CONCENTRATION OF ELEMENTS IN TEETH OF ROE DEER (*Capreolus capreolus* L.) AND MOSSES IN SLOVAK INDUSTRIAL POLLUTED SITES

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Abstract

Maňkovská B., Oszlányi J., Goryanova Z.I., Frontasyeva M.V.: Regional variation in environmental element concentrations in Slovakia derived from analysis of roe deer teeth (*Capreolus capreolus* L.)
Values emanating from element analysis in roe deer teeth from 3 polluted sites and the control locality in the Nízke Tatry National Park ("Low Tatra Mountains Nat. Pk."--NAPANT) are discussed herein. We found the following highest concentrations in the roe deer teeth: As, Ba, Br, Cl and Na in Žiar nad Hronom; Cd, Cu, Hg and Zn in Spiš and Al, Ca, Co, Fe, Mg, Mn, Pb, Rb, Sb and Sr in Orava. Statistically significant difference was established between the concentration of of As, Cd, Co, Cu, Hg, Na, Pb, Rb, Sr and Zn in roe deer teeth from Žiar, Spiš and Orava sites and the control locality of NAPANT. This obtained data is a useful reference point for comparison with future measurements of air pollution in the examined area, whenever hazards due to heavy metal accumulation in the food chain are assessed.

Key words: roe deer teeth, heavy metals, bio-indication

Introduction

It was shown that Slovakia (SK) has been affected by an intense atmospheric depositional load of elements (Maňkovská et al., 2003; Suchara et al., 2007). It is assumed that a large gradient of the atmospheric deposition load of elements exists in Slovakia because part of this territory lies in the most polluted area of central Europe known as the 'Black Triangle II'. The outflow of elements and compounds from anthropogenic sources of pollution leads to their accumulation in all parts of exposed ecosystems (Maňkovská, 1995, 1996; Maňkovská et al., 2003; Čurlík, Ševčík, 1999). Bio-indicator organisms provide beneficial results in assessment of environmental pollution because these organisms give spatial and temporal insight into the exposure and effects of pollutants on ecosystems.

Human and animal teeth are considered reliable historical bio-indicators of environmental pollution from substances which include radionuclides, heavy metals and pesticides. Elements are incorporated into the mineral phase of dental tissues formed during the time of their exposure to pollution. Unlike bones, in which the mineral phase periodically reproduces, teeth provide a permanent, cumulative and relatively stable record of environmental exposure from the time of their formation. Toxic elements such as lead and strontium readily accumulate in teeth where they substitute for calcium (Kierdorf, H., Kierdorf, U., 2002).

The results of numerous investigations showed that herbivorous mammals, and Cervids in particular, are beneficial experimental models for ecological studies (Conder, Lanno, 1999; Medvedev, 1995). The content of some elements and compounds in organs and tissues, such as the kidney, liver, hair, bones and antlers of these animals correlates with their input to the

ecosystem, and therefore they indicate emissions which are hazardous for people living in the vicinity of the pollution sources.

Roe deer (*Capreolus capreolus*) represent a perfect bioindicator with obvious advantages over other wild European ungulates. Meier (1976) investigated the roe deer movements and estimated their migration to be 1–2 km. Roe deer is the most abundant deer species in Europe with wide geographical range and ecological optimum in ecosystems. It can be found even in areas greatly modified by anthropogenic activity. However, an individual home range of roe deer is less than that of the other European wild ungulates (25–40 ha). The ecological and physiological characteristics of the species are well known, which enables easily standardized sampling and analytical methodology (Kierdorf, H., Kierdorf, U., 2002; Pokorny et al., 2002, 2004). Many studies have established the correlation between pollutants in the ecosystem and their accumulation in the roe deer organs and tissues (Bowen, 1979; Maňkovská, 1980, 1988, 1991; Holm, Wester, 1988; Pokorny et al., 2002, 2004).

The objective of this paper is to determine the concentration of 19 elements in the teeth of roe deer from the three polluted areas of the Žiarska dolina valley, the Central Spiš and the Upper Orava region, and also in the Nízke Tatry National park control area (NAPANT) which is far removed from industrial pollutant sources. The difference in concentration of these elements in the teeth of roe deers was compared between these sites, and also with their concentration in mosses at the different sites.

Material and methods

The following material was collected from the four Slovak sites: 27 samples from Orava (Čierne, Beskydy/Korna, Zborov, Bystrica, Turzovka, Velký Vreteň, Radola, Klokočov, Horelica, LSR Beskydy, Polom, Povina, 3 kopce Rudina, Polana/Neslušia, Makov, Vysoká, OZ LSR Husárik, Vadičov, Krásno, Zákopčie, Skalité, Serkov, OZ LSR Zvadivá, Brizjarky, Olešná, Starý diel, OZ LSR Tichá, Riečnica), 16 samples from Žiar valley (Lutila, Bartošova Lehôtka, Lehôtka pod Brehy and Trubín), 5 samples from Central Spiš (Nálepkovo), 9 samples from control sites in the NAPANT.

The samples were received from specialists in the wildlife department of Zvolen Forest Research Institute, who had studied the roe deer habits from 1977 to 2003. All animals were shot under the regular legal conditions. Each animal was assigned basic data such as sex, age, class, body weight, date and death location. The teeth of roe deer were taken from 4 whole molar teeth from the left jaw of each roe deer. Samples were homogenized in an agate bowl for analysis and the dry material was determined separately.

Neutron activation analysis (NAA) of the 39 elements of Al, As, Au, Ba, Br, Ca, Ce, Cl, Co, Cr, Cs, Fe, Hf, K, La, Mg, Mn, Na, Ni, Mo, Rb, Se, Sb, Sc, Sm, Sr, Ta, Tb, Th, U, V, W and Zn was performed in the Frank Laboratory of Neutron Physics in Dubna, Russia. For short-term irradiation, samples of approximately 300 mg weight were pelletized in simple press forms and heat-sealed in polyethylene foil. For epithermal neutron activation analysis, samples prepared in the same way were packed in aluminium cups for long-term irradiation. The samples were then irradiated in the IBR-2 fast-pulsed reactor, in channels equipped with a pneumatic system. The neutron flux characteristics are shown in Table 1. The following two types of analysis were performed: (1) the samples were irradiated for 3 minutes in the second channel (Ch2) to determine short-lived radionuclides and (2) samples were irradiated for 100 hours in the cadmium screened Channel 1(CH1) to determine elements associated with long-life radionuclides. Following irradiation, the gamma-ray spectra were recorded twice for each irradiation using a high-purity Ge detector: the first one after decay periods of 2÷3 minutes for 5 minutes, the second one for 20 minutes, 9÷10 minutes following the short irradiation. For

long irradiation, samples were repacked into clean containers and measured after 4÷5 days for 45 minutes and 20÷23 after days for 3 hours (Frontasyeva, Pavlov, 2000).

T a b l e 1: Flux parameters of irradiation positions.

Irradiation position	Neutron flux density, [n× cm $^{-2}$ × s $^{-1}$]×1012		
	Thermal (E = 0÷0.55 eV)	Resonance (E = 0.55÷105 eV)	Fast (E = 105÷25.106 eV)
Ch1 (Cd-screened)	0.023	3.3	4.2
Ch2	1.23	2.9	4.1

The VARIAN SPECTRA A-300 atomic absorption spectrometer and AMA-254 mercury analyzer were used to determine Cd, Cu, Hg, and Pb concentrations.

Results were assessed statistically by one-factor variance analysis and then the Student's t-test at 0.5 level of significance. This gave 95% reliability in the assessment of the averages differences. The accuracy of these chemical analytical methods has been verified by 109 independent laboratories and also tested in IUFRO (Hunter, 1994). Current statistical methods of factor and correlation analysis were used to assess.the teeth and vegetation.

Results and discussion

Data on the concentration of Al, As, Ba, Br, Ca, Cd, Cl, Co, Cu, Fe, Hg, Mg, Mn, Na, Pb, Rb, Sb, Sr, Zn in roe deer teeth from the four Slovak sites of Orava, Žiar nad Hronom, Spiš and NAPANT, together with the F test are presented in Table 2. There is a statistically significant difference between the concentrations of As, Cd, Co, Cu, Hg, Na, Pb, Rb, Sr and Zn in roe deer teeth from the Orava, Žiar, Spiš and NAPANT control sites. The following highest concentrations were recorded in the roe deer teeth: As, Ba, Br, Cl and Na in Žiar nad Hronom; Cd, Cu, Hg and Zn in Spiš, and Al, Ca, Co, Fe, Mg, Mn, Pb, Rb, Sb and Sr in Orava.

Statistical assessments from the comparison of pair levels are presented in Table 3. A statistically significant difference was registered between Orava/Žiar (Cd, Cu, Rb, Sr, Zn); Orava/Spiš (As, Cu, Hg, Pb, Rb, Sr, age of roe deer teeth); Orava/NAPANT (Cu, Pb); Žiar/Spiš (Cd, Cu, Pb, Zn); Orava/NAPANT (Na, Rb, Sr) and Žiar/NAPANT (Cd, Cu, Pb, Zn). No statistically significant difference was established between the concentration of other elements in the roe deer teeth and that in all sites.

The Orava area is affected by the Katowice, smelter complex in Poland and by the Ostrava-Karviná agglomeration in the Czech Republic. The Slovakian portion, however, does not have factories. In 1996, this locality was referred to as the "second black triangle of Europe".

Problems with environmental pollution in the Žiarska dolina valley since 1953 have been connected with aluminium production. An aluminium plant is situated on the bank of the river Hron at 250m altitude, with average annual temperature of 8.3 °C and extreme temperatures from -30 °C in January to +37.5 °C in July. The total precipitation is 706 mm. The Žiarska dolina valley is mostly enclosed in the following manner; by the Pohronský Inovec Mts in the south-west, by the Vtáčnik and Kremnické vrchy Mts in the west and north and also by the Štiavnické vrchy Mts in the east and south-east. This area experiences very unfavourable meteorological conditions, due, in part, to the level of pollution in the ground layer of air from industrial sources. Approximately one third of the year is without wind, and in this period the valley is badly aerated. In 1967, a further stage of plant construction was implemented for secondary production, and a new heating plant was also erected in 1986. Annual consumption of raw materials was 600,000 t (bauxite, coke), 300,000 t of coal, 150 million m^3 of natural gas, 1300 GWh of electric energy and 14 million m^3 of water. Annual production of Al was

66,000 t with the production of 315,000 t of solid and liquid wastes, fallout including fly ash, Al_2O_3, calcined coke and anthracite, and 17,000 t of gaseous emission (SO_2, NO_x, F, Cl, Hg). The Total aluminium production reached 2 million tons. Evacuation from the Horné Opatovce municipality situated near the plant proved the injurious effects of emission from this site. The reconstruction of the aluminium plant began in 1986, and a new chapter in the history of the aluminium plant commenced on the 29th of February 1996,when the last electrolyzer from the old series based on Söderberg aluminium production technology was shut down. The "Hydro Aluminium" Norwegian company provided new production technology. The implementation of this project contributed to a significant reduction in emissions, mainly due to increased efficiency in the pollutant capture. The emission of solid pollutants, fluorine (Fc as HF, Fg as HF, Fs), SO_2, NO_x and CO released into the air was then measured at emission sources. This reduction in emissions markedly reflected a great reduction in vegetation loading by fluorine (Maňkovská, 2000, 2001a, 2001b). Pollution deposition types were characterized as acidic, with fluorine and smelter dust (Maňkovská, 1996).

The Central Spiš area is historically linked with exploitation and processing of non-ferrous metals. Since the bronze age this has been the location of copper exploitation and later also of ferrous ores, and this production history has been documented since 1290. In addition to ferrous metal exploitation, a parching mill for ores was constructed in Bindt in 1903 and also ironworks in Markušovce. During the 19th century, small manufacturing progressed into large industrial production. Pollution problems occurred in the 5 km^2 Central Spiš forest ecosystem resulting from 100 year long operation of the three Krompachy, Rudňany and Spišská Nová Ves smelter complexes. The effects of trans-border air pollutants from Katowice in Poland are also evident here on the northern aspects (Maňkovská, 2001a, b; Maňkovská, Oszlányi, 2008; Markert et al., 1996, 2003). The main source of emission in Central Spiš emanated from the Krompachy Metallurgical Factory (MFK) The following emissions in 1997 in t.year^{-1} were recorded, with the amounts for 1998 in brackets: 296(151) solid pollutants; 7237(2543) SO_2; 99.4(97.9) NO_x ,690(530) CO]; Finiš Spišská Nová Ves [141 (114) of solid pollutants; 230(172) SO_2; 29(21) NO_x; 67(42) CO] and Želba Rudňany [11(8.2) of solid pollutants; 43.3(10.9) SO_2; 6.1(6.1) NO_x; 11.5(2.1) CO]. These emission reductions are connected with a decline in industrial production. The parent rock is middle-upper Triassic consisting mainly of dolomites, with limestones and shales in some places. Specific rated emission is as follows: 11.32t.km^2 SO_2, O.31 NO_x t. km^2, 2.38 t.km^2 of solid fallout. The pollution depositional types were characterized as acidic together with smelter dust (Maňkovská, 1996). This territory's altitude ranges from 350 m to 1120 m, and forest communities are situated from the 3rd to the 6th altitudinal vegetation zones. The 4th altitudinal vegetation zone has the highest distribution with the following tree species composition: beech 44%, fir 25%, spruce 23%, birch 2% and other tree species 6%.

The loading by elements is as follows; Ag, Al, As, Au, Ba, Br, Ca, Cd, Ce, Cl, Co, Cr, Cs, Cu, Fe, Hf, Hg, I, In, K, La, Mg, Mn, Mo, N, Na, Ni, Pb, Rb, S, Sb, Sc, Se, Sm, Sr, Ta, Tb, Th, Ti, U, V, W, Yb, Zn, Zr in the particular sites of Žiar nad Hronom, Central Spiš, Orava and control number the least polluted central part of Norway) by mosses *P. schreberi, H. splendens* and *Dicranum* sp. (Suchara et al., 2007). The valid equation for moss concentrations in mg.kg^{-1} is 4x atmospheric deposition mg.m^{-2}.year^{-1} (Steinnes et al., 2001). The analytic results were then interpreted in the form of contamination factors K_F as the rates median value of elements in Slovak mosses C_{iSl} vs. Norway mosses C_{iN} ($K_F = C_{iSl}/C_{iN}$), with the Median Norway value C_{iN} taken from Steinnes et al. (2001, 2005). Excesses in the concentration of elements in mosses compared with those in Norway are expressed by the loading coefficient of air pollutants (K_F) and classified into 4 classes; class 1 – elements in normal standard concentration where K_F does not exceed the value of 1; class 2 – slight

loading where K_F ranges from 1 to 3; class 3 – moderate loading where K_F ranges from 3 to 5 and class 4 with heavy loading where K_F exceeds 5 (Table 4).

T a b l e 4: Excesses of element concentrations in mosses.

Locality	Normal <= 1	Light loading <1–3>	Moderate loading <3–5>	Heavy loading >5	K_F
Žiar nad Hronom	Br,I,In,Mn, Mg,	As,Ba,Ca,Cl,Co,Cr,Cu, Hg,K,Na,Ni,Rb,Se,U,Zn	Cs,Fe,La,Pb,V,W	Al,Cd,Hf,Mo,Sb,Sc,Sr,Ta, Tb,Th,Yb	8.1
Orava	Br,I,In,Mg,	As,Ba,Ca,Cl,Co,Cu,K,M n,Na,Ni,Rb,Se,U,Zn	Al,Cr,Cs,Fe,La,V	Cd,Hf,Hg,Mo,Pb,Sb,Sc,Sr, Ta, Tb, Th,W,Yb	8.5
Spiš	Br,I,In,Se,	As,Ba,Ca,Cl,Cu,K,Mn, Mg,Na,Ni,Rb,U,Zn	Co,Cr,Fe,Sr,V	Al,Cd,Cs,Hf,Hg,La,Mo,Pb, Sb, Sc,Ta, Tb,Th,W,Yb	13

No important local pollution sources were observed in the NAPANT control site, which is located far from industrial pollutant sources.

Higher concentrations of Cd, Cu, Hg and Zn in roe deer teeth in the Spiš region reflect their high concentrations in some other media (Tables 2, 4). Thus, local soils contain a markedly increased concentration of Hg in comparison with its limit values (Čurlík, Ševčík 1999). While Cd, Cu, Pb and Zn concentrations do not exceed the limits, the Cu and Hg contents in Spiš are higher than in Orava and Žiar nad Hronom.. In comparison with the Norwegian limit values, used because Central Norway is one the least polluted regions in Europe, there are excess concentrations in moss for Cd and Zn (Suchara et al., 2007). Such elements as Pb, Cd and Hg are non-essential trace elements with their biological implications related to their abundance in the environment, but other elements, including Zn and Cu, are essential trace elements involved in many different physiological processes. These elements are effectively regulated within organisms, so they do not accumulate in mammal tissues in proportion to their environmental levels. Therefore, despite relatively high concentrations of Zn and Cu in Spiš soils and plants, it is impossible to definitely conclude that Zn and Cu teeth burdens reflect their local anthropogenic contamination.

Concentrations of Al, Ca, Co, Fe, Mg, Mn, Pb, Rb, Sb and Sr in roe deer teeth in Orava sites reflect their high concentrations in some other media (Tables 2, 4). Although Orava is a forest region without industrial complexes, there was no significant difference in Pb teeth content between Orava and Spiš. This can be explained by the cross-border pollution from Poland and from the Czech Republic. The, Sr teeth level content in the Orava region is the highest statistically, and this correlates with the Sr content in local mosses, which is more than 5 times the limit values (Suchara et al., 2007).

The higher concentrations of As, Ba, Br, Cl and Na recorded in roe deer teeth in Žiar nad Hronom reflect their high concentrations in some other media (Tables 2, 4). The statistically higher concentrations of Na in the three other investigated areas compared to Žiar nad Hronom, and the high concentrations of Ba, Br, Cl in Orava teeth there, which were proven to be statistically insignificant, may be caused by increased content of these compounds in the atmosphere (from 1 to 3 times the limit concentrations in mosses for Cu, Cl, Na and from 3 to 5 times the limit values for Ba) (Tables 2, 4). Nevertheless, as with Cu and Zn, Na, Cl, Ba and Br are essential elements in organisms, and these are so well regulated that they may not correlate with the atmospheric pollution.

The balance of individual elements in an organism is necessary for normal growth. Similar chemical properties which proceed from approximately the same ionic radicals and charges most likely cause similar interactions noted between individual elements in animal organisms. Both synergetic and antagonistic relationships exist between individual elements, and these are disturbed by polluted air. A mutual correlation with “r” equal to or higher than $\pm$ 0.5 was established for the following element pairs : Ca/Cl; Cd/Cu and As/age of roe deer, plus Pb/site and Sr/site (Table 5).

The method of main components was utilized to process the data and to determine the interrelationships between the element concentrations accumulated in roe deer teeth (Table 6).

Of the total variability, 86.7% was explained by means of 11 factors. All weightings from the first main component (PC1) to the last (PC11) are comparable in all sites.

The individual components from PC1 to PC10 designate various functions: PC1 explains 16.4% of communality and exhibits the highest negative value of Pb. PC2 explains 29.5% of communality for As and age of roe teeth. PC 3 explains 40.9% of communality and exhibits the highest value for Cd, Cu,Hg and Zn, and PC4 (50.3% of communality) for Mg and Sr. PC 5 explains 58.8% of communality and exhibits the highest value for Ba, and Sb. PC6 (65.5% of communality) for Ba. In contrast, components numbered PC7 to PC11 explain less than 5 % of total communality for Br, Hg, Fe, Co, Ca, and Al.

Conclusion

A statistically significant difference was established between the concentration of of As, Cd, Co, Cu, Hg, Na, Pb, Rb, Sr and Zn in roe deer teeth from Orava, Žiar, Spiš and the NAPANT control site. The following highest concentrations were registered in the roe deer teeth : As, Ba, Br, Cl and Na in Žiar nad Hronom; Cd, Cu, Hg and Zn in Spiš and Al, Ca, Co, Fe, Mg, Mn, Pb, Rb, Sb and Sr in Orava. Additionally,mutual correlation existed for the following pairs: Ca/Cl; Cd/Cu and As/age of roe deer, plus Pb/all sites and Sr/all sites. The main components method was used to process data and to determine the interrelationships between elemental concentrations accumulated in roe deer teeth, and 86.7 % of the total variability was explained by these 11 factors.

This obtained data is expected to be very beneficial as a reference level for comparison with future measurements of air pollution in the examined area, and also in biodiversity study.

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T a b l e 2: Concentrations of elements in the teeth of roe deer from the region of Žiar nad Hronom, Horná Orava , Spiš and control (NAPANT) in $mg.kg^{-1}$.

Site		**Al**	**As**	**Ba**	**Br**	**Ca**	**Cd**	**Cl**	**Co**	**Cu**	**Fe**	**Hg**	**Mg**	**Mn**	**Na**	**Pb**	**Rb**	**Sb**	**Sr**	**Zn**
Orava	x	14.6	0.055	232	2.15	262926	2.73	520	0.080	12.5	26.7	0.68	7018	189	5830	17.8	4.923	0.012	187	103
n =27	SD	2.0	0.001	33	0.84	30912	0.69	524	0.049	1.4	5.6	0.19	850	179	592	2.2	21.20	0.015	27	10
A	min	11.4	0.055	161	1.14	195000	1.55	53.6	0.05	10.3	24.5	0.15	5640	24.6	4350	14.6	0.300	0.005	140	80
	max	22.6	0.055	281	4.64	312000	4.22	1480	0.216	15.6	49.9	0.96	9200	826	7240	21.6	111	0.066	239	129
	med	14.6	0.055	233	2.0	265000	2.61	99.5	0.053	12.5	25	0.69	7060	134	5660	17.6	0.869	0.005	184	104
Žiar	x	14.9	0.066	230	1.82	269125	2.48	438	0.05	7.0	25	0.81	7101	119	6582	10.3	0.841	0.014	146	103
n = 16	SD	0.9	0.020	94	1.63	14056	0.52	576	0.001	2.5	1.0	0.18	907	92	895	2.7	0.388	0.024	60	17
B	min	13.2	0.055	123	0.49	237000	1.68	33.6	0.05	2.9	25	0.49	5330	27.7	5830	6.9	0.300	0.005	72	73
	max	16.6	0.109	434	7.10	299000	3.65	1530	0.05	11.0	27	1.05	8540	398	8940	15.6	1.850	0.098	235	134
	med	14.8	0.055	205	1.31	271500	2.55	87.95	0.05	7.0	25	0.83	7250	96.3	6220	9.4	0.801	0.005	147	104
Spiš	x	15.2	0.055	231	1.55	261200	4.9	256	0.07	20.8	25	1.1	6986	89.2	5944	18.5	1.14	0.005	137	129
n = 5	SD	0.6	0.001	29	1.84	25173	0.7	433	0.05	2.3	1.0	0.3	1074	66.5	1021	1.8	1.127	0.001	19	16
C	min	14.7	0.055	208	0.38	241000	3.9	45	0.05	17.8	25	0.75	5940	17.9	4540	16.4	0.341	0.005	115	117
	max	16.1	0.055	278	4.80	303000	5.7	1030	0.161	24.1	27	1.66	8400	198	7320	21	3.120	0.005	154	156
	med	15.0	0.055	218	0.89	250000	4.9	60	0.05	20.8	25	0.99	6780	83.3	5890	18.9	0.684	0.005	147	120
Control	x	14.3	0.083	191	1.380	265000	2.57	62	0.05	5.9	25	0.81	7335	135	6282	12.5	0.758	0.005	193	100
n = 9	sd	0.6	0.019	24	0.787	12000	0.63	21	0.00	0.7	0	0.16	712	55	296	1.8	0.223	0.000	31	15
D	min	13.2	0.055	157	0.485	237000	1.68	34	0.05	4.1	25	0.49	5720	28	5860	10.1	0.300	0.005	140	73
	max	14.9	0.099	236	2.960	287000	3.65	93	0.05	7.1	25	1.05	8540	237	7170	15.6	1.150	0.005	235	134
	med	14.6	0.065	189	0.973	263000	2.48	63	0.05	5.9	25	0.84	7385	148	6195	12.3	0.801	0.005	205	98
F test	Cal.	$0,64^{N}$	3.85*	0.34^{N}	1.10^{N}	0.56^{N}	28.1^{*}	0.95^{N}	3.13^{*}	71.1^{*}	1.03^{N}	8.75^{*}	0.10^{N}	1.78^{N}	4.50^{*}	60.2*	18.2^{*}	0.63^{N}	62.4*	6.75^{*}
	Theor.	2.779	2.779	2.779	2.779	2.779	2.779	2.779	2.779	2.779	2.779	2.779	2.779	2.779	2.779	2.779	2.779	2.779	2.779	2.779

Notes: x –average, SD – standard deviation, min – minimum, max –maximum, med – median, Cal. –calculated, Theor. – theoretical, * – statistical significance at $p < 0.005$, N – non statistical significance.

T a b l e 3: Statistical analysis ANOVA – Pair comparison of the pairs of levels – method by Scheffé.

Element	Com.	Differ.	Sign.	Probability	Element	Com.	Differ.	Sign.	Probability	Element	Com.	Differ.	Sign.	Probability	Element	Com.	Differ.	Sign.	Probability
Al	A-B	-0.550	N	0.9040	As	A-B	-0.0007	N	0.9990	Br	A-B	0.593	N	0.7970	Ba	A-B	1.35	N	1.0000
	A-C	-0.220	N	0.9750		A-C	-0.0010	*	0.0260		A-C	0.329	N	0.8620		A-C	2.15	N	1.0000
	A-D	-0.615	N	0.7710		A-D	-0.0007	N	0.9990		A-D	0.625	N	0.6160		A-D	-25.19	N	0.8440
	B-C	0.330	N	0.9800		B-C	-0.0105	N	0.2960		B-C	-0.264	N	0.9800		B-C	0.80	N	1.0000
	B-D	-0.064	N	1.0000		B-D	0.0001	N	0.9990		B-D	0.032	N	1.0000		B-D	-26.53	N	0.9330
	C-D	-0.394	N	0.9410		C-D	0.0105	N	0.1308		C-D	0.296	N	0.9510		C-D	-27.33	N	0.8430
Ca	A-B	1726	N	0.9990	Cd	A-B	-2.131	*	0.0001	Cl	A-B	264	N	0.8090	Co	A-B	0.008	N	0.9770
	A-C	-6199	N	0.8840		A-C	0.251	N	0.6290		A-C	82	N	0.9730		A-C	0.030	N	0.0900
	A-D	-10185	N	0.7580		A-D	0.317	N	0.6000		A-D	-209	N	0.8070		A-D	0.030	N	0.2170
	B-C	-7925	N	0.9390		B-C	2.381	*	0.0001		B-C	-181	N	0.9370		B-C	0.022	N	0.7090
	B-D	-11911	N	0.8570		B-D	2.448	*	0.0001		B-D	-473	N	0.5040		B-D	0.022	N	0.7600
	C-D	-3986	N	0.9840		C-D	0.067	N	0.9950		C-D	-292	N	0.6570		C-D	0.000	N	1.0000
Cu	A-B	-8.216	*	0.0001	Fe	A-B	1.678	N	0.8580	Hg	A-B	-0.407	*	0.0020	Mg	A-B	31	N	1.0000
	A-C	5.513	*	0.0001		A-C	1.678	N	0.6140		A-C	-0.126	N	0.2740		A-C	-82	N	0.9930
	A-D	4.411	*	0.0001		A-D	1.678	N	0.7480		A-D	-0.119	N	0.5060		A-D	114	N	0.9910
	B-C	13.729	*	0.0001		B-C	0.000	N	1.0000		B-C	0.280	N	0.0700		B-C	-114	N	0.9960
	B-D	12.627	*	0.0001		B-D	0.000	N	1.0000		B-D	0.288	N	0.0970		B-D	82	N	0.9990
	C-D	-1.102	N	0.6620		C-D	0.000	N	1.0000		C-D	0.008	N	0.9990		C-D	197	N	0.9640
Mn	A-B		N	0.5600	Na	A-B	-114.4	N	0.9910	Pb	A-B	-0.696	N	0.9423	Rb	A-B	1.006	*	0.0450
	A-C	69.8	N	0.4930		A-C	-380.4	N	0.5410		A-C	7.535	*	0.0001		A-C	1.305	*	0.0001
	A-D	70.4	N	0.6480		A-D	-953.7	*	0.0140		A-D	8.881	*	0.0001		A-D	1.361	*	0.0001
	B-C	-29.8	N	0.9820		B-C	-266.0	N	0.9250		B-C	8.231	*	0.0001		B-C	0.299	N	0.8760
	B-D	-29.2	N	0.9870		B-D	-839.3	N	0.2390		B-D	9.578	*	0.0001		B-D	0.355	N	0.8440
	C-D	0.5	N	1.0000		C-D	-573.3	N	0.3810		C-D	1.347	N	0.8030		C-D	0.056	N	0.9980
Sb	A-B	0.007	N	0.9080	Sr	A-B	-131.7	*	0.0001	Zn	A-B	-25.44	*	0.0060	Age	A-B	0	N	1
	A-C	-0.001	N	0.9990		A-C	-140.7	*	0.0001		A-C	0.440	N	0.9990		A-C	-1.500	*	0.0103
	A-D	-0.008	N	0.8150		A-D	-99.2	*	0.0001		A-D	-0.950	N	0.9990		A-D	-0.556	N	0.7660
	B-C	-0.009	N	0.8860		B-C	-9.0	N	0.9759		B-C	25.88	*	0.0080		B-C	-1.500	N	0.2066
	B-D	-0.015	N	0.6330		B-D	32.5	N	0.1960		B-D	24.49	*	0.0280		B-D	-0.556	N	0.9081
	C-D	-0.007	N	0.8960		C-D	41.5	N	0.0951		C-D	-1.390	N	0.9960		C-D	0.944	N	0.4264

Notes: Com. – comparison. * – statistical significance at $p < 0.005$. N – non statistical significance. Differ. – difference. A – Orava. B – Žiar. C – Spiš. D – Control NAPANT

T a b l e 5: Correlation matrix (all sites).

Ele-ment	**Age**	**Al**	**As**	**Ba**	**Br**	**Ca**	**Cd**	**Cl**	**Co**	**Cu**	**Fe**	**Hg**	**Mg**	**Mn**	**Na**	**Pb**	**Rb**	**Sb**	**Sr**	**Zn**
Site	0.152	0.162	0.021	0.133	-0.211	0.126	0.105	0.075	-0.269	-0.167	-0.172	0.340	-0.043	-0.226	0.410	-0.686	-0.112	0.099	-0.708	0.178
Age	1.000	-0.086	0.668	-0.171	-0.099	-0.033	-0.131	-0.351	-0.170	-0.338	-0.081	0.236	0.308	0.004	0.048	-0.356	-0.053	0.012	0.027	0.230
Al	-	1.000	-0.115	0.068	0.033	0.385	0.083	0.156	-0.292	0.127	-0.230	0.004	0.158	-0.335	0.316	-0.117	0.054	0.022	-0.156	0.059
As	-	-	1.000	-0.197	-0.278	-0.088	-0.135	-0.230	-0.136	-0.352	-0.064	0.165	0.202	0.018	0.001	-0.234	-0.043	-0.114	0.030	0.040
Ba	-	-	-	1.000	0.082	0.026	-0.033	-0.118	-0.012	-0.041	-0.123	0.055	0.257	0.195	0.087	-0.139	-0.004	0.307	0.055	0.149
Br	-	-	-	-	1.000	0.149	0.076	0.134	-0.020	-0.064	-0.106	-0.017	-0.050	-0.085	0.164	0.068	0.032	-0.078	0.308	-0.102
Ca	-	-	-	-	-	1.000	-0.046	0.534	-0.332	0.009	-0.210	-0.163	0.347	-0.364	0.269	-0.122	0.249	0.063	-0.043	-0.003
Cd	-	-	-	-	-	-	1.000	-0.080	-0.018	0.648	-0.068	0.314	0.051	-0.171	-0.035	0.316	-0.051	-0.131	-0.157	0.309
Cl	-	-	-	-	-	-	-	1.000	0.006	0.067	-0.166	-0.286	-0.097	-0.160	0.070	0.015	0.170	0.108	-0.166	-0.279
Co	-	-	-	-	-	-	-	-	1.000	0.180	0.234	-0.167	-0.153	0.425	-0.290	0.193	-0.078	-0.093	0.238	0.085
Cu	-	-	-	-	-	-	-	-	-	1.000	0.011	0.162	0.015	-0.026	-0.188	0.614	0.135	0.015	-0.046	0.452
Fe	-	-	-	-	-	-	-	-	-	-	1.000	0.125	0.037	0.107	-0.264	0.131	-0.034	-0.080	-0.009	-0.052
Hg	-	-	-	-	-	-	-	-	-	-	-	1.000	-0.019	0.043	-0.088	-0.114	0.020	0.138	-0.329	0.307
Mg	-	-	-	-	-	-	-	-	-	-	-	-	1.000	-0.229	0.197	-0.129	0.362	0.139	0.123	0.227
Mn	-	-	-	-	-	-	-	-	-	-	-	-	-	1.000	-0.393	0.011	-0.057	0.161	0.068	-0.045
Na	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.000	-0.460	0.026	0.070	-0.048	0.228
Pb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.000	-0.023	-0.244	0.435	0.046
Rb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.000	0.433	0.039	0.025
Sb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.000	-0.137	0.003
Sr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.000	-0.062

Note: Marked correlations are significant at $p < 0.005$.

T a b l e 6: WARIMAX rotated PC analysis of the first eight factors on roe deer teeth collected in the territory of Slovakia -all sites.

Component	1	2	3	4	5	6	7	8	9	10	11
Rel.Var. (%)	16.4	13.0	11.5	9.3	8.5	6.8	5.0	4.8	4.4	3.7	3.2
Cum.var.(%)	16.4	29.5	40.9	50.3	58.8	65.5	70.6	75.4	79.8	83.5	86.7
Site	0.3988	-0.0604	0.1929	0.3394	-0.0163	-0.0618	-0.0926	0.0361	0.1260	-0.0516	0.1572
Age	0.1975	-0.3814	0.0463	-0.3283	-0.0972	0.0763	0.0618	-0.1787	0.1490	-0.1476	-0.1064
Al	0.2303	0.2761	0.0296	-0.0436	-0.0996	-0.0456	-0.0922	0.0076	-0.3632	-0.0102	**-0.7665**
As	0.1346	-0.3835	-0.0144	-0.2770	-0.1713	0.2151	-0.0286	-0.2433	-0.0290	-0.1227	0.0170
Ba	0.0696	0.0471	0.0374	-0.0136	0.4564	-0.4504	-0.0648	0.0714	-0.3623	-0.3266	0.2187
Br	-0.0434	0.1957	-0.1570	-0.1005	-0.0451	-0.3967	**0.5552**	0.0510	0.4102	-0.2001	-0.1114
Ca	0.2477	0.3366	-0.1652	-0.1450	-0.0218	0.1755	-0.0757	-0.0092	0.0737	-0.4435	-0.0396
Cd	-0.0529	0.2092	0.4638	-0.0378	-0.1537	-0.0346	0.1398	-0.0845	0.0485	-0.1015	0.2810
Cl	0.0656	0.3464	-0.2399	0.2158	-0.0027	0.2793	-0.0867	-0.1929	0.2667	-0.3040	0.0580
Co	-0.3201	-0.0735	0.0099	0.0788	0.1566	-0.0815	-0.4144	-0.0341	0.4522	-0.1165	-0.1884
Cu	-0.1937	0.3270	0.4293	-0.0612	0.0315	0.1154	-0.1090	-0.1342	0.0201	0.0420	-0.0425
Fe	-0.1838	-0.1351	0.0587	0.0450	0.0502	0.2217	-0.0184	**0.7978**	0.1198	-0.0917	-0.1558
Hg	0.1077	-0.1464	0.4230	0.0426	0.0693	0.0498	0.4745	0.0645	0.0594	-0.1729	-0.2163
Mg	0.1813	0.0486	0.0310	-0.4942	0.2011	0.0913	-0.1885	0.2714	-0.0561	-0.2395	0.1993
Mn	-0.2327	-0.2461	-0.0101	0.1486	0.3620	-0.0698	-0.0387	-0.2858	0.0268	-0.2512	-0.2484
Na	0.3440	0.1198	-0.0556	-0.0711	-0.0729	-0.3420	-0.1611	0.0955	0.2596	0.3960	-0.0059
Pb	-0.4109	0.2238	0.1139	-0.1770	-0.1345	0.0979	0.0573	-0.0666	-0.2011	0.0178	0.0641
Rb	0.0730	0.1694	-0.0433	-0.2309	0.3887	0.3786	0.1671	-0.0103	0.2068	0.3090	-0.0018
Sb	0.1316	0.0367	-0.0037	0.0318	**0.5772**	0.1367	0.1821	-0.1407	-0.0305	0.2733	-0.0396
Sr	-0.2617	0.0197	-0.2423	-0.4401	-0.0076	-0.2519	0.0316	-0.0410	0.0137	0.1008	-0.0309
Zn	0.0703	0.0135	0.4348	-0.2336	0.0678	-0.1869	-0.3091	-0.0715	0.2691	0.0514	-0.1250

Note: Eight main source types were identified. Characteristic elements for the sources types are marked in bold type.
Rel.var. – relative variability; Cum.var. – cumulative variability.