# CORRELATION OF SPECTOMETRIC METHODS IN HARD TISSUE HEAVY ELEMENTS CONCENTRATION STUDY

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*Monitorizarea şi cercetarea mediului şi a expunerii oamenilor la factori poluanţi se bazează pe măsurători directe pe probe prelevate din ţesuturi umane şi din mediu. Autorii şi-au propus o analiză a concentraţiei de Sr, Pb, Cd, Hg, Zn, Fe, Ni, Cr, Mo, Al şi Cu în ţesuturi dentare (dinţi extraşi) pe probe prelevate de la persoane expuse în timpul istoriei recente din aceeaşi zonă geografică. Folosind metoda complementară EDS cuplată metodei SEM este posibilă o analiză compoziţională cantitativă a elementelor mai uşoare decât Na, care completează rezultatele obţinute prin EDPXRF. Metabolismul metalelor grele în ţesuturile umane dure poate fi evaluat prin studii individuale efectuate pe grupuri ţintă din aceeaşi zonă.*

*Environment monitoring and exposure research are based on direct measurements in humans on samples taken from human tissues and from the environment. The authors have proposed an analysis of Sr, Pb, Cd, Hg, Zn, Fe, Ni, Cr, Mo, Al and Cu concentration in dental tissues (extracted teeth) on samples taken from individuals exposed during recent history in the same area. Using the complementary EDS method coupled to SEM method allows a quantitative compositional analyzes regarding elements that are lighter than Na, which completes the results obtained by EDPXRF. The heavy metal metabolism in the human hard tissues can be assessed by individual studies made on targeted groups from the same living area.*

**Keywords**: SEM/EDS, EDPXRF, heavy metals, human hard tissues

## 1. Introduction

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Many chemical elements are essential to the human body functions while others are toxic. Due to the toxicity of some chemical elements the need to control their levels in human organs and tissues appears [1]. Knowing the relationship between observable toxic effects and concentration of elements in the human body and the environment becomes essential [2, 3].

Environment monitoring and exposure research are based on direct measurements in humans on samples taken from human tissues and the environment [2]. Throughout childhood and a great part of adult life, exposure to the elements due to environmental and occupational sources lead to increasing concentrations in calcified tissues, their study reflecting the integrated or the cumulative exposure [4, 5].

Considering the data analysis and the facts summarized above, the authors have proposed an Sr, Pb, Cd, Hg, Zn, Fe, Ni, Cr, Mo, Al and Cu concentration in dental tissues (extracted teeth) on samples taken from individuals exposed during recent history in the same area. This may result in the hazard estimation due to the activities carried out in recent decades in this area. In addition to the mentioned elements, the concentrations of major elements (Ca, P, Na, Mg, K, Si) in teeth were determined. In order to obtain a comprehensive traceability, tissue samples from 12 individuals of both sexes divided into three groups, were analyzed. The first group consisted of 4 young people under the age of 8 years, second group included people aged between 25 and 35 years, and the last group consists of people older than 65 years. Chronically ill people, smokers or persons occupationally exposed to metals were excluded from the analysis. It has also been taken into consideration, that most authors test the metal concentrations in whole teeth without differentiating their characteristic parts [6].

To achieve these goals, two methods based on energy dispersive spectrometry (EDS) technique were used. The first method was based on X-ray microanalysis coupled to scanning electron microscopy (SEM), which allows identification of elemental concentrations from B to U, and the second method involved energy dispersive spectrometry with fluorescence polarized radiation (EDPXRF), used for the determination of trace elements from Na to U. The two selected methods complementarity's allowed a complete elemental compositional analysis of hard tissues collected for this purpose.

The wavelength dispersion radiation fluorescence spectroscopy (WDXRFS) seems to be the most suitable for the determination of heavy metals due to the X-ray power sources and its better resolution, but newly, by EDPXRFS method, comparable results at a lower level equipment price were obtained [7-9]. EDPXRF method can be considered as one of the most useful non-destructive analytical techniques for estimating metal pollution of soils, but also to obtain a relationship between soil composition, food and heavy metal concentration in human bones [10].

Depending on the type of hard tissue, heavy metals bone remanence is from two to 30 years, while in the blood is approximately one month [11, 12]. Heavy metal toxicity pharmacokinetics means that from the age of two heavy metals present in the blood begin to fix in the bone cells and by age of four their remanence in bone exceeds the one burden back into the blood [13]. After this age, the measured quantities of heavy metals in blood indicates recent exposure, while heavy metals from the teeth and bones indicates long-term exposure [14]. By adolescence, approximately 75% of heavy metals is deposited in bones [15]. At maturity this figure rises to 90-95% for those who were exposed outside professional activity [14].

## 2. Materials and Methods

SEM/EDS method was chosen to assess the morphology of the studied bones and Ca/P ratio. XRF method is proposed for the heavy elements analysis and its accuracy is improved by usage of polarised radiation [16].

XRF method can be used due to their quantitative advantages compared with other analytical methods. The method has advantages related to the rapid and simple sample preparation, the simultaneous determination of elements from Na to U, the analite concentration in large scale from 1ppm to 100% and very low equipment cost. Ideally XRF measurements should give reproducible results well correlated with the heavy metal human body intoxication [6]. The EDPXRF can be considered as one of the most useful non-destructive analytical techniques to estimate heavy metal pollution, and to obtain a good relation between the soil pollution, food products and heavy metal concentration in human bones [8].

The EDPXRF instrumentation used for bone elemental analysis was a SPECTRO equipped with a 50W Rh and a Si-drifted detector with a resolution of 148eV (1000cps Mn Kα) (from University Politehnica from Bucharest). The XEPOS 3D geometry is designed for exciting sample X-ray fluorescence with polarized radiation aimed to improve analytical sensitivities [10]. This design leads to a better peak to background ratio and therefore better sensitivity. The sample chamber was purged with helium during data acquisition to lower the radiation absorption and scattering. The obtained spectra were evaluated with TURBOQUANT software package matrix effects which will occur are taken into account. The higher analysis exactness of this method is based on three ways of fluorescence excitation e.g. the light elements Na-V are excited using a HOPG target (intense monochromatic polarized X-rays), the elements Cr-Zr and Pr-U are excited using a Mo secondary target (intense monochromatic non polarized X-rays) and the high-energy elements Y-Ce are excited using a Barkla $Al_2O_3$ target (intense polychromatic polarized X-rays) [17].

After sterilization, the samples were grounded in ball mill in three stages. The imaging analysis after each of the stage is illustrated in the Fig. 1. Each stage allowed reducing gradually the dental tissue particle dimensions until reaching the optimal size for XRF method analysis, in order to significantly reduce the matrix factor [18, 19].

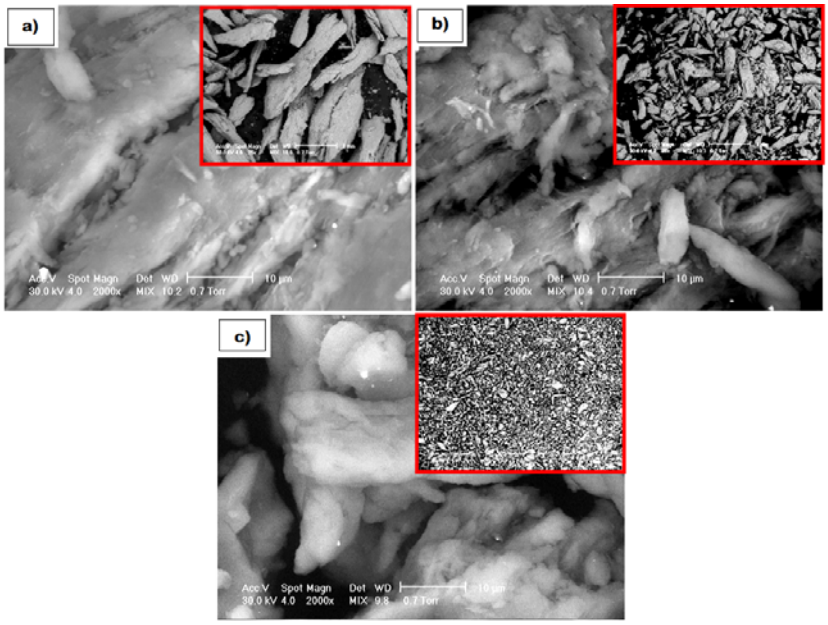


Fig. 1: ESEM morphological aspect of the samples prepared using W milling balls after first step a), intermediary step b), and final step c).

Following a first stage mill grinding, elongated particle of millimetre order length were obtained. Secondary stage grinding reduced the particle to submillimetre size and milling continued. In the final stage we obtained optimum particles size for XRF analysis. Also, the finally obtained powder presented rounded particle morphology. After grinding step, the samples were placed in special sample holders with Mylar window in order to avoid artefacts appearance during EDS analysis [19, 20]. The same samples were investigated in terms of chemical composition by X-ray microanalysis; X-ray radiation emission spectra obtained are shown in Fig. 1. EDS spectral results presented in Fig. 2, confirm the stoichiometric composition of the standard samples and provide a Ca/P ratio of the studied hard tissue samples. In order to assess the heavy elements concentration, 12 human sampled teeth were analysed by EDPXRF using the same procedure. Each sample was analysed three times in repetitive condition to assess the precision of the analytical results. Each analytical data for a sample is

provided on the base of the three spectra that are normalized before estimating the elemental concentrations.

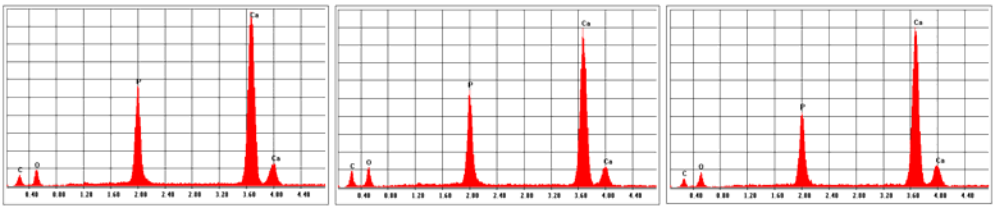

Fig. 2: EDS spectral results examples on each analyzed group.

The typical spectra obtained from one of the subjects bone samples, is shown in Fig. 3.

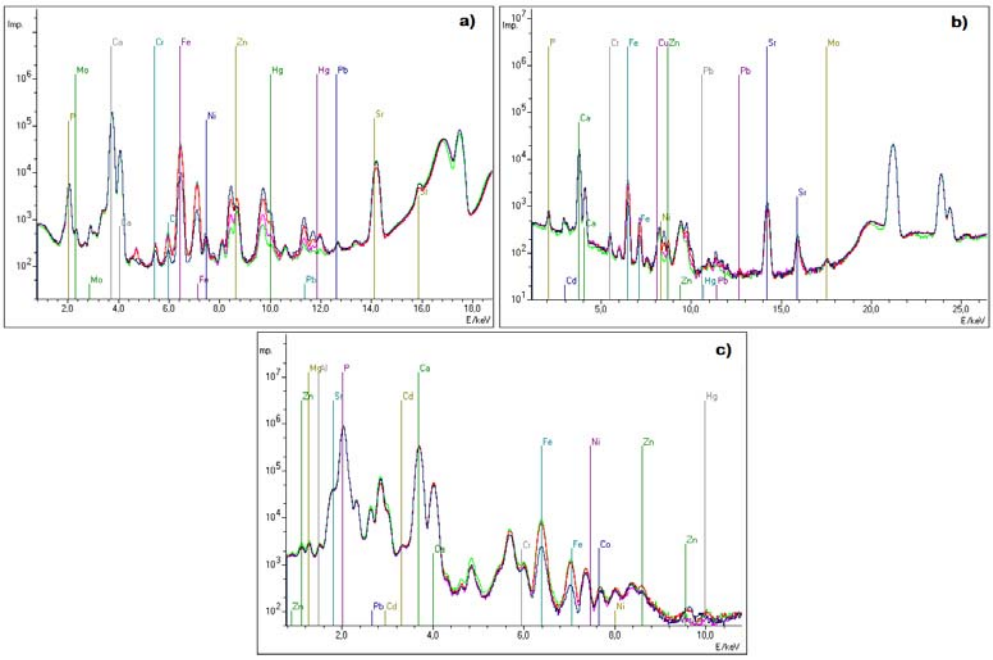


Fig. 3: EDPXRF spectra obtained using different secondary targets: a) $Al_2O_3$; b) Mo; c) HOPG.

The Turboqunt program takes into account the fundamental parameters of fluorescence excitation and the coincidence of the characteristics lines in all the above spectra for a higher exactness assessing of elemental concentrations. From these spectra, it is clear that hard tissue sample contains the well known major elements as Ca, K, Si, Cl, S, Na, Mg, but minor ones as Fe, Ni, Cu, Zn, Al, Sr, Pb, Cd, Hg, etc. Figure 4 shows EDPXRF spectra obtained on a sample from first group (<8 years), compared with one obtained from the second group (24÷35 years), respectively with the third one (>65 years).

## 3. Results

The average elemental concentrations of the samples give a significant picture of the heavy metal burden of the population in the region but more important suggest metabolic differences among younger and older population regarding heavy metal storage in hard tissues. Thus the Hg, Pb but Fe, Ni burden of older people samples is greater than that of younger ones. The RSDs of older people concentrations are frequently greater than the younger correspondent ones. It means that the variability of the metabolism of hard tissues of older people sample is greater than that of younger ones. The EDPXRF average elemental concentration of the three group samples is presented in Table 1.

*Table 1:*

EDPXRF average elemental concentration

| Group | Sample | Na | Mg | K | Si | P | Ca | Al | Fe |
|---|---|---|---|---|---|---|---|---|---|
| | | [%] | | | | | | [ppm (mg/kg)] | |
| Group 1 | 1 | 0.515 | 0.602 | 0.199 | 1.23 | 12.02 | 24.14 | 320 | 836 |
| | 2 | 0.569 | 0.424 | 0.210 | 1.34 | 13.34 | 26.16 | 166 | 659 |
| | 3 | 0.446 | 0.702 | 0.203 | 1.25 | 12.18 | 25.37 | 416 | 621 |
| | 4 | 0.400 | 0.654 | 0.203 | 1.23 | 11.69 | 25.07 | 144 | 855 |
| Group 2 | 5 | 0.314 | 0.145 | 0.192 | 1.32 | 10.32 | 24.38 | 117 | 509 |
| | 6 | 0.410 | 0.219 | 0.192 | 1.03 | 12.55 | 24.24 | 95 | 760 |
| | 7 | 0.451 | 0.457 | 0.193 | 1.26 | 9.62 | 22.68 | 119 | 725 |
| | 8 | 0.174 | 0.354 | 0.196 | 1.24 | 11.21 | 23.66 | 112 | 429 |
| Group 3 | 9 | 0.281 | 0.083 | 0.231 | 1.11 | 12.57 | 24.36 | 95 | 183 |
| | 10 | 0.370 | 0.082 | 0.192 | 0.94 | 10.74 | 24.56 | 63 | 113 |
| | 11 | 0.298 | 0.085 | 0.185 | 0.99 | 10.69 | 23.13 | 91 | 262 |
| | 12 | 0.380 | 0.180 | 0.186 | 1.25 | 12.31 | 24.20 | 97 | 98 |
| Group | Sample | Zn | Cu | Cr | Ni | Mo | Sr | Pb | Cd |
| | | [ppm (mg/kg)] | | | | | | | |
| Group 1 | 1 | 161 | 15.7 | 6.7 | 5.5 | 4.1 | 251 | 8.1 | 3.12 |
| | 2 | 142 | 17.0 | 5.7 | 5.0 | 5.3 | 291 | 8.5 | 2.31 |
| | 3 | 135 | 17.6 | 11.2 | 14.4 | 4.7 | 235 | 9.5 | 4.62 |
| | 4 | 182 | 17.8 | 4.5 | 6.9 | 4.2 | 239 | 9.1 | < 2.0 |
| Group 2 | 5 | 95 | 12.7 | 3.9 | 3.5 | 3.0 | 222 | 8.8 | < 2.0 |
| | 6 | 91 | 13.3 | 4.1 | 4.7 | 3.7 | 202 | 7.5 | < 2.0 |
| | 7 | 94 | 10.8 | 4.2 | 3.4 | 3.5 | 221 | 7.1 | < 2.0 |
| | 8 | 104 | 12.8 | 4.3 | 4.8 | 3.9 | 203 | 7.3 | < 2.0 |
| Group 3 | 9 | 77 | 10.3 | 2.1 | 1.7 | 2.7 | 127 | 6.3 | < 2.0 |
| | 10 | 68 | 9.0 | 2.2 | 0.9 | 2.8 | 144 | 6.7 | < 2.0 |
| | 11 | 81 | 10.3 | 3.7 | 2.5 | 2.4 | 86 | 6.2 | < 2.0 |
| | 12 | 73 | 9.0 | 1.6 | 2.5 | 2.9 | 100 | 6.6 | < 2.0 |

On the first group (<8 years) in the case of heavy elements concentration we obtained a mean value of 2.5 ppm in the Cd case, 8.8 ppm Pb and 0.9 ppm Hg. On the second group (25÷35 years) Cd was not detected, a mean value of 7.6 ppm for Pb, the presence of Hg also being not detected for this group. Regarding the third group (>65) Cd and Hg was not detected, and a mean value of 6.5 ppm was found in the case of Pb.

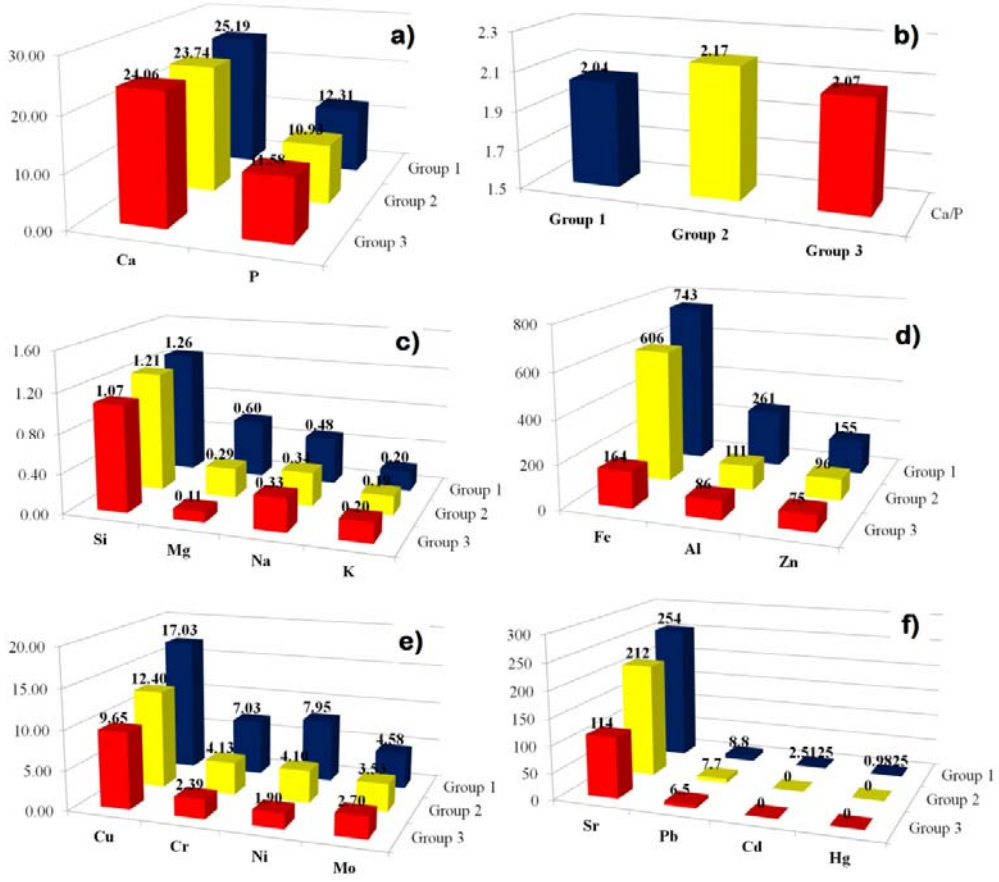


Fig. 4. Comparative results on the detected elements by groups.

The comparison of the values obtained for these selected groups for the Cd, Hg, Pb and Sr concentration in the analyzed samples is presented in Figure 4.f). Through the same method we analyzed also the P, and Ca concentration, the mean value comparison for the two groups being presented in figure 4,a).

The other detected elements comparison between the mean values for each group is presented in figure 4c, d, e. Cadmium average concentration taken from our results is higher than the ones determined in the bones by other authors [21, 22]. The lead concentration in the hard tissues assayed in our investigations varies much for the three groups but its concentrations reported by different authors also range widely [23]. For example, the average lead concentrations presented in other paper varies from 6.2 to 9.5ppm [24, 25]. The average concentration of iron in the teeth was similar to that assayed by other authors [24] and several times higher than the one found in country side population [22]. The concentration of copper in the teeth ranged from 9.0μg/g to 17.8μg/g, reaching 12.4μg/g on

average, and was much higher than the values assayed by other authors [22, 25] of 3.6μg/g. The average Ni content in teeth of the analyzed population was close to that observed in other investigations [22].

The EDPXRF obtained results show that there is a significant difference between the three selected groups on one hand in the case of heavy metals concentration and also on the other detected elements. In the case of heavy elements we can notice higher values for the third group meaning that the teeth elements accumulation in time is bigger than the elements elimination.

## 4. Discussions and conclusions

One of the limitations of XRF method is given by the amount of detected scattered photons. Because the human body is composed of light elements, incoherent scattering is the dominant photon interaction. The low Z number matrixes scattering, leads to a background signal having high width and depth. The concentration of the element of interest is usually small, of the μg/g order, which implies a very small signal/background ratio because the characteristic X-ray peaks overlap to a large distribution of the background signal scattered radiation. It is therefore essential to improve this ratio by increasing the net signal and/or to reduce the background signal. However, the constructive use of the incoherent scattered photons, it can be tried. For most of the biological materials and also when heavy elements from the human hard tissues are determined, the amount of incoherent scattered photons is proportional to the analysed volume mass, so a normalization can be performed; than, the coherent scattering can be used to measure the bone tissue mineral content.

Reducing background signal may be done by the use of partially polarized photons (e.g. produced by coherent primary photons scattering at 90°). However, the incoherent scattering has been described as one of the best ways to improve the relationship between the fluorescence and scattered radiation for low Z matrixes.

XRF technique can be used to determine the heavy metals concentration in human hard tissues and its relation to more traditional indicators of exposure to heavy metals (blood and urine). In the case of suspected heavy metals poisoning their bone concentration determination is very important. In the case of lead poisoning, XRF technique gives an idea of the amount of lead remanence in bone, indicating the risk of future lead bone endogenous exposure. In the case of cadmium and mercury the XRF technique still has some limitations, but surely they will disappear in time. The applied method in the case of cadmium has the lowest dispersion and can already be used for measurements on the general population. In the case of mercury is a bit more delicate, because only the subjects with very high exposure have shown detectable levels.

Using the complementary EDS method coupled to SEM method allows a quantitative compositional analyzes regarding elements that are lighter than Na, which completes the results obtained by EDPXRF.

The heavy metal metabolism in the human hard tissues can be assessed by individual studies made on targeted groups from the same living area. The interpretation of the data in order to assess a conclusion related to the influence of the living area of the subject on the metallic level induced the need of a data base creation that should contain pre-operative data recording such as: age, weight, previously diseases, diagnosis, smoker or non-smoker. The choice of the three well defined groups helped us to conclude there is a certain influence of the age of the subject on the quantity of heavy metal in particularly as this represented the goal of this paper but also of the rest of metallic elements detected in the bone structure.

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