DETERMINATION OF TRACE HEAVY METALS IN HUMAN TEETH USING PIXE

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Abstract Human teeth samples at the EG-5 experimental basis of FLNP were used to determine heavy metal accumulation in people of various professions and living in various conditions. PIXE analysis, using a 2 MeV proton beam of 1.5 mm diameter, apart of the determination of Ca, allowed an optimised detection of Cr, Cu, Fe, and Zn above detection limits by use of Al and Mylar filters. The results showed enhanced, but relatively constant, heavy metal levels in the dental enamel. This was consistent with their history of long term-exposure to elevated anthropogenic heavy metal sources and with the hypothesis that the dental enamel can be used as an archive of the human's exposure to heavy metals during their life.

Keywords: heavy metals, dental enamel, PIXE, long-term exposure, pollution sources

1. INTRODUCTION

Heavy metal concentrations in human teeth reflect people's exposure to the heavy metals during their life [1]. Absorbed elements continuously adds to that deposited in the enamel/dentin of the teeth. Thus, these tissues are expected to contain a heavy metal concentration which reflects the integrated heavy metal exposure during the time from completion of tooth formation to tooth extraction or shedding.

During this investigation we want to gather information on the level of heavy metal exposure in Bihor county inhabitants, and look for differences in exposure in different areas.

The proton-induced X-ray emission (PIXE) technique has been shown to be a method well suited for the quick multielement, near surface analysis of biological samples as human teeth, reported here [2, 3]. The analysis is contamination-free for elements heavier than calcium in dental hard tissues.

In this work, we present the first results of a research of trace heavy metal composition of dental enamel, using PIXE in association with RBS, in a set of 20 dental samples.

2. THEORETICAL BACKGROUND

In PIXE analysis the proton-induced X-rays are detected by a Si(Li) detector, and spectra with peaks corresponding to emitted characteristic X-rays are obtained. The position of a peak in the spectrum gives after calibration the X-ray energy of the element in sample matrix. The number of pulses, N, under a peak of an element with atomic number Z is given by [4]

$$N = n_p C \omega k \Omega (4\pi)^{-1} \varepsilon T_1 \int_{E_0}^{E_1} \sigma(E) T_2(\xi) (-dE/dx)^{-1} dE \qquad (1)$$

where n_p is the total number of incident protons, C is the concentration of Z atoms per cm^3, ω the fluorescence yield, k the relative transition probability, Ω and ε the solid angle and

respectively, the efficiency of the detector, T_1 the transmission of X-rays through filter material between the sample surface and detector, σ the cross-section for the ionization of specific shell in cm^2, $T_2(\xi) = exp(-\mu_m \xi)$ is the transmission through the sample, μ_m is the mass absorption coefficient in cm^2/g and ξ is the free path of X-rays in the sample, in g/cm^2, dE/dx is the stopping power of the matrix, E_0 is the initial energy of the proton and E_1 is the final energy of the proton (here it is always 0).

Secondary production of characteristic X-rays by proton-induced characteristic X-rays, secondary electrons and bremsstrahlung are neglected. In a tooth matrix enhancement effects are expected only for elements with absorption edges below 3.69 KeV (k_α line of Ca) [6].

The stopping power and mass absorption coefficients in equation (1) are calculated from the enamel matrix which consists of major constituents as hydroxyapatite, $(Ca_{10}(PO_4)_6(OH)_2)$, 1% organic material and 4% water [5].

The 50 μm thick Mylar filter (C10H8O4, r = 1.38 g/cm2) reduces very low energy X-rays and bremsstrahlung, until k_α of P has a 7% transmission [6].

3. EXPERIMENTAL DETAILES

3.1. SAMPLE COLLECTION AND PREPARATION

Teeth of Oradea municipal town's inhabitants were sampled at two stomatologic clinics in Oradea during the 2004 year. Teeth were collected from patients presenting themselves for tooth extraction at the clinic. Prior to extraction of the teeth, a detailed investigation of dental history and examination was carried out in each individual.

Immediately after extraction each tooth was dissolved in oxygenated water (to clean the organic material from their surface) into an individual polyethylene container. Then samples were stored in a deep freeze (at - 20 ^{0}C).

3.2. ANALYSIS

Samples were irradiated in vacuum with a 2 MeV proton beam, 1.5 mm diameter, from the 3 MV Van der Graaff accelerator of EG-5 experimental basis of FLNP. The experimental arrangements are shown in Figure 1. The sample was placed with the surface to be irradiated at 45^0 with respect to beam direction and the X-rays were detected by a Si(Li) detector mounted at right angle to the beam direction (according to Figure 1). The sample to detector gap included 10 mm of air and absorbers. The energy resolution of the system was 260 eV FWHM at 5.89 KeV Mn K_α line. The analyzing time was typically 1h 30min. The spectra were calibrated using the ^{241}Am source [7].

The elemental composition is deduced directly from the measured relative X-ray intensities, using known X-ray production cross sections and correcting for proton energy loss, X-ray attenuation in the sample and detector efficiency. The analysis procedure for human teeth was checked by analysis of thin samples of known composition.

With optimized PIXE procedures precisions of 1 - 2% and an accuracy of better than 5% are obtainable, whereas the detection limits are down to 0.1 microgram/g.

The teeth samples were analyzed for 20 major and trace elements by PIXE accompanied by the Rutherford backscattering spectroscopy (RBS). In this study we retained further only four trace heavy metals, namely Cr, Cu, Fe, and Zn, since their presence was well established

in almost all measured samples. Figures 2 and 3 show the spectra from the analysis of the external email of an upper female canine.

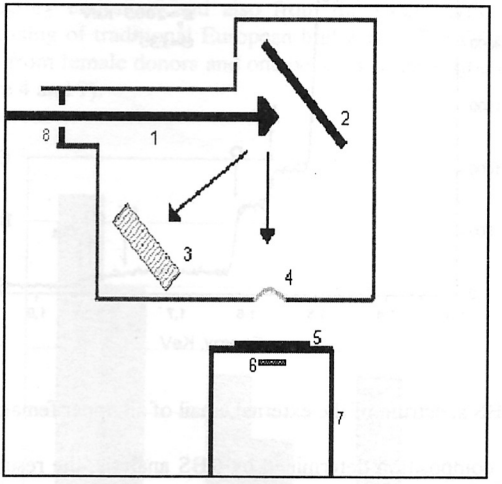

Figure 1. The experimental layout for X-ray analysis: 1 – proton beam, 2 – target, 3 – charged particle detector, 4 – Mylar (Al metallized) exit window (25 μm), 5 – Be window (4mm), 6 – X-ray detector, 7 – detector cryostat, 8 – beam defining slit (1.5 mm).

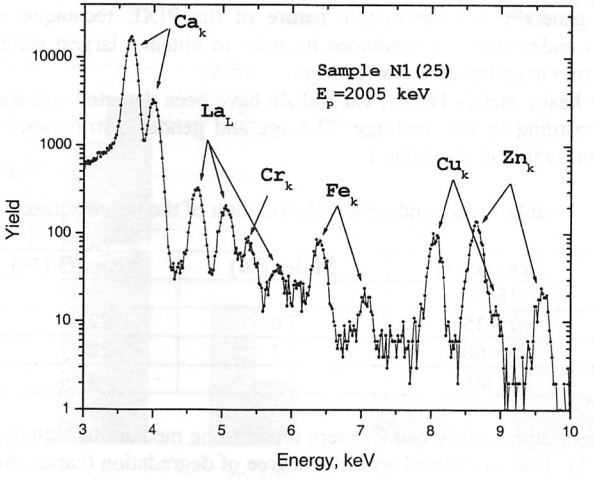

Figure 2. PIXE spectrum of the external email of an upper female canine

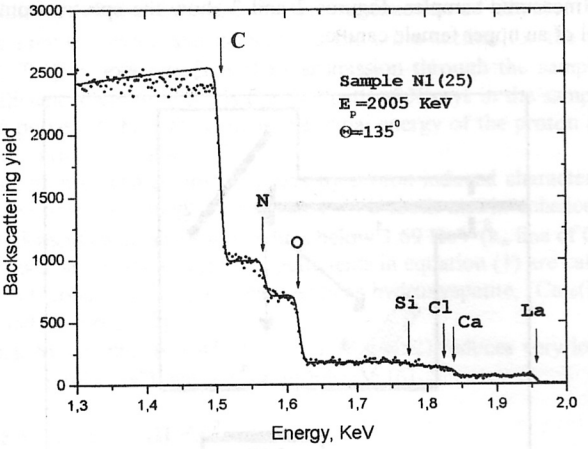

Figure 3. RBS spectrum of the external email of an upper female canine

Using the matrix composition determined by RBS analysis, the results were normalized to Ca concentration of 38%.

RESULTS AND DISCCUSION

The results presented in this section can be considered as a pilot study on the trace metals in tooth enamel. They have been presented in terms of a limited suite of trace elements. The generally non-destructive nature of the PIXE technique allows performing several analyses under different conditions in order to obtain a largest suite of elements. The experimental errors in all the cases varied from 3 - 15%.

The trace heavy metals Fe, Cr, Cu and Zn have been detected and statistically analyzed by grouping according to sex, and age. The age and gender distribution of the investigated individuals are presented in the Table 1.

Table 1. Age and gender distribution of the tested subjects

Age (years)	Males (%)	Females (%)
18-25	13	9.2
25-35	5.6	22
35-60	7.4	28
> 60	11	4

The concentrations of Fe and Cr were found to be medium to high in the analyzed teeth (Figures 4 and 5). They correlated with the degree of degradation (carious/non-carious) of the analyzed teeth. Further these results can be connected with the fact that the inhabitants of Oradea city are exposed to the local pollution (exposure/food intake). If we'll represent log-

log dependence of Cr - Fe (Figure 6), we obtain strong positive correlations ($R > 0.93$) of the two trace metals for all age groups. One possible explanation of the recorded Fe and Cr levels in teeth is that such contaminants may derive from the "CET" power plants and "Electrometal" factory emissions and also from the local traffic density (as the town is situated at the crossing of traditional European highways). The Fe and Cu levels seem to be lower in the teeth from female donors and one believes to be attributed to the specific women physiology (Figure 4 and 7).

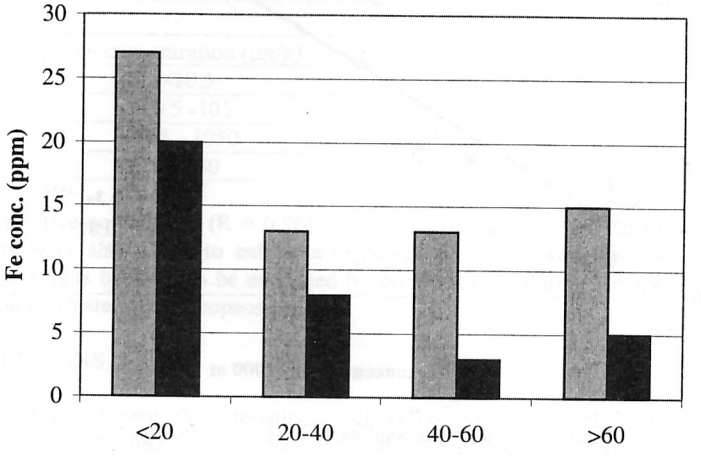

Figure 4. The distribution of iron concentration in teeth of the tested subjects

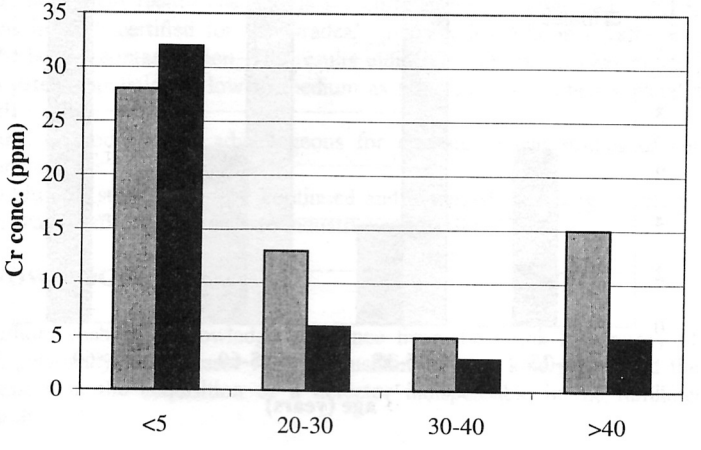

Figure 5. The distribution of chromium concentration in teeth of the tested subjects

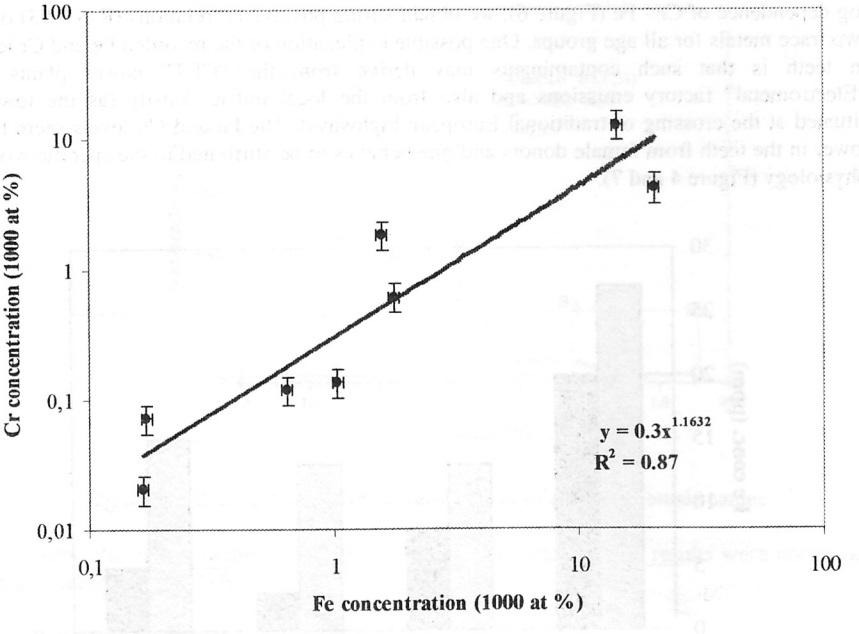

Figure 6. Log-log dependence Cr vs. Fe in analyzed teeth

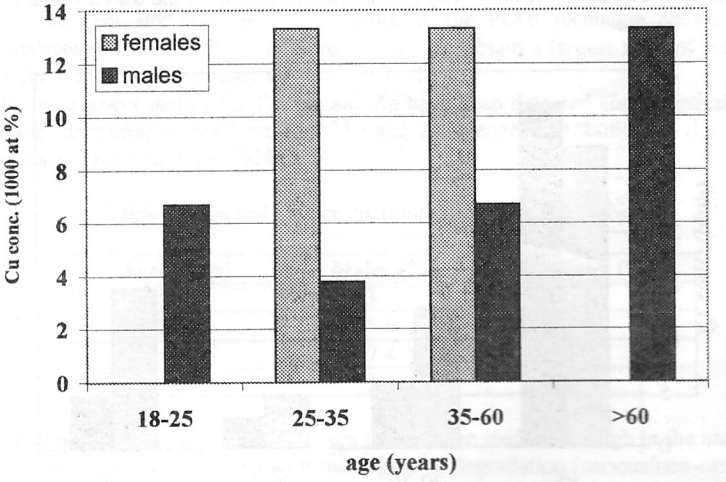

Figure 7. The distribution of copper concentration (10^3 at %) in tested subjects according to age and gender

In Table 2 is presented the distribution of Zn concentration (μg/g) by groups of age in tested individuals. The Zn in enamel of the tooth analyzed encountered a broad range of values. As zinc in usual concentrations is an essential substance in human organism, we'll consider only the last two population samples as contaminated by zinc. This decision is connected with the observed carious degree of the decayed teeth. The teeth contamination can reflect the general health of the individual and it may be contaminated from the specific anthropogenic activities (as local power plant emissions).

Table 2. The zinc concentration distribution in teeth of the tested subjects

Zn concentration (μg/g)	Subjects (%)
<10.5	33.4
10.5 -105	26.7
105 - 1050	33.4
> 1050	6.7

Strong positive correlations (R = 0.96) were found between Ca and Zn for all groups; these elements were also found to exhibit a negative correlation with age for teeth from female donors. This is believed to be explained by decalcification during the specific woman life and physiology (maternity, menopause, etc.).

CONCLUSIONS

Because of its inherent characteristics, PIXE offers great potential for trace element analysis in teeth and this was demonstrated through selected examples through the present study. This research performed a realistic estimation of the cumulative contamination of the tested population sample to heavy metal emissions in the town of Oradea. The specific atmospheric pollutants emitted by local industry are characterized by the high retention capacities of the human teeth. The significant correlations between pairs of heavy metal concentrations in teeth certified for the Oradea's industry and regional traffic emissions as sources of the human contamination. The results indicated that human heavy metal exposure of the investigated population is low to medium as was reflected by heavy metal content in analyzed teeth.

Then this method appears advantageous for epidemiological studies of heavy metal toxicity in humans.

The monitoring study should be continued and extended, including people living close to roads with heavy traffic and/or close to industrial companies.

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