Original Article

Multielemental Chemical Analysis of Elements in Mandibular Bone and Teeth in the Rat

(chemical elements / bone / teeth / mandible / laboratory rat)

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Abstract. The purpose of the study was to test the hypothesis of different distribution spaces of elements in the rat mandibular bone and teeth. We used six adult males of Wistar laboratory rats for the study. After killing the animals, we extracted the molars and removed incisor crowns. The mandibular bone was divided into four parts (mesial-central-distal-ridge). Inductively coupled plasma mass spectrometry was used to determine the presence of 41 elements in the bone and tooth. Evidence of 14 elements was found in all samples (incisors-molars-bone). Generally, significant differences between the left and right side were found for K and Rb in the bone locations. As regards statistically significant differences in incisors-molars-bone locations, the elements for which these differences were found for all comparisons are listed as incisors versus individual molars, incisors versus bone locations, and individual molars versus bone locations: a) incisors-molars: Ba, Mn, Mo, Sr, Zn, K, Mg and Rb; b) incisors-bone: Fe, K, Mg, Mn, Na, Zn and Ba; c) molars-bone: Mn, Mo, Na and Mg. Statistically significant differences were also found between molars for Fe, Mg, Mn, and Sr and between bone locations for Ba, Ca, Mn, Sr, K, Rb, Zn, Mo, Mg, and Na. The elements Cu, Ni and Co were without pronounced differences. Twenty-seven elements were below the detection limit. Our results indicate different distributions of some elements in the rat mandibular incisors-molars-bone. We assume that the knowledge of chemical element contents in the laboratory rat bone and teeth will prove useful in experimental research of both these hard tissues.

Introduction

Chemical elements are part of all tissues of the living organisms. Some elements are physiologically essential components and play an important role for the function of tissues and organs (Fischer et al., 2013). Some elements may have an essential function or be toxic depending on their levels in the organism (Dermience et al., 2015).

Various authors have studied elements in human bones (Katić et al., 1991; Smrčka, 2005; Zaichick et al., 2009; Zaichick and Zaichick, 2010a, b; Zaichick et al., 2011; Lanocha et al., 2012). Other studies analysed elements present in the teeth (Curzon and Cutress, 1983; Vrbič et al., 1987; Lane and Peach, 1997; Reitznerová et al., 2000; Fischer et al., 2009, 2013; Ghadimi et al., 2013) or focused on elements in animal models (Hirayama et al., 2011; Oliveira et al., 2012; Maciejewska et al., 2014). The importance of elements with respect to the bone metabolism has also been studied by a number of authors (Yamaguchi et al., 1987, 2000; Schneiderka et

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Abbreviations: LOD – limit of detection, SD – standard deviation.

al., 2004; Sarko, 2005; Parelman et al., 2006; Rude et al., 2009; Žofková, 2012; Cianferotti, et al. 2013; Dermience et al., 2015). For example, Dermience et al. (2015) described the roles of 30 elements in the bone metabolism.

Our study presents an analysis of 41 elements in the rat mandibular bone and teeth: sodium (Na), magnesium (Mg), aluminium (Al), potassium (K), calcium (Ca), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), rubidium (Rb), strontium (Sr), yttrium (Y), molybdenum (Mo), cadmium (Cd), tin (Sn), antimony (Sb), barium (Ba), lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), lutetium (Lu), thallium (Tl), lead (Pb), bismuth (Bi), and uranium (U).

The purpose of our study was to test the hypothesis of different distribution spaces for chemical elements in the rat mandibular bone and teeth.

Material and Methods

Animals

We used six adult males of Wistar laboratory rat, age 12 weeks, for the study. The animals were obtained from the breed of the Institute of Physiology, First Faculty of Medicine, Charles University. The experiment was performed in compliance with applicable regulations for the use of laboratory animals – EU Council Directive 86/609/EEC. The animals were kept in boxes at 20–23 °C with the standard 12-h light-dark cycle. The animals received standard food (ST-1; www.velaz.cz/en/product/st-1/) and water available *ad libitum*.

Tissue preparation

The animals were killed by their overdosing with intraperitoneally administered thiopental. The left and right parts of the mandible were prepared. Incisor crowns were collected and molars M1, M2, M3 were extracted. Incisor roots were gradually removed as they were not included in the elemental analysis. The teeth were mechanically cleaned and washed in *aqua pro injectione*. The mandibular bone was divided in four parts. Osteotomy was performed vertically, mesially from the extracted first molar and distally from the extracted third molar. In addition, a sample was taken from the middle part of the mandibular branch, where the mandible widens and passes into the articular projection. In total, four bone samples were taken from every side, labelled as follows: mesial, central, distal, and ridge (Fig. 1).

Chemical analysis

The weighed amount of 10–130 mg of the dried bone sample and the sample of individual teeth was inserted to a 10 ml volumetric flask; a value of 0,5 ml of concentrated HNO_3 was added; subsequently, the sample was dissolved by careful heating of the glass on the heating plate at approx. 100 °C. After cooling, deionized water was added to the mark of the volumetric flask. A blank samples were prepared for every series of 20 samples. The measurement quality was tested by analyzing the standard reference material (SRM 1400, Bone Ash, National Institute of Standards and Technology, USA). Differences between the measured and certified valuess were lower than the 10% RSD (relative standard deviation). All the acids used in the dissolution procedure were reagent grade (Merck, Darmstad Germany). Deionized water from MilliQPlus (Millipore, USA) were used to prepare the solutions. The contents of Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Y, Mo, Cd, Sn, Sb, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Tl, Pb, Bi, and U in the mineral residues were determined using inductively coupled plasma mass spectrometry (X Series II, Thermo-analytical Germany) under the following conditions: ICP 1350 W, "peak jump" measurement mode, measurement time 3 × 50 s, ion optics parameters optimized with Ge, Re, an Rh solution, gas flows 13.5 l/min (cooling), 0.7 l/min (additive), 0.65 l/min (nebulizer). Measured isotopes of ^{72}Ge, ^{103}Re, ^{185}Rh 20 µg l^{-1} Astasol solutions (Analytika, Czech Republic) were used as internal standards.

Statistical analysis

Forty-eight bone samples, 36 molars and 12 incisor crown samples collected from six animals were used for the statistical analysis. Relationships between element concentrations, side and location were evaluated by the ANOVA model consisting of a subject factor explaining the inter-individual variability between animals, Side factor explaining the differences between the left and right side, Location factor explaining differences between the sites of measurement and also including Side × Location interaction testing whether there are significantly different patterns for location differences between the left and right side. Least significant difference multiple

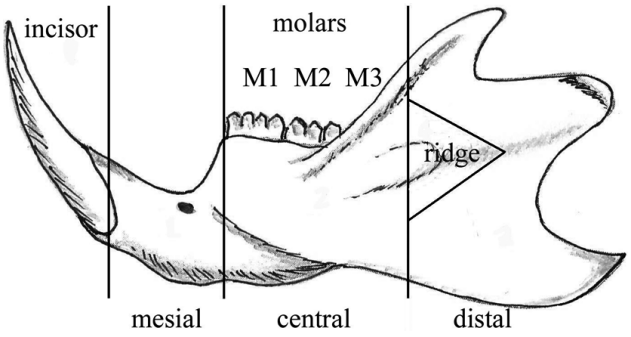

Fig. l. Scheme of bone sample and teeth collection from the left side of the mandible (samples from the right side were collected identically). Teeth are labelled as: incisor, molars (M1-M3). The black line indicates the site of osteotomy. Bone samples are labelled as mesial, central, distal, and ridge.

comparisons followed the ANOVA testing. For differences between locations (eight levels of factor Location), the Bonferroni correction for multiplicity was applied. Due to mostly skewed data distribution, the original data were transformed by power transformations to attain data symmetry and constant variance before the ANOVA testing (Meloun et al., 2000). The results obtained in the transformed data with the use of ANOVA model (means with 95% confidence intervals) were retransformed to original scale in tables and figures. Homogeneity of the transformed data was tested using residual analysis as described elsewhere (Meloun et al., 2002, 2004).

The limit of detection (LOD) was calculated as follows: LOD = 3 × SD based on 10 measurements of the blind experiment; SD = standard deviation. Standard statistical software was used for the calculation. Values of element contents in individual samples below this limit were replaced with 2/3 LOD. Comparisons of element concentrations in the samples were performed for elements whose mean values were higher than LOD (Fig. 2).

Results

In total, 41 elements were analysed. The presence of 14 elements was found in all samples. As regards statistically significant differences incisors-molars-bone locations, the elements for which these differences were found for all comparisons are listed as incisors versus individual molars, incisors versus bone locations, and individual molars versus bone locations. All comparisons of incisors versus individual molars (M1-3) were significant for: Ba, Mn, Mo, Sr, and Zn (with a higher content of the element in the molars) and for the elements: K, Mg, and Rb (with a higher content of the element in the incisors). Furthermore, we demonstrated statistically significant differences between M1 and M3 for the elements: Fe, Mg, and Mn, with a lower content of the elements found in M3; and Sr, with a lower content found in M1. A statistically significant difference between M2 and M3 was found for: Fe, Mg, and Mn (with their higher contents in M2) and Sr (with its higher content in M3). All comparisons for incisors versus individual bone locations (mesial, central, distal, and ridge) showed significant differences in: Fe, K, Mg, Mn, and Na, with a higher content of the elements found in the incisors; for: Zn and Ba, with a higher content of the elements found in the bone.

All comparisons of individual molars (M1-3) versus individual bone locations (mesial, central, distal, and ridge) showed a statistically significant difference for:

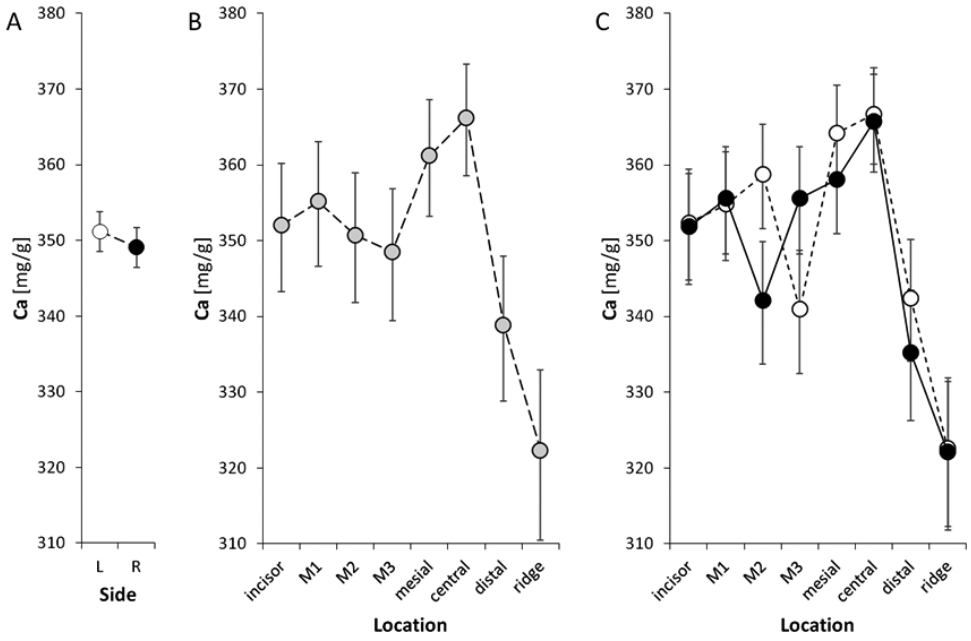

Fig. 2. Relationships between Ca (mg/g) concentrations, side and location as evaluated by the ANOVA model consisting of a subject factor explaining inter-individual variability between animals, Side factor explaining differences between the left and right side, Location factor explaining differences between the sites of measurement and also including Side × Location interaction testing if there are significantly different patterns for location differences between the left and right side. Panel **A** illustrates the influence of Side (Side factor), Panel **B** shows differences between locations, and panel **C** illustrates whether there are significant differences between the left and right side in individual locations. Error bars represent 95% confidence intervals of the mean values. The 95% confidence intervals in panel B take into account the Bonferroni correction for multiplicity. Empty and full circles in panel C designate the left and right side, respectively. The significance of individual factors and between-factor interaction was as follows: Side: $F(1, 75) = 0.6$, $P = 0.435$, Location: $F(7, 75) = 12$, $P < 0.001$, Subject: $F(5, 75) = 20.7$, $P < 0.001$, Side × Location: $F(7, 75) = 1.4$, $P = 0.227$. F represent F-statistics, while P is the significance of the factor. Values in parentheses show the degrees of freedom for individual factors.

Mn, Mo, and Na (with their contents higher in the molars); for Mg, with the content higher in the bone. As regards individual samples taken from the bone, statistically significantly higher contents of elements in the mesial part and central part of the mandible were found for: Ba, Ca, Mn, and Sr. In the distal part of the mandible, a higher content of K was found compared to the mesial part and the ridge. For Zn, a higher content was found in the central part and the ridge. The contents of Mg and Na were lower in the central and distal parts of the mandible compared to the mesial part and the ridge. A higher content of Rb was found in the central and distal parts compared to the mesial part and the ridge. At the same time, we demonstrated statistically significant differences between the distal part and the ridge for: K, Mg, Mn, Mo, Na, Rb, and Zn (Figs 2-15B). Generally, significant differences (Bonferroni multiple comparisons) were found between the left and right side in the bone locations for the elements K and Rb. Significant interaction in K may be interpreted as a significant distinction in the profile of between-location differences between the left and right side (Figs 2-15A,C). Significant differences between the left and right side in any of the locations were found for Ca, Co, Na, and Ni (for M2); Cu and Mo (for M3); Sr and Ba (for part of the bone samples) (Figs 2-15C). The other 27 elements were below the limit of detection in the bone and teeth.

Discussion

Trace elements enter the human body from different sources such as food, water, air, etc. (Ghadimi et al., 2013). As reported by Fischer et al. (2013), toxic elements spread in various organs and tissues. Soft tissues are characterized by dynamic changes of the elements. Hard tissues, which form our bones and teeth, accumulate most heavy metals absorbed by the body (Fischer et al., 2013*)*. Reitznerová et al. (2000) described different distributions of some elements in individual layers of tooth enamel. The authors believe this concentration gradient to be related to the contact of teeth with the saliva (Reitznerová et al., 2000). Zaichick (2010a) states that the knowledge of chemical element contents in bones plays a decisive role in understanding the aetiology and pathogenesis of bone diseases including osteoporosis. That is why it is necessary to know them in intact bone. These authors have analysed chemical elements in human iliac crests that were obtained post mortem from intact cadavers (Zaichick and Zaichick, 2010a). The degree of mineralization is dependent on the metabolic state of the whole organism, not only on the physicochemical processes in the place of mineralization (Maciejewska et al., 2014). Bone stretch depends on the bone matrix volume, the microarchitectural distribution of this volume, and the degree of mineralization (Boivin and Meunier, 2003).

Given that the monitoring of elements in human bones is difficult, an animal model needs to be used (Maciejewska et al., 2014). For example, rat is relevant for the study of osteoporosis in men as the age-related bone mass loss is similar (Wang et al., 2001). Hirayama et al. (2011) determined the concentrations of 29 elements in the rat femur. Maciejewska et al. (2014) determined the concentrations of Zn, Sr and Fe in the rat (age: 7, 14 and 28 days) bone (mandible, cranium, femur and tibia) and incisors. The authors found higher Zn and Sr contents in the incisors compared to their contents in the mandible. Furthermore, as reported by the authors, the highest Zn and Sr contents in incisors probably result from their being rubbed off during eating, and thus there is a necessity to rebuild them even in older individuals (Maciejewska et al., 2014).

Ca is one of essential elements in the bone and teeth. Ca is present especially in the form of hydroxyapatite in these tissues. A calcium intake deficit results in its reduced content in the bone and in reduced bone density. A long-term deficit leads to rickets, osteomalacia and osteoporosis (Dermience et al., 2015). For Ca, in our study we found a statistically significant decrease of the contents of the element in the bone from the mesial and central parts toward the distal part and the ridge (Fig. 2B).

Na is a major extracellular element (Smrčka, 2005). Although bone diseases are not associated with elevated or reduced sodium levels, concerns have been increasing as regards any impact of hyponatremia on osteoporosis in the elderly. Several studies have indicated an increased risk of osteoporosis and fractures in connection with hyponatremia. Hyponatremia may stimulate osteoclastic proliferation and mobilize Na stored in the bone (Dermience et al., 2015). In our study, we found a lower content of Na in the bone compared to the incisors and molars. As regards the bone, a decrease of this element was apparent in the central and distal parts compared to the mesial part and the ridge (Fig. 3B).

K is a major intracellular element. On the surface of the bone, there is a layer enriched in K. The elements K and Na are responsible for keeping the membrane potentials.

Rb has many chemical and biological properties similar to K, including its distribution in mammalian tissues. This element can substitute K in the sodium-potassium pump (Smrčka, 2005). In our study, we showed decreased contents of K in the molars and in the bone compared to the incisors. For Rb, its decrease was found only in the molars compared to the incisors. Furthermore, a statistically significant decrease of this element was found in the mesial part and the ridge compared to the incisors. In the bone, a higher Rb content was apparent in the central and distal parts compared to the mesial part and the ridge. A higher content of K was apparent in the distal part compared to the mesial part and the ridge (Fig. 4B, Fig. 5B).

Mg has a protective effect on the skeleton (approximately 60 % of this element is contained in the bone). Similarly as Cu and Zn, Mg is also a co-factor of enzymes that regulate the Ca metabolism. The osteopro-

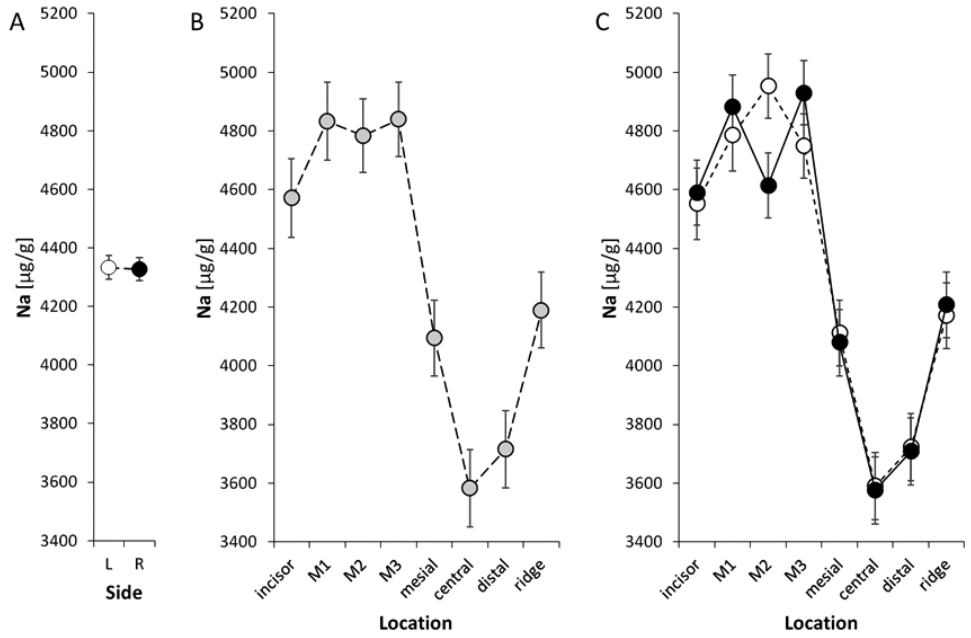

Fig. 3. Relationships between Na (µg/g) concentrations, side and location as evaluated by the ANOVA model. For detailed description see Fig. 2. Side: $F(1, 73) = 0.0$, $P = 0.88$, Location: $F(7, 73) = 79.5$, $P < 0.001$, Subject: $F(5, 73) = 94.4$, $P < 0.001$, Side × Location: $F(7, 73) = 1.9$, $P = 0.086$.

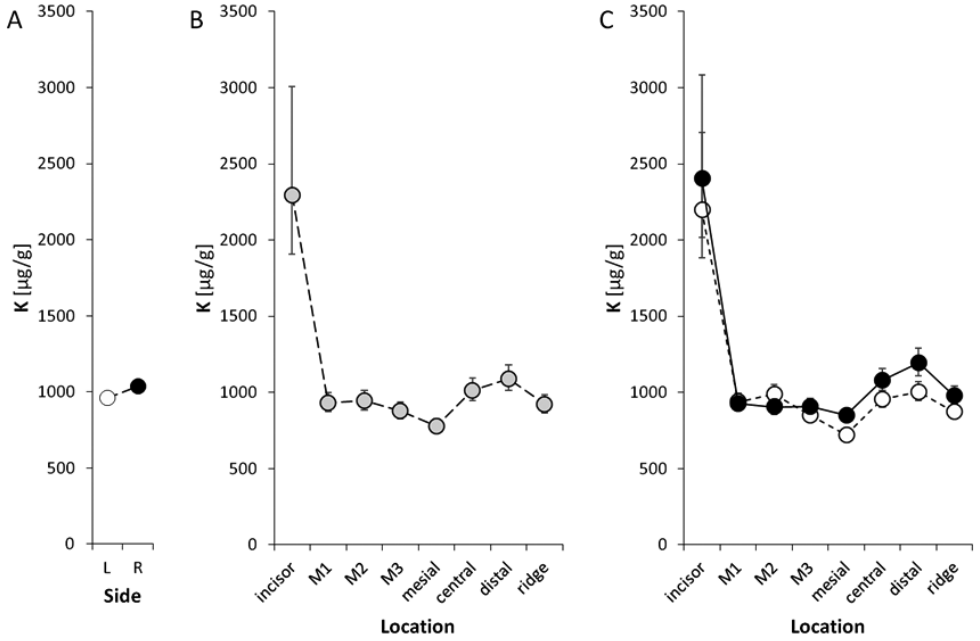

Fig. 4. Relationships between K (µg/g) concentrations, side and location as evaluated by the ANOVA model. For detailed description see Fig. 2. Side: $F(1, 75) = 12.1$, $P < 0.001$, Location: $F(7, 75) = 59.1$, $P < 0.001$, Subject: $F(5, 75) = 34.2$, $P < 0.001$, Side × Location: $F(7, 75) = 2.5$, $P = 0.024$.

tective effect of Mg consists in bone resorption attenuation and in an improvement of bone quality (Žofková, 2012). A deficit of Mg results in growth disorders, osteopenia, osteoporosis, and increased bone fragility (Rude et al., 2009). For Mg, we have shown a decreased content in the molars and in the bone compared to incisors. Mg content was higher in the bone compared to the molars. In the bone, a decrease of this element was seen in the central and distal parts compared to the mesial part and the ridge (Fig. 6B).

Sr increases cartilage matrix secretion and osteoblastic proliferation, improves bone mineralization, and inhibits osteoclastic differentiation and resorption activity (Henrotin et al., 2001; Cianferotti, et al., 2013; Dermience et al., 2015). In their study, Oliveira et al. (2012) report a different Sr content in the bone and teeth of rats re-

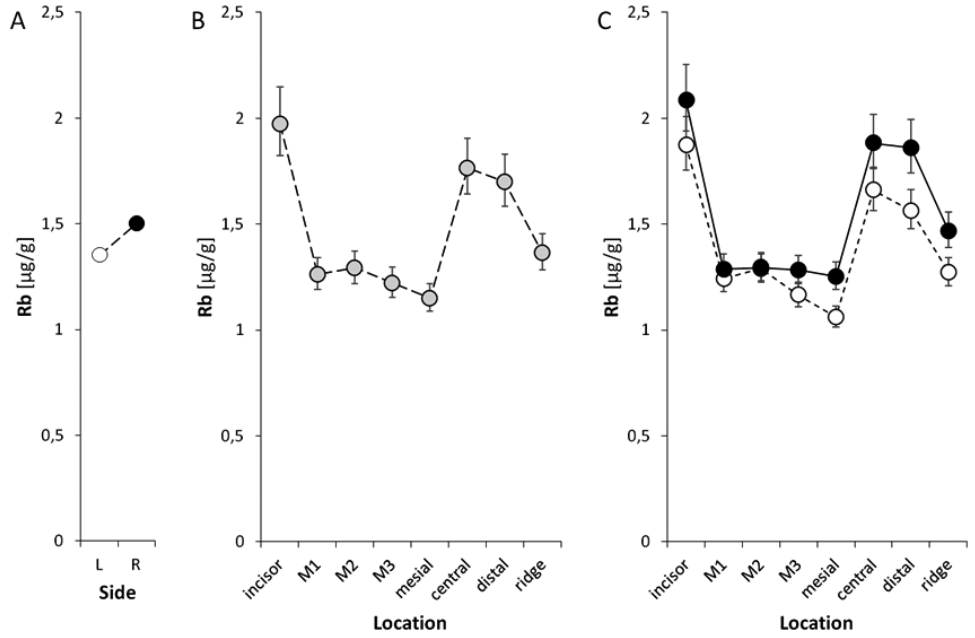

Fig. 5. Relationships between Rb (µg/g) concentrations, side and location as evaluated by the ANOVA model. For detailed description see Fig. 2. Side: $F(1, 75) = 28$, $P < 0.001$, Location: $F(7, 75) = 48$, $P < 0.001$, Subject: $F(5, 75) = 5.2$, $P < 0.001$, Side × Location: $F(7, 75) = 1.2$, $P = 0.315$.

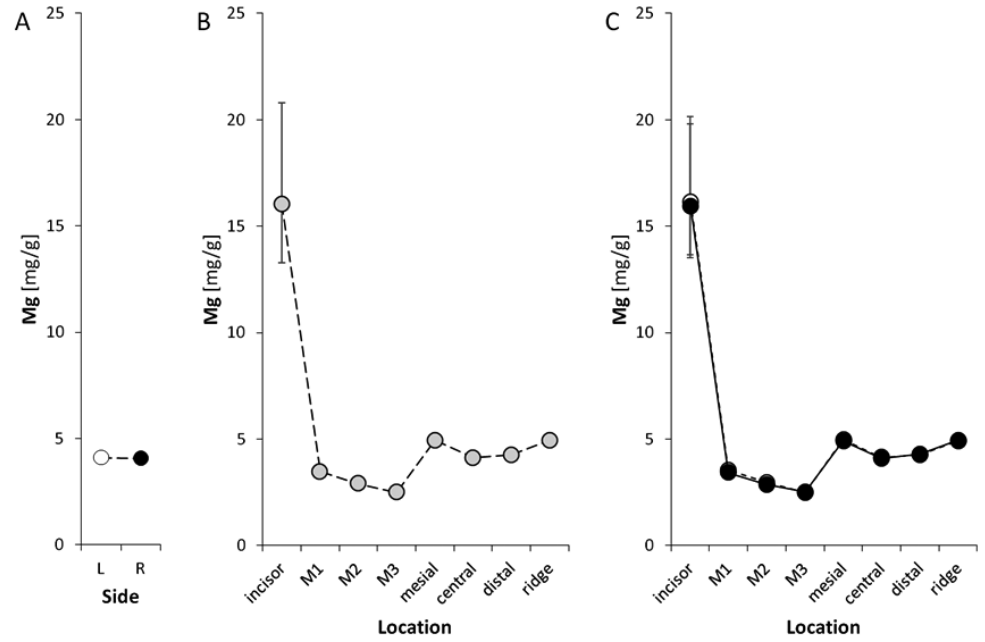

Fig. 6. Relationships between Mg (mg/g) concentrations, side and location as evaluated by the ANOVA model. For detailed description see Fig. 2. Side: $F(1, 74) = 0.4$, $P = 0.558$, Location: $F(7, 74) = 413.5$, $P < 0.001$, Subject: $F(5, 74) = 18.4$, $P < 0.001$, Side × Location: $F(7, 74) = 0.3$, $P = 0.965$.

ceiving strontium renalate. As reported by the authors, the incisor tooth presented high strontium incorporation levels, with strontium found in both the enamel and dentin along the whole extension of the tooth. The Sr content of the molar tooth was negligible. Distinct regions of the alveolar bone also seemed to present different Sr levels (Oliveira et al. 2012). In our study, we demonstrated a statistically significantly lower content of this element in incisors compared to molars. At the same time, a distinct increase of its content could be observed from the incisor towards the mesial and central parts of the bone. Additionally, a decrease in its content was apparent in the distal part of the bone compared to the incisors. Furthermore, we demonstrated a higher content of this element in M3 compared to M1 and M2. As regards bone samples, a statistically significant decrease of the

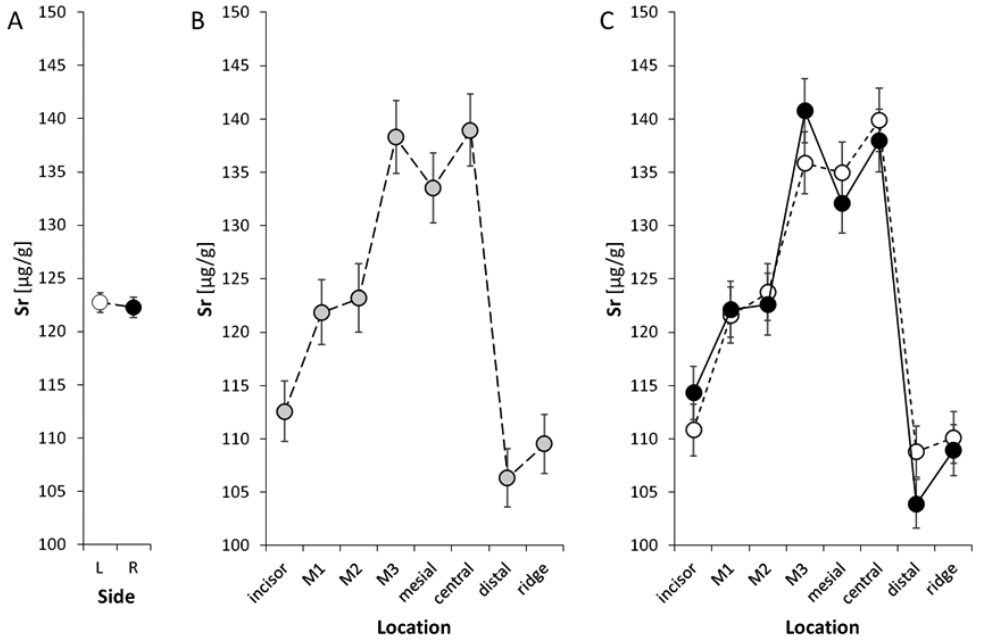

Fig. 7. Relationships between Sr (µg/g) concentrations, side and location as evaluated by the ANOVA model. For detailed description see Fig. 2. Side: F (1, 74) = 0.2, P = 0.632, Location: F(7, 74) = 94.4, P < 0.001, Subject: F(5, 74) = 9.6, P < 0.001, Side × Location: F(7, 74) = 1.5, P = 0.171.

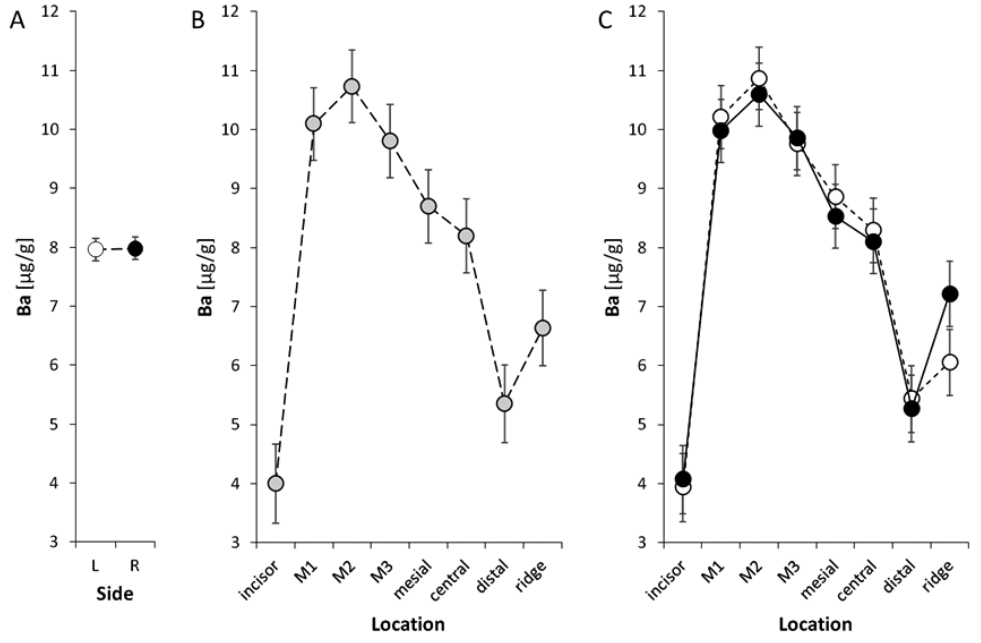

Fig. 8. Relationships between Ba (µg/g) concentrations, side and location as evaluated by the ANOVA model. For detailed description see Fig. 2. Side: F(1, 75) = 0.0, P = 0.918, Location: F(7, 75) = 75, P < 0.001, Subject: F(5, 75) = 6.1, P < 0.001, Side × Location: F(7, 75) = 0.8, P = 0.614.

content of this element was apparent from the mesial and central parts toward the distal part and the ridge (Fig. 7B).

Ba is contained in various tissues. Ninety-one % of this element is found in the bone tissue (Curzon and Cutress, 1983; WHO, 1990; Fischer et al., 2014). The physiological meaning of this element for the body remains unexplained (Fischer et al., 2014). In our study, we demonstrated a higher content of Ba in the molars and the bone compared to incisors. In the bone, a statistically significant decrease of its content was found in the distal part and the ridge compared to the mesial and central parts (Fig 8B).

Mn stimulates bone matrix synthesis and provides a general calcification effect (Žofková, 2012). Its deficit causes abnormal development of the skeleton (Aschner and Aschner, 2005). In our study, a statistically signifi-

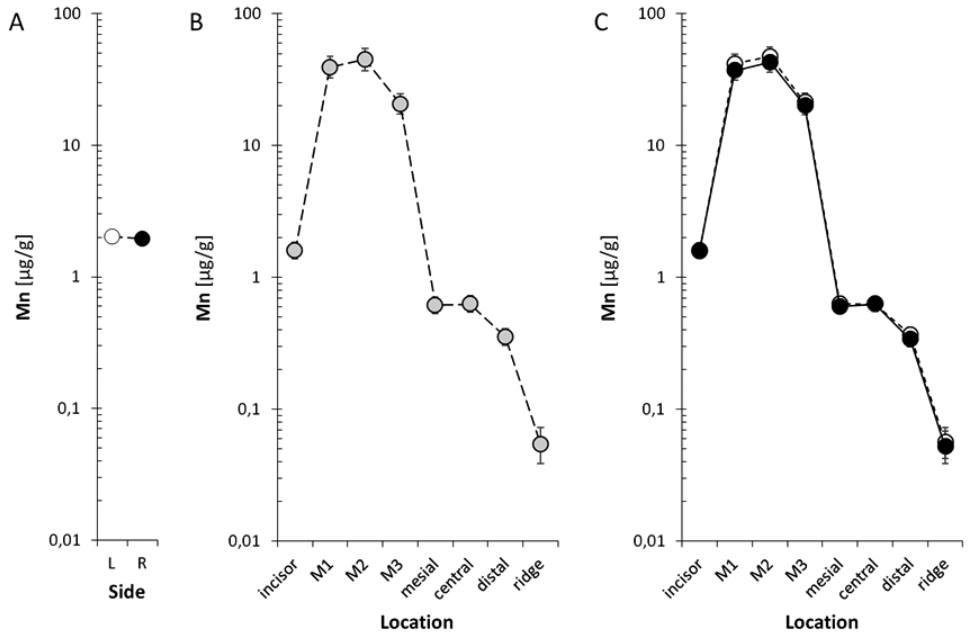

Fig. 9. Relationships between Mn (µg/g) concentrations, side and location as evaluated by the ANOVA model. For detailed description see Fig. 2. Side: $F(1, 73) = 1.1$, $P = 0.31$, Location: $F(7, 73) = 1178.3$, $P < 0.001$, Subject: $F(5, 73) = 4$, $P = 0.003$, Side × Location: $F(7, 73) = 0.1$, $P = 0.999$.

cantly lower content of Mn was shown in the incisors compared to the molars. At the same time, the Mn content was higher in the incisors compared to the bone. A decrease in the Mn content was apparent from M1 and M2 towards M3. Furthermore, a decrease of this element was noted from the mesial and central parts toward the distal part and the ridge (Fig. 9B).

Fe as an enzymatic co-factor is involved in bone matrix synthesis and in the D-hormone synthesis. Through the D-hormone, it stimulates calcium absorption in the intestine (Žofková, 2012). The skeleton of rats with a Fe deficit is insufficiently mineralized. The trabecular microarchitecture of the bone became damaged and its strength decreased (Parelman et al., 2006). An increased Fe intake may cause osteoporosis with an increased risk of fractures (Dermience et al., 2015). We demonstrated a Fe decrease from the incisors towards the molars (M2, M3). At the same time, a statistically significantly higher content of the element was found in M2 compared to M3. Furthermore, a statistically significantly higher content of the element was found in the incisors compared to the bone (Fig. 10B).

Co is an essential trace element that forms part of vitamin B12. This element enables the vitamin to activate a number of enzymes (Schneiderka et al., 2004; Dermience et al., 2015). Thirteen % of cobalt in the human body is contained in the bones (Schneiderka et al., 2004). For Co, we showed a statistically significantly lower content in the incisors compared to M3. At the same time, a higher content was found in M3 compared to the mandibular bone (Fig. 11B).

Zn stimulates growth, being a co-factor of enzymes that activate DNA and RNA synthesis and of protein synthesis enzymes. This element activates osteoblasts in the bone and increases their formation; it also supports collagen synthesis and attenuates osteoclastic resorption (Žofková, 2012). At the same time, Zn increases bone strength (Dermience et al., 2015). Other authors have studied the effect of reduced Zn intake with food on mandibular development. In their study, they report reduced bone density in the mandible in connection with reduced Zn content in the diet (Maki et al., 2002). As reported by Yamaguchi et al. (1987), zinc had a direct stimulatory effect on bone mineralization *in vitro*, and bone protein synthesis was a necessary component of this response. We demonstrated a statistically significantly lower Zn content in the incisors compared to the molars and the bone. In the bone, a higher content of the element was found in the central part and the ridge compared to the mesial and distal parts of the mandible (Fig. 12B).

After Fe and Zn, **Cu** is the third most widely represented essential trace element in the human body (Schneiderka et al, 2004). Cu attenuates bone resorption. Reduced skeleton strength can be found in animals exposed to insufficient Cu intake (Žofková, 2012). A deficit of this element reduces bone mineralization, leading to bone hypoplasia and deformation. An increased Cu content causes reduced bone density, rickets and anomalous osteophytes in Wilson's disease (Dermience et al., 2015). We did not show any statistically significant differences in the samples based on our analysis (Fig. 13B).

Ni is contained predominantly in soft tissues, but has also been found in bones. Ni affects the bone metabolism. In animals, it causes a growth disorder and is in-

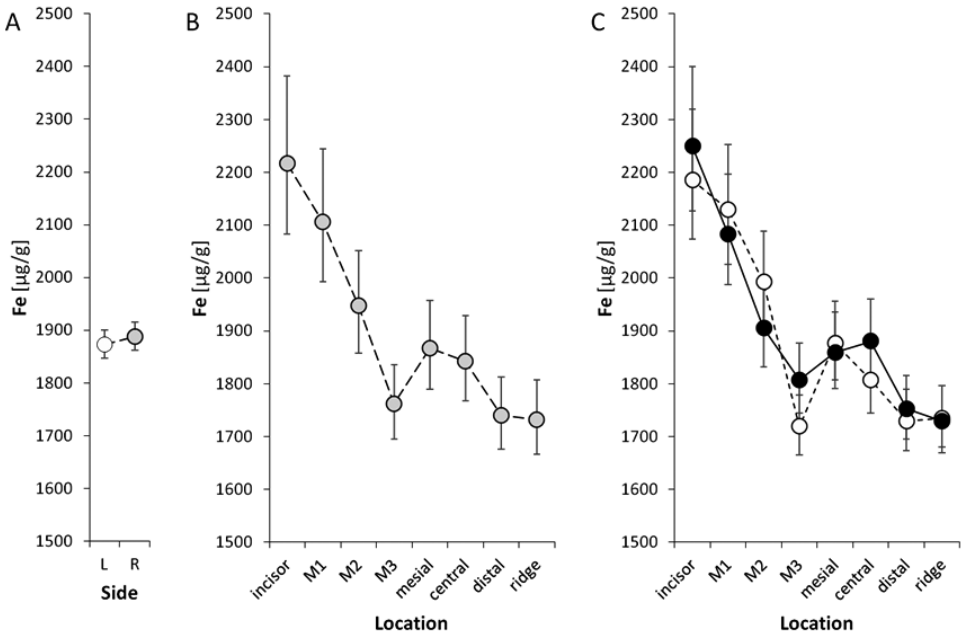

Fig. 10. Relationships between Fe (µg/g) concentrations, side and location as evaluated by the ANOVA model. For detailed description see Fig. 2. Side: $F(1, 74) = 0.3$, $P = 0.581$, Location: $F(7, 74) = 17.9$, $P < 0.001$, Subject: $F(5, 74) = 6$, $P < 0.001$, Side × Location: $F(7, 74) = 0.7$, $P = 0.71$.

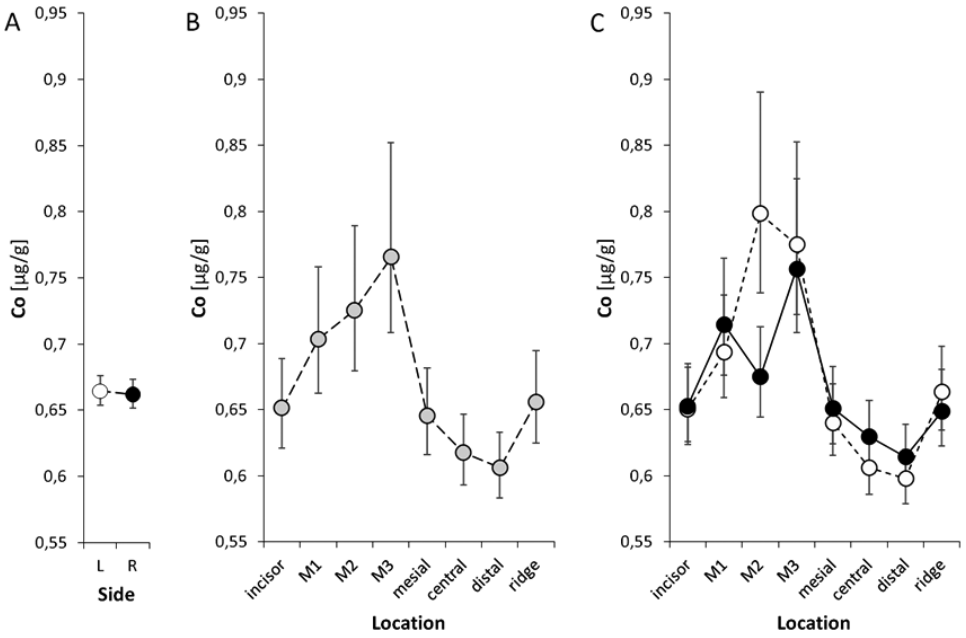

Fig. 11. Relationships between Co (µg/g) concentrations, side and location as evaluated by the ANOVA model. For detailed description see Fig. 2. Side: $F(1, 75) = 0.1$, $P = 0.832$, Location: $F(7, 75) = 10.3$, $P < 0.001$, Subject: $F(5, 75) = 2$, $P = 0.082$, Side × Location: $F(7, 75) = 1.2$, $P = 0.291$.

volved in marrow hyperplasia (Roczniak et al., 2017). A statistically significantly higher content of this element was found only in M1 compared to the central part of the mandible (Fig. 14B).

Mo forms part of flavoenzymes (Schneiderka et al., 2004). As shown by animal experiments, a Mo deficit reduces the rate of growth in early stages of development. High levels of Mo cause symptoms similar to foetal development inhibition, reduced rate of growth and development of skeleton deformations (Dermience et al., 2015). An excessive intake of Mo may lead to increased Cu loss in the urine (Schneiderka et al., 2004; Dermience et al., 2015). This element is applied in the treatment of Wilson's disease (Brewer, 1995, 2009). We showed a statistically significantly lower Mo content in the incisors compared to the molars. At the same time, higher

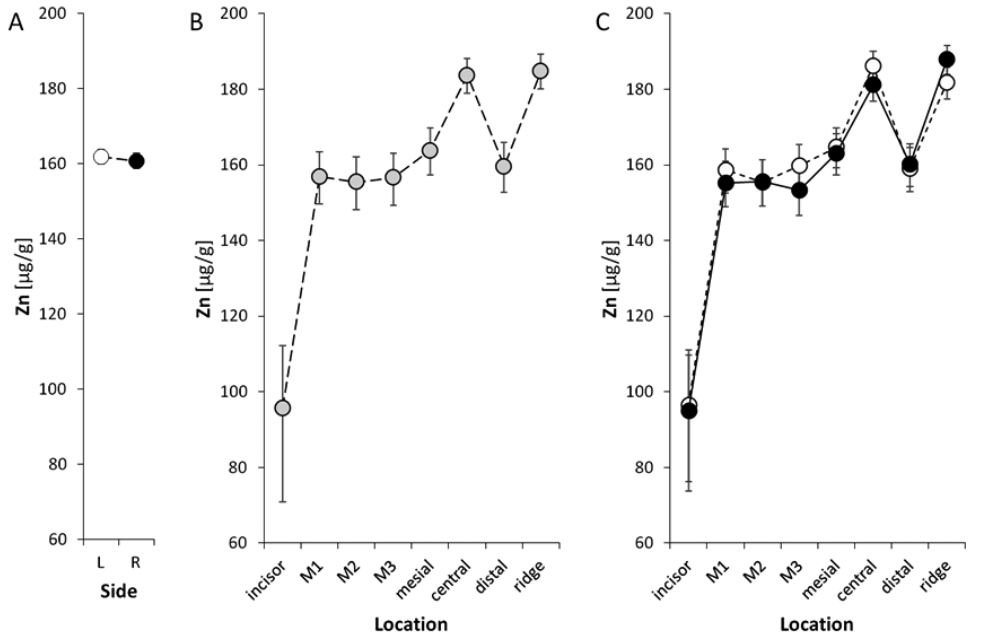

Fig. 12. Relationships between Zn (µg/g) concentrations, side and location as evaluated by the ANOVA model. For detailed description see Fig. 2. Side: $F(1, 75) = 0.3$, $P = 0.574$, Location: $F(7, 75) = 58.9$, $P < 0.001$, Subject: $F(5, 75) = 7.1$, $P < 0.001$, Side × Location: $F(7, 75) = 0.7$, $P = 0.643$.

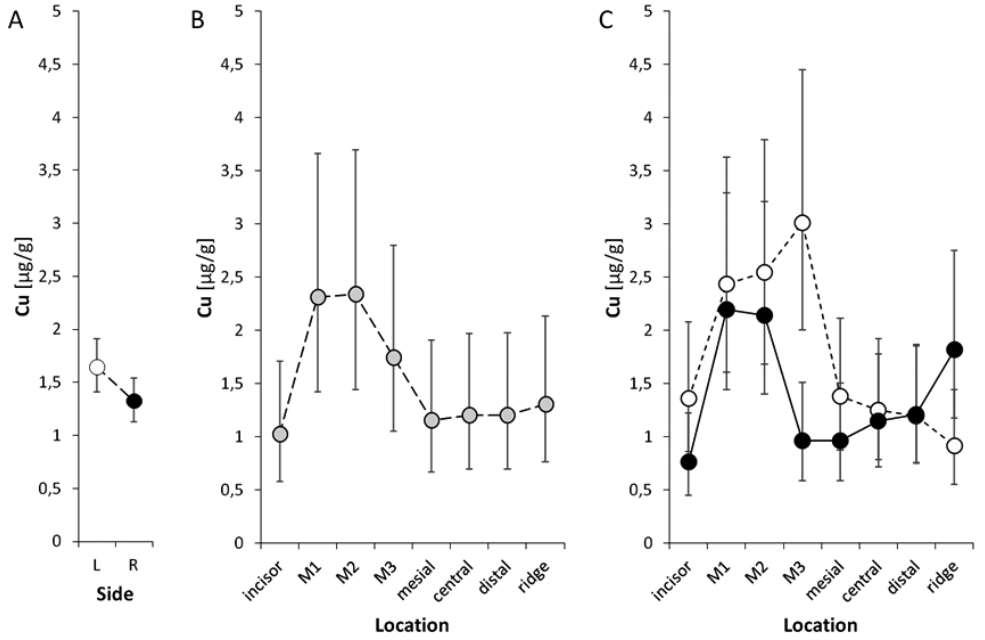

Fig. 13. Relationships between Cu (µg/g) concentrations, side and location as evaluated by the ANOVA model. For detailed description see Fig. 2. Side: $F(1, 75) = 2$, $P = 0.163$, Location: $F(7, 75) = 2.2$, $P = 0.04$, Subject: $F(5, 75) = 6.8$, $P < 0.001$, Side × Location: $F(7, 75) = 1.4$, $P = 0.214$.

contents of this element were shown in the molars compared to the bone. Additionally, a significant decrease of Mo was seen in the ridge compared to the incisors. As regards the bone, the Mo content decreased from the central part toward the distal part and the ridge (Fig. 15B).

In accordance with the literature, we believe that higher contents of some elements in the teeth may be caused by their contact with saliva (Reitznerová et al., 2000). The most abundant trace elements in the saliva (Na, Mg, K and Zn) are also the most abundant trace elements in tooth enamel (Ghadimi et al., 2013). Various studies have indicated the importance of mandibular loading for bone mineralization (Tanaka et al., 2007; de Jong et al., 2013; Hichijo et al., 2015). We believe that the varying distribution of elements in the mandibular bone (Ba, Ca, Mn, Sr, K, Rb, Mo, Na, Mg, and Zn) shown

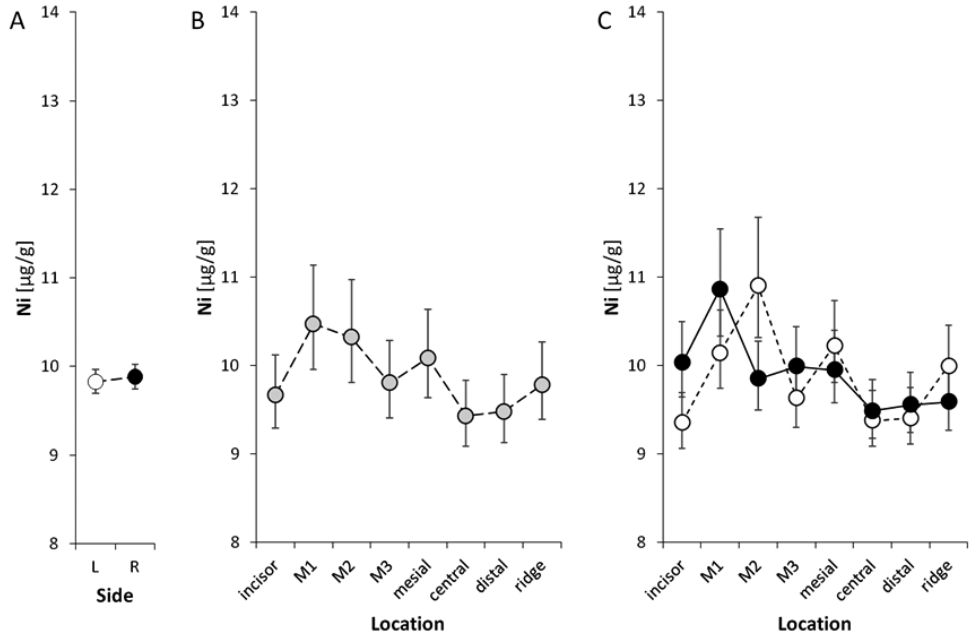

Fig. 14. Relationships between Ni (µg/g) concentrations, side and location as evaluated by the ANOVA model. For detailed description see Fig. 2. Side: $F(1, 74) = 0.2$, $P = 0.692$, Location: $F(7, 74) = 3.5$, $P = 0.003$, Subject: $F(5, 74) = 38.9$, $P < 0.001$, Side × Location: $F(7, 74) = 1.8$, $P = 0.108$.

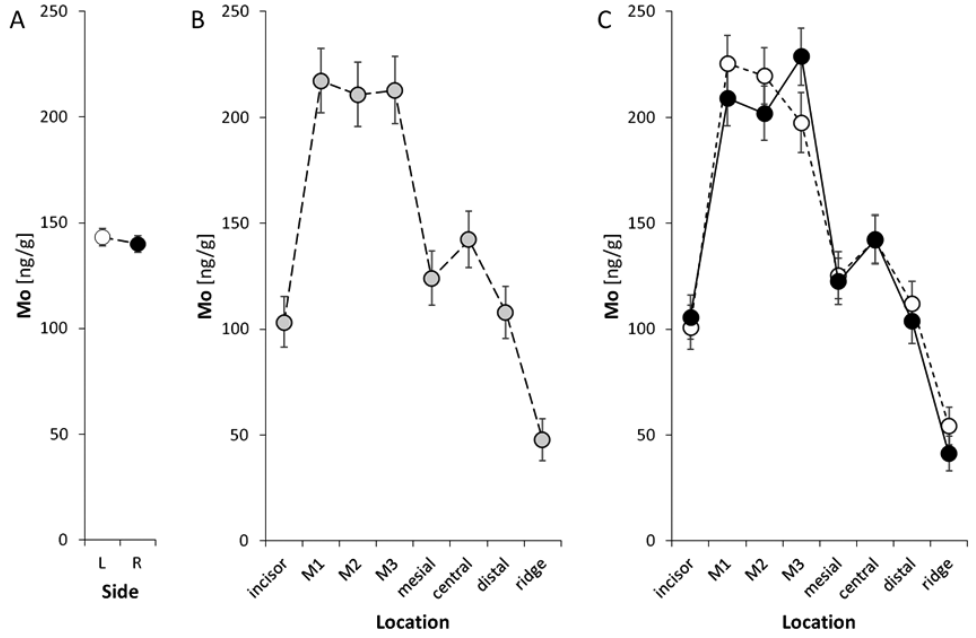

Fig. 15. Relationships between Mo (ng/g) concentrations, side and location as evaluated by the ANOVA model. For detailed description see Fig. 2. Side: $F(1, 74) = 0{,}6$, $P = 0.431$, Location: $F(7, 74) = 118.9$, $P < 0.001$, Subject: $F(5, 74) = 7.9$, $P < 0.001$, Side × Location: $F(7, 74) = 1.5$, $P = 0.17$.

in our study may be caused by different functional loads of the individual parts of the mandible. We expect that the different loading may also be the cause of different distributions of some elements (Fe, Mg, Mn, Sr) in individual molars. At the same time, we assume that different distributions of elements in the mandibular bone and in the teeth may be related to mandibular growth and gradual emergence of teeth.

Furthermore, we believe that the difference between the contents of some elements determined in incisor crowns and in the molars (Ba, Mn, Mo, Sr, Zn, K, Mg, and Rb) may be related to the different anatomy and functions of the incisors and molars. Another reason can be growing up of incisors. Elemental analysis only in the incisor crowns may be another cause given that entire teeth were used in the analysis of molars.

Although mandibular ossification and development differ from the same processes in long bones, we believe that a certain relationship of our results can actually be seen with the study published by Smrčka (2005). The author found an inhomogeneous distribution of elements in the long bones. Based on an analysis of elements contained in the femur and tibia of cadavers, the author observed accumulation of the following elements in the epiphysis: Zn, V, Ni, Cr, Pb, Mn, Co, and Sn. On the other hand, Ca, Sr, Na, and K occur predominantly in the central part of the diaphysis. According to this author, the inhomogeneous distribution of elements in the long bones is related to the development and growth of the skeleton. Based on this fact, the author concluded that elements in the centre influence construction of the skeleton and those in the epiphysis take part in the growth (Smrčka, 2005). In our study, we demonstrated decreased contents of the following elements: Ba, Ca, Mn, and Sr toward the distal part and the ridge. The K and Rb contents were higher in the central part, distal part and the ridge compared to the mesial part. A higher Rb content was seen in the central and distal parts of the mandible. A decrease in Na and Mg contents was seen in the central and distal parts of the mandible. Looking for a similarity in the distribution of elements between long bones and the mandible, the higher contents of Ca and Sr in the mesial part of the mandible would correspond to their higher contents in the diaphysis of cadaveric long bones. On the other hand, as reported by Oliveira et al. (2012) in their study of rats receiving strontium renalate, the strontium levels found in the femoral neck were higher than in the femoral diaphysis.

Conclusion

The study shows different distributions of the contents of some elements in the mandibular bone and teeth in the rat. In total, 14 elements were determined in the collected bone and tooth samples. In accordance with the literature, we believe that the higher contents of some elements in the teeth may be due to their contact with the saliva. Furthermore, we believe that the different contents of some elements between incisor crowns and molars may be related to the anatomical shapes and functions of the respective teeth. Another reason can be growing up of incisors. Furthermore, we believe that the varying distribution of elements in the mandibular bone demonstrated in our study may be caused by different functional loading of individual parts of the mandible. It is assumed that different loading may also be the cause of varying distributions of some elements in individual molars. At the same time, we believe that the varying distribution of elements in the mandibular bone and in the teeth may be related to mandibular growth and gradual emergence of the teeth. We assume that the knowledge of chemical element contents in the laboratory rat bone will prove useful in experimental bone disease research including osteoporosis.

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