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Patterns of select element accumulation in tissues and feces of *Rupicapra rupicapra tatrica*

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Abstract. We analyzed 352 samples of chamois tissue and feces and found lead in most of the samples, confirming the high frequency of lead occurrence in the alpine zone. In juvenile bones, lower Sr and P values were found than in adult individuals, which may be related to faster metabolism in younger chamois. In female bone samples, the elements P, Ca, Rb, Ba, Mn, Sr were found in higher concentrations than in male bones. In tongue tissue, Rb and Ca content was higher in adult individuals than juveniles and were also found in higher concentrations in females than males. Liver tissue had higher Cu concentrations and lower Zn concentrations in males.

Key words: Rupicapra rupicapra tatrica, heavy metal and other element accumulation, Tatra Mountains

Introduction

The Tatra chamois as a glacial relict and endemic subspecies, is a significant element of Tatras fauna. Chamois remain active throughout the year in the Tatra Mountains - even in the coldest winter conditions. These animals live in the alpine and subnival vegetation zone year-round.

During the vegetative season, chamois prefer herbs, particularly grasses and juicy plants, and they like to graze on plant buds. They do not tear the plants, but catch them gently with their lips and consume only the upper juicier parts of plants. During the growing season, chamois graze up to 117 plant species. The vegetation of the alpine and subalpine zones is rich in many juicy and fragrant plants, which are popular with these animals. Chamois living in the mountains are often subject to human influences and air pollution.

Many heavy metals accumulate on the ridges of mountains where chamois live as a result of atmospheric deposition, as confirmed by many studies. Gábriš (1998) writes that several factors influence metal toxicity, including: the quantity, form, properties, and reactivity of the metals; environmental conditions (pH, temperature and others); and the individual ability of each living organism to accumulate the metal (element) in underground and above-ground organs, plant seeds, or more precisely, in the body of an animal. Some of this content may be excreted in urine, excrement, and the like. In terms of toxicity of chemical elements in eatables, Brtková (1991) ranks cadmium, lead and mercury among the most toxic heavy metals. As the concentration of heavy metals increases in nature, animal toxicity increases as well. Heavy metals such as Cd, Pb, Hg and As are most toxic to animals, humans and the environment. Excess of these elements can cause serious problems. Heavy metals result in a lack of minerals in the body. Decreasing Ca concentration can lead to osteoporosis, tooth decay, periodontal disease, heart disease, muscle spasms and colic. Al relocates Ca so it is not available for the formation of teeth, bones and muscles (including heart muscle), and these body structures weaken. Heavy metals can enter the body through the skin, respiratory tract, or through intestinal absorption. Following absorption, heavy metals can be distributed to various organs, including glands and CNS. Some of the elements may be deposited in the skeletal system or in teeth (Athar and Vohora 1995). The availability and mobility of heavy metals in the terrestrial environment is influenced by soil pH, soil type, oxidation-reduction potential and cation exchange. Metals first enter the food chain through abosorption by plants, which are subsequently ingested by both humans and herbivores, (Hrudey *et al.* 1995). While some of these grazed plants are poisonous to humans, as well as to animals, (e.g., white hellebore (*Veratrum*)), chamois often consume young shoots of this poisonous plant without being harmed by the poisonous alkaloids contained in the whole plant (Teren 1987). Heavy metals accumulate in most of these plants, which can affect chamois life. The plant can absorb substances and metals through the root system or leaf surfaces. In order for metals to penetrate leaves, they must either pass through the epidermis of the leaf, which is covered with a waxy cuticle, or through the vents. The vents are equipped with a mechanism that regulates the penetration of substances into the leaves by changing the diameter of the vent hole. This mechanism determines the entry of substances into the leaf based on molecular weight (Kvesitadze *et al.* 2006). Plant root activity has a major impact on soil properties, including water dynamics, as well as solutes and gases in the soil (Hinsinger *et al.* 2009). The plant root system takes on metals through two phases; the initial rapid phase, when metals penetrate from the surrounding rhizosphere into the root system by diffusion, and the subsequent slower phase of metal accumulation in plant tissues (Puckett *et al.* 2010). Metals received by root cells are accumulated in the root or are translocated through xylene to tissues and organs in the aerial parts of the plant (Prasad 2004; Jabeen *et al.* 2009).

Because metal ions enter root system cells accompanied by water, the speed of their uptake by the root is also dependent on current soil moisture; and thus impacted by the capacity of soil to hold water. Soil moisture plays an important role in its use, and in decision-making within land and soil resources related to the bioavailability of metals in soil. Significant sorption (binding) of metals takes place in soils rich in organic matter (e.g., humic and fulvic acids) (Kvesitadze *et al.* 2006; Marschner 2011).

The goal of this paper is to determine element concentrations in the tissue and feces of chamois.

Material and Methods

Sample collection

Chamois specimens used for this analysis were naturally deceased, due to avalanche conditions or predation. Often, chamois are startled by human interactions (e.g., rescue helicoptors, or tourists), resulting in a slip and fall, followed by predation by the wolf population. Samples were labeled with the date, location, and the name of the researcher they were found by.

Most chamois were from the TANAP state forests. Samples were moved to the Institute of High Mountain Biology (IHMB) in Tatranská Javorina. Samples were taken of organs including: liver, heart, lungs, hair, bone, muscle, spleen, kidney, tongue and feces. We moved the samples to Petri dishes and dried them in the dryer for 10-13 hours. Subsequently, samples were x-rayed using spectrophotometers. We examined 352 samples of Tatra Chamois.

X-ray fluorescence spectrometry

Organ samples from the Tatras were analyzed by the hand-held XRF spectrometer DELTA CLASSIC (USA), using the DELTA portable workstation. Samples of organs were mechanically processed. Every sample was transferred to a small plastic cuvette with a clear bottom and analyzed by the spectrometer. The device was calibrated using the certificated reference of 'Beef liver standard NCS ZC 71001' (Haizhou 2015). When a sample was large or unformed we had to fit its shape it was cut down to fit. Each sample was analyzed for 240 seconds in three 80 second measurements, and the results were averaged. The data was processed using the EveryWAN Remote Support (Personal Edition) program.

Statistical analyses

We used Principal component analysis (PCA); a mathematical statistical method that uses orthogonal transformation to convert elements of a set of observations that are potentially correlated to elements of a set of values that are linearly uncorrelated. Amounts of elements were statistically compared using one way ANOVA and Unequal N HSD test at the 95% confidence level ($p < 0.05$). One-way ANOVA of principal component scores was used to test the differences in mutual concentration of more elements in the tissues and feces of chamois. Another statistical method that was used was the Mann-Whitney U Test, which was suitable for evaluating an insufficient number of samples in individual tissues and feces. We also used Fisher's exact test on 2 x 2 contingency tables with data of positive and negative measurements of elements in samples divided by the research area in where the sample was taken. This last method was used on elements found at all localities but not in all samples.

Results

The following elements were detected in our samples: P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Rb, Sr, Zr, Mo, Cd, Sn, Sb, Ba, and Pb. Other elements that were measured but not found in any of our samples were Ag, and Hg. Elements belonging to heavy metals like As, Cd, and Se were measured, but their content was less than the detection limit in most of our samples. We found evidence of Pb, Ba, Sr and Rb, which are classified as toxic heavy metals. Out of the heavy metals (Ti, Ni, Zr, Cd, Sn, Sb and Pb) that were not measured in significant amounts in our samples, only Pb was found in enough samples for statistical evaluation. Fig. 1 shows higher accumulation of Pb in feces when compared to all other studied tissues of Tatra chamois. Contingency tables (Table 3 and 4.) show differences in lead accumulation between individual localities. The highest lead accumulation was in High Tatras, which contributed the most samples of chamois. Table 1 shows a comparison of the average values of elements in sex and generation. Table 2 shows a comparison of the average values of elements in individual tissues and feces.

Bones

PCA of bones revealed eight factors. The first six of these show a mutual relationship of elements in the bone with 81 % variance (Appendix 1). Factor 1 is in strong positive correlation with Ca, P and Rb. These elements have increased values in bone tissue of female Tatra chamois (Fig. 2). At factor 2, a strong mutual relationship among elements (Zn, Sr, Cu, Pb and Fe) was found. Factor 3 expresses a strong negative correlation of the biogenic elements S, Cl, and K that rises or decreases simultaneously. Factor 4 is a bipolar vector, which

	Female	Male	Adult	Juvenile
P	170188.998	74757.284	99867.531	51127.922
S	12792.547	9698.365	17352.623	11937.831
Cl	8701.386	6691.526	7064.692	6202.369
K	13792.870	12922.762	9918.507	12152.855
Ca	69209.373	56135.628	78554.451	47346.490
Ti	10.904	22.296	17.543	30.775
Cr	56.545	45.047	54.871	42.028
Mn	109.861	102.640	90.169	99.914
Fe	7066.479	5364.395	4399.107	4211.290
Co	0.614	0.001	0.209	0.001
Ni	40.219	22.106	23.925	15.989
Cu	38.692	47.734	31.985	37.768
Zn	193.850	199.742	188.855	174.239
As	0.001	0.001	0.001	0.001
Se	0.801	1.142	0.659	0.821
Rb	42.396	33.771	31.751	34.904
Sr	16.344	16.610	39.894	17.990
Zr	0.148	0.598	0.306	0.822
Mo	6.154	6.223	5.131	5.917
Ag	0.001	0.001	0.001	0.001
Cd	0.657	0.775	0.693	0.319
Sn	0.001	0.768	0.764	0.001
Sb	2.227	3.444	2.831	3.154
Ba	32.758	25.466	38.763	27.198
Hg	0.001	0.001	0.001	0.001
Pb	5.818	8.935	5.358	8.135

Table 1. Average values of elements between sex and generation.

states that when Fe and Cu increase, toxic elements Pb and Ba decrease and vice versa. Factors 2, 3 and 4 did not manifest between age groups and sexes. Factor 5 is in negative correlation with Mo. Mo decreased with a simultaneous increase of Ba in the bones of females, whereas in males, Ba decreases when Mo increases (Fig. 3). Factor 6 shows us the difference between the accumulation of Mn in male and female samples. While higher concentrations are found in males (Fig. 4), a negative correlation is observed in Mn at factor 7 with simultaneously increasing Cr in males and the opposite in females (Fig. 5). Factor 8 is in negative correlation with P and Sr (Table 5). These elements increased in female bones when compared to male bones (Fig. 6). Juveniles showed lower contents of Sr and P than adults, in whom these elements increased (Fig. 7).

Kidneys

PCA revealed that the first three factors show a mutual relation of elements in the kidney tissues of Tatra chamois with variability of 67.2 %. First factor showed variability, of which the highest instance was a unipolar vector, in which elements

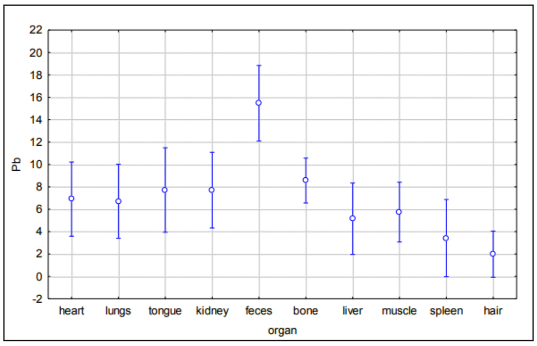

Fig. 1. Concentrations of Pb (ppm) in the feces and tissues of Tatra chamois (One-way ANOVA, F (9, 342) = 6.1057, p = 0.00000). Feces significantly differ from hair (p = 0.000014) and spleen (p = 0.000061), Unequal N HSD test, MS = 0.78723, df = 342.00.

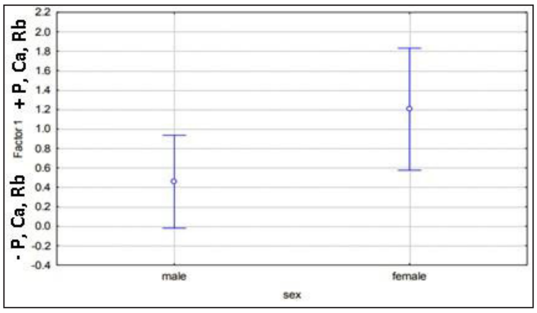

Fig. 2. Differences between sexes in concentration of elements (P, Ca, Rb) in bones of Tatra chamois, based on PCA values (One-way ANOVA , F (1, 28) = 3.7534, p = 0.06285). The means of the principal components scores (PC1) with their 95 % confidence limits are compared.

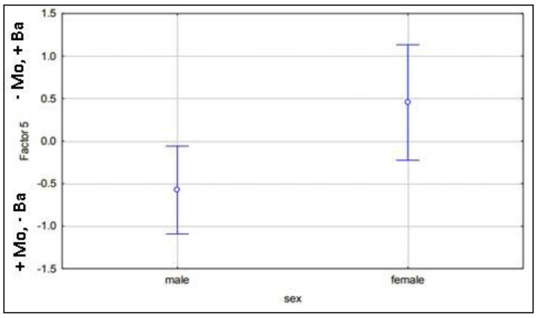

Fig. 3. Differences between sexes in elements (Mo, Ba) accumulation in bones of Tatra chamois, based on PCA values (One-way ANOVA , F (1, 28) = 6.1162, p = 0.01973). The means of the principal components scores (PC1) with their 95 % confidence limits are compared.

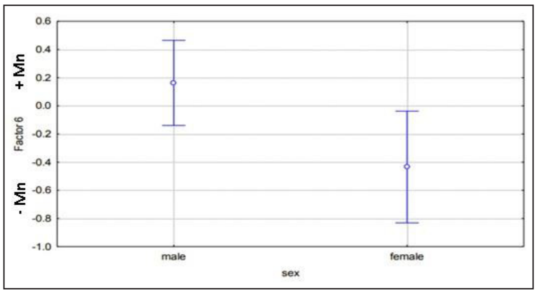

Fig. 4. Comparison of the amounts of Mn between male and female bones of Tatra chamois based on PCA values (One-way ANOVA, F (1, 28) = 6.0207, p = 0.02062. The means of the principal components scores (PC1) with their 95 % confidence limits are compared.

	Bone (71) mean	Kidney (25) mean	Tongue (20) mean	Feces (25) mean	Liver (28) mean	Spleen (24) mean	Muscle (40) mean	Heart (26) mean	Lungs (26) mean	Hair (68) mean
P	383471.41	31771.91	8586.88	10694.24	21974.50	4798.14	14055.62	4066.97	17370.75	23192.73
S	889.25	6489.30	6302.30	2218.73	4358.33	4996.77	5075.42	5807.76	6229.46	69951.91
Cl	1743.53	9470.57	6780.53	2144.53	6534.32	7637.41	5650.59	7103.51	15544.75	11176.52
K	2264.58	18198.78	13719.92	10682.35	11323.47	15231.36	11490.08	13203.24	19578.21	9964.77
Ca	329184.88	3075.39	2872.97	15359.53	3095.63	1135.64	10415.86	1200.08	2337.00	16093.65
Ti	3.85	N/D	5.25	211.56	N/D	N/D	1.51	0.79	N/D	26.02
Cr	16.66	29.26	33.60	20.22	18.59	16.09	24.89	24.60	43.75	180.29
Mn	66.99	90.27	37.31	587.40	63.11	42.18	32.43	36.76	51.78	53.21
Fe	211.18	2420.83	914.83	2297.56	2843.52	50753.45	688.36	1713.61	3752.58	955.16
Co	0.80	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Ni	16.11	9.22	4.35	4.29	4.41	N/D	0.92	8.17	14.75	87.29
Cu	0.59	31.97	4.69	21.42	335.17	N/D	7.23	26.58	15.35	3.74
Zn	197.83	263.70	172.87	281.15	295.21	160.27	262.40	114.46	117.75	109.07
As	0.20	N/D	N/D	0.42	N/D	N/D	N/D	N/D	N/D	N/D
Se	0.09	9.75	N/D	N/D	0.20	N/D	N/D	N/D	N/D	N/D
Rb	6.93	57.47	41.91	48.01	43.91	39.70	43.99	44.27	46.70	17.58
Sr	151.96	0.70	N/D	47.67	0.53	2.63	3.82	N/D	N/D	2.72
Zr	0.29	N/D	N/D	5.53	N/D	N/D	N/D	N/D	N/D	N/D
Mo	3.48	6.50	5.90	5.02	6.51	5.07	5.05	5.76	6.22	6.61
Ag	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Cd	0.51	5.04	N/D	0.61	0.48	N/D	N/D	N/D	N/D	0.25
Sn	N/D	N/D	N/D	N/D	0.52	N/D	2.35	N/D	N/D	1.42
Sb	1.37	2.48	4.25	2.90	2.96	3.27	4.54	4.50	2.00	2.74
Ba	114.65	8.07	1.81	50.53	2.45	N/D	5.37	6.34	7.21	36.95
Hg	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Pb	8.21	7.83	7.27	15.75	5.12	3.04	5.60	6.92	6.71	1.30

Table 2. Average values of elements. The number in brackets shows the number of examined samples.

Sample type	HT	WT	Total
Frequencies of positive samples	136	2	138
Percent of total	43.871 %	0.645 %	44.516 %
Frequencies of negative samples	159	13	172
Percent of total	51.290 %	4.194 %	55.484 %
Column totals	295	15	310
Percent of total	95.161 %	4.839 %	

Table 3. Contingency table of Pb measurements with statistical values between localities: HT - High Tatras and WT - West Tatras (Fisher's exact test, p = 0.0102).

Sample type	HT	BT	Total
Frequencies of positive samples	136	4	140
Percent of total	42.633 %	1.254 %	43.887 %
Frequencies of negative samples	159	20	179
Percent of total	49.843 %	6.270 %	56.113 %
Column totals	295	24	319
Percent of total	92.476 %	7.524 %	

Table 4. Contingency table of Pb measurements with statistical values between localities: HT - High Tatras and BT - Belianske Tatras (Fisher's exact test, p = 0.0037).

(P, S, Cl, K, Ca, Mn, Cu, Zn, and Mo) simultaneously increased or decreased. The effect of factor 1 was not significantly different in adults than juveniles (Fig. 8) nor between generations (One-way ANOVA, $F(1, 23) = 0.44832$, $p = 0.50980$). Concentrations of P and Cl at factor 2 increased with a simultaneous decrease in Mo and Pb. We also noted an increasing concentration of Cd and Se in kidneys with a simultaneous decrease in Ca, Mn and Ba (Appendix 2, factor 3).

Tongue

There was no significant difference between generations and sexes among factors 1, 2, 3 and 4 (Mann-Whitney U Test, $p < 0.05000$). Appendix 3 shows that factor 5 showed the most significant relationship between the content of elements in a generation ($p = 0.02$) and sex ($p = 0.01$). Higher negative correlation occurred in juvenile individuals. Greater prevalence of positive correlation was seen in adult individuals

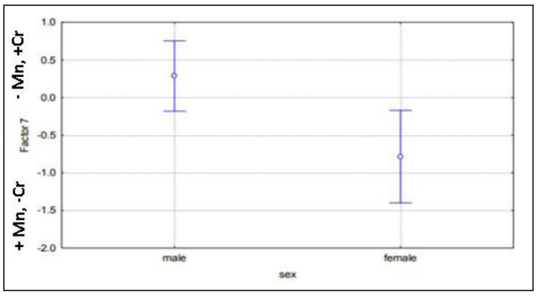

Fig. 5. Concentrations of Cr, Mn in male and female bones of Tatra chamois, based on PCA values (One-way ANOVA F (1, 28) = 8.0707, p = 0.00829. The means of the principal components scores (PC1) with their 95 % confidence limits are compared.

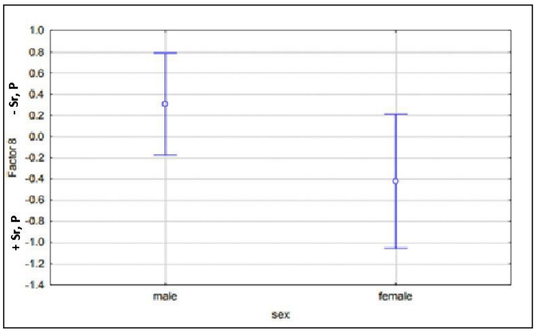

Fig. 6. Comparisons of concentrations of elements (Sr, P) between male and female sex in bones of Tatra chamois, based on PCA values (One-way ANOVA F (1, 69) = 4.0112, p = 0.04913). The means of the principal components scores (PC1) with their 95 % confidence limits are compared.

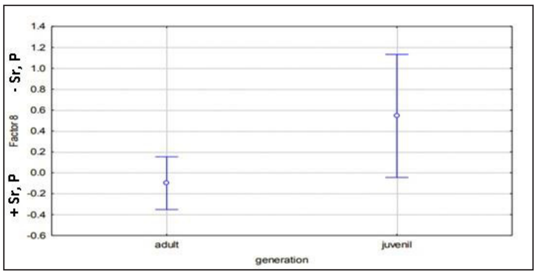

Fig. 7. Difference in concentration of element (Sr, P) between adult and juvenile generation in bones of Tatra chamois, based on PCA values (One-way ANOVA F (1, 69) = 4.0112, p = 0.04913). The means of the principal components scores (PC1) with their 95 % confidence limits are compared.

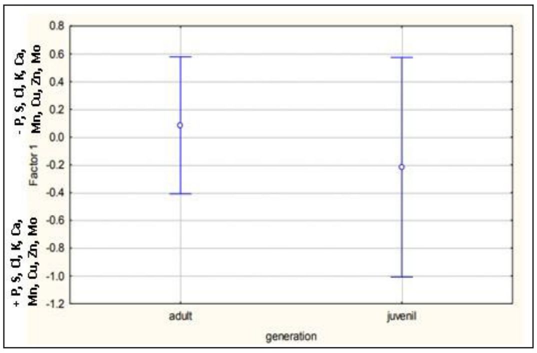

Fig. 8. Mutually decreasing or increasing concentrations of elements (P, S, Cl, K, Ca, Mn, Cu, Zn, Mo) in kidneys of Tatra chamois based on principal component coordinates of PCA analysis (One-way ANOVA, F (1, 23) = 0.44832, p = 0.50980).

(Fig. 10). Expression of factor 5 was also significant within individual sexes (p = 0.01). Male values showed a negative correlation in more than 75 % of the investigated samples (Fig. 9). Approximately 75 % of female values had a positive correlation. Some elements, including Ca, Rb and Ba were higher in females than in males.

Feces

In the analysis of feces samples, the only significant result was the effect of factor 2 between sexes (Mann-Whitney U Test, p = 0.03). There was no significant relationship between the generations. Females had higher amounts of Ca, Zn and Sr and lower concentrations of Cr than males, who exhibited the opposite correlation (Fig. 11). Factor 2 had a variance of 17.98 %. A negative correlation was seen in Cr and a positive correlation was observed in Ca, Zn, and Sr (Appendix 4).

Liver

The effect of factor 6 was the most notable. The variance of the samples was 4.58 %. This factor was characterized by the negative correlation of Zn and the positive correlation of Cu (Appendix 5). Factor 6 did not show up significantly between generations, nor between sexes, but it does have an important effect. In male tissues, the greater content of Cu is reflected in Fig. 12. These results indicate that copper content is higher in males than in females, while the content of Zinc is higher in females than in males.

We did not find differences between sexes or age groups in synergic contamination of elements when we examined spleen, muscle, heart, lung or hair tissues.

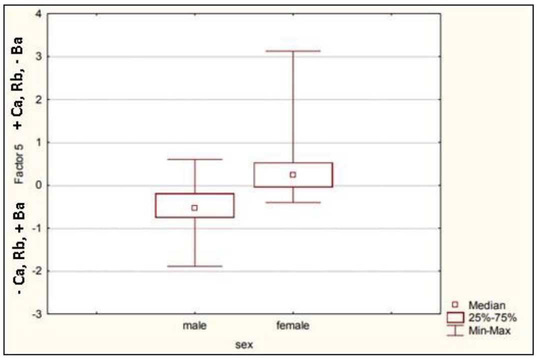

Fig. 9. The box plot graph compares median factor coordinates. Rb, Ca and Ba were higher in females compared to males.

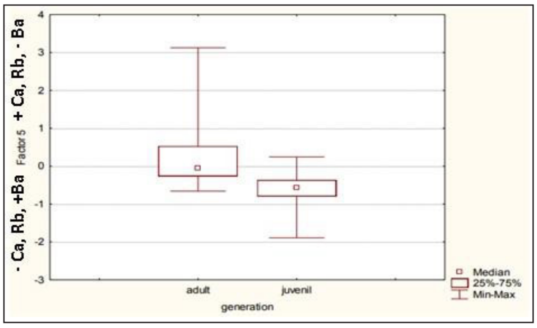

Fig. 10. The box plot graph compares median factor coordinates. Ca and Rb were higher in adult individuals.

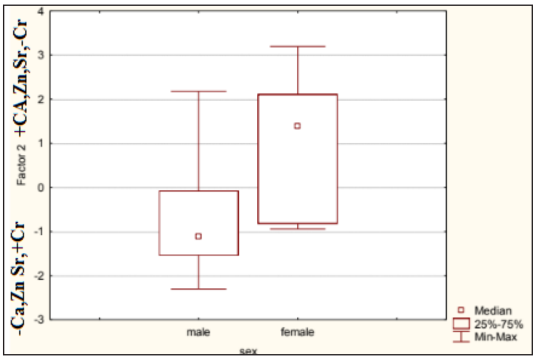

Fig. 11. The box plot graph compares median factor coordinates. Ca, Zn and Sr was higher in females.

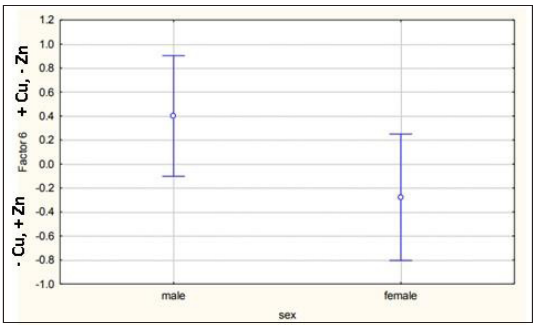

Fig. 12. LS Means with 0,95 confidence limits of factor coordinates. Male liver contained proportionally more Cu and Zn than female liver tissue (One-way ANOVA, $F(1, 19) = 3.7851$, $p = 0.06666$).

Discussion

Concentration of elements in hard tissues (bones)

We found significant increases of P, Ca and Rb in the bones of chamois. The metabolism of calcium and phosphorus is linked, so these two elements should not be considered separately. Both are very important in tooth and bone metabolism. Calcium is an essential nutrient, necessary for growth and development. It builds the skeleton and helps to prevent many skeletal disorders during adolescence and childhood. Phosphorus, along with other elements such as Zn, Pb, Sr, Ca, are accumulated in higher concentration in skull bones than in teeth (Matkovic 1991, 1992; Matkovic *et al.* 1990). The increased amount of Rb can be a result of a higher concentration in the Earth's crust at a given location, where it contributes to the soil and is ingested by animals. The lowest Rb content of the flora was determined on keeper weathering soils. The Rb content of plants is subject to remarkable variation based on the geological origin of the soil when compared to other trace elements (Nyholm and Tyler 2000). In factor 2, strontium follows the physiological pathway of calcium, with 99 % of its content present in bone, it is thus considered essential and associated with the occurrence of calcium (Wadhwa and Care 2000). The animal body absorbs strontium as if it was calcium. The metabolism of strontium is similar to calcium, but in general, animals utilize and retain strontium less effectively than calcium (Comar 1967; Comar and Wasserman 1964). Strontium is thought to play a critical role in bone health. It tends to mi-grate to sites where active remodeling is taking place and promotes mineralization of bones and teeth. In fact, because of its similarities, strontium is capable of replacing a small proportion of calcium in calcified crystals of bone and teeth. As it appears, strontium adds strength to these tissues, making them more resistant to breakdown. Strontium also appears to draw extra calcium into the bone. The presence of these elements (Ca, P and Sr) is linked (Alina 2013). Another interesting element we measured in bones was Rb. Elements which are absorbed in the body through digestion don't only have a plant origin. Elements from the soil, including lead, are absorbed through the intestines of organisms.

Mn and Cr were also detected the bones of chamois. For plants, manganese availability in soil increases with decreasing soil pH and decreases with increasing organic content (Schulte and Kelling 1999). The primary source of manganese to chamois was plants. Schroeder *et al.* (1966) confirm in their study that all human tissues contain manganese in concentrations remarkably constant throughout most of life. Chromium is an element that occurs naturally in both animals and humans, and is necessary for life. Chromium is responsible for preserving bone material by reducing the loss of calcium in urine, promoting collagen production, increasing adrenal DHEA levels and improving insulin regulation. It helps the skeleton regulate energy metabolism. This complex process includes osteocalcin (a hormone secreted by the bone-building osteoblast cells) acting on the pancreas to enhance insulin production and in peripheral tissues to increase glucose (Anderson 1987). In female chamois, when Mn concentration in bones increases, chromium decreases and males present the opposite correlation. Because these elements are essential for bodily function, interactions between them may represent complex metabolic and physiologic processes.

Heavy metals in bones

The main factors affecting the accumulation of potentially-toxic metals by grazing animals are the presence of the metal, its concentration at the soil surface or in herbage, and the duration of exposure to the contaminated pasture and soil. With regard to heavy metals, we only found Cd in two bone samples and As in one bone sample out of 352 total chamois samples. Hg was not found in any samples. Lead was present in most samples. Lead accumulation in plants and animals in the alpine zone is a highly discussed topic in many studies. Lead accumulates in vascular plants primarily through the leaves and to a lesser degree in the roots (Kabata-Pendias and Pendias 1984). Janiga (1998) describes the role of lead as a pollutant that be transported several thousand kilometers depending on meteorological conditions. Wind speed and prevailing direction play a very important role in heavy metal transport in the mountains. In high altitude habitats, where chamois occur, north-western and western winds mainly prevail in the West and High Tatras (Konček *et al.* 1973). Janiga *et al.* (1998) confirmed that lead is deposited in the Tatras, which represents a barrier to pollutants from the northern part of central and east Europe. About 50 % of lead is physiologically accumulated in vascular plants through their tissues and the other 50 % is washed away (Bednářová and Bednář 1978). Additionally, most heavy metals stored in soil are not natural but have an anthropogenic origin. Heavy metals (cadmium and lead) are found more frequently in the upper levels of soil than in the lower levels, due to emissions (Ewers and Schlipkoter 1991). Lead as the only heavy metal present in multiple samples, and in general is considered to be a major contaminants that enters the food chain. It has a negative impact on the environment, so its use has been banned in most countries (Greenwood 1993). We can also confirm high lead deposition in the West Carpathians in ruminant *Rupicapra rupicapra tatrica* populations. In the contingency tables (Table 3 and 4) we processed the frequency of lead accumulation according to localities. The highest lead accumulation was recorded in samples from the High Tatras. Pb levels in Tatra lake sediments increased in the samples from the 20th century, and were likely deposited as a result of air pollution due to the development of road transportation (Szarlowicz 2013). Pb, as a pollutant, can be transported from Zn-Pb smelters due to north-westernly winds in the Tatra mountains (Steblez 2005). Lead has not bee mined recently in surrounding areas (including the Czech Republic), and mining and smelting of lead were recently reduced in Poland.

Concentration of elements in soft tissues and feces.

The majority of kidney samples came from juvenile individuals. We surmise that stress hormones could be affecting young individuals to a greater degree in their interactions with tourists, skiers or rescue helicopters and further impact could also have an environmental burden. However, such an impact would depend on an increased metabolic rate in young individuals, and is as yet unconfirmed in Blagojević *et al.* (2012), where the authors state that at early age stages, due to increased metabolic rate, heavy metals are more rapidly accumulated. Bioaccumulation may also be affected by the general fitness of young animals. Therefore, it is not surprising that bioaccumulation is more effective at an unpolluted locality, due to the better condition of the animals (on average). At that age (neonates and juveniles) bioaccumulation is mainly affected by the high rate of metabolism, no matter how high the concentration of heavy metals is in the environment. Moreover, the higher metabolic rate of juveniles, implying a high uptake of food, may explain the increased amounts of xenobiotics, such as Cd and Pb, from polluted areas (Blagojević *et al.* 2012). With regard to cadmium, plant absorption has been shown to be significantly easier than absorption of lead, which may also contribute to greater foodborne cadmium toxicity (Ye *et al.* 2015). In tongue tissue, we found males had higher Ba content and lower Ca and Rb content, while females had higher Ca and Rb content in the tongue and lower Ba content. Prasad (2004) claim significant variation in arsenic intake occurs between sexes in bank voles, where they found stomach arsenic concentrations to be higher in females than in males. Interestingly, this gender difference couldn't be explained on the basis of dietary preference, as both sexes of bank vole ingest similar diets. The impact of sex on trace element concentrations in sample species cannot be fully attributed to behavioural characteristics and thus requires further investigation. In feces, the accumulation of Ca and Cr. Ca and Cr was found, which are essential elements that the body regularly excludes. We cannot infer why the content of these elements correlated with sex, and to do so a further study would be required.

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Appendix 1

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8
P	**0.809**	0.045	0.117	-0.195	0.319	-0.049	-0.157	**-0.329**
S	-0.367	0.001	**-0.787**	-0.115	0.065	0.124	-0.033	-0.263
Cl	-0.194	-0.116	**-0.796**	-0.063	0.313	-0.098	0.049	0.257
K	0.570	0.012	**-0.645**	-0.054	-0.031	-0.065	-0.313	-0.090
Ca	**0.870**	-0.178	0.204	0.087	0.229	-0.011	-0.121	-0.184
Cr	0.639	-0.240	-0.067	-0.273	0.397	0.255	**0.396**	0.094
Mn	-0.303	-0.370	0.184	-0.063	0.225	**0.669**	**-0.447**	0.159
Fe	0.330	**-0.474**	-0.005	**-0.703**	-0.143	0.105	0.240	0.084
Cu	-0.202	**-0.595**	0.198	**-0.522**	-0.185	-0.300	-0.230	0.067
Zn	-0.196	**-0.885**	-0.117	0.042	-0.184	-0.091	-0.051	-0.003
Rb	**0.783**	-0.198	-0.166	0.268	-0.171	-0.182	-0.168	0.266
Sr	-0.462	**-0.693**	0.085	0.043	0.178	-0.214	0.062	**-0.328**
Mo	0.657	-0.326	-0.071	0.311	**-0.432**	0.088	0.068	0.098
Ba	-0.102	-0.442	0.136	**0.482**	**0.634**	-0.230	0.027	0.195
Pb	0.006	**-0.525**	-0.142	**0.532**	-0.246	0.322	0.189	-0.222
TV %	25.81	18.04	12.65	10.67	8.45	5.99	4.57	4.06
CV %	25.80	43.85	56.50	67.17	75.62	81.61	86.18	90.24

Appendix 1. Factor coordinates of the variables, based on correlations of elements in bones of Tatra chamois (TV % - total variance %; CV % - cumulative variance %). The most important correlations are in bold.

Appendix 2

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9
P	**-0.688**	**0.606**	0.134	-0.127	-0.168	0.191	-0.044	0.004	-0.108
S	**-0.767**	0.314	0.169	0.268	0.059	-0.007	0.213	0.285	-0.096
Cl	**-0.584**	**0.695**	-0.049	0.016	-0.282	0.168	-0.126	0.019	0.038
K	**-0.876**	0.286	-0.055	-0.147	-0.305	0.111	-0.013	0.019	-0.036
Ca	**-0.601**	-0.290	**-0.605**	0.185	0.190	0.175	0.119	0.039	-0.217
Cr	-0.589	0.142	0.134	**0.531**	0.013	-0.293	-0.241	-0.411	-0.047
Mn	**-0.718**	-0.379	**-0.523**	0.047	0.132	-0.001	0.087	-0.066	-0.073
Fe	-0.408	0.224	0.063	**-0.688**	0.184	-0.011	0.366	-0.357	0.029
Cu	**-0.765**	-0.312	0.288	-0.174	-0.013	-0.157	-0.121	0.222	0.154
Zn	**-0.783**	-0.309	0.364	-0.169	0.139	0.074	-0.127	0.014	0.112
Se	-0.412	-0.009	**0.664**	0.308	0.178	-0.267	0.381	0.043	-0.046
Rb	-0.432	0.106	0.112	-0.160	**0.782**	0.155	-0.327	0.043	-0.039
Mo	**-0.730**	**-0.523**	0.157	0.061	-0.284	0.011	-0.121	-0.149	-0.024
Cd	0.295	-0.177	**0.442**	0.387	0.054	**0.690**	0.124	-0.142	0.078
Ba	-0.546	0.271	**-0.565**	0.329	0.154	-0.016	0.140	-0.007	0.380
Pb	-0.574	**-0.735**	-0.025	-0.134	-0.279	0.109	0.063	0.025	0.036
TV %	39.83	15.35	12.04	8.53	7.15	5.06	3.85	3.01	1.71
CV %	39.83	55.18	67.22	75.75	82.90	87.96	91.81	94.82	96.53

Appendix 2. Principal component weights of the first nine factors of PCA of elements in kidneys of Tatra chamois (TV % - total variance %; CV % - cumulative variance %). The most important correlations are in bold. Factor 1 express 39.83 % of total variance.

Appendix 3

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9
S	**-0.800**	0.355	0.047	0.161	-0.164	-0.138	-0.209	0.207	-0.249
Cl	-0.426	**0.792**	-0.204	-0.070	-0.056	0.043	0.303	0.029	0.182
K	**-0.764**	0.401	-0.290	0.153	-0.105	0.132	0.324	0.058	0.009
Ca	-0.014	-0.122	**0.654**	0.058	**0.629**	-0.082	0.303	0.239	-0.013
Cr	-0.251	**0.657**	0.467	-0.291	-0.034	-0.190	0.103	-0.340	-0.187
Mn	**-0.933**	-0.060	0.057	0.184	-0.065	-0.047	-0.119	0.199	-0.011
Fe	-0.530	0.532	0.282	-0.237	0.226	0.060	-0.416	0.002	0.241
Cu	-0.339	-0.449	-0.122	**-0.547**	-0.191	**-0.543**	0.131	0.092	0.089
Zn	**-0.794**	-0.494	-0.077	-0.126	0.144	0.003	-0.141	-0.088	0.185
Rb	0.013	0.228	-0.507	0.474	**0.480**	**-0.449**	-0.073	-0.164	0.022
Mo	**-0.814**	-0.417	0.140	0.061	0.155	0.195	0.060	-0.246	0.006
Ba	0.051	-0.106	**0.601**	**0.561**	**-0.468**	-0.223	0.019	-0.079	0.172
Pb	**-0.781**	-0.558	-0.060	0.104	-0.021	0.073	0.142	-0.120	0.004
TV %	35.75	20.35	11.71	8.50	7.90	5.21	4.61	2.96	1.76
CV %	35.75	56.10	67.81	76.31	84.21	89.42	94.03	96.99	98.75

Appendix 3. Factor coordinates of the variables, based on correlations of elements in tongue of Tatra chamois (TV % - total variance %; CV % - cumulative variance %). The most important correlations are in bold. Factor 5 express 7.90 % of total variance.

Appendix 4

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9
S	-0.331	-0.113	**0.691**	**-0.535**	-0.063	0.211	-0.005	0.058	-0.146
Cl	**-0.703**	0.219	-0.311	-0.293	0.112	-0.218	0.314	0.312	0.037
K	**-0.800**	0.134	0.241	-0.344	0.022	-0.311	0.120	-0.042	0.054
Ca	-0.146	**0.862**	0.008	-0.079	0.261	0.020	-0.266	0.006	-0.249
Cr	-0.474	**-0.501**	0.375	-0.201	0.255	**0.458**	-0.034	-0.082	0.137
Mn	**-0.724**	0.448	-0.298	-0.034	-0.035	-0.054	-0.075	-0.359	0.127
Fe	**-0.797**	-0.377	0.144	0.272	-0.140	0.017	-0.231	0.103	-0.037
Cu	-0.060	0.346	-0.217	-0.324	**-0.796**	0.277	-0.049	0.037	0.021
Zn	-0.314	0.584	0.151	0.337	0.081	**0.443**	0.435	-0.084	-0.069
Rb	0.024	0.201	**0.821**	0.055	-0.189	**-0.450**	0.064	-0.135	0.020
Sr	0.187	**0.560**	0.536	**0.495**	-0.084	0.068	-0.096	0.221	0.180
Mo	**-0.821**	-0.282	-0.089	0.363	-0.135	0.001	0.190	0.008	-0.031
Ba	**-0.822**	0.313	-0.108	-0.034	0.174	0.074	-0.311	0.102	0.136
Pb	**-0.800**	-0.320	-0.040	0.389	-0.159	-0.095	-0.088	-0.023	-0.179
TV %	34.18	17.98	13.85	9.77	6.64	6.43	4.23	2.40	1.53
CV %	34.18	52.16	66.01	75.78	82.42	88.85	93.08	95.48	97.00

Appendix 4. Factor coordinates of the variables, based on correlations of elements in feces of Tatra chamois (TV % - total variance %; CV % - cumulative variance %). The most important correlations are in bold. Factor 2 express 17.98 % of total variance.

Appendix 5

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9
P	**0.886**	-0.189	0.080	-0.208	0.155	-0.060	0.059	-0.254	0.003
S	**0.727**	0.132	-0.235	-0.368	-0.304	-0.081	0.344	-0.058	-0.156
Cl	**0.880**	-0.076	-0.087	-0.165	0.203	0.158	-0.268	0.047	-0.042
K	**0.925**	0.061	0.213	0.062	0.099	0.094	-0.132	-0.128	-0.117
Ca	0.106	-0.122	**0.696**	**-0.498**	0.407	-0.114	0.140	0.057	0.181
Cr	**0.751**	-0.426	-0.172	0.110	-0.321	0.002	0.158	0.133	0.196
Mn	**0.876**	-0.206	0.068	0.355	0.014	-0.086	-0.051	0.100	-0.005
Fe	**0.626**	0.478	-0.099	**-0.502**	-0.091	0.100	-0.107	0.256	-0.021
Cu	0.243	**0.771**	-0.140	0.202	0.102	**0.459**	0.133	-0.096	0.185
Zn	0.253	**0.759**	-0.104	0.014	-0.155	**-0.517**	-0.167	-0.081	0.139
Rb	0.027	0.134	**-0.676**	0.119	**0.658**	-0.182	0.160	0.093	-0.062
Mo	**0.769**	-0.138	0.118	**0.578**	0.066	-0.085	0.043	0.036	0.046
Pb	0.034	0.563	**0.701**	0.336	-0.002	-0.038	0.153	0.109	-0.167
TV %	41.55	15.85	12.61	10.41	7.07	4.58	2.83	1.70	1.54
CV %	41.55	57.39	70.00	80.41	87.48	92.06	94.89	96.59	98.13

Appendix 5. Factor coordinates of the variables, based on correlations of elements in liver of Tatra chamois (TV % - total variance %; CV % - cumulative variance %). The most important correlations are in bold. Factor 6 express 4.58 % of total variance.