ABOUT ORAL HEALTH OF ROMANIAN CHILDREN FROM VARIOUS POLLUTED AREA DUE TO HEAVY METALS

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În cadrul acestei lucrări este prezentat un studiu referitor la procesul de demineralizare al dinților primari, întâlnit la copiii proveniți din zone cu riscuri mediu diferit. Probele dentare au fost prelevate de la subiecți ce locuiesc în ora□e cu nivel ridicat de poluare. Determinarea ionilor de metale grele din dinții temporari, s-a realizat utilizând spectrometrul de masă cu plasmă cuplată inductiv (ICP-MS); rezultatele sunt discutate în legătură cu caracteristicile de suprafață evaluate prin microscopie de forță atomică (AFM) □i microscopie electronică de baleiaj (SEM).

This paper presents a study regarding the process of human primary teeth demineralization, found in children from areas with different environmental risks. The dental samples were taken from children who live in areas with high level of pollution. The determination of heavy metals ions in primary teeth was carried out by inductively coupled plasma mass spectrometry (ICP-MS); the results are discussed in connection with surface features evaluated from atomic force microscopy (AFM) and scanning electron microscopy (SEM).

Keywords: Oral health; Demineralization process; Primary teeth; Heavy metals; Pollution level

1. Introduction

Heavy metals can be present in significant concentrations in air, water sources and food which affect oral health of both adults and children. In fact, contamination of soil [1], water, and agricultural products by heavy metals resulting from industrial activities has caused major concern all over the world [2]. Chemicals released to the environment may distribute in water, air and soil. From there, they may be taken up into plants, accumulate in the human food chain and affect the health of humans, and in particular of children [3, 4]. The exposure of children to environmental chemicals is greater than that of adults, and in terms of risk assessment, children should be regarded as a particularly vulnerable group [5].

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Humans usually have 20 deciduous teeth. Ten are found in the upper maxilla and the other ten in the lower mandible. In the primary set of teeth, there are two types of incisors, centrals and laterals, and two types of molars, first and second. They can provide information about development, nutrition, and physiological stress, exposure to disease, pollution and residential mobility [6]. The mineralization of the deciduous teeth starts at an early stage of intrauterine life, many studies indicating that the concentrations of the elements in the deciduous teeth reflect the mineral balance of the organism resulting from intrauterine nutrition. Numerous authors have observed elevated concentrations of metals in the placentas of women living in environmentally hazardous - areas or those smoking cigarettes [7].

Dental caries are an ecological disease. Its case is increasingly associated with dietary changes in modern society, and it was still one of the common chronic diseases in the world [8]. Hippocrate, the father of European medicine (V a. hr. century) defined health as an “equilibrium between mind, body and environment“. In the ancient world the concept “a healthy mind in a healthy body” was accepted as a problem of strategy in life [9].

A number of metals may be accumulated in calcified tissues. Interestingly, small amounts of heavy metals elements are common in our environment and diet and are actually necessary for a good health, but large amounts of any of them may cause acute or chronic toxicity [10].

Calcium can be partially substituted by a small amount of heavy metals, like in a bone. The way metals are accumulated in calcified tissue also reflects the interaction between elements [11, 12].

As dental hard tissues are relatively stable, the metals stored in teeth during the mineralization are to a large extent retained. Thus, the level of metals in teeth can be used to estimate especially the long term environmental exposure.

The negative influence of the heavy metals on the teeth of human beings, especially young, is determined by the dosage of heavy metals, the exposure time and the subject’s individual immunity [13].

Biocompatibility assays have showed that the deciduous teeth are good markers for estimating the long term environmental exposure. In the carious teeth there are toxic substances which affect fibroblast viability in a time depending manner, and the level of teeth degradation is related to the environmental pollution.

As the teeth decay such as carries could be associated with demineralization with calcium substitution by heavy metals, it is possible to presume that the levels of selected metals differ significantly inside the roots of carious and non–carious teeth. It is not yet a clear answer regarding this question and is a need of more research in this field, but some literature data sustain the association of heavy metal content with dental health factors [14, 15].

In this context, the present paper is focused on the influence of heavy metals on primary teeth and on the dissolution and demineralization process caused by different kind of drinks.

Atomic force microscopy (AFM) provides not only three–dimensional information about morphology, but quantitative information regarding surface morphology and mechanical properties, with minimal sample preparation [16-18]. Studies on the morphological changes in enamel surface have been widely investigated through scanning electron microscopy (SEM) [19, 20].

2. Experimental part

2.1 Methods

2.1.1 Preparation of the specimen

The research material consisted of 93 primary teeth from patients within 5–12 years old. Teeth were collected by dentists from University of Medicine "Carol Davila", Bucharest, Romania.

The teeth were collected from groups of children living in two regions with various pollution areas regarding the level of heavy metals (48 from Pătârlagele city and 45 from Slatina city). As reference area with less pollution, was selected a small village in a mountain region (Pătârlagele). As an area with environmental risk, Slatina city (industrial area with high level pollution risk) was chosen.

The collected teeth were soaked 24 h in distilled deionized water to remove any debris. The samples were dried at a room temperature of 23°C and then they were put into Teflon vessels. Each tooth was divided in two parts.

The first part was used for ICP–MS measurements, in order to observe the heavy metals concentration.

Each specimen was digested in 50 mL concentrated nitric acid ULTRAPURE, Fa. Merck). After digestion, the sample was diluted and liquid fraction was analyzed by ICP-MS.

The second part was used for the study of physical process of dissolution and demineralization. From the second part were selected only teeth with the values of heavy metals almost the same as the average values of such metals. Selected teeth were soaked in a drink with sugar and in a sugar free drink (pH ~ 2.8) for 15 minutes. Tooth specimens were weighed before and after immersion, using a laboratory analytical balance. They ranged from 0.189 to 0.735g. After immersion, the topographical analysis of each specimen was carried out by Atomic force microscopy (AFM) and Scanning Electron Microscope (SEM) measurements [21].

2.1.2 ICP-MS measurements

The determination of calcium, copper, lead, cadmium, chromium, iron, manganese and zinc concentrations was performed using a Perkin Elmer ELAN DRC-e - inductively coupled plasma mass spectrometry (ICP–MS). Calibration function for the ICP-MS spectrometer was generated using a multi–element standard [22, 23]. In this way were determined the differences in the calcium concentration of the drinks arising from calcium released from the tooth pieces during immersion.

2.1.3 AFM measurements

Surface roughness of the specimen was evaluated with atomic force microscopy (AFM) in order to complete confocal microscopy information about the investigated teeth [24]. Contact mode AFM images were obtained using an atomic force microscope from APE Research, Italia. Data acquisition and image processing were performed with Gwyddion software.

2.1.4. SEM analysis

For SEM analysis an Enviromnental Scanning Electron Microscope FEI/Phillips XL30 ESEM was used.

2.1.5. Statistical results

Statistical results were calculated using Data Analysis from Microsoft Office Excel. The correlation matrix of the metal concentrations present in carious and non–carious deciduous teeth was obtained using „Correlation" function from Analysis Tools – Microsoft Office Excel.

3. Results and discussion

3.1 ICP-MS results

Experimental ICP-MS data obtained from primary teeth samples are presented in Table 1 for both target groups with specification of tooth level degradation (carious and non-carious teeth).

As can be seen from Table 1, the experimental data has shown that the amount of heavy metals released from teeth is greater in industrial area than in reference area. The higher concentrations of zinc found in teeth could be also related to incidence of caries. This result is in agreement with this of Harris *et al.* [25]. By quantitative X-ray fluorescence imaging of sections of human teeth they found an increase of zinc concentrations in carious regions of teeth when compared with unaffected portions of the tooth.

Table 1

Average values of metals concentration (ppb) in temporary teeth

Metal	Pătârlagele, n = 48		Slatina, n = 45	
	Carious teeth	Non–carious teeth	Carious teeth	Non–carious teeth
Ca	38221	41675	33712	37529
Cd	0.06	0.04	0.13	0.08
Co	0.07	0.02	0.102	0.09
Cr	1.22	0.8	1.27	1.10
Cu	0.3	0.08	0.72	0.41
Fe	81.35	99.02	68.025	97.23
Mg	3389	4373	3009.68	3744.8
Mn	0.54	0.41	1.54	1.05
Pb	0.05	0.02	0.075	0.063
Zn	221.36	435.78	199.44	385.60

A correlation analysis was carried out (Table 2 and 3) in order to show the nature of correlations between the elements in the structure of hard tissues in carious and non–carious deciduous teeth.

Table 2

Co-occurrence of metals in deciduous carious and non-carious teeth from Pătârlagele

	Pb	*Cd*	*Cu*	*Cr*	*Mn*	*Fe*	*Zn*	*Mg*	*Ca*
Pb	1								
Cd	-1	1							
Cu	-1	1	1						
Cr	-1	1	1	1					
Mn	1	-1	-1	-1	1				
Fe	1	-1	-1	-1	1	1			
Zn	1	-1	-1	-1	1	1	1		
Mg	1	-1	-1	-1	1	1	1	1	
Ca	1	-1	-1	-1	1	1	1	1	1

The resulting statistically significant positive correlation coefficients indicate that the elements exert a mutual effect on their concentrations in the hard tissues of the teeth. It can be seen an inversely proportional correlation between the concentrations of cadmium and zinc, cadmium and magnesium, cadmium and iron, chromium and manganese, chromium and zinc, chromium and magnesium and a positive correlation between the main elements that build teeth– calcium and magnesium.

Table 3

Co-occurrence of metals in deciduous carious and non-carious teeth from Slatina

	Pb	*Cd*	*Cu*	*Cr*	*Mn*	*Fe*	*Zn*	*Mg*	*Ca*
Pb	1								
Cd	1	1							
Cu	1	1	1						

Cr	1	1	1	1					
Mn	-1	-1	-1	-1	1				
Fe	-1	-1	-1	-1	1	1			
Zn	-1	-1	-1	-1	1	1	1		
Mg	-1	-1	-1	-1	1	1	1	1	
Ca	-1	-1	-1	-1	1	1	1	1	1

3.2 Demineralization and dissolution results

According to literature data [26] the surface of apatite crystals (inorganic parts of tooth) is in dynamic equilibrium with the adjacent aqueous phase. The following series of equilibrium describe the process:

$$\begin{aligned} &Ca_{10}(PO_4)_6(OH)_2 \leftrightarrow 10Ca^{2+} + 6PO_4^{3-} + 2HO^- \\ &PO_4^{3-} + 3H^+ \leftrightarrow HPO_4^{2-} + 2H^+ \leftrightarrow H_2PO_4^{3-} + H^+ \leftrightarrow H_3PO_4 \end{aligned} \quad (1)$$

Moreover, is known that for a pH<5.5 apatite can be dissolved and a process, known as demineralization. Saliva can act as a natural buffer medium, serving to neutralize acid and to restrict the dissolution process. For pH >5.5 apatite can be established from salivary calcium and phosphate (PO_4^{3-}). This is the remineralization process [26].

To assess the effect of drink with sugar or free of sugar on healthy teeth, gravimetric analysis was used for the determination of the average loss rate of material according to the relation 2. In table 4 weight loss in time is listed.

$$r = \Delta m / S \cdot t \quad (2)$$

Table 4

Average loss rate of material

Average loss rate of material (g/mm^2 year)	Slatina		Pătârlagele	
	Carious	Non–carious	Carious	Non–carious
Drink with sugar	0.00285	0.00190	0.00282	0.00187
Free of sugar drink	0.00278	0.00178	0.00276	0.0176

As can be seen from Table 4, the weight loss in time is more pronounced when sugar drinks are used; if the tooth is from a zone with a higher level of pollution then it is even more evident. The loss for carious teeth was higher than these of healthy teeth. Similar results were obtained by Sollbohmer *et al.,* [27] using AFM technique.

Regarding calcium evaluation with ICP–MS, both types of drinks have an initial concentration of calcium of about 32000 ppb. The concentrations after immersion are presented in Table 5.

Table 5

Metals concentration (ppb) of drinks after tooth immersion

Metals	Carious tooth immersed in free of sugar drink	Carious tooth immersed in drink with sugar	Non–carious tooth immersed in free of sugar drink	Non–carious tooth immersed in drink with sugar
Ca	32284	29932	35562	33427
Cd	0.18	0.22	0.08	0.11
Co	0.35	0.70	0.12	0.10
Cr	1.29	1.27	1.21	1.88
Cu	1.09	2.32	1.00	0.42
Fe	42.20	66.21	23.05	42.20
Mg	2660.9	2162.9	2754.2	2545.5
Mn	0.93	1.41	0.73	1.21
Pb	0.15	0.23	0.09	0.10
Zn	105.41	85.12	185.17	115.11

According to Table 5 data, the calcium concentration increased in the drinks after teeth immersion. This fact is due to the dissolution of hydroxyapatite from the tooth in this solution. The composition of the drinks was not constant during the experimental procedure. Thus, when hydroxypatite is dissolved, calcium phosphate and hydroxide ions will be released from the tooth substance into the drinks. The pH of drinks changes consequently from an initial value of about 2.8 to 5.75.

Results for enamel dental tissues have shown no significant differences between non-carious and carious teeth for the elements calcium and magnesium. For enamel tissues the concentrations of manganese and zinc were higher in carious teeth than those obtained for healthy ones. This result may be related to the superior structuring of the hard tissue in the non–carious teeth comparing to the carious teeth.

3.3 AFM results

AFM technique was used in order to evaluate the evolution of surfaces roughness before and after immersion for 15 minutes in drinks (figures 1–4). Roughness parameters are presented in Table 6 and 7.

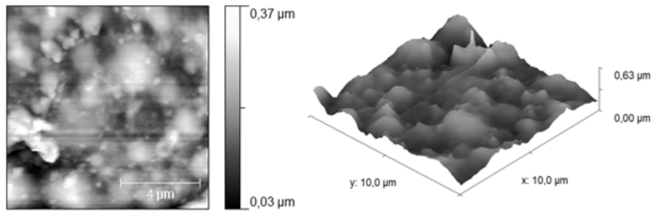

Fig.1. AFM images (2D, 3D) of a carious tooth before immersion in drink with sugar

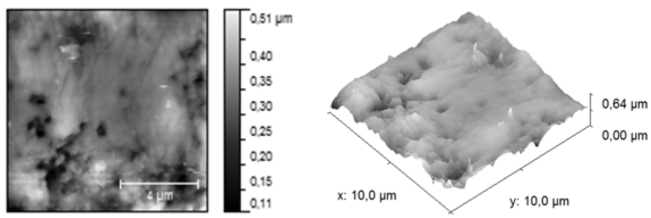

Fig.2. AFM images (2D, 3D) of a non - carious tooth before immersion in drink with sugar

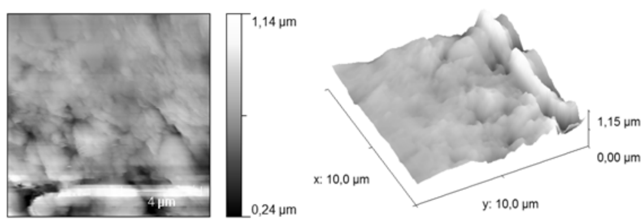

Fig.3. AFM images (2D, 3D) of a carious tooth after immersion in drink with sugar

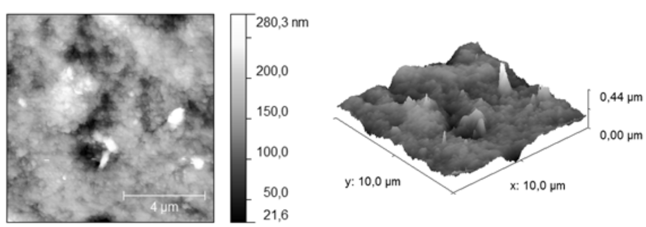

Fig.4. AFM images (2D, 3D) of a non - carious tooth after immersion in drink with sugar

Table 6

Roughness parameters before a tooth immersion from reference area

AFM Parameters	Carious tooth	Non–carious tooth
Selected area [µm]	10 x 10	10 x 10
Roughness Ra [nm]	74.5	0.196
Square average roughness Rms [nm]	95.1	0.254

Table 7

Roughness parameters after a tooth immersion from reference area

AFM Parameters	Carious tooth	Non–carious tooth
Selected area [µm]	10 x 10	10 x 10
Roughness Ra [nm]	93.5	0.314
Square average roughness Rms [nm]	112.6	0.379

As can be seen, the roughness is higher for carious tooth after immersion (figure 3) than for carious tooth before immersion (figure 1). We can conclude that unlike free of sugar drinks, sugar drinks cause a higher increase in roughness.

3.4 SEM results

Roughness parameters for the tooth surface, before and after immersion are presented in Table 6 and 7. Basically surface roughness is defined as the change in the profile of the surface in which the height and the depth of ridges and valleys vary in the nanometer order.

The figures 5 (a) and (b) obtained using SEM show images of a non-carious tooth before and after immersion in drink with sugar. Figure 6 shows enamel from primary teeth before and after one week immersion in drinks with sugar.

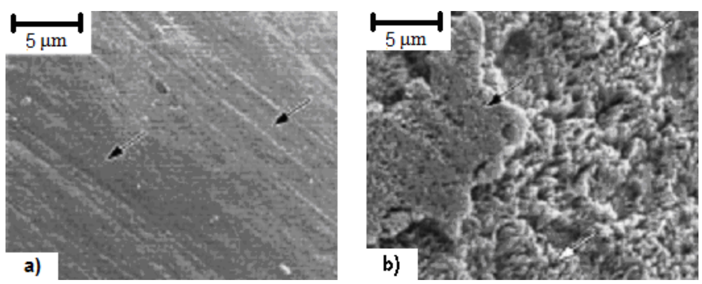

Fig.5. SEM images for a non-carious tooth before (a) and after immersion (b)

Figure 5 (a) shows the results for a non–carious tooth. The initial surface is generally smooth, while various scratches can be observed in a specific direction according to arrows. Figure 5 (b) revealed an extensively eroded enamel surface for healthy tooth, after immersion in drink with sugar. The higher magnification showed enhanced porosity and prism irregularity caused by the action of sugar.

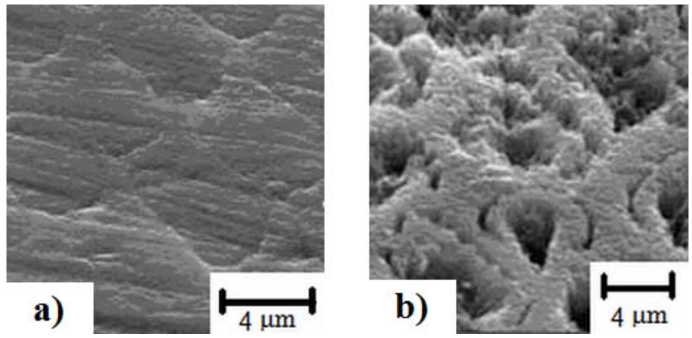

Fig.6. SEM images of enamel (a) before and (b) after 1 week of immersion in sugar drinks

For carious teeth, SEM examination of the resulting primary enamel surfaces showed that demineralization is initiated at core (prism or rod)/wall (prism sheath) interfaces, as can be seen in figure 6 (b).

4. Conclusions

A comparison between tooth samples coming from a reference non-polluted area and those coming from area with environmental heavy metals risks shows that heavy metal concentrations in primary teeth are bigger in the area with higher level of pollution; furthermore, significant differences in heavy metals were observed for enamel of carious and non-carious teeth.
Average concentration of lead in teeth from children living in industrial area is approximately 48% higher than that corresponding to the reference region.

The average loss of material in time after immersion in various drinks is more pronounced in the case of sugar drinks. It is even more evident if the tooth is from a zone with a higher level of pollution.
A higher weight loss was observed in the case of carious teeth than that of healthy teeth. This result may be related to the superior structuring of the hard tissue in the non–carious teeth comparing to the carious ones.

The calcium concentration increase in the drinks during the teeth immersion is due to dissolution of hydroxyapatite from tooth and it is an argument of demineralization process.

The association between environmental risk, sugar drinks and level of oral children health suggests that the accurate knowledge of children nutrition could help in preventing children dental carries especially in the more polluted area.

Acknowledgements

The authors wish to acknowledge the CNMP supporting the above research through the Grant PN2 41-005/2007. The first author wishes to thank the

Sectorial Operational Programme Human Resources Development 2007-2013 of the Romanian Ministry of Labour, Family and Social Protection through the Financial Agreement POSDRU/88/1.5/S/60203.

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