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Variation in the accumulation of chemical elements in the bones of chamois during their two-year exposure in the field

S. BRECELJ[1], M. JANIGA[1], Z. KOMPIŠOVÁ BALLOVÁ[1] and G. CHOVANCOVÁ[2]

[1]Institute of High Mountain Biology, Žilina University, Tatranská Javorina 7, SK-059 56, Slovak Republic; [2]Research Station and Museum of the TANAP, Tatra National Park Administration, Tatranská Lomnica 66, SK-059 60, Slovak Republic; e-mail: ballova1@uniza.sk

Abstract. The accumulation of chemical elements in the bones of Tatra chamois (*Rupicapra rupicapra tatrica*) in Tatranská Javorina in the area of the Belianske Tatras were studied. Over a two year period, bones taken from the same site were sampled four times. During each sampling, chemical elements were measured, and then the bones were put back in their original position. The site was at an altitude of 1000 m a.s.l. We investigated the effect of time on the concentration of elements in the bones of deceased chamois. It is clear from our research that the elements behave differently over time. It can be said that if bone samples are taken directly from the field, the results may differ due to element exchanges, mainly between bones, precipitation water and air. Elements like P, Cr, Mn, Fe, Zn, Rb, Sr, Mo, Sb, Ba, and Pb tended to increase or do not change concentrations in the bones over time, while concentrations of Ca, K, Cl decreased.

Key words: bones, chamois, Tatras, elements, measurement, XRF spectrometry

Introduction

Elements can be stored in the bones of an animal throughout its life (Ballová *et al.* 2019). Bone of a dead animal is a material that can absorb trace elements from the surrounding soil and act as a funnel for these trace elements. However, it appears that bone content does not always match soil content (for some elements), suggesting that bone may be affected by factors other than soil, such as moisture, porosity, pH, reactive conditions, climate and time (Krajcarz 2019). Therefore, the information obtained will not be reliably usable in other conditions in which the experiment was performed.

The concentration of elements in the bones of dead wild ruminants reflects the environmental conditions in which these animals lived, as well as post-mortem changes in the levels of the elements. It has been shown that the content of heavy metals increases with altitude and heavy metals can have adverse effects on animal health. We therefore consider ruminants living in alpine ecosystems to be a good indicator of environmental contamination by heavy metals from the atmosphere (Ballová *et al.* 2019). Increased pollution in our environment has exposed flora and fauna to the harmful effects of toxic substances (Wavita 2018) and this pollution can also be seen in the study of bones from dead individuals. Heavy metals that can be observed in bones include: lead, mercury, arsenic, cadmium, nickel, or chromium.

This study focuses on assessing the impact of the natural external environment over a relatively short time (approximately 2.5 years) on the concentration of elements in the bones of dead Tatra chamois (*Rupicapra rupicapra tatrica*). The influence of the environment on the concentrations of elements in the bones can manifest itself, for example, as a chemical change in the main component of the bone; trace metal sorption; and adhesion of fine dirt particles to the bone surface (López-Costas *et al.* 2016). Furthermore, a large number of microcracks are likely to increase bone fragility, which provides space for the accumulation of elements (Jans *et al.* 2002). When the bones are warm, the dry environment affects them almost immediately. Exposure to a temperature-controlled environment results in dramatic changes in the internal composition of the elements in the bones, but much more slowly than is shown for bones exposed to warmer conditions (Karr and Outram 2015).

Material and Methods

Study area

The experiment was carried out using freshly prepared bones (ribs and pelvic bones) from Tatra chamois (*Rupicapra rupicapra tatrica),* which died in the winter of 2017/2018 and were stored in freezers at a temperature of -70° C. The site where the bones were stored is located on the land belonging to the Institute of Hight Mountain Biology (IHMB), at an altitude of 1000 m a.s.l. within an alder (*Alnus*) forest. It is a place affected by different conditions and the climate is closest to the conditions of a deciduous forest, though it is comprised of mountain alder floodplain forests with gray alder (*Alnus incana*). This type of floodplain forest occurs along mountain streams and at springs up to 1200 m a.s.l. Throughout our research, all the bones were placed at the same location, at the coordinates: N 49.266262°, E 20.141956°. The samples were placed at the site during the summer (Fig. 1a) and winter seasons (Fig. 1b). Bones were labeled using a sterile bandage to allow precipitation and climatic conditions to affect it. All bones were placed in a mesh to protect them from predators.

Sample preparation and laboratory analyses

Samples from Tatra chamois (n = 30) used for this study came from naturally deceased Tatra chamois found in their natural habitat (1600 – 2100 m a.s.l.) during the winter season (2017/2018). Animals were recovered by the staff of the State Forests of Tatra National Park. We obtained bone samples by dissecting these dead chamois. Subsequently, we removed the pelvis and rib bones from each individual. After dissecting the chamois bones, we cleaned off as much soft tissue as possible. The chamois bone samples included 30 pelvic bones and 30 rib bones, so that together there were 60 bone samples. At the beginning of the experiment, we cut a small piece of each bone sample using a hand saw. The remains of the bones were placed in mesh in the alder forest, where they were affected by environmental conditions. At certain intervals from October 2019 to December 2021 (October 2019, May 2020, June 2021, December 2021), we examined the next piece of each bone. We obtained a total of 4 samples from each bone during the 2.5 years of the experiment. These bone pieces were dried in a laboratory Incubator IF 160 Plus (Memmert, Germany) at 80 °C for 8 hours.

Subsequently, these dried samples were ground using a cryomill (Retsch, Germany) at a frequency of 30 oscillations per second for 50 seconds, to a powder. Powder samples were continuously analyzed immediately after sawing for elemental concentrations using a hand-held XRF spectrophotometer Delta (Olympus, Innov-x Systems, USA). Measurements run in closed Delta XRF Portable WorkStation. Samples were analyzed in plastic cuvettes with plastic foil at the bottom for 240 s in three 80 s intervals, from which the average was calculated. A spectrometer was calibrated for bones by using certificated reference Bone Meal standard (SRM 1486, Maryland). The detection limits were determined continuously for each measurement and for each element by software using Compton Normalization method.

Statistical analyses

The results processed by the XRF spectrometer were stored in an Excel spreadsheet. Subsequently, statistical analyses were performed with Statistica 12 software for Windows (Stat Soft CR, Prague, Czech Republic). Elements that had many values below the detection limit of the XRF spectrophotometer were excluded from statistical analyses: sulfur, titanium, cobaltum, niccolum, cuprum, arsenicum, selenium, zirconium, argentum, cadmium, stannum, hydrargyrum. Selected elements that we used in statistical analyzes were: P, Cl, K, Ca, Cr, Mn, Fe, Zn, Rb, Sr, Mo, Sb, Ba, Pb. Because the distribution of observed levels of elements between groups was non-normal according to the Shapiro-Wilk test, we used non-parametric tests. Differences in elemental concentrations in the two bone types (pelvis and rib) between different months of exposure were compared using the non-parametric Kruskal-Wallis test. Elemental concentrations were also compared between rib and pelvic bones using the Kruskal-Wallis test. As the concentration of elements in the pelvic and rib bones did not differ for most ele-

Fig. 1. **a)** Place of storage of samples in summer. **b)** Place of storage of samples in winter (Photo: S. Brecelj, 2019/2020).

ments other than Fe, both bone types were used in one cluster to investigate the differences in the concentration levels of elements in different sexes and in juvenile and adult individuals. These differences were analyzed using the least squares average method.

Results

Phosphorus. The level of phosphorus in the pelvis and ribs ranged from 40,000 to 80,000 µg/g. Its amount increased after exposure of the bone to the outdoor environment from 8 to 19 months. Phosphorus began to decline slightly 26 months after the bone was placed in the external environment. This means that phosphorus levels increased after the first and second winter periods (Fig. 2).

Chlorine. The amount of chlorine reaches a standard lower level immediately after the first winter and then did not change significantly for the remainder of the observation period (Fig. 3).

Potassium behaves in the same way as chlorine in both the ribs and the pelvis (Fig. 4). It is very likely that it leached out of the bones in the external environment in the form of salts (KCl).

Calcium. Figure 5 shows a decrease in the amount of calcium in the pelvis after the first winter with a subsequent increase following the second winter. At the end of 26 months, calcium was leached from the pelvis due to environmental conditions. Calcium in the ribs did not decrease after the first winter or after the second winter. Similarly to the pelvis, the calcium content increased after 19 months and then decreased after 26 months. The trend is not identical but in the time horizon after 19 months, it is similar to the variability of phosphorus in bones.

Chromium. After the first winter, the concentration of chromium in the ribs increased, but the concentration of chromium in the pelvic bones remained unchanged throughout the experiment (Fig. 6).

Manganese is also a biogenic element. The concentrations of this element in the ribs increased significantly during the experiment. There was also a slight increase in Mn in the pelvic bones, but this was not significant (Fig. 7).

Iron. The concentrations of iron did not change significantly for either type of bone (Fig. 8).

Zinc concentrations increased significantly in the pelvic and rib bones during the experiment (Fig. 9). The measured values ranged from 79 to 5604 µg/g.

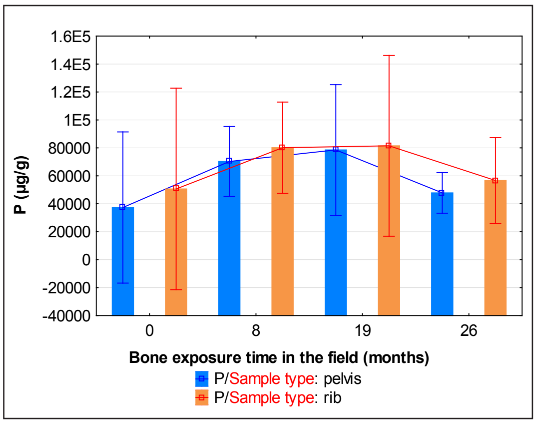

Fig. 2. Mean concentrations of phosphorus in the bones of Tatra chamois. Amount of phosphorus significantly increased in both types of bones from 8 to 19 months after the exposure (pelvis: KW-H (3,67) = 27.4, p = 0.00000, rib: KW-H (3,85) = 24.5, p = 0.00002).

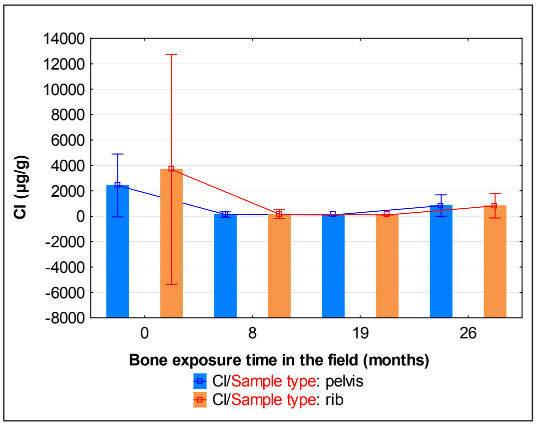

Fig. 3. Mean chlorine concentrations in chamois bones. The amount of chlorine decreased in both bone types from 0 to 8 months after exposure (pelvis: KW-H (3,62) = 50.9, p = 0.0000, rib: KW-H (3,77) = 66.1, p = 0.0000).

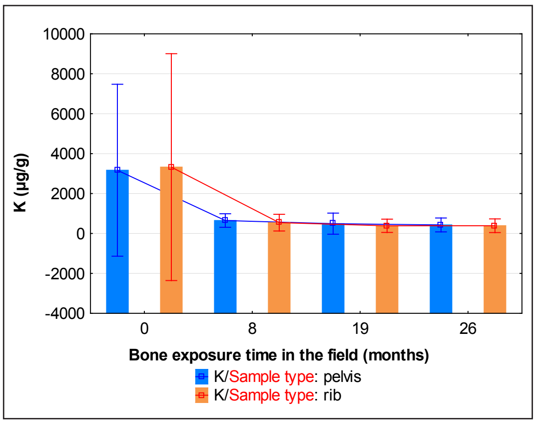

Fig. 4. Mean potassium concentrations in chamois bones. The amount of potassium decreased in both bone types from 0 to 8 months after exposure (pelvis: KW-H (3,66) = 27.9, p = 0.0000, Rib: KW-H (3,84) = 20.2, p = 0.0002).

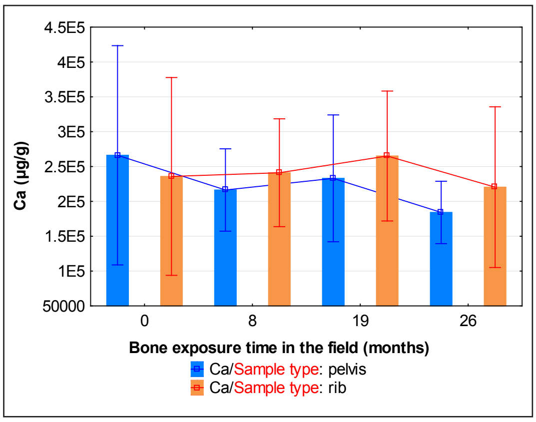

Fig. 5. Mean calcium concentrations in chamois bones (pelvis: KW-H (3,67) = 14.0, p = 0.0029, rib: KW-H (3,87) = 7.6, p = 0.0556).

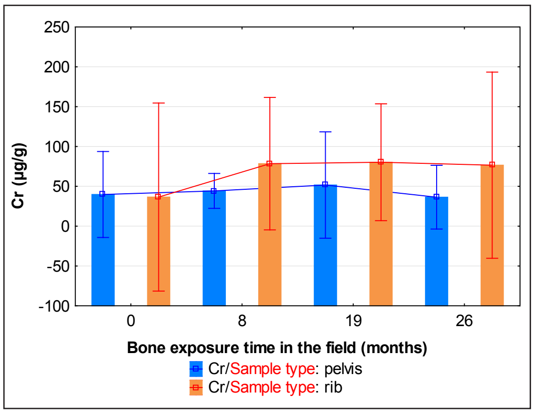

Fig. 6. Mean chromium concentrations in chamois bones (pelvis: KW-H (3,67) = 6.3, p = 0.0958, rib: KW-H (3,84) = 32.1, p = 0.0000).

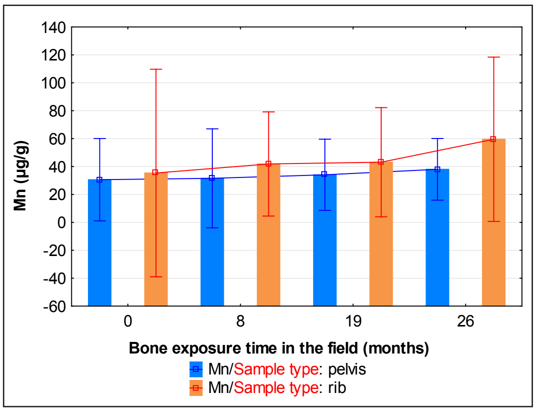

Fig. 7. Mean manganese concentrations in chamois bones (pelvis: KW-H (3,84) = 7.2, p = 0.0658, rib: KW-H (3,84) = 17.3, p = 0.0006).

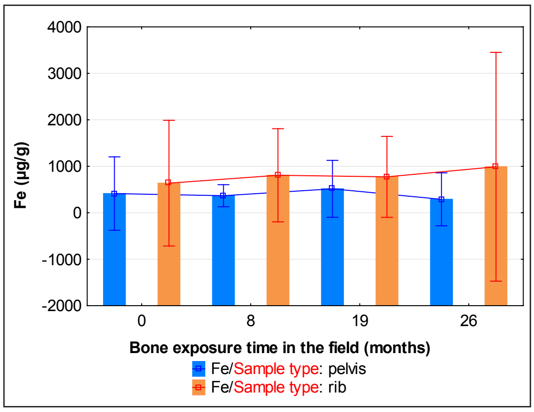

Fig. 8. Mean iron concentrations in chamois bones (pelvis: KW-H (3,67) = 8.5, p = 0.0368, rib: KW-H (3,84) = 5.3, p = 0.1499).

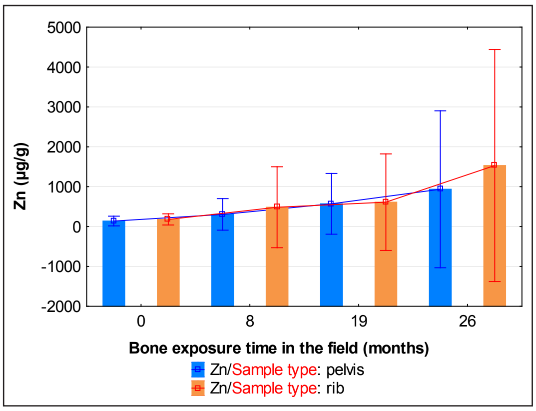

Fig. 9. Mean concentrations of zinc in the bones of chamois. The amount of zinc significantly increased in both types of bones from 0 to 26 months after the exposure (pelvis: KW-H (3,67) = 45.4, p = 0.0000, rib: KW-H (3,84) = 49.1, p = 0.0000).

Rubidium. The course of accumulation of rubidium levels is different for the pelvic bones and for the rib bones as a whole. In the pelvic bones, the level of rubidium was lower at the beginning of our study than at the end at 26 months. In the rib bones, the rubidium level was higher at the beginning than at the end of the experiment (Fig. 10).

Strontium. Changes in strontium levels were not significant in the ribs or pelvic bones (Fig. 11).

Molybdenum. Molybdenum levels increased over time in both types of bone. In the pelvic bones, the level of molybdenum first decreased slightly (within 8 months) and then rose until the 26th month, when it exceeded the original values. Similarly on the rib bones, the molybdenum levels dropped slightly until the 19th month and then rose by the 26th month (Fig. 12).

Antimony. Changes in antimony levels were not significant in the ribs or pelvic bones in different measured periods (Fig. 13).

Barium. The concentration of barium in the rib bone increased after 8th and then after 26th month. However, in the pelvis, changes in the concentration of this element were not significant (Fig. 14).

Lead. The concentration of lead rose significantly for two years, with a visible curved line. This means that the amount of lead in the bones constantly increased over time (Fig. 15).

Elements in the bones of dead chamois

Levels of element concentration in the pelvic and rib bones of chamois were not significant in most cases (Table 1). Higher levels of iron were measured in the rib bones compared to the pelvis.

Interesting differences were found between juveniles and adults older than 2 years, where the juveniles were compared before the first winter of survival. Juveniles had the same calcium content as adults (Table 1). However, they had also more biogenic elements in their bones (P, Fe, Zn) compared to adults. In addition, they had higher levels of metals such as Sr and Sb.

After excluding juveniles from the measured set, concentration of elements did not differ between sexes (Table 1).

Discussion

We found that the phosphorus in the bones of the Tatra chamois had values from 40,000 to 80,000 µg/g. Hancock *et al.* (1989) have phosphorus data in their work ranging from 180,000 to 191,000 µg/g in human archeological bones. Further, Zaichick *et al.* (2009) have values of 123,000 and 122,730 µg/g in their work about rib bones in healthy humans. Farswan and

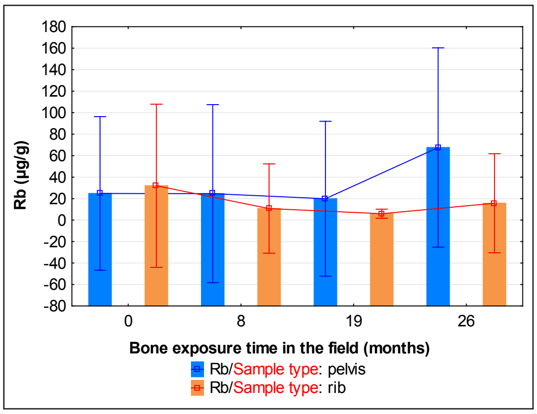

Fig. 10. Mean rubidium concentrations in chamois bones (pelvis: KW-H (3,67) = 16.3, p = 0.0010, rib: KW-H (3,84) = 28.9, p = 0.00000).

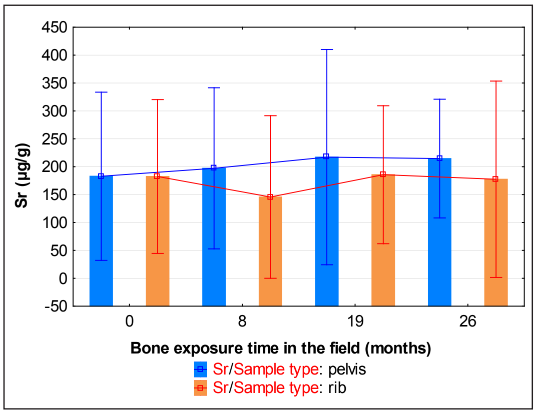

Fig. 11. Mean strontium concentrations in chamois bones (pelvis: KW-H (3,67) = 2.9, p = 0.4007, rib: KW-H (3,84) = 3.7, p = 0.3015).

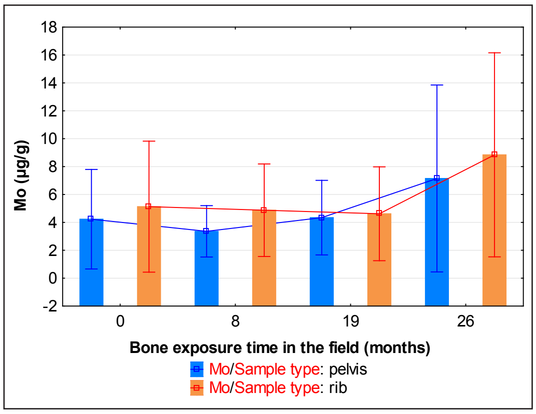

Fig. 12. Mean molybdenum concentrations in chamois bones (pelvis: KW-H (3,51) = 7.1, p = 0.0681, rib: KW-H (3,75) = 16.5, p = 0.0009).

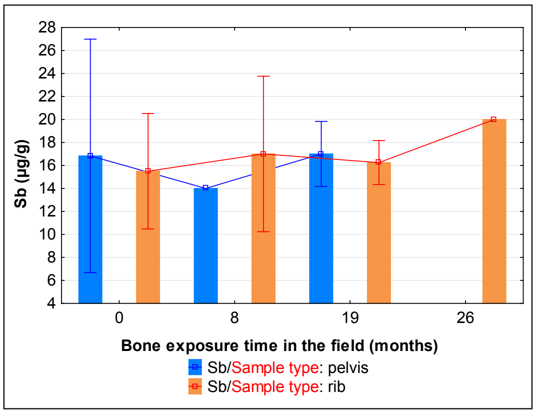

Fig. 13. Mean antimony concentrations in chamois bones. The amount of antimony did not differ among different periods of exposure. (pelvis: KW-H (2,9) = 2.2, p = 0.3316, rib: KW-H (3,19) = 2.7, p = 0.4428).

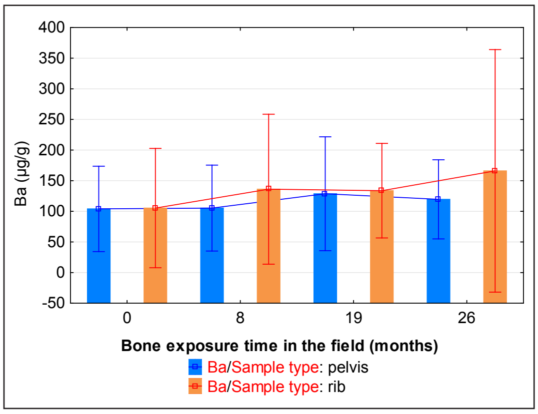

Fig. 14. Mean barium concentrations in chamois bones after different time of exposure of bones in the field (pelvis: KW-H (3,62) = 3.3, p = 0.3462, rib: KW-H (3,81) = 7.9, p = 0.0478).

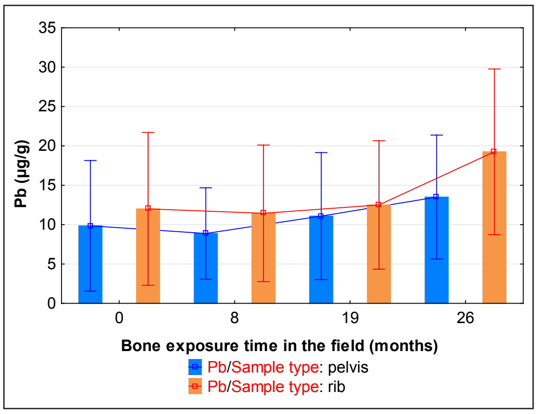

Fig. 15. Mean lead concentrations in chamois bones (pelvis: KW-H (3,4) = 6.7, p = 0.0832, rib: KW-H (3,62) = 13.7, p = 0.0033).

Nautiyal (1997) state that the phosphorus value in mountain soils can be from about 400 to 1400 µg/g. Phosphorus is a strontium-related element and can enter the bones after death through snails that feed on these bones because there is up to 10,000 µg/g of phosphorus in the slug mucus (Greistorfer *et al.* 2017).

Average chlorine in our bone samples ranged from approximately 100 to 4000 µg/g. Kilburn *et al.* (2021) report a value of chlorine of about 200 µg/g in archaeological human skeletal remains. In work by Blondiaux *et al.* (1992), on bones from the 6th century, case data are reported as: compact bone 213 µg/g; trebacular bone 465 µg/g; and new bone 373 µg/g. Chlorine values of human archeological bones are reported as follows: 118.6 µg/g, 127.7 µg/g, 254.9 µg/g, 683 µg/g and 934 µg/g (Edward 1990). Chlorine is an element that is absorbed by bone mainly during the life of the animal. Following death, or environmental exposure, the bones quickly lose chlorine to almost zero values (Piga *et al.* 2013).

Element		**Rb**	**Ca**	**P**	**K**	**Cr**	**Mn**	**Fe**
Sample type	Pelvis	24.8 ± 6.8 (29) a	266,147.2 ± 13,933.8 (29) a	3167.8 ± 465.7 (29) a	39.8 ± 8.4 (29) a	30.6 ± 5.2 (29) a	412.9 ± 101.9 (29) a	138.4 ± 12.2 (29) a
	Ribs	31.9 ± 7.1 (27) a	235,778.0 ± 14,440.7 (27) a	3323.0 ± 482.6 (27) a	36.5 ± 8.7 (27) a	35.4 ± 5.4 (27) a	637.3 ± 105.6 (27) a	179.4 ± 12.6 (27) b
	Males	21.4 ± 6.2 (32) a	244,049.1 ± 13 649.4 (32) a	3516.9 ± 447.1 (32) a	35.9 ± 8.3 (32) a	36.9 ± 5.1 (32) a	554.0 ± 102.3 (32) a	160.1 ± 12.3 (32) a
	Females	35.0 ± 8.0 (19) a	260,346.6 ± 17,713.8 (19) a	3049.8 ± 580.2 (19) a	45.3 ± 10.8 (32)a	27.2 ± 6.6 (19) a	483.5 ± 132.8 (19) a	155.5 ± 16.0 (19) a
	Adults	31.6 ± 6.7 (32) a	243,266.0 ± 13,550.0 (32) a	2493.6 ± 342.4 (32) a	31.4 ± 4.6 (32) a	26.2 ± 2.0 (32) a	372.7 ± 60.4 (32) a	138.1 ± 9.8 (32) a
	Juveniles	24.3 ± 9.4 (16) a	242,675.3 ± 19,162.6 (16) a	4122.3 ± 484.2 (16) b	37.9 ± 6.5 (16) a	32.4 ± 2.7 (16) a	576.1 ± 85.5 (16) a	186.6 ± 13.9 (16) b
Element		**Zn**	**Sr**	**Mo**	**Sb**	**Ba**	**Pb**	**Cl**
Sample type	Pelvis	182.9 ± 13.4 (29) a	4.2 ± 0.4 (23) a	16.8 ± 1.6 (6) a	104.0 ± 8.1 (27) a	9.9 ± 1.0 (20) a	2424.5 ± 606.0 (29) a	2424.5 ± 606.0 (29) a
	Ribs	182.4 ± 14.0 (27) a	5.1 ± 0.5 (20) a	15.5 ± 1.6 (6) a	105.3 ± 8.4 (25) a	12.0 ± 1.0 (22) a	3680.9 ± 628.1 (27) a	3680.9 ± 628.1 (27) a
	Males	179.3 ± 12.8 (32) a	4.5 ± 0.4 (27) a	16.2 ± 1.3 (10) a	99.7 ± 8.0 (29) a	11.4 ± 1.0 (23) a	3253.3 ± 613.2 (32) a	3253.3 ± 613.2 (32) a
	Females	191.5 ± 16.6 (19) a	5.0 ± 0.6 (13) a	16.0 ± 2.9 (2) a	108.3 ± 9.9 (19) a	10.5 ± 1.3 (14) a	2882.7 ± 795.8 (19) a	2882.6 ± 795.8 (19) a
	Adults	176.7 ± 10.5 (32) a	3.8 ± 0.3 (24) a	14.9 ± 0.7 (8) a	87.3 ± 5.1 (32) a	9.5 ± 0.9 (21) a	2539.3 ± 214.3 (32) a	2539.3 ± 214.3 (32) a
	Juveniles	236.9 ± 14.8 (16) b	5.7 ± 0.5 (12) b	16.0 ± 1.1 (3) a	118.1 ± 7.2 (16) b	11.8 ± 1.0 (15) a	2767.6 ± 303.1 (16) a	2767.6 ± 303.1 (16) a

Table 1. Least square means with standard errors of element concentrations (μg/g) in the bones of dead Tatra chamois (One-way ANOVA, different letters in a column denote the significant differences between groups, $p < 0.05$, number of samples is in the parentheses).

In our results, mean potassium in chamois bones has values of about 3000 μg/g at the first measurement and later drops to almost zero. Work by Blondiaux *et al.* (1992), states that the bones from the 5th and 6th century case contain compact bone values of 255 μg/g, trebacular bone values of 270 μg/g and per new bone of 1155 μg/g. Values of rib bone of healthy humans are 412 and 427 μg/g (Zaichick *et al.* 2009). Potassium in the human skeleton is 810 μg/g (Brätter 1977). According to Yamagata (1962), in human tissues such as bones, potassium values increase from 0.98 to 20.0 μg/g. It is probably stored in the bones of animals mainly during their lives and after death, when their bones are exposed to external environmental influences, potassium levels drop rapidly to almost zero (Follis *et al.* 1942).

In our results, average calcium in chamois bones was found at values between 270,000 μg/g and 190,000 μg/g. According to Hancock *et al.* (1989), values of 396,000, 400,000 and 402,000 μg/g calcium were found in human archeological bones. Zaichick *et al.* (2009), in turn reported calcium values of 265,800 and 267,300 μg/g in rib bones of healthy humans and Blondiaux *et al.* (1992) report compact bone values of 30, trabecular bone values of 31, and new bone values of 32 μg/g in bones from the 5th and 6th century. Calcium values were from 200,000 to 240,000 μg/g in human archeological bones (Edward 1990). Calcium is an element that occurs naturally in animals and is an important part of bones. This element is important to bone strength. Calcium is leached from the bone after death. According to Vass (2001), calcium concentration can be used to determine the length of time since death because it is leached from the bone at a rate determined primarily by temperature and exposure to moisture. This is corroborated by our results, where the level of calcium was lower in warmer months than in the colder months.

Chromium increased more significantly in rib bones. In the pelvic bones, changes in chromium levels are less noticeable. Bones as a sorbent can absorb chromium well from aqueous solutions (Chojnacka 2005). Average manganese in our bone samples reached a maximum value of 60 µg/g. Blodiaux *et al.* (1992) recorded manganese values of 3.4, 65 and 252 µg/g, in their samples from the 5th and 6th century, which are quite consistent with our work. Conversely, other studies, (Zaichick *et al.* 2009) showed manganese values in human rib bones was as high as 1000 to 1020 µg/g. Kilburn *et al.* (2021) recorded manganese values of 1000 µg/g in archaeological human skeletal remains.

In our results, mean iron reached a level of 1000 µg/g in chamois bones. Iron levels in archaeological human skeletal remains were recorded at a maximum of 30,000 µg/g, or conversely, Kilburn *et al.* (2021) recorded valuesa of 1000 µg/g. In Zaicshick *et al.* (2009) iron values in rib bone of healthy humans were 92,000 and 99,000 µg/g.

In our results, maximum average zinc concentrations were measured at 1500 µg/g. Zaichick *et al.* (2009) recorded zinc values in rib bones of healthy humans at concentrations of 143,000 and 147,000 µg/g. Work by Blondiaux *et al.* (1992) reported zinc values in archeological bones at 82 µg/g for compact bone, 128 µg/g for trebacular bone and 350 µg/g for new bone. Zinc was almost identical in the pelvic and rib bones. Although there were some differences, they were less significant than in other elements. It appears that zinc was only present in small amounts in the bones and concentrations increased over time. This increase may be due to diffusion from the soil, as this element occurs in the environment due to zinc smelters (Wierzbicka and Pielichowska 2004).

Rubidium in chamois bones was measured at values 60 µg/g and less. Yamagata (1962) records rubidium values in human tissues such as bones at 4.9 µg/g, 5.4 µg/g, 9.7 µg/g and 10.5 µg/g (the work contained more bones numbered as a type). Thus, rubidium is already present in bone tissue, but in small quantities (Yamagata 1962). Rubidium is normally found in animal and human bones, and Yamagata (1962) found 7.6 µg/g of rubidium in human bone ash.

Based on our results, strontium exists in bones at values around 200 µg/g. Zaichick *et al.* (2009) recordss values in rib bone of healthy humans at 264,000 and 251,000 µg/g. Blondiaux *et al.* (1992) write that strontium values in bones from the 5th and 6th century are 220 for compact bone, 185 for trabecular bone and 257 µg/g for new bone. Furthermore, Edward (1990) mentions values in human archeological bones such as 85.7 µg/g, 90.1 µg/g, 90.7 µg/g, 99.3 µg/g, 100.2 µg/g. This may be due to food intake, even in the case of plant foods (Price 2002).

In our results, molybdenum has the highest average value of approximately 9 µg/g in bones. Hidiroglou *et al.* (1982) studies fresh long bones of the left thoracic limb, and reported molybdenum values averaging 1.66 µg/g, 1.69 µg/g and 1.74 µg/g. Other molybdenum values are also mentioned, which are: 46, 48 and 49 µg/g (Hidiroglou *et al.* 1982).

Antimony is an element found in the bones that highest average values are 20 µg/g. Another study (Heydorn 1967) reported antimony values for hair in men, namely: 0.153 µg/g. Antimony levels in the bones, as with many other elements, generally increased. High levels of antimony, which were measured at the first measurement, can be taken up into the bones through food or from the atmosphere (Nixon 1969). Antimony concentration generally increased, but this was mainly observed in the rib bones. Measurements for pelvic bones at 26 months were unavailable. Bones (such as teeth) absorb less antimony than other parts of the body (such as soft tissues) (Friedrich *et al.* 2012).

Barium has the highest average value in bones, with concentrations above 150 µg/g. According to Zaichick *et al.* (2009), the barium value in rib bones of healthy humans is 270,000 µg/g and Brätter (1977) mentions a barium value in the human skeleton of 71 µg/g. Again, according to Kilburn *et al.* (2021) the barium value in archaeological human skeletal remains is around 200 µg/g. The level of barium in the bones generally increased, as with other elements. As with other elements, the levels in the pelvis and rib bones do not match at all. Barium is an element that is most likely to be absorbed from the soil (Carvalho *et al.* 2004), which can also be seen in the graph.

Manganese is an element that generally does not have high uptake in ruminants (Ballová *et al.* 2019), which can also be seen in the results.

The highest average lead was shown by our data as just below 20 µg/g. According to Brätter (1977), lead values in human skeleton reach 3.43 µg/g. Lead levels have generally risen sharply. Lead levels were higher in the rib bones than in the pelvic bones. Lead can also enter the environment due to road pollution (Minoranskij 1990). According to Ballová *et al.* (2019), lead and zinc contamination in the High Tatras is prevalent. Pollution or contamination by these elements is most likely caused due to the influence of the coal and mining industries. The sources of these pollutants do not neccessarily have to be in close proximity to the High Tatras, but can travel long distances (e.g., Moravia in the Czech Republic).

The mountains are a good identifier of air pollution concentrations (Ba, Mn, Pb, Sr, Zn) in the bones and teeth of wild ruminants from the West Carpathians and the Tian-Shan Mountains, as evidenced by Ballová *et al.* (2019). These high pollution concentrations likely have had a significant effect on element concentrations in chamois bones. Further, bones, as calcified tissues, are good bioindicators of long-term accumulation of elements. Pollution in the mountains can also be related to emissions from transport, which means that large cities are also significant polluters (Ballová *et al.* 2019).

Air pollution from transport emissions is mainly a source of lead and barium. According to Krajcarz (2019), the most important lithological features of the soil in terms of chemical changes in metal concentrations in bone are the content of organic matter and sediment moisture. Both factors acting together are necessary to significantly change the chemical composition of bones. Further, other elements such as sulfur, titanium, cobaltum, niccolum, cuprum, arsenicum, selenium, zirconium, argentum, cadmium, stannum, hydrargyrum, had poorly measured levels in chamois bones (*Rupicapra rupicapra tatrica*). Therefore, due to their non-quantified levels, they were not included in the processing of data on the synergistic behavior of the elements, even though these elements are sufficiently interesting from a research point of view.

Calcium is leached from the bone after death, as confirmed by Vass (2001). Calcium is used as an element to determine the length of time since death because it is leached from the bone at a rate determined primarily by temperature and moisture exposure. Zinc was present in the bones only in small amounts and increased over time. This increase may be due to diffusion from the soil, as this element occurs in the environment due to zinc smelting (Wierzbicka and Pielichowska 2004). According to Ballová *et al.* (2019), environmental contamination by lead as well as zinc is strong in the High Tatras. Pollution is most likely caused by the coal and mining industries.

Furthermore, bones as calcified tissues are good bioindicators of long-term accumulation of elements. Pollution in the mountains can be related to emissions from transport, which means that even large cities are significant polluters (Ballová *et al.* 2019). Air pollution from transport emissions consists mainly of the elements lead and barium. Elements such as sulfur, titanium, cobalt, nickel, copper, arsenic, selenium, zirconium, silver, cadmium, tin, hydrargyrum had low levels in chamois (*Rupicapra rupicapra tatrica*) bones. If bone samples are taken directly from the field to compare concentrations between mountain ranges, results may differ due to these element exchanges mainly between bones, soil and air. A duration of 26 months is enough to change the chemical composition of the bone to a detectable level, if environmental conditions are suitable (Krajcarz 2019).

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