RESEARCHES BY MULTIVARIATE STATISTICAL METHODS OF INORGANIC ELEMENTAL CONTENT IN HUMAN TEETH

C. Oprea, M.V. Gustova, I.A. Oprea, V. Buzguta

[1]*Joint Institute for Nuclear Research (JINR), Dubna 141980, Russian Federation*

[2]*Bucharest State University, 76900 Bucharest, Romania*

[3]*Faculty of Sciences, University in Oradea, 3700 Oradea,Romania*

Abstract

X-ray fluorescence spectrometry (XRFS) was used as a multielement method of evaluation of human teeth for their amounts of trace elements. Measurements were carried out on dental tissues, and some differences in tooth matrix composition were noted. In addition, the elemental concentrations determined in teeth from subjects of different ages, nutritional states, professions and gender, living under various environmental conditions, were included in a comparison by multivariate statistical analysis (MVSA) methods. The statistical analyses performed seem to indicate that deciduous teeth might be a suitable indicator of environmental exposure to several trace heavy metals.

Keywords: *human teeth, XRFS, trace heavy metals, multivariate analysis*

Introduction

The municipal town of Oradea, located in the north-western part of Romania, on the Crisul Repede river bank and about 18 kilometres east of the frontier with Hungaria, is a medium industrial centre crossed by the traditional European highways. The environmental situation of this urban area, including the exposure to the ambient emissions of its inhabitants, is enough complexly since the accelerated development of the town throughout the industrial advancement. The urban activity in Oradea is undergoing transition since the closure of some industrial plants in the late 1990s and due to the all processes of modernization connected with the adhering of Romania to the European Union.

Usually the concentrations of trace heavy metals in teeth are higher in urban areas compared with those found in the teeth of people living in rural areas. As toxic heavy metals

are easily deposited in tooth tissues by replacing the mineral tooth compounds during the human life, they can be used as indicators of cumulative long-term contamination with heavy metals of the studied population.

We, a group of researchers of the Joint Institute for Nuclear Research and affiliated research institutions started monitoring environmental studies of this area under the agreement of National Agency for Scientific Research (Romania) in 2003. During this period, we collected data on various environmental and ecological parameters in this region. Then we investigated the application of nuclear and atomic methods to trace element analysis of human teeth from Oradea's population, and published several papers on these materials. The heavy metal content in deciduous teeth of the population segment under study was found to refer to some environmental parameters indicating recent industrial pollution.

Materials and methods

The experimental material used in this study was prepared based on decayed permanent teeth from urban inhabitants of Oradea and from 5 of the residents leaving in a remote area located at 60 km north of Oradea, as it was previously reported. The teeth survey comprises a total of 160 teeth collected from several nonsmoking donors of different age, gender and profession residing in a given area all their life. The patient history included information about habit, medication, possible chronic diseases and others. The teeth sampling included the all tooth types recorded from males as well as from female subjects. The decayed teeth chosen for research are represented only by their own physical and chemical characteristics as they were not subjected to any stomatological treatment in the past. The general characteristics of the teeth survey from which the data were obtained are summarized in Table 1.

The teeth were cleaned by any extraneous material (i.e. tartar, caries and soft tissue) and further rinsed with distilled water. Then they were dried until constant weight in a thermostat at 105^0C and stored in individual polyethylene containers. Experimental samples of dental enamel, dentine and cementum tissues were cut in thin layers and used further in the analytical procedures. The whole tooth and tooth tissue measurements were made by using a XRFS set-up which was proven to provide concentration data which were suitable enough for statistical analysis. Dental standard materials of $Ca_{10}(PO_4)_6(OH)_2$ and $Ca(OH)_2$ were measured in the same experimental conditions as the dental tissue samples and Ca from the

both materials was chosen as a standard for quality control of the analytical results. Results of the analyses for all element concentrations are expressed in μg/g of tooth dry weight.

TABLE 1. Characteristics of the human teeth survey

Tooth tissue	Group	N	Age range	Tooth type							
				Incisor		Canine	Premolar		Molar		Wisdom tooth
				11	12	13	14	15	16	17	18
Enamel	Total	160	18-72	7	9	14	19	26	30	41	14
	Female	91	18-68	5	8	8	9	13	11	28	9
	Male	69	18-72	2	1	6	9	14	13	19	5
Dentine	Total	58	18-65	2	3	5	7	10	11	15	5
	Female	22	18-55	1	1	2	3	4	6	4	1
	Male	36	18-65	2	1	3	4	6	9	7	4
Cementum	Total	23	18-58	2	1	2	2	5	7	2	2
	Female	10	18-52	1	1	1	1	2	3	1	-
	Male	13	18-58	1	-	1	1	3	4	1	2

Results and discussion

The results of element concentrations determination (whole tooth measurements) in the teeth of urban population segment were normalized to the element concentrations of the control group (Figure 1). Accordingly, they are represented as ratio R of the average element concentration in both human groups.

The concentrations for most of the metals were significantly higher for the Oradea inhabitants compared with those of rural group. The highest ratios were found for Zn (6.65), Fe (5.68), In(Cd) (4.75), Nd (3.83), As (3.67) and Cr (3.36).

The results of the most significant element concentrations determined in tooth tissues for the tooth from which we were able to separate the three slices, namely enamel, dentine and cementum, also reflect the changes in tooth matrix composition (Figure 2).

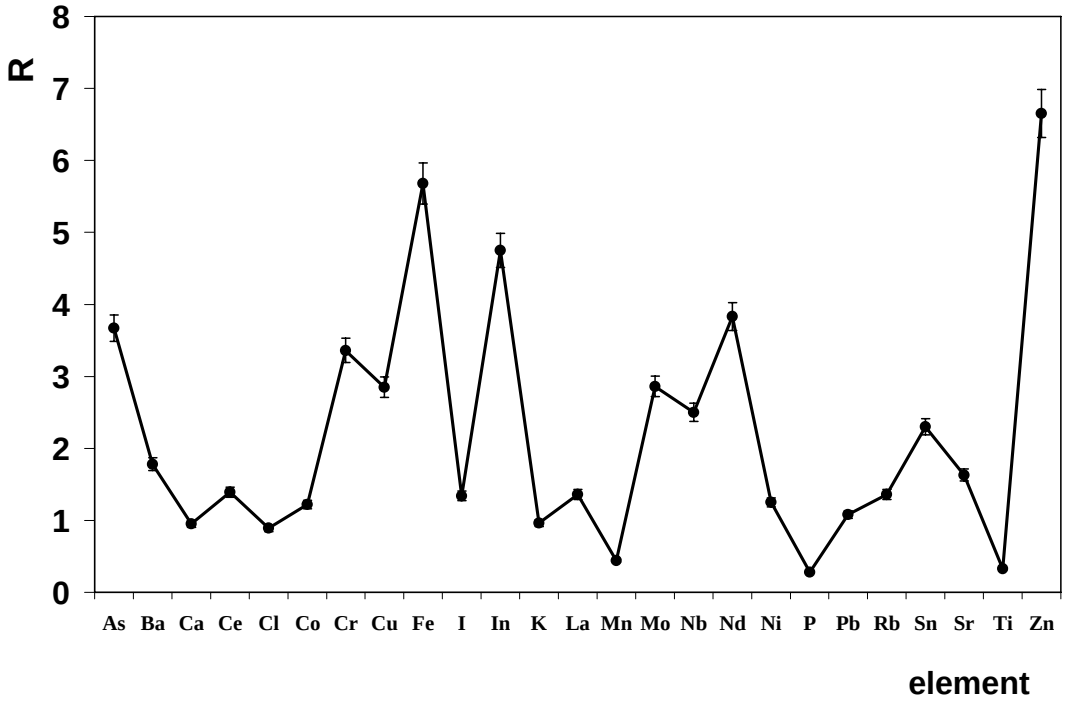

Figure 1. Average element concentrations in deciduous teeth of urban population versus control population

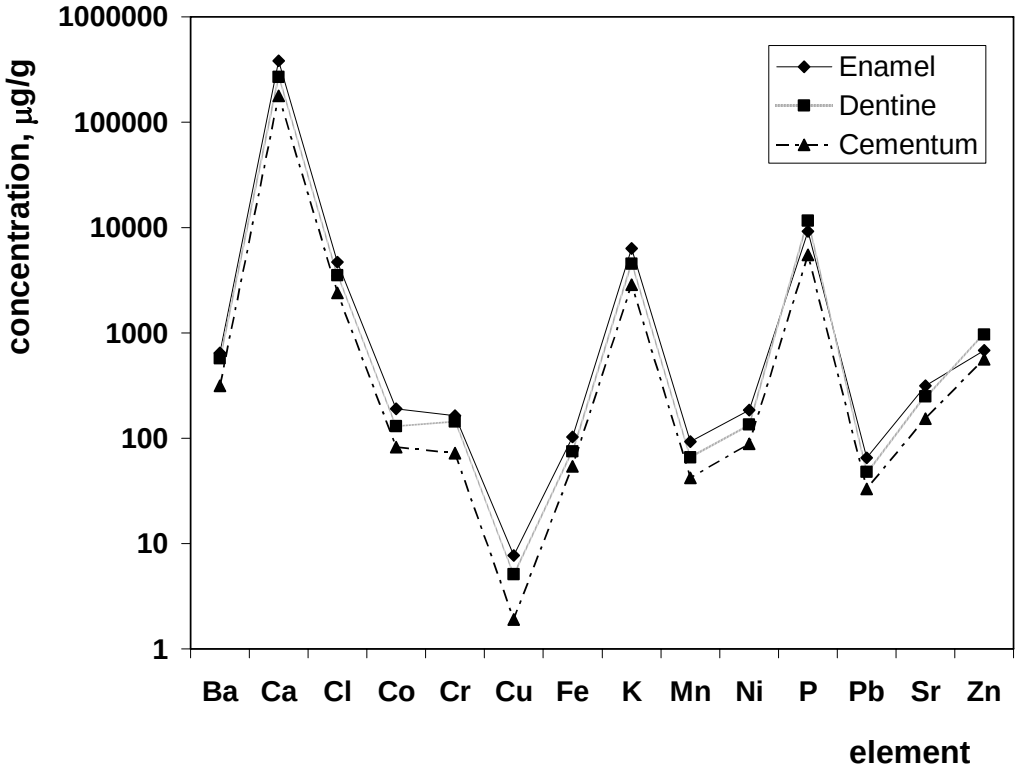

Figure 2. The mean concentrations of the most significant inorganic compounds in tooth tissues

It is wellknown that the mineral component of enamel is 95%, of dentine is 67% and that of cementum is about 45-50% and the rest of tissue material has an organic nature. This support the tendency reflected by the determined concentration, that ($conc_{enamel}$>$conc_{dentine}$>$conc_{cementum}$) for almost all inorganic elements. Exception from this behavior is done by Zn and P, for which ($conc_{dentine}$>$conc_{enamel}$).

The last mostly relevant 14 elements from the 25 determined experimentally will be further subjected to multivariate modeling.

Multivariate analysis

In order to reveal the contribution to the variance of the results obtained for the measurements of the human teeth, the intrinsic statistical variance due to the process of trace heavy metal incorporation obtained during the life time should be controlled. The statistical methods used for multivariate modeling of concentration data were exploratory factor analysis by R-mode intercorrelations. The calculations were performed by using Microsoft Excel program and SPSS program package for Windows.

The Oradea residents of various ages were assigned the following groups: 1) 18-25; 2) 25-35; 3) 35-60 and 4) >60 years old. These ranks were further used as coded values for the variable “age” in multivariate statistical evaluations. The variables supposed to statistical analysis were 14 chemical elements, and also age, gender and toot tissue.

The multivariate statistical results are characterized by product-moment R-mode correlation matrix based on the interrelationships between the analyzed variables that is listed in Table 2. In a future work more detailed results will be available.

Age of the examined population segment was well correlated with Ca, P, K, Pb and Fe. This dependence reveals an intrinsic mineralization factor and also an industrial factor. The group of urban residents was chosen since of the expected higher environmental exposure to trace metals and other elements in their teeth. The Zn, Cu and Ca content of teeth has been found to correlate with gender following the own physiological human parameters. Tooth tissue were high correlated by Ca, P, K and follow up the own mineralogical composition and characteristics of the tooth tissues. The significant correlation of the tooth tissue with age draws a behavior that human deciduous teeth can be used as habitat pollution bioindicators.

Table 2. Product-moment R-mode correlation matrix of the concentrations of significant elements, age, gender and toot tissue in deciduous teeth of Oradea population segment.

Variable	Cr	Cu	Zn	Mn	Ni	Co	Sr	Ba	Pb	Cl	Ca	P	K	Age	Gender	Tooth tissue
Fe	**0.79**	**0.50**	**0.35**	0.23	**0.41**	**0.55**	0.25	0.24	0.18	-0.09	-0.21	-0.23	-0.14	**-0.38**	0.12	0.09
Cr		**0.41**	**0.30**	0.15	-0.20	**0.38**	-0.20	0.17	0.10	-0.13	-0.16	-0.01	0.07	0.04	0.05	0.16
Cu			**0.59**	**0.36**	**0.39**	**0.31**	-0.11	0.20	**0.35**	0.20	**0.32**	0.17	0.20	0.11	**0.48**	0.18
Zn				**0.48**	**0.31**	**0.35**	0.13	**0.52**	**0.32**	0.12	**0.40**	0.24	0.15	-0.17	**0.52**	0.21
Mn					-0.16	0.27	0.05	**0.33**	0.16	0.24	0.19	0.03	-0.09	0.07	-0.11	-0.13
Ni						0.09	**-0.33**	0.19	-0.11	-0.03	0.18	0.17	0.25	0.21	0.27	0.07
Co							-0.26	**0.34**	0.08	0.25	**0.39**	0.12	-0.12	0.14	-0.10	-0.16
Sr								-0.28	0.04	-0.07	0.14	-0.05	0.02	0.23	0.28	0.11
Ba									0.01	-0.21	0.17	-0.07	-0.01	0.14	0.15	-0.04
Pb										0.06	-0.10	-0.15	-0.13	**0.31**	-0.17	0.10
Cl											0.11	0.08	0.20	0.10	0.20	0.19
Ca												**0.31**	**0.36**	**-0.35**	**0.31**	**0.54**
P													0.21	-0.22	-0.13	**0.47**
K														-0.20	0.09	**0.38**
Age															0.03	**0.31**
Gender																-0.16

The bold values correspond to significant correlation at $p \leq 0.05$.

Conclusion

If the hypothesis of human teeth contamination is rejected by a suitable statistical test at a given significance level, usually the additional degree of variability is added to the uncertainty of the concentration data set as result of a random distribution of observations.

The results obtained by our multivariate statistical approach based on analytical concentration data measured by XRFS method shows the efficiency of this method especially when the investigation includes a limited number of observations with a large degree of variability. The results seem to be in agreement with the physiological properties of the human teeth including their uptake of trace heavy metals from the living habitat. Once more our results prove that deciduous human teeth might be a good indicator of environmental exposure to several trace metals.

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