The Effect of Age and Gender on Calcium, Phosphorus, and Calcium-Phosphorus Ratio in the Roots of Permanent Teeth

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Abstract: *Objectives*: The aim of the current study was to evaluate quantitatively the effect of age and gender on calcium, phosphorus, and calcium-phosphorus ratio in the roots of permanent teeth.

Methods: Calcium (Ca) and phosphorus (P) mass fractions as well as Ca/P mass fraction ratio were estimated in intact tooth root(s) samples from apparently healthy humans, 38 women and 46 men, aged from 16 to 55 years. For Ca and P mass fractions measurements, instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides was used.

Results: Mean values (mean ± standard error of mean, on dry mass basis) for the investigated parameters in two age groups (16-35 and 36-55 years) of females were: Ca 300 ± 12 g/kg, P 163 ± 7 g/kg, Ca/P ratio 1.85 ± 0.07 and Ca 277 ± 8 g/kg, P 145 ± 4 g/kg, Ca/P ratio 1.94 ± 0.06, respectively. The investigated parameters in the same age groups of males were: Ca 266 ± 13 g/kg, P 143 ± 7 g/kg, Ca/P ratio 1.88 ± 0.04 and Ca 278 ± 10 g/kg, P 152 ± 6 g/kg, Ca/P ratio 1.84 ± 0.04, respectively. The mean values for Ca, P and Ca/P ratio in the tooth root(s) were within a very wide range of reference data for dentin and close to their median.

Conclusions: A tendency for reduced Ca and P mass fraction in roots of female teeth after 35 years of age as well as for raised Ca mass fraction in female tooth root(s) up to 35 years of age as compared to male was observed.

Keywords: Neutron activation analysis, calcium, phosphorus, Ca/P ratio, human tooth root(s).

INTRODUCTION

Since the development of the ruby laser in 1960, several types of lasers have been introduced in the dental clinic to remove carious dental hard tissues or cavity preparations in anticipation of replacing the high speed dental drill [1]. There is many evidence to support the further development of 'laser drill" that are stable in use and commercially viable, to deliver more efficient hard tissue ablation with less risk of collateral thermal damage [2]. However, before application of new lasers in the dental clinic, several other considerations need to be evaluated, especially on the compositional changes of dentin with age, because dentin forms the bulk of the tooth and the interaction of laser energy with dentin depends from level of its mineralization [1-4].

Dentin consist of an inorganic calcium phosphate mineral approximated by hydroxylapatite ($[Ca_{10}(PO_4)_6(OH)_2]$), matrix proteins and water. The unique physical and chemical properties of these "bioapatite" crystals are required for fulfilling the biological functions of teeth. Calcium (Ca) and phosphorus (P) are the main chemical elements of hydroxylapatite. The mass fraction of Ca in pure hydroxyapatite is 39.9%, the mass fraction P - 18.5%, and stoichiometric Ca/P mass fraction ratio - 2.15. Because dentin is a tissue consisting mainly not only of hydroxyapatite, but proteins and water also, the Ca and P contents in it may not correspond to hydroxyapatite values. Since dentin is a biomaterial that is structurally adapted to different chewing functions and loading situations in tooth, the exact composition may very depending on sex, age, type of tooth and its site, but also with alterations known to occur in dental diseases.

Teeth and bones have many similar physical and chemical properties [5]. As was shown by us in previous studies [6-20] the Ca and P mass fractions in different bones depend from age and gender. These findings allow us assume also the age- and gender dependence of Ca and P mass fractions in permanent teeth.

The tooth root was chosen for our investigation because it consisting mainly not only of dentin, but a very thin layer of cementum, that covers the root, and the pulp canal(s) located in the center of the root(s) also. Thus, the Ca and P contents in tooth root tissue reflect the situation during 'laser drill" more adequately than mass fractions of these elements in only dentin.

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Moreover, if use the tooth root as the subject of study there is no necessity to separate any tooth tissue. To our knowledge, only one report is available on Ca mass fraction in roots of permanent teeth [21]. No data are available for the P mass fraction, Ca/P ratio, and age- and gender-dependence of these parameters in roots of permanent teeth.

Dentin comprises the main portion of the tooth root(s). There are many studies regarding Ca and P determination in tooth dentin, using chemical techniques and instrumental methods [21-69]. However, the majority of these data are based on measurements of processed tissue. First of all, dentin samples can be chemically contaminated in the course of sampling [70-71]. In many studies dentin samples are ashed before analysis. In other cases, dentin samples are treated with solvents (distilled water, ethanol, formaldehyde etc) and then are dried at high temperature for many hours. There is evidence that certain quantities of chemical elements, including Ca and P, are lost as a result of such treatment [71-73]. Moreover, only few of these studies employed quality control using certified reference materials (CRM) for determination of the chemical element mass fractions.

In our previous reports it was shown that neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) is an adequate analytical tool for the non-destructive precise determination of Ca and P mass fraction in intact teeth crowns [74].

The primary purpose of this study was to determine reference values for the Ca and P mass fraction and Ca/P mass fraction ratio in the intact roots of permanent teeth using INAA-SLR. The second aim was to evaluate the quality of obtained results. The third aim was to compare the mean values of Ca and P mass fraction and Ca/P mass fraction ratio in different age groups in the period of life from 16 to 55 years. The final aim was to estimate the difference between Ca and P mass fraction and Ca/P mass fraction ratio in the teeth roots of males and females.

All studies were approved by the Ethical Committee of the Medical Radiological Research Center, Obninsk.

MATERIALS AND METHODS

Samples

Non carious permanent teeth were collected at the Department of Forensic Medicine of the Obninsk Hospital. The molars and premolars were extracted early after death at necropsy (within 24 hours) from 38 women and 46 men (age range 16 - 55 years). One tooth was obtained from each subject. The typical causes of death in most of these subjects included traffic accident, occupational injury and domestic trauma. All the deceased were citizens of Obninsk. None of those who died a sudden death had suffered from any systematic or chronic disorders before.

Sample Preparation

After extraction teeth were immediately frozen at -18°C until use. A titanium tool was used to cut and to scrub soft tissue and blood off the roots. After separating the roots from crowns with a titanium knife, samples were freeze dried until constant mass was obtained. Only the roots were used in this study. After drying roots were weighed and sealed in thin polyethylene films washed with acetone and rectified alcohol beforehand. The sealed samples were then placed in labeled polyethylene ampoules.

Method and Reference Materials

A horizontal channel in the pneumatic rabbit system of the WWR-c research nuclear reactor was used to determine Ca and P mass fractions by INAA-SLR. Ten subsamples of the standard reference material NIST SRM1486 bone meal and certified reference material IAEA H-5 animal bone, were analyzed under the same conditions as teeth roots samples to estimate the precision and accuracy of results.

The basement of INAA is the irradiation of stable atoms in the sample by neutrons, the transmutation of atoms in radionuclides, and the spectrometry of their self-radiations. Details of nuclear reactions, radionuclides, gamma-energies, methods of analysis and the results of quality control were presented in our earlier publications concerning the chemical elements of human bones [6-14, 16-20].

Computer Programs and Statistic

A dedicated computer program of INAA-SLR mode optimization was used [75]. Using the Microsoft Office Excel program to provide a summary of statistical results, the arithmetic mean, standard deviation, and standard error of mean were calculated for Ca and P mass fractions and Ca/P mass fraction ratio obtained. The reliability of difference in the results between all age groups and between males and females was evaluated by Student's parametric *t*-test. For the estimation of the Pearson correlation coefficient between the Ca and P mass fractions the Microsoft Office Excel program was also used.

RESULTS

Table **1** shows INAA-SLR results for Ca and P mass fractions (g/kg, dry mass basis) in ten sub-samples of the standard reference material NIST SRM1486 bone meal and certified reference material IAEA H-5 animal bone compared to certified values.

Table **2** depicts our data for mean values (mean ± standard error of mean) of Ca and P mass fractions and Ca/P mass fraction ratio in the teeth roots of healthy women and men from 16 to 55 years in subgroups of 5 years.

In order to estimate the effect of age on the investigated parameters we used also two combined groups: one with young people, 16-35 years, and one with older people, 36-55 years, because in our previous study was found that Ca and P mass fractions in

Table 1: Instrumental Neutron Activation Analysis Data of Ca and P Mass Fractions (g/kg, Dry Mass Basis) in the CRM IAEA H-5 Animal Bone and SRM NIST 1486 Bone Meal Reference Materials Compared to Certified Values

Element	CRM IAEA H-5 certified values		This work results (n=10)	SRM NIST 1486 certified values		This work results (n=10)
	Mean	Type	Mean±SD	Mean	Type	Mean±SD
Ca	212	**C**	208±2	266	C	271±8
P	102	C	94.3±0.8	123	C	119±3

Mean –arithmetic mean, SD – standard deviation, C - certified values.

Table 2: Mean Values (M±SEM) of Ca and P Mass Fractions and Ca/P Mass Fraction Ratio in the Intact Roots of Permanent Teeth Depending on Age (g/kg, Dry Mass Basis)

Age, years	Female				Male			
	n	Ca	P	Ca/P	n	Ca	P	Ca/P
16-20	5	280±15*	153±3	1.84±0.10	5	283±27	145±12	1.97±0.08*
21-25	4	291±42	177±27	1.65±0.10	5	301±24	171±12	1.75±0.04
26-30	4	291±25	151±10	1.95±0.19	5	247±34	131±18	1.88±0.09
31-35	5	336±18	171±12	1.98±0.15	7	247±17	130±12*	1.92±0.08
36-40	4	285±23	154±9	1.84±0.05	5	280±14	148±10	1.92±0.10
41-45	5	254±5**	133±11*	1.97±0.19	8	286±24	155±15	1.87±0.06
46-50	6	303±18	146±6	2.08±0.14	5	268±12	151±11	1.80±0.08
51-55	5	264±12**	147±7	1.80±0.05	6	272±24	155±13	1.77±0.09

Mean –arithmetic mean, SEM – standard error of mean, * $p<0.05$ and ** $p<0.01$, Student's *t*-test (compared to data for age group 31-35 years of females and 21-25 years of males, respectively).

Table 3: Effect of Age on Mean Values (M±SEM) of Ca and P Mass Fractions (g/kg, Dry Mass Basis) and Ca/P Mass Fraction Ratio in the Intact Roots of Permanent Teeth of Healthy Humans

Parameter	Female			Male		
	16-35 year n=18	36-55 year n=20	*p* Student's *t*-test	16-35 year n=22	36-55 year n=24	*p* Student's *t*-test
Ca	300±12	277±8	N.S.	266±13	278±10	N.S.
P	163±7	145±4	$p≤0.05$	143±7	152±6	N.S.
Ca/P	1.85±0.07	1.94±0.06	N.S.	1.88±0.04	1.84±0.04	N.S.

M - arithmetic mean, SEM – standard error of mean, N.S. - not significant.

Table 4: Effect of Gender on Mean Values (M±SEM) of Ca and P Mass Fractions (g/kg, Dry Mass Basis) and Ca/P Mass Fraction Ratio in the Intact Roots of Permanent Teeth of Healthy Humans

Age group years	Parameter	Gender		*p* Student's *t*-test	Females and males (combined)
		Females	Males		
16-35	Ca	300±12	266±13	N.S.	278±10
	P	163±7	143±7	N.S.	150±6
	Ca/P	1.85±0.07	1.88±0.04	N.S.	1.87±0.04
36-55	Ca	277±8	278±10	N.S.	278±7
	P	145±4	152±6	N.S.	149±4
	Ca/P	1.94±0.06	1.84±0.04	N.S.	1.88±0.04
16-55	Ca	286±7	272±8	N.S.	278±6
	P	152±4	148±5	N.S.	149±3
	Ca/P	1.91±0.05	1.86±0.03	N.S.	1.88±0.03

M - arithmetic mean, SEM – standard error of mean, N.S. - not significant.

different bones begin to decrease after 35 years [19, 20]. For female and male, results are shown in Table **3**.

All data for female and male separately were used to detect if there are any differences related to gender in three age groups 16-35, 36-55, and 16-55 years (Table **4**). In the right column of this Table there is additional information concerning the same parameters for female and male together in each age group.

Comparison of reference data with our results for Ca and P mass fractions as well as for Ca/P mass fraction ratio is shown in Tables **5** to **7**, respectively. All reference data for Ca and P are given in Tables **5** and **6** on dry mass basis. Some values for Ca and P mass fraction in Table **5** and **6** were not given by the authors on dry mass basis but were calculated by us using the median values of water and ash content in the dentine, 10% and 72% respectively, taken from reference data [54, 67, 76-79]. Some values for Ca/P ratio shown in Table **7** were also calculated by us using the mean of Ca and P mass fraction reported by the same authors (Table **5** and **6**).

DISCUSSION

The means for Ca and P mass fractions in ten sub-samples of the IAEA H-5 animal bone and NIST SRM1486 bone meal reference materials determined by INAA-SLR were in a good agreement with mean values of the certificates (Table **1**). Good agreement with the certified data of CRM indicated an acceptable accuracy of the results obtained in the study of Ca and P mass fractions in teeth roots presented in Tables **2-4**.

The Ca mass fraction in tooth root(s) of female increased in the second to fourth decades and reached a maximum at about the age of 33 years (Table **2**). In the period of life from 30 to 35 years the Ca mass fraction was 1.2 and 1.3 times higher than in teeth roots of females aged 16-20 and 51 to 55 years, respectively and this differences were statistically significant. The same tendency of age-dependency was found for P mass fraction in female teeth roots. The Ca and P mass fraction in tooth root(s) of male reached a maximum at about the age of 23 years, i.e. ten years earlier than in tooth root(s) of female (Table **2**). However, the statistical significance of this maximum was not confirmed. The means of Ca/P mass fraction ratio were maintained in the range 1.65-2.08 in female teeth roots and 1.75-1.97 in male teeth roots and did not change with age.

A tendency for reduced Ca and P mass fraction by age was observed in the comparison between two combined age groups (15-35 and 36-55 years) of females (Table **3**). In spite of the fact that statistically significant differences ($p \leq 0.05$) were only detected in P mass fraction, this tendency and data presented in Table **2** suggested that relative losses of Ca and P in female teeth roots develop after 35 years of age.

Significant differences on the investigated parameters related to gender were not found in all age groups (Table **4**), however, it should be noted that in the period of life from 16 to 35 years the mean values of Ca and P mass fractions in female teeth roots were 13-14% higher than in teeth roots of males.

For almost lack of reported data of chemical element contents in tooth root(s) we compared our results with published data on tooth dentin. It was acceptable because a tooth root mainly consists of

Table 5: Reference Data of Ca Mass Fraction (g/kg, Dry Mass Basis) in Dentin (or Root) of Sound Permanent Teeth

Reference	Method	n	Age years	Treatment of samples	Sample	Ca mass fraction	
						M±SD or range of M	Range of individual results
[22]	Chem	-	14	Ash, AD	Dentin	278	-
[23]	Chem	1	15	AD	Dentin	292	-
[24]	Chem	1	Children	PF, F, Ash, AD	Dentin	270	-
[25]	Chem	20	Adult	PF, F, Ash, AD	Dentin	262±3	-
[26]	Chem	6	17–41	PF, Ash, AD	Dentin	278	263 – 288
[27]	Chem	52	M34	PF, Ash, AD	Dentin	265	253 – 279
[28]	Chem	13	Adult	PF, AD	Dentin	273	-
[29]	Chem	50	Adult	Ash, AD	Dentin	264	-
[30]	Chem	1	44	PF, Ash, AD	Dentin	275	248 – 314
[31]	Chem	-	-	-	Dentin	309	-
[32]	Chem	28	11–-60	PF, Ash, AD	Dentin	293±1	-
[33]	Chem	-	Adult	PF, Ash, AD	Dentin	267	-
[34]	Chem	20	Adult	PF, Ash, AD	Dentin	268 –302	-
[35]	Chem	-	17–21	AD	Dentin	270	-
[36]	Chem	96	Adult	PF, Ash, AD	Dentin	245 – 271	-
[37]	Chem	-	15–30	Ash, AD	Dentin	312 – 367	-
[38]	Chem	6	10–12	AD	Dentin	259	246 – 270
[39]	GAA	15	14–16	F, D	Dentin	282±12	-
[40]	EMPA	-	15–30	PF, AD	Dentin	386 ± 30	-
[41]	Chem	-	-	-	Dentin	313	-
[42]	EMPA	-	Adult	Em, P	Dentin	-	333 – 344
[43]	INAA	8	Adult	D	Dentin	262±15	-
[44]	INAA	175	10–90	F, D	Dentin	262 – 274	-
[45]	INAA	-	Adult	D	Dentin	260±15	-
[46]	PIXE	1	Adult	P	Dentin	300	-
[48]	INAA	25	<14	D	Dentin	291±11	-
[49]	Chem	18	7–18	-	Dentin	264±21	-
[50]	PIXE	30	Adult	P	Dentin	347±22	-
[51]	EMPA	-	Children	FF, Em, P	Dentin	-	150 – 221
[52]	Chem	7	Adult	Ash, AD	Dentin	309±52	-
[54]	-	-	-	-	Dentin	392	-
[56]	EMPA	34	Adult	P	Dentin	113±17	-
[57]	PIXE	6	Adult	-	Dentin	356	-
		9	Adult	-	Dentin	353	-
[58]	AAS	-	Adult	-	Dentin	142	-
[59]	LIBS	1	Adult	-	Dentin	66	-
[60]	EMPA	3	Adult	-	Dentin	57.7±18.3	-
[61]	EMPA	15	Adult	F, D, AD	Dentin	327 ± 37	-
[62]	LAICPMS	11	14–77	P, CC	Dentin	321 – 343	-
[21]	AAS	155	18–34	D, AD	Root	63.1±10.0	46.4 –78.4

(Table 5). Continued.

Reference	Method	n	Age years	Treatment of samples	Sample	Ca mass fraction	
						M±SD or range of M	Range of individual results
[64]	EMPA	7	Adult	PF	Dentin	304±7	-
[65]	INAA	10	Adult	F	Dentin	238±20	206 – 277
[66]	INAA	9	Adult	F	Dentin	290±27	238 – 324
[67]	INAA	30	Adult	F, D	Dentin	256 ±11	-
		30	Adult	G	Dentin	276 ±11	-
[68]	ICP-AES	6	Adult	F, AD	Dentin	224±4	-
[69]	ICP-MS	80	Adult	AD	Dentin	237±41	-
This work	INAA	84	16–58	Intact	Root	278±49	150 – 418
All references (n=46)						Median of means	Range of means
						276	57.7 – 392

"**-**" – no information, Chem – chemical method, GAA – gamma activation analysis, EMPA – electron microprobe analysis, INAA – instrumental neutron activation analysis, PIXE – proton induced X-ray emission, AAS – atomic absorption spectrophotometry, LIBS – laser-induced breakdown spectroscopy, LAICPMS – laser ablation ICP-MS, ICP-AES – inductively coupled plasma atomic emission spectrometry, ICP-MS – inductively coupled plasma mass spectrometry, Ash. – ashing, AD – acid digestion, PF – pulp (fat, protein) free, F – flotation (washing), D – drying at high temperature, Em – embedding in balsam or plastic, P – polishing, FF – fixation by formalin, CC – carbon coating, G –grinding.

dentin. As it was shown in Table **5** there was a coincidence between our mean value of Ca mass fraction in tooth root(s), 278±49 (SD) g/kg on dry mass basis, and the median reference value, 276 g/kg on dry mass basis. Our mean value for P mass fraction, 149±29 (SD) g/kg on dry mass basis, was also close to the median reference one, 141 g/kg on dry mass basis (Table **6**).

The median reference value for Ca/P mass fraction ratio in dentin, 2.05, was lower than the stoichiometric value for hydroxyapatite, 2.15, but higher than ours for roots, 1.88±0.23 (SD). The differences were due to the presence of organic matrix in dentin, which increased the P mass fraction and decreased the Ca/P mass fraction ratio in dentin, respectively. Particularly it was concern the teeth roots because they included such non-mineralized component of tooth as the pulp.

Standard deviations found for Ca and P mass fractions in tooth root(s) were respectively large but laid in the same level as some published data for dentin (Tables **5** and **6**). This was the consequence of the very wide individual variation in Ca and P mass fractions in intact roots (Tables **5** and **6**).

The lower standard deviations obtained for Ca/P mass fraction ratio (Table **7**) than for Ca and P mass fraction separately were due to the strong correlation between Ca and P mass fractions. This correlation for males and females aged 16 to 35 years was r=0.951 (p<0.0001) and r=0.627 (p<0.001), respectively. By aging the correlation becomes lower. So, for males and females aged 36 to 55 years, the respective values were r=0.846 (p<0.0001) and r=0.497 (p<0.01).

Tables **5-7** present a very wide range for Ca and P mass fractions and Ca/P mass fraction ratio. These values can not be explained only on the fact that various investigators have used dentin from the sound permanent teeth of different type and from different part of teeth (crowns or roots). We consider that other reasons account for this. The majority of data shown in these Tables were based upon techniques in which the dentin sample was subjected to various treatments in order to remove organic components by washing in different solvents, or depleting the whole organic matrix by dry ashing. These treatments lead to miscalculations of the inorganic component of dentin. For example, according to Pellegrino and Biltz [80] when organic components were removed from bone by chloroform-ethanol mixtures, Ca was removed too. Other evidence [71, 72, 81-83] shows that upon dry ashing a great amount of minerals and other biological materials are lost.

Information from other sources referring to the impact of age on the Ca and P mass fraction in dentin is very limited and contradictory. The evidence presented by Derise *et al*. [44] demonstrated the slight increase of Ca content in dentin of permanent teeth extracted from males and females ages 10 to 25 years. Arany *et al.* [62] reported the inverse Pearson's correlation between macro elements in dentin and age. In period of life from 14 to 77 years the correlation

Table 6: Reference Data of P Mass Fraction (g/kg, Dry Mass Basis) in Dentin (or Root) of Sound Permanent Teeth

Reference	Method	n	Age years	Treatment of samples	Sample	P mass fraction	
						M±SD or range of M	Range of individual results
[22]	Chem	-	14	Ash, AD	Dentin	138	-
[23]	Chem	1	15	AD	Dentin	141	-
[24]	Chem	1	Children	PF, F, Ash, AD	Dentin	131	-
[25]	Chem	20	Adult	PF, F, Ash, AD	Dentin	127±5	-
[26]	Chem	6	17– 41	PF, Ash, AD	Dentin	138	129 – 145
[27]	Chem	52	M34	PF, Ash, AD	Dentin	127	121 – 134
[28]	Chem	13	Adult	PF, AD	Dentin	130	-
[29]	Chem	50	Adult	Ash, AD	Dentin	131	-
[30]	Chem	1	44	PF, Ash, AD	Dentin	132	108 – 162
[31]	Chem	-	-	-	Dentin	183	-
[32]	Chem	28	11–-60	PF, Ash, AD	Dentin	140±0.4	-
[33]	Chem	-	Adult	PF, Ash, AD	Dentin	129	-
[34]	Chem	20	Adult	PF, Ash, AD	Dentin	125 – 132	-
[35]	Chem	-	17–21	AD	Dentin	130	-
[36]	Chem	96	Adult	PF, Ash, AD	Dentin	116 – 126	-
[37]	Chem	-	15–30	Ash, AD	Dentin	157 – 179	-
[38]	Chem	6	10–12	AD	Dentin	122	115 – 128
[39]	RNAA	15	14–16	F, D	Dentin	135±28	-
[40]	EPMA	-	15–30	PF, AD	Dentin	187±32	-
[41]	Chem	-	-	-	Dentin	150	-
[42]	EMPA	-	Adult	Em, P	Dentin	-	156 – 178
[44]	Chem	175	10–90	F, D	Dentin	127 – 135	-
[46]	PIXE	1	Adult	P	Dentin	211	-
[48]	INAA	25	<14	D	Dentin	141±10	-
[49]	Chem	18	7–18	-	Dentin	164±51	-
[50]	PIXE	30	Adult	P	Dentin	156±20	-
[51]	EMPA	-	Children	FF, Em, P	Dentin	-	79.9 – 106
[52]	Chem	7	Adult	Ash, AD	Dentin	178±46	-
[54]	-	-	-	-	Dentin	191	-
[55]	INAA	244	Adult	-	Dentin	208±191	-
[56]	EMPA	34	Adult	P	Dentin	66±17	-
[57]	PIXE	6	Adult	-	Dentin	156	-
[60]	EMPA	3	Adult	-	Dentin	48.9±1.1	-
[61]	EMPA	15	Adult	F, D, AD	Dentin	162 ± 27	-
[62]	LAICPMS	11	14–77	P, CC	Dentin	149 – 161	-
[64]	EMPA	7	Adult	PF	Dentin	148±3	-
[67]	INAA	30	Adult	F, D	Dentin	140 ±6	-
		30	Adult	G	Dentin	136 ±6	-
[68]	ICP-AES	6	Adult	F, AD	Dentin	107±7	-
[69]	ICP-MS	80	Adult	AD	Dentin	127±11	-
This work	INAA	84	16–58	Intact	Root	149±29	79 – 237
All references (n=40)						Median of means	Range of means
						141	48.9 – 211

"-" – no information, Chem – chemical method, RNAA – radiochemical neutron activation analysis, EMPA – electron microprobe analysis, INAA – instrumental neutron activation analysis, PIXE – proton induced X-ray emission, LAICPMS – laser ablation ICP-MS, ICP-AES – inductively coupled plasma atomic emission spectrometry, ICP-MS – inductively coupled plasma mass spectrometry, Ash. – ashing, AD – acid digestion, PF – pulp (fat, protein) free, F – flotation (washing), D – drying at high temperature, Em – embedding in balsam or plastic, P – polishing, FF – fixation by formalin, CC – carbon coating, G –grinding.

Table 7: Reference Data of Ca/P Mass Fraction Ratio in Dentin (or Root) of Sound Permanent Teeth

Reference	Method	n	Age years	Treatment of samples	Sample	Ca/P mass fraction ratio	
						M±SD or range of M	Range of individual results
[22]	Chem	-	14	Ash, AD	Dentin	2.01	-
[23]	Chem	1	15	AD	Dentin	2.07	-
[24]	Chem	1	Children	PF, F, Ash, AD	Dentin	2.07	-
[25]	Chem	20	Adult	PF, F, Ash, AD	Dentin	2.05	-
[26]	Chem	6	17–41	PF, Ash, AD	Dentin	2.03	2.03 – 2.04
[27]	Chem	52	M34	PF, Ash, AD	Dentin	2.08	2.01 – 2.25
[28]	Chem	13	Adult	PF, AD	Dentin	2.10	-
[29]	Chem	50	Adult	Ash, AD	Dentin	2.03	-
[30]	Chem	1	44	PF, Ash, AD	Dentin	2.08	1.84 – 2.29
[31]	Chem	-	-	-	Dentin	1.69	-
[32]	Chem	28	11–-60	PF, Ash, AD	Dentin	2.1±0.1	-
[33]	Chem	-	Adult	PF, Ash, AD	Dentin	2.04	-
[34]	Chem	20	Adult	PF, Ash, AD	Dentin	2.19 – 2.28	-
[35]	Chem	-	17–21	AD	Dentin	2.08	-
		-	17–21	Ash, AD	Dentin	2.01	-
[36]	Chem	96	Adult	PF, Ash, AD	Dentin	1.94 – 2.29	-
[37]	Chem	-	15–30	Ash, AD	Dentin	1.92 – 2.14	-
[38]	Chem	6	10–12	AD	Dentin	2.14	2.04 – 2.17
[39]	RNAA	15	14–16	F, D	Dentin	2.10	-
[40]	EPMA	-	15–30	PF, AD	Dentin	2.12±0.2	-
[41]	Chem	-	-	-	Dentin	2.09	-
[42]	EMPA	-	Adult	Em, P	Dentin	-	1.89 – 2.28
[44]	INAA	175	10–90	F, D	Dentin	~2.05	-
[46]	PIXE	1	Adult	P	Dentin	1.42	-
[47]	EMPA	2	Adult	Em, P	Dentin	-	2.0 – 2.1
[48]	INAA	25	<14	D	Dentin	2.06	-
[49]	Chem	18	7–18	-	Dentin	1.61	-
[50]	PIXE	30	Adult	P	Dentin	2.30±0.18	-
[51]	EMPA	-	Children	FF, Em, P	Dentin	-	1.88 – 2.08
[52]	Chem	7	Adult	Ash, AD	Dentin	1.74	-
[53]	PIXE	1	71	P	Dentin	-	1.0 – 2.5
[54]	-	-	-	-	Dentin	2.05	-
[56]	EMPA	34	Adult	P	Dentin	1.71	-
[57]	PIXE	6	Adult	-	Dentin	2.28	-
[60]	EMPA	3	Adult	-	Dentin	1.16±0.11	-
[61]	EMPA	15	Adult	F, D, AD	Dentin	2.02	-
[62]	LAICPMS	11	14–77	P, CC	Dentin	2.17±0.26	-
[63]	XRF	32	Adult	-	Dentin	-	2.85 – 3.35
[64]	EMPA	7	Adult	PF	Dentin	2.08±0.06	-
[67]	INAA	30	Adult	F, D	Dentin	1.83	-
		30	Adult	G	Dentin	2.25	-
[68]	ICP-AES	6	Adult	F, AD	Dentin	2.08±0.05	-
[69]	ICP-MS	80	Adult	AD	Dentin	1.84	-
This work	INAA	84	16–58	Intact	Root	1.88±0.23	1.40 – 2.70
All references (n=42)						Median of means	Range of means
						2.05	1.16 – 2.30

"-" – no information, Chem – chemical method, RNAA – radiochemical neutron activation analysis, EMPA – electron microprobe analysis, INAA – instrumental neutron activation analysis, PIXE – proton induced X-ray emission, LAICPMS – laser ablation ICP-MS, XRF – X-ray fluorescence analysis, ICP-AES – inductively coupled plasma atomic emission spectrometry, ICP-MS – inductively coupled plasma mass spectrometry, Ash. – ashing, AD – acid digestion, PF – pulp (fat, protein) free, F – flotation (washing), D – drying at high temperature, Em – embedding in balsam or plastic, P – polishing, FF – fixation by formalin, CC – carbon coating, G – grinding.

coefficient for Ca and P was -0.278 ($p \leq 0.05$) and -0.424 ($p < 0.01$), respectively. These results are very close to our data (Tables **2** and **3**). However among the donors from Hsinchu City, Taiwan [69] no changes in Ca and P content in dentin of permanent teeth were found up to the age of 68.

The influence of gender on the Ca and P mass fraction in dentin has also received little attention. Söremark and Lundberg [39] have found no significant variation with respect to gender. Derise [1973] and Derise *et al*. [44], however, have found significant gender-related differences in dentin for Ca mass fraction. The females had some higher levels of Ca as compared to males. The result obtained by Derise *et al*. agrees with our findings for females aged 16 to 35 years (Table **4**).

In conclusion, INAA-LLR has been demonstrated to be an adequate analytical tool for the non-destructive determination of Ca and P mass fractions in the intact roots of human teeth. The mean values (M ± SD) of Ca and P mass fractions (g/kg, dry mass basis) as well as Ca/P mass fractions ratio in intact roots of apparently healthy 16 - 55 years old women and men were: 278 ± 6, 149 ± 3, and 1.88 ± 0.03 respectively. A tendency for reduced Ca and P mass fraction in roots of female teeth after 35 years of age as well as for raised Ca mass fraction in female tooth root(s) up to 35 years of age as compared to male was observed. The mean values for Ca, P and Ca/P ratio in tooth root(s) were within a very wide range of reference data for dentin and close to their median.

Data obtained in our study expands the knowledge of physiology of dental tissues and may be used for diagnostic, therapeutic and preventive purposes. Moreover, elemental analysis, including Ca content, of human teeth is often used in paleoanthropology for dietary and environment reconstruction to assess the social and economic status of human groups [85]. It is therefore evident that for all of these applications it is necessary to establish the normal levels and gender- and age-related changes of chemical elements in a large scale study of teeth.

CONFLICT OF INTEREST AND SOURCE OF FUNDING STATEMENT

The study was self-financed by the authors. The authors report no conflicts of interest related to this study.

REFERENCES

[1] Hossain M, Nakamura Y, Tamaki Y, Yamada Y, Murakami Y, Matsumoto K. Atomic analysis and knoop hardness measurement of the cavity floor prepared by Er,Cr: YSGG laser irradiation *in vitro*. J Oral Rehabil 2003; 30: 515-552. http://dx.doi.org/10.1046/j.1365-2842.2003.01113.x

[2] Parker S. Surgical lasers and hard dental tissue. Br Dent J 2007; 202 (8): 445-454. http://dx.doi.org/10.1038/bdj.2007.294

[3] Nagai K, Kinoshita JI, Kimura Y, Matsumoto K. Morphological and compositional changes in human teeth following 9.6-μm CO_2 laser irradiation *in vitro*. J Oral Laser Application 2006; 6: 265-276.

[4] Dilber E, Malkoc MA, Ozturk AN, Ozturk F. Effect of various laser irradiations on the mineral content of dentin. Eur J Dent 2013; 7(1): 74-80.

[5] Boskey A. Mineralization of bones and teeth. Elements 2007; 3: 387-393. http://dx.doi.org/10.2113/GSELEMENTS.3.6.385

[6] Zaichick V, Dyatlov A, Zaichick S. INAA application in the age dynamics assessment of major, minor, and trace elements in the human rib. J Radioanal Nucl Chem 2000, 244(1): 189-193. http://dx.doi.org/10.1023/A:1006797006026

[7] Tzaphlidou M, Zaichick V. Neutron activation analysis of calcium/phosphorus ratio in rib bone of healthy humans. Appl Rad Isotop 2002; 57: 779-783. http://dx.doi.org/10.1016/S0969-8043(02)00171-9

[8] Tzaphlidou M, Zaichick V. Calcium, Phosphorus, Calcium-Phosphorus ratio in rib bone of healthy humans. Biol Trace Elem Res 2003; 93: 63-74. http://dx.doi.org/10.1385/BTER:93:1-3:63

[9] Tzaphlidou M., Zaichick V. Sex and age related Ca/P ratio in cortical bone of iliac crest of healthy humans. J Radioanal Nucl Chem 2004; 259(2): 347-349. http://dx.doi.org/10.1023/B:JRNC.0000017316.20693.45

[10] Zaichick V, Tzaphlidou M. Determination of calcium, phosphorus, and the calcium/phosphorus ratio in cortical bone from the human femoral neck by neutron activation analysis. Appl Rad Isotop 2002; 56: 781-786. http://dx.doi.org/10.1016/S0969-8043(02)00066-0

[11] Zaichick V, Tzaphlidou M. Calcium and phosphorus concentrations and calcium/phosphorus ratio in trabecular bone from femoral neck of healthy humans as determined by neutron activation analysis. Appl Rad Isotop 2003; 58: 623-627. http://dx.doi.org/10.1016/S0969-8043(03)00092-7

[12] Zaichick V. INAA application in the age dynamics assessment of Ca, Cl, K, Mg, Mn, Na, P, and Sr contents in the cortical bone of human femoral neck. J Radioanal Nucl Chem 2004; 259(2): 351-354. http://dx.doi.org/10.1023/B:JRNC.0000017317.64992.04

[13] Zaichick V. NAA of Ca, Cl, K, Mg, Mn, Na, P, and Sr contents in the human cortical and trabecular bone. J Radioanal Nucl Chem 2006; 269(3): 653-659. http://dx.doi.org/10.1007/s10967-006-0281-8

[14] Zaichick V. INAA application in the assessment of selected elements in cancellous bone of human iliac crest. J Radioanal Nucl Chem 2007; 271(3): 573-576. http://dx.doi.org/10.1007/s10967-007-0308-9

[15] Zaichick V, Zaichick S, Karandashev V, Nosenko S. The Effect of Age and Gender on Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Sr, V, and Zn Contents in Rib Bone of Healthy Humans. Biol Trace Elem Res 2009; 129(1-3): 107-115. http://dx.doi.org/10.1007/s12011-008-8302-9

[16] Zaichick S, Zaichick V. The effect of age and gender on 38 chemical element contents in human iliac crest investigated by instrumental neutron activation analysis. Journal of Trace Elements in Medicine and Biology 2010; 24(1): 1-6. http://dx.doi.org/10.1016/j.jtemb.2009.07.002

[17] Zaichick S, Zaichick V. The effect of age and gender on 38 chemical element contents in human femoral neck investigated by instrumental neutron activation analysis. Biol Trace Elem Res 2010; 137: 1-12. http://dx.doi.org/10.1007/s12011-009-8554-z

[18] Zaichick S, Zaichick V. Human bone as a biological material for environmental monitoring. Int. J Environment and Health 2010; 4: 278-292. http://dx.doi.org/10.1504/IJENVH.2010.033714

[19] Zaichick V. Data for the Reference Man: skeleton content of chemical elements. Radiat Environ Biophys 2013; 52(1): 65-85. http://dx.doi.org/10.1007/s00411-012-0448-3

[20] Zaichick V. Chemical elements of human bone tissue investigated by nuclear analytical and related methods. Biol Trace Elem Res 2013; 153: 84-99. http://dx.doi.org/10.1007/s12011-013-9661-4

[21] Malara P, Kwapulinski J, Malara B. Do the levels of selected metals differ significantly between the roots of carious and non-carious teeth? Sci Total Environ 2006; 369(1-3): 59-68. http://dx.doi.org/10.1016/j.scitotenv.2006.04.016

[22] Bowes JH, Murray MM. The chemical composition of teeth. II. The composition of human enamel and dentine. Biochem J 1935; 29: 2721-2727.

[23] Logan M.A. The composition of bone, cartilage, dentin and enamel. J Biol Chem 1935; 110: 375-389.

[24] Murray MM, Bowes JH. The composition of enamel, dentine and root in caries and pyorrhea. Brit Dent J 1936; 61: 473-477.

[25] Armstrong WD, Brekhus PJ. Chemical constitution of enamel and dentin. 1. Principal components. J Biol Chem 1937; 120: 677-687.

[26] French EL, Welch EA, Simmons EJ, LeFevre ML, Hodge HC. Calcium, phosphorus and carbon dioxide determination on all the dentin from sound and carious teeth. J Dent Res 1938; 17: 401-410. http://dx.doi.org/10.1177/00220345380170050701

[27] Dragiff DA, Karshan M. Effect of pregnancy on the chemical composition of human dentin. J Dent Res 1943; 22: 261-265. http://dx.doi.org/10.1177/00220345430220040401

[28] Oskerse T. Chemical composition of enamel and dentin in high and low caries areas in South Africa. J Dent Res 1943; 22: 441-446. http://dx.doi.org/10.1177/00220345430220060201

[29] Bird MJ, Gallup H, Gandino J, Hodge HC. A comparison of two methods of ashing enamel and dentin. J Dent Res 1948; 27: 693-704. http://dx.doi.org/10.1177/00220345480270060601

[30] Bird MJ, Kelman E, Lerner H, Rosenfeld D, Totah V, Hodge HC. Chemical analysis of teeth showing unusual wear. J Dent Res 1948; 27: 629-634. http://dx.doi.org/10.1177/00220345480270051201

[31] Cherkasova LC, Merezhinskii MF. Chemical composition of dental tissues and internal biochemical processes. Stomatologiya 1950; 29(4): 19-27.

[32] McClure FJ. Fluorine, ash, calcium and phosphorus in human teeth. J Dent Res 1950; 29: 315-319. http://dx.doi.org/10.1177/00220345500290031001

[33] McCann HG, Bullock FA. The reaction of the fluoride ion with powdered enamel end dentin. J Dent Res 1955; 34: 59-67. http://dx.doi.org/10.1177/00220345550340011601

[34] Asgar K. Chemical analysis of human teeth. J Dent Res 1956; 35: 742-748. http://dx.doi.org/10.1177/00220345560350051201

[35] Peckham SC, Losee FL, Ettelman I. Ethylenediamine vs. KOH-glycol in the removal of the organic matter of dentin. J Dent Res 1956; 35: 947-949. http://dx.doi.org/10.1177/00220345560350061701

[36] Burnett GW, Zenewitz JA. Studies of the composition of teeth. VIII. The composition of human teeth. J Dent Res 1958; 37: 590-600. http://dx.doi.org/10.1177/00220345580370040401

[37] Verbraeck L. Analyse quantitative du taux de calcium et de phosphore dans la dentine au Congo belge. Ann Soc belge Med 1958; 38: 1075-1083.

[38] Ohmori I. Biochemical studies on deciduous tooth substances. Part 1. Application of silver nitrate. Bull Tokyo Med Dent Univ 1961; 8: 83-95.

[39] Söremark R, Samsahl K. Gamma-ray spectrometric analysis of elements in normal human dentine. J Dent Res 1962; 41: 603-606. http://dx.doi.org/10.1177/00220345620410031201

[40] Frank RM, Capitant M, Goni J. Electron Probe Studies of Human Enamel. J Den Res 1966; 45(S3): 672-682. http://dx.doi.org/10.1177/00220345660450033401

[41] Little MF, Casciani FS. The nature of water in sound human enamel. A preliminary study. Archs Oral Biol 1966; 11: 565-571. http://dx.doi.org/10.1016/0003-9969(66)90222-6

[42] Besic FC, Knowles CR, Wiemann MR, Keller O. Electron probe microanalysis of noncarious enamel and dentin and calcified tissues in mottled teeth. J Dent Res 1969; 48(1) 131-139. http://dx.doi.org/10.1177/00220345690480010501

[43] Retief DH, Cleaton-Jones PE, Turkstra J, De Wet WJ. The quantitative analysis of sixteen elements in normal human enamel and dentine by neutron activation analysis and high-resolution gamma-spectrometry. Arch Oral Biol 1971; 16(11): 1257-1267. http://dx.doi.org/10.1016/0003-9969(71)90029-X

[44] Derise NL, Ritchey SJ, Furr A. Mineral composition of normal human enamel and dentine and the relation of composition to dental caries: I. Macrominerals and comparison of methods of analyses. J Dent Res 1974; 53(4): 847-852. http://dx.doi.org/10.1177/00220345740530041501

[45] Turkstra J, Retief DH, Cleaton-Jones PE. Activation analysis of biological material. S Afr Med J 1975; 49: 191-196.

[46] Ahlberg M, Akselsson R. Proton-induced X-ray emission in the trace analysis of human tooth enamel and dentine. Appl Rad Isotop 1976; 22(5/6): 279-290. http://dx.doi.org/10.1016/0020-708X(76)90145-9

[47] Zach GA. X-ray diffraction and calcium-phosphorous analysis of irradiated human teeth. J Dent Res 1976; 55(5): 907-909. http://dx.doi.org/10.1177/00220345760550053301

[48] Lakomaa EL, Rytömaa I. Mineral composition of enamel and dentin of first and permanent teeth in Finland. Scand J Dent Res 1977; 85: 89-95.

[49] Vihm N, Kokk H. The study of mineral composition of hard tissues of hypoplastic teeth and the prevalence of caries among them. Proceedings of the Tartu University 1978; 478: 139-145.

[50] Cohen DD, Clayton E, Ainsworth T. Preliminary investigations of trace element concentrations in human teeth. Nucl Instr Meth 1981; 188(1): 203-209. http://dx.doi.org/10.1016/0029-554X(81)90340-2

[51] Knychalska-Karwan Z, Pawlicki R, Karwan T. Ultrastructural analysis quantitative distribution of macro- and

microelements in young teeth hard tissues by the use of an X-ray microanalysis. Folia Histochem Cytochem (Krakow) 1981; 19(2): 87-91.

[52] Borovskii EB, Rusuddinov C, Maximovskaya LN, Remizov CM, Smirnova TA. Calcium and phosphorus content in teeth, blood, saliva as well as the microhardness of enamel and dentin in workers of phosphoric manufacture. Stomatologiya 1987; 66(3): 7-9.

[53] Sommer F, Engelmann CH. Possibilities for using the nuclear microprobe to determine carbon, nitrogen and other elements in teeth. Nucl Instr Meth 1987; B22(1-3): 128-132. http://dx.doi.org/10.1016/0168-583X(87)90309-0

[54] Molleson T. Trace elements in human teeth. In: Grupe G, Herrmann B, Eds. Trace Elements in Environmental History. Berlin: Springer-Verlag 1988; pp. 67-82. http://dx.doi.org/10.1007/978-3-642-73297-3_6

[55] Gomerschtein VA, Maximovskii YuM. Zinc and caries. Stomatologiya 1989; 68(6): 52-54.

[56] Lagutina NYa, Vorob'ev VC, Kulagin AP, Popov AV, Schah GE. Impact of the pulp removal on the status of hard dental tissues. Stomatologiya 1990; 69(3): 13-16.

[57] Chaudhri MA. Nuclear analytical methods for trace element studies in calcified tissues. Biol Trace Elem Res 1990; 26-27: 149-159. http://dx.doi.org/10.1007/BF02992668

[58] Perez-Jordan MY, Salvador A, Guardia M. Determination of Sr, K, Mg and Na in human teeth by atomic spectrometry using a microwave-assisted digestion in a flow systems. Anal Lett 1998; 31: 867-877. http://dx.doi.org/10.1080/00032719808002824

[59] Samek O, Beddows DCS, Telle HH, Kaiser J, Liska M, Caceres JO, Gonzales Urena A. Quantitative laser-induced breakdown spectroscopy analysis of calcified tissue samples. Spectrochimica Acta Part B 2001; 56: 865-875. http://dx.doi.org/10.1016/S0584-8547(01)00198-7

[60] Del Pilar Gutiérrez-Salazara M, Reyes-Gasgaa J. Microhardness and Chemical Composition of Human Tooth. Materials research 2003; 6(3): 367-373. http://dx.doi.org/10.1590/S1516-14392003000300011

[61] Hossain M, Nakamura Y, Tamaki Y, Yamada Y, Murakami Y, Matsumoto K. Atomic analysis and knoop hardness measurement of the cavity floor prepared by Er,Cr: YSGG laser irradiation *in vitro*. J Oral Rehabil 2003; 30: 515-52. http://dx.doi.org/10.1046/j.1365-2842.2003.01113.x

[62] Arany S, Yoshioka N, Ishiyama D, Mizuta T. Investigation of trace element distribution in permanent root dentine by laser ablation inductively coupled plasma mass spectrometry. Akita J Med 2004; 31: 107-112.

[63] Antunes A, Salvador VLR, Scapin MA, de Rossi WZezell DM. Nanosecond Nd: YAG laser on dental enamel: compositional analysis by X-ray fluorescence. Laser Phys Lett 2005; 2(6): 318-323. http://dx.doi.org/10.1002/lapl.200410181

[64] Nagai K, Kinoshita JI, Kimura Y, Matsumoto K. Morphological and compositional changes in human teeth following 9.6-μm CO2 laser irradiation *in vitro*. J Oral Laser Application 2006; 6: 265-276.

[65] Soares MAB, Adachi EM, Saiki M. INAA of enamel and dentine samples of a group of children and adults: A comparative study. J Radioanal Nucl Chem 2008; 276(1): 49-52. http://dx.doi.org/10.1007/s10967-007-0408-6

[66] Saiki M, Adachi LK, Adachi EM. Elemental comparison in sound and carious human teeth by instrumental neutron activation analysis. J Radioanal Nucl Chem 2009; 282(1): 29-32. http://dx.doi.org/10.1007/s10967-009-0262-9

[67] Zenóbio MAF, Tavares MSN, Zenóbio EG, Silva TA. Elemental composition of dental biologic tissues: study by means of different analytical techniques. J Radioanal Nucl Chem 2011; 289: 161-166. http://dx.doi.org/10.1007/s10967-011-1067-1

[68] Dilber E, Malkoc MA, Ozturk AN, Ozturk F. Effect of various laser irradiations on the mineral content of dentin. Eur J Dent 2013; 7(1): 74-80.

[69] Liu H, Chao J, Chuang C, Chiu H, Yang C, Sun Y. Study of P, Ca, Sr, Ba and Pb Levels in Enamel and Dentine of Human Third Molars for Environmental and Archaeological Research. Advances in Anthropology 2013; 3: 71-77. http://dx.doi.org/10.4236/aa.2013.32010

[70] Zaichick V., Zaichick S. Instrumental effect on the contamination of biomedical samples in the course of sampling. J Anal Chem 1996; 51(12): 1200-1205.

[71] Zaichick V. Sampling, sample storage and preparation of biomaterials for INAA in clinical medicine, occupational and environmental health. In: Harmonization of Health-Related Environmental Measurements Using Nuclear and Isotopic Techniques. Vienna: IAEA 1997; pp. 123-133.

[72] Zaichick V. Losses of chemical elements in biological samples under the dry ashing process. Trace Elements in Medicine 2004; 5(3): 17-22.

[73] Zaichick V. Medical elementology as a new scientific discipline. J Radioanal Nucl Chem 2006; 269: 303-309. http://dx.doi.org/10.1007/s10967-006-0383-3

[74] Zaichick V. Neutron activation analysis of Ca, Cl, K, Mg, Mn, Na, P, and Sr contents in the crowns of human permanent teeth. J Radioanal Nucl Chem 2009; 281(1): 41-45. http://dx.doi.org/10.1007/s10967-009-0083-x

[75] Korelo AM, Zaichick V. Software to optimize the multielement INAA of medical and environmental samples. In: Activation Analysis in Environment Protection. Dubna, Russia: Join Institute of Nuclear Research 1993; pp. 326-332.

[76] LeFevre ML, Manly RS. Moisture, inorganic and organic contents of enamel and dentin from carious teeth. J Amer Dent Ass 1938; 25: 233-242.

[77] Losee FL, Leopold RS, Hess WC. Dentinal protein: recovery in pure form. J Dent Res 1951; 30: 565-574. http://dx.doi.org/10.1177/00220345510300040601

[78] Burnett GW, Zenewitz JA. Studies of the composition of teeth. VII. The moisture content of calcified tissues. J Dent Res 1958; 37: 581-589. http://dx.doi.org/10.1177/00220345580370040301

[79] Elliott JC. Structure and chemistry of the apatites and other calcium orthophosphates. Amsterdam: Elsevier 1994.

[80] Pellegrino ED, Biltz RM. The composition of human bone in uremia. Medicine 1965; 44(5): 397-418. http://dx.doi.org/10.1097/00005792-196509000-00002

[81] Grynpas MD, Pritzker KPH, Hancock RGV. Neutron activation analysis of bulk and selected trace elements in bone using low flux SLOWPOKE reactor. Biol Trace Elem Res 1987; 13: 333-344. http://dx.doi.org/10.1007/BF02796644

[82] Edward JB, Benfer RA, Morris JS. The effects of dry ashing on the composition of human and animal bone. Biol Trace Elem Res 1990; 25: 219-231. http://dx.doi.org/10.1007/BF02990417

[83] Kuo H-W, Kuo S-M, Chou C-H, Lee T-C. Determination of 14 elements in Taiwanese bones. Sci Total Environ 2000; 255(1-3): 45-54. http://dx.doi.org/10.1016/S0048-9697(00)00448-4

[84] Derise NL. The mineral composition of normal human enamel and dentine and the relation of composition to dental caries. Ph.D. Thesis, Virginia Polytechnic Institute and State University, 1973.

[85] Szostek K. Chemical signals and reconstruction of life strategies from ancient human bones and teeth - problems and perspectives. Anthropological Review 2009; 72: 3-30. http://dx.doi.org/10.2478/v10044-008-0013-5

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