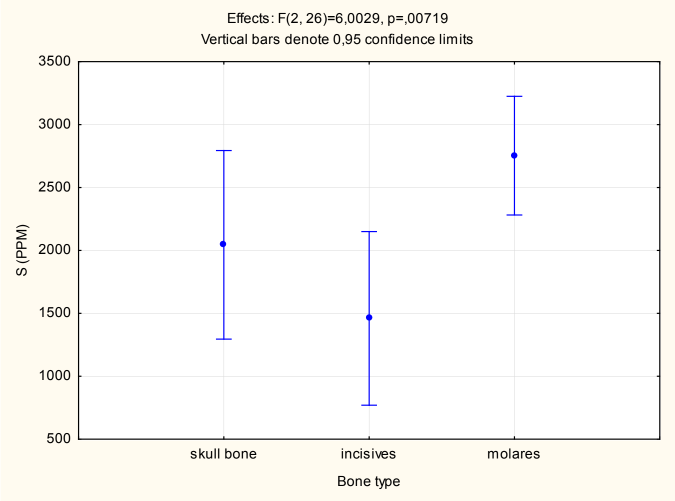

Fig. 9. The highest level of sulphur occurs in molar teeth. Incisors contained the least amount of sulphur. (F=0.6, P=0). No significant deviations occurred between age groups, N=31.

Discussion

Undetected elements

The group of undetected elements included: Hg, Sb, Mo, Ag, Cd, Se, Rb, Zr. The most toxic elements in this group are cadmium and mercury.

In our environment, cadmium generally appears together with zinc. The largest sources of cadmium are: burning fossil fuels; metallurgical engineering and metal processing (Zn, Pb, Cd); atmospheric pollution of metallurgic origin (Pb, Zn); waste containing cadmium; and use of fertilizers containing cadmium (Frankovská *et al.* 2010). Emitted cadmium accumulates in soil and water, and thereby enters the food chain (Ďurža and Khun 2002). Cadmium does not contribute to any essential biological function, and is highly toxic for plants and animals. The reason that cadmium was not detected in bones could be that it tends to accumulate mainly in the liver and kidneys as stated by Eisler (1985).

Another harmful and highly toxic element is mercury, which was not detected in any of the samples. A very dangerous characteristic of mercury is its ability to change from one form to another within the body, as per (Ďurža and Khun 2002). Mercury gradually enters the food chain following methylation of mercury in anaerobic conditions within lakes, rivers and seas. It is present in the tissues of all organisms, but the concentration of mercury is generally less in terrestrial animals than in aquatic ones (Kabata-Pendias and Mukherjee 2007). The main target organs of mercury accumulation are kidneys and liver (Ďurža and Khun 2002).

Selenium is both an essential and a toxic element. In general, selenium has a protective effect in organisms. According to Parízek and Ostádalová (1967), selenium has the ability to protect organisms from the acute toxicity of mercury. Selenium compounds have similar toxic effects to arsenic compounds. However, selenium is also an essential element for organisms, in small quantities. The toxicity of selenium is mainly caused by its interaction with other elements (Barceloux 1999). The absence of these elements in our samples may be attributed to their tendency to accumulate in tissues other than bone.

Detected elements in all the samples

Only Zn, Sr, P and Ca were detected in all 88 samples. The highest amount of zinc was detected in skull bones, with significantly less found in molars and incisors. Old animals contained more zinc in their bones than middle-aged and young marmot. The concentration of zinc was increased with age (Fig. 8). According to Aitken (1976), skeletal zinc is retained better than calcium later in the animal's lifespan. The highest zinc concentrations were found in the skull bones of old marmot from Žiarska Valley (516 ppm). Samples taken from polluted areas like Kolíňany and Nováky (Martiniaková *et al.* 2012), had a much lower zinc level, not exceeding 219.42 mg kg^{-1}. We observed that marmots from Žiarska Valley contained significantly more zinc than rodents from well-known polluted areas. According to Mishima *et al.* (1997) and Jemai *et al.* (2007), zinc has a preventative effect against toxicants such as cadmium. An important source of zinc emissions include burning of coal and other fossil fuels, metallurgy, mining, and application of artificial fertilizers and pesticides. Zinc is released into the atmosphere in large quantities through metallurgical processes (Alloway 1995). Zinc is an essential trace element involved in many different mechanisms - including its important role in protein and nucleic acid metabolism - and is found in relatively high concentrations throughout the body. Zinc is concentrated in the bones of mammals (150 ± 250 μg^{-1}), as they constitute a large proportion of total body weight, but can also be found in the wool or fur of these animals (Underwood 1977).

The highest amount of strontium was measured in the skull bones of young animals. Old animals contained strontium mainly in their incisors, and medium age marmots contained strontium mainly in their molars. The animal body absorbs strontium as if it was calcium. Our results represent different accumulations in each bone type. The measured mean value in our study was 96.1 ppm. Comparatively, Appleton *et al.* (1999) measured a mean strontium concentration of 84.6 μg.g^{-1} in the molar teeth of the bank vole of the Wolski Forest. Another study supports the same theory, stating that once absorbed, strontium is distributed throughout the body but is preferentially deposited in bones and teeth (Johnson *et al.* 1968). The metabolism of strontium is similar to calcium, but in general, animals utilise and retain strontium less effectively than calcium (Comar 1967; Comar and Wasserman 1964). Strontium follows the physiological pathway of calcium, with 99% of its content present in bone, for which it is likely essential (Wadhwa and Care 2000).

Metabolism of phosphorus and calcium is linked, so these two elements should not be considered separately. Both are important in bone and tooth metabolism. Phosphorus, together with other elements as Zn, Pb, Sr and Ca are accumulated in higher concentration in skull bones than in teeth.

Calcium is an essential nutrient, required for growth and development. It builds the skeleton and helps to prevent many skeletal disorders during childhood and adolescence (Matkovic 1991, 1992; Matkovic *et al.* 1990). Across all bone types, the concentration of calcium varied based on age group (Fig. 4). The highest concentration of calcium was measured in the molar teeth of middle-aged marmot while old animals showed the highest accumulation in skull bones and incisors. We expected the calcium level to decrease in older specimens. As stated by Chan and Duque (2002), bones tend to loose important minerals such as calcium as animals age. However, our results could be explained by other studies which have shown marmot bones to retain strength throughout their life span, as they preserve calcium during hibernation (Doherty *et al.* 2012; Wojda *et al.* 2012).

Elements detected in some samples

Manganese is an essential nutrient and it has been experimentally shown that its lack causes skeletal anomalies and impairment of growth (Priest and Van de Vyver 1990). The use of methylcyclopentadienyl manganese tricarbonyl as a replacement for tetraethyl lead as a fuel additive may influence rising manganese concentrations in the environment (Priest and Van de Vyver 1990). Maňkovská *et al.* (1996) stated that the most substantial manganese mobilization takes place at higher altitudes. Manganese is important for normal processes in the body, but adverse health effects have been noted at higher concentrations. Excessive manganese exposure, predominantly reported in adults exposed occupationally via inhalation, has been associated with adverse central nervous system effects. In experimental animal studies, very high doses of manganese were shown to impair male fertility resulting in foetal toxicity (Elbetieha *et al.* 2001). Our graph (Fig. 7) shows that molars contained significantly more manganese than skull bones and incisors. There were considerably higher concentrations measured in the molar teeth of seven young marmots from Žiarska Valley, where the concentration rose from 1,075 - 3,167 ppm.

Our results reflected the presence of chlorine in incisors and molar teeth but not in skull bones. Molars contained more chlorine than incisors. Chlorine content in teeth did not differ across age groups (Fig. 8). Chlorine is used in paper pulp and cellulose bleaching, and many persistent pesticides (fungicides) also contain chlorine. The use of chlorinated pesticides and polychlorinated biphenyls pose a great threat to the environment as all the compounds transfer over long distances and afterwards accumulate in the fat of mammals (Kabata-Pendias and Mukherjee 2007).

Distribution of potassium was similar to other measured elements, with the highest amount present in molar teeth. There was no difference found between age groups. In incisors, the lowest levels of potassium were detected (Fig. 6). Potassium is mainly an intracellular element, and is important for many metabolic mechanisms. Bone metabolism is resistant to lack of potassium (Dermience *et al.* 2015).

Arsenic is a wide-spread toxic metal in natural ecosystems. It is released into the atmosphere through metallurgical processing of Pb, Ag, Cu, Fe (among others) and by burning coal in power plants. It makes its way into the soil through anthropogenic means from the atmosphere through precipitation. Arsenic is dangerous due to its ability to transform within the body into various toxic compounds, such as arsenic trioxide (Frankovská *et al.* 2010). Arsenic is present in the soil in the form of aluminium and iron arsenites and arsenates. Arsenates are toxic to plants. In our samples, arsenic was detected in molar teeth of two young and one medium-age marmots found in Žiarska Valley, ranging from six to nine ppm.

Copper is one of the essential elements important for bone metabolism. Copper deficiency in animals caused deformed bones, frequent fractures, hypoplasia and brittle bones (Dollwet and Sorenson 1988, Keen *et al.* 1998, Sarazin *et al.* 2000). In our samples copper was found in four cases, all from Žiarska Valley sample location, in molar teeth. Copper is absorbed primarily in the stomach (Dodds-Smith *et al.* 1992), and subsequently stored in the liver.

Nickel was primarily concentrated in the molars and incisors across all age groups. The highest concentrations of nickel were found in young marmots, and the lowest in old marmots. Nickel was detected in all sample locations throughout the Tatra Mountains. Natural sources of nickel in soil include volcanic activity, salt particles, forest fires, and meteor dust. Anthropogenic sources of this metal include coal, fuel and oil combustion, mining and metallurgy of nickel, and sewage sludge (Alloway 1995).

Titanium accumulated mostly in the teeth, but in a few cases we detected titanium in skull bones. The highest amount of titanium was measured in incisors of young marmots from Tichá Valley. Values ranged between 97 and 9,507 ppm.

Similarly to titanium, iron accumulated in higher concentrations in the teeth than in skeletal bones, but the highest value was detected in the skull bone of old marmots from Belianske Tatra Mountains.

The results of this study show that elements accumulate primarily in skull bones and molar teeth of sample marmots. Molars and incisors differ in their composition; incisors are comprised of enamel on the front surface and dentine on the back, while molars are largely composed of dentine. Incisors of rodents grow throughout life due to their continuous attrition (Klevezal and Anufriev 2013). Molars remain static and so reflect the exposure to elements and toxins more effectively than incisors.

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References

Aitken, J.M. 1976: Factors affecting the distribution of zinc in the human skeleton. *Calcified Tissue Research,* **20**: 23-30.

Alloway, B.J. 1995: Heavy Metals in Soils. Chapman & Hall, London.

Appleton, J., Lee, K.M., Sawicka-Kapusta, K. and Cooke, M. 1999: The heavy metal content of the teeth of the bank vole (*Chionomys nivalis*) as an exposure marker of environmental pollution in Poland. *Environmental Pollution*, **110:** 441-449.

Ballová, Z. and Šibík, J. 2015: Microhabitat utilization of the Tatra marmot (*Marmota marmota latirostris*) in the Western Carpathian Mountains, Europe. *Arctic, Antarctic, and Alpine Research,* **47:** 169-183.

Barceloux, D.G. 1999: Selenium. *Journal of Toxicology: Clinical Toxicology,* **37**: 145-172.

Budd, P., Montgomery, J., Cox, A., Krause, P., Barreiro, B. and Thomas, R.G. 1998: The distribution of lead within ancient and modern human teeth: implications for long-term and historical exposure monitoring. *Science of the Total Environment,* **220:** 121-136.

Chan, G.K. and Duque, G. 2002: Age-related bone loss: old bone, new facts. *Gerontology,* **48:** 62–71.

Comar, C.L. 1967: Some principles of strontium metabolism: implications, applications, limitations. In: *Strontium Metabolism* (eds. J.M.A. Lenihan., J.F. Loutit and J.H. Martin), pp. 17–31. Academic Press, London.

Comar, C.L. and Wasserman, R.H. 1964: Strontium. In: *Mineral metabolism* (eds. C.L. Comar and F. Bronner), pp. 523-571. Academic Press, London.

Curzon, M.E.J. and Cutress, T.W. 1983: Trace elements and dental disease (Postgraduate dental handbook series). John Wright PSG INC., Bristol.

Dermience, M., Lognay, G., Mathieu, F. and Goyens, P. 2015: Effects of thirty elements on bone metabolism. *Journal of Trace Elements in Medicine and Biology*, **32:** 86-106.

Dollwet, H.H.A. and Sorenson, J.R.J. 1988: Roles of copper in bone maintenance and healing. *Biol. Trace Elem. Res.*, **18**: 39-48.

Dodds-Smith, M.E., Johnson, M.S. and Thompson, D.J. 1992: Trace metal accumulation by the shrew *Sorex araneus* II. Tissue distribution in kidney and liver. *Ecotoxicology and Environmental Safety,* **24:** 118-130.

Doherty, A.H., Frampton, J.D. and Vinyard, C.J. 2012: Hibernation does not reduce cortical bone density, area or second moments of inertia in woodchucks (*Marmota monax*). *J Morphol.*, **273:** 604-617.

Ďurža, O. and Khun, M. 2002: Environmentálna geochémia niektorých ťažkých kovov. Univezita Komenského, Bratislava.

Eisler, R. 1985: Cadmium hazards to fish, wildlife and invertebrates: A synoptic review. *U.S. Fish Wild Serv. Biol. Rep.,* **85:** 1–46.

Elbetieha, A., Bataineh, H., Darmani, H. and Al-Hamood, M.H. 2001: Effects of long-term exposure to manganese chloride on fertility of male and female mice. *Toxicol Lett.,* **119:** 193-201.

Frank, R.M., Sargentini-Maier, M.L., Leroy, M.J.F. and Turlot, J.C. 1989: Distribution of lead strontium and zinc in human enamel and dentin. In: *Tooth Enamel V. Proceedings of the 5th International Symposium on the Composition, Properties and Fundamental Structure of Tooth Enamel and Related Issues* (eds. R.W. Fernhead), pp. 384-392. Florence.

Frankovská, J., Slaninka, I., Kordík, J., Jurkovič, Ľ., Greif, V., Šottník, P., Danadaj, I., Mikita, S., Dercová, K. and Jánová, V. 2010: Atlas sanačných metód environmentálnych záťaží, Štátny geologický ústav Dionýza Štúra, Bratislava.

Frase, B.A. and Armitage, K.B. 1989: Yellow-bellied marmots are generalist herbivores. *Ethology Ecology & Evolution,* **1**: 353–366.

Jemai, H., Messaoudi, I., Chaouch, A. and Kerkeni, A. 2007: Protective effects of zinc supplementation on blood antioxidant defence system in rats exposed to cadmium. *Journal of Trace Elements in Medicine and Biology,* **21**: 269-273.

Jenkins, D.W. 1981: Biological monitoring of toxic trace elements. EPA Report 600/S 3-80-090, Las Vegas.

Johnson, A.R., Armstrong, W.D. and Singer, L. 1968: The incorporation and removal of large amounts of strontium by physiologic mechanisms in mineralized tissues. *Calcif Tissue Res.,* **3:** 242-252.

Kabata-Pendias, A. and Mukherjee, B. 2007: Trace elements from soil to human. Springer Science and Business Media, Berlin.

Karč, P. 2006: Príspevok k poznaniu populácie svišťa vrchovského (*Marmota marmota* L.) v západnej časti národného parku Nízke Tatry (Prašivá-Ďumbier). *Naturae tutela*, **10:** 79–94.

Keen, C.L., Uriu-Hare, J.Y., Hawk, S.N., Jankowski, M.A., Daston, G.P., Kwik-Uribe, C.L. and Rucker, R.B. 1998: Effect of copper deficiency on prenatal development and pregnancy outcome. *Am. J. Clin. Nutr.,* **67:** 1003–1011.

Klevezal, G.A. and Anufriev, A.I. 2013: Variation in increments and "Hibernation zone" on the incisor surface in marmots (*Marmota*). *Zoologicheskii Zhurnal,* **92:** 1333–1348.

Lee, K.M., Appleton, J., Cooke, M., Sawicka-Kapusta, K. and Damek, D. 1999: Development of a method for the determination of heavy metals in calcified tissues by Inductively Coupled Plasma-Mass Spectrometry. *Fresenius J. Anal. Chem.,* **364**: 245-248.

Mann, C.S., Macchi, E. and Janeau, G. 1993: Alpine marmot (*Marmota marmota L.*). *IBEX J. M. E.,* **1:** 17-31.

Maňkovská, B. 1996: Geochemical atlas of Slovakia, part 2, Forest biomass. Geology services of Slovakia, Bratislava.

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. *Polish Journal of Environmental Studies,* **1:** 153-158.

Matkovic, V., Fontana, D., Tominac, C., Goel, P. and Chesnul, CH. 1990: Factors that influence peak bone mass formation: a study of calcium balance and the inheritance of bone mass in adolescent females. *Am J Clin Nutr,* **52**: 878-888.

Matkovic, V. 1991: Calcium metabolism and calcium requirement during skeletal modelling and consolidation of bone mass. *Am J Clin Nutr,* **54:** 245-260.

Matkovic, V 1992: Calcium intake and peak bone mass. *New Engl J Med,* **327**: 119-120.

Mishima, A., Yamamoto, C., Fujiwara, Y. and Kaji, T. 1997: Tolerance to cadmium cytoxicity is induced by zinc through non-metallothionein mechanisms as well as metallothionein induction in cultured cells. *Toxicology,* **118:** 85-92.

Parízek, J. and Ostádalová, I. 1967: The protective effect of small amount of selenite in sublimate intoxication. *Experientia,* **23:** 142-143.

Priest, N.D. and Van De Vyver, F.A. 1990: Trace metals and fluoride in bones and teeth. CRC Press, Florida.

Sarazin, M., Alexandre, C. and Thomas, T., 2000: Influence of trace element, protein, lipid, carbohydrate, and vitamin intakes on bone metabolism. *Rev. Rhum.*, **67:** 486–497.

Sargentini-Maier, M.L., Frank, R.M., Leroy, M.J.F. and Turlot, J.C. 1998: A method of lead determination on human teeth by Energy Dispersive X-Ray Fluorescence (EDXRF). *J. Trace Elem. Electrolytes Health Dis.*, **2:** 221-226.

Sheffield, S.R., Sawicka-Kapusta, K., Cohen, J. B. and Rattner, B.A. 2001: Rodentia and lagomorpha. In *Ecotoxicology of wild mammals* (eds. R.F. Shore and B.A. Rattner), pp. 215-315. John Wiley & Sons, Chichester.

Shore, R.F. 1995: Predicting cadmium, lead and fluoride levels in small mammals from soil residues and by species-species extrapolation. *Environmental Pollution*, **88**: 333-340.

Talmage, S.S. and Walton, B.T. 1991: Small mammals as monitors of environmental contaminants. *Rev. Environ. Contam. Tox.*, **119:** 47-145.

Underwood, E.J. 1977: Trace Elements in Human and Animal Nutrition, 4th Edition. Academic Press, London.

Wadhwa, D.R. and Care, A.D. 2000: Description of strontium on the absorption of calcium, magnesium and phosphate ions from the ovine reticulo-rumen. *Journal of Comparative Physiology B*, **170:** 225-229.

Wojda, S.J., McGee-Lawrence, M.E., Grindley, R.A., Auger, H., Black, L. and Donahue, S.W. 2012: Yellow-bellied marmots (*Marmota flaviventris*) preserve bone strength and microstructure during hibernation. *Bone*, **50:** 182-188.

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