Analysis of Trace Elements in Teeth by ICP-MS: Implications for Caries

Mohamed A. Amr* and Abdul Fattah I. Helal

Nuclear Physics Department, NRC, Atomic Energy Authority, Cairo, 13759, Egypt

*****Corresponding author: amrewis@hotmail.com

Abstract: *Teeth are good indicators of environmental exposure to heavy metals and of nutritional status. Inductively coupled plasma-mass spectrometry (ICP-MS) was used to compare the content of trace elements in primary and permanent teeth. For this purpose, primary teeth were collected from 64 children and 112 permanent teeth were collected from 40- to 60-year-old adults. The data were assessed statistically using t-tests. We found that, in comparison to primary teeth, permanent teeth contained significantly higher concentrations of Na, Mg, Al, Fe, Ni, Cu, Sr, Cd, Ba, Pb and U and significantly lower concentrations of Mn, Co, As, Se, Mo and Bi. In addition, a comparison of the concentrations of trace elements in the pulps of individuals with healthy vs. carious teeth showed that the mean concentrations of Na, Al, Cr, Mn, Co, Cu, Zn, Mo, Ag, Bi and U were lower in those with carious teeth. However, the concentrations of Mg, Cd and Pb in the pulps were higher in individuals with carious teeth than in those with healthy teeth.*

Keywords: ICP-MS, teeth, trace elements, caries, teeth pulps

1. INTRODUCTION

Biomonitoring of trace elements in human teeth has become an important tool to evaluate an individual's nutritional and environmental status.[1–5] Primary teeth are easily obtained because they naturally exfoliate as the permanent teeth erupt. Variations in the content of trace elements in the teeth have been previously demonstrated.[1] Trace elements can be ingested by humans via different routes, including ingestion in the food and water or by deliberate consumption of soil and by dermal absorption. The presence and/or absence of trace elements in the environment influences their availability to humans.[1] For example, in the rural areas of Finland, the concentrations of Zn and Mg found in human dentine correlate with their concentrations in the soil,[6] and dental fluorosis has been linked to the concentrations of fluoride (F^-) and Pb present in the drinking water.[1,4]

The concentration of Pb in the teeth can be used as an index of environmental pollution.[7] This element is preferentially incorporated and stored in calcified tissues, such as the teeth.[8] A Pb concentration above 4 mg/kg in the teeth has been suggested as being indicative of Pb toxicity.[5,9] Here, we have investigated the importance of the location of trace elements in the teeth, with a focus on Pb. For this purpose, we have used various techniques, including the use of microprobes. Our results indicate that trace elements are systematically but inhomogeneously distributed within the enamel and dentine of teeth.[10]

Sources of Cd include emissions from fuel combustion, tobacco smoke, phosphate fertiliser, sewage sludge, metal smelting, and disposal of metal waste. Other sources include the use of Cd for industrial applications, such as in the production of pigments, stabilisers, and alloys. In addition, Cd is present in trace amounts in certain foods, such as leafy vegetables, potatoes, grains and seeds, liver and kidney, and crustaceans and molluscs. Exposure to environmental Cd has been linked with an increased risk of dental caries.[11]

Other studies have demonstrated that nutritional deficiencies can be detected by analysing the chemical composition of teeth and that such deficiencies can affect teeth during dentition, which is a critical growth period for teeth.[6–8,10–15] Differences in the availability of foods and food choices, which are often influenced by social and cultural practices, may be important factors in determining which trace elements are ingested by humans and can complicate any attempts at changing dietary habits to alter the intake of trace elements. Shashikiran *et al.*[15] found that, among different ethnic groups, there is a lot of variation in the concentrations of trace elements in teeth enamel.

Teeth pulp occupies the centre of each tooth and consists of soft connective tissue (Fig. 1). Adult individuals normally have 32 pulp organs in the permanent teeth, while primary teeth contain 20 pulp organs.[16] The primary and permanent teeth pulps have a number of characteristics that are morphologically similar. Each pulp organ resides in a pulp chamber that is surrounded by dentin and which contains the peripheral extensions of the cells that formed it. Ide-Ektessabi *et al.*[17] examined the distribution of trace elements in the layers and in the pulp of two teeth by X-ray fluorescence using synchrotron radiation microbeams. They found that the border of the dentine and the pulp contain high concentrations of Ca, Pb and Zn. Pinheiro *et al.*[18] investigated how the environment and dietary habits influence the accumulation of trace elements in the teeth. For this purpose, they determined the concentrations of different elements in the enamel, dentine crown, dentine root and the pulp in nine teeth using synchrotron microprobe radiation. Also using synchrotron microprobe radiation, Carvalho *et al.*[19] analysed 40 molar teeth that were previously restored with dental amalgams to determine how the major components of the amalgam (Cu, Zn and Hg) diffuse into the structures of the teeth. Curson and Crocker[20] determined the concentrations of Zn and Pb in the pulp of the teeth.

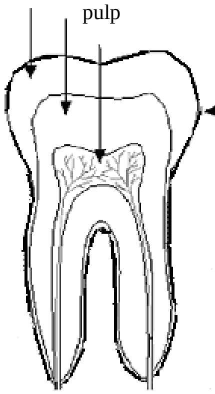

Figure 1: Schematic diagram of a human molar.

El-Kanayat City, Egypt is a rural area, and its inhabitants obtain their livelihood from agriculture. In addition, the inhabitants of this city drink underground water, and 99% of the population cooks their meals using metallic utensils of poor quality. As such, the content of trace elements in the environment and the foods grown there are likely to be enriched in certain elements, such as Mg. The aim of this study was to examine the relationship between exposure to trace elements and dental caries in teeth collected from inhabitants of El-Kanayat City. We compared primary and permanent teeth as well as the pulps of carious and healthy, permanent teeth.

2. EXPERIMENTAL

2.1 Instrumentation

An inductively coupled plasma-mass spectrometer (ICP-MS) was used to measure the concentrations of trace elements. ICP-MS (JMS-PLASMAX2, JOEL, Japan) was optimised by using a standard ^{89}Y solution at a concentration of 10 ppb in 2% HNO_3. The same 2% HNO_3 solution, without ^{89}Y, was used to clean the tubes between measurements. To perform the measurements, we used a microconcentric nebuliser with a desolvation introduction system (ARDIUS, CETAC, USA). Table 1 shows the operating parameters used for the ICP-MS measurements.

Table 1: Experimental conditions used for the ICP-MS measurements.

RF power	950 W
Reflected RF power	< 2 W
Sampling depth	5 mm
Coolant gas flow rate	14 l/min
Auxiliary gas flow rate	0.3 l/min
Nebuliser gas flow rate	0.95 l/min
Sample uptake rate	0.6 l/min
Mass resolution (m/Dm)	300
Accelerating voltage	6 kV applied on the sampler and skimmer
Acquisition mode	Peak area
Introduction system	Desolvation (ARDIUS)
Optimisation	Maximum ion intensity of 10 ppt of ^{89}Y

2.2 Collection and Preparation of Primary and Permanent Teeth

A total of 64 primary teeth of all types (incisors, canines and molars) were collected from 5 to 12-year-old children and 112 permanent teeth from 40- to 60-year-old adults; all participants lived in EI-Kanayat, Egypt. Table 2 summarises the details of the number and health status of the teeth collected. Whenever possible, we used caries-free primary and permanent teeth to compare the trace elements concentrations in both types. The teeth were soaked in H_2O_2 to remove connective tissue, washed with deionised water and dried overnight. The teeth were individually ground and weighed and then digested in 10 ml of HNO_3 using a microwave digestion system (Ethos 1600, Milestone Inc., USA).

Table 2: Summary of the number of teeth and teeth pulps analysed.

	Incisors	Canines	Molars
Primary teeth	11	8	45
Healthy permanent teeth	2	3	36
Carious permanent teeth	3	7	61
Healthy teeth pulps	2	3	36
Carious teeth pulps	–	4	58

2.3 Extraction and Preparation of Teeth Pulps

The permanent teeth were classified into two groups; healthy and carious. Immediately after their extraction from the adult subjects, the crowns of the teeth were removed and the pulps were collected. As shown in Figure 2, the pulp tissues were removed using a broach file (size 20). The pulps were then washed with 1% ultra-pure HNO_3, dried at 60°C and digested in a mixture of

Figure 2: Shematic diagram of tooth pulp extraction obtained by broach filling.

Table 3: Concentrations of tace elements (ppm) in bovine liver (NIST1577b).

Element	Certified value	Measured value	Error (%)
Na	2420±60	2428±45	0.33
Ca	116±4	113±5	2.59
K	9940±20	9899±22	0.41
Mg	601±28	609±24	1.33
Al*	3	2.9±0.5	3.33
Mn	10±0.5	10.5±0.6	5.00
Co	0.25	0.27±0.08	8.00
Fe	184± 5	185±11	0.54
Cu	160±8	159±17	0.63
Zn	127±16	126±19	0.79
As*	0.05	0.04±0.006	20.0
Se	0.73±0.06	0.77±0.05	5.48
Rb	13.7±1.1	14.2±1.5	3.65
Sr	0.136±0.001	0.127±0.001	6.62
Mo	3.5±0.3	3.4±0.5	2.86
Ag	0.039±0.007	0.042±0.006	7.69
Cd	0.5±0.03	0.48±0.05	4.00
Pb	0.129 ± 0.004	0.133±0.019	3.10

*Note:** Non-certified values.

HNO_3 (250µl, 69%) and H_2O_2 (100µl, 30%) using a microwave digestion system. After digestion, the pulp samples were cooled in a water bath and brought up to a volume of 1 ml using deionised water (18.2 MW). Bovine liver (NIST1577b), obtained from National Institute of Standard and Technology (NIST) was used as a standard for quality assurance. Table 3 shows the concentrations of trace elements in bovine liver.

2.4. Statistical Analysis

The data were assessed using the Tukey test with a 95% confidence level. *p* values of <0.05 were considered statistically significant. Outlying values, which were rejected based on their D-values [$D = (X_n - \bar{X})/S$; where X_n is a value, $\bar{X}$ is the mean, and S is the standard deviation], were omitted from the analysis in order to make general conclusions.

3. RESULTS AND DISCUSSION

3.1 Trace Elements in Primary and Permanent Teeth

The results obtained from the analysis of trace elements in primary and permanent teeth are summarised in Table 4. Table 4 shows the concentrations of known essential elements (such as Cu, Mg and Zn), possible essential elements (such as Sr) and elements with no known biological role and/or that are potentially toxic (Al, Cd, Ba, Pb and U) found in primary and permanent teeth. We found a statistically significant difference in the concentrations of Na, Mg, Al, Zn, Sr, Pb, Cd and Ba between permanent and primary teeth. The concentrations were higher in permanent teeth compared to primary teeth. Additionally, the concentration of Mn, Mo, Ag and Bi also showed statistically significant differences in primary teeth compared to permanent teeth. The concentrations were lower in permanent teeth compared to primary teeth. We found no statistically significant differences in the concentrations of Cr, Fe, Cu, Ag and U between primary and permanent teeth.

Table 4: Concentrations of trace elements (ppm) in healthy primary and permanent teeth.

Element	Primary teeth	Permanent teeth	*p*-value
	Mean ± SD (range)	Mean ± SD (range)	
Na	5454±950 (4211–7280)	9000±120 (7054–10030)	0.0003*
Mg	1755 ± 340 (1043–2750)	2800 ± 478 (1500–3700)	0.0009*
Al	17.9±12.3 (3–69.5)	51.4±18.2 (27.5–84)	0.0013*
Cr	0.04± 0.01 (0.005–2)	0.05±0.03 (0.03–0.11)	0.1992
Mn	5.5±2 (2.5–8.5)	0.27±0.11 (0.09–1.2)	0.0033*
Fe	80.1± 16.5 (28.9–135)	94±23 (65.3–122)	0.0915
Cu	6.4 ± 4.8 (2–11.3)	9.2 ±11.4 (1.4–26.1)	0.1257
Zn	133 ± 30 (85–166)	178±44.6 (124.6–235.7)	0.0011*
Sr	87±11.3 (60.9–114)	101.2±24.3 (70.2–130)	0.0167*
Mo	1.8±0.29 (1.02–2.33)	0.044±0.012 (0.031–0.067)	0.0007*
Ag	0.08± 0.03 (0.004–1.9)	0.009±0.002 (0.005–0.012)	0.1583
Cd	0.00011±0.00001 (0.00007–0.00014)	0.012±0.004 (0.0084–0.016)	0.0012*
Ba	7.8±3.2 (2.06–13.8)	9.5±5.4 (5.11–17.97)	0.042*
Pb	1.2±0.89 (0.34–4.01)	6.26±1.24 (0.6–9.23)	0.0061*
Bi	23±2.34 (16.3–28.5)	0.22±0.09 (1.10–3.01)	0.0061*
U	0.005±0.002 (0.001–0.052)	0.011±0.005 (0.0012–0.041)	0.3485

*Note: *p ≤ 0.05*

3.2 Trace Elements in the Pulps of Healthy and Carious Teeth

The pulp contains the nerves and blood vessels of the teeth. The blood vessels feed the tooth and keep it alive and healthy. The nerve endings inside the pulp send signals to the brain about the activity that occurs in the tooth. The concentrations of trace elements found in the teeth often provide information on deficiency or disease status, poisoning or contamination. The trace elements most widely encountered in the teeth include Al, Cd, Ba, K, Li, Mg, Mn, Na, Pb and Sr. Some of these, such as Cd and Pb, can potentially be toxic. Table 5 shows the concentrations of trace elements found in the pulps of the teeth.

Table 5: Concentration of trace elements (ppm) in permanent healthy and carious teeth pulps.

Element	Healthy teeth pulp	Carious teeth pulp	*p*-value
Na	1793±342	1385±705	0.2881
Ca	82336±8980	121814±1718	0.0186*
Mg	2733±589	6285±1981	0.0015*
Al	1029±172	1413±422	0.0069*
Cr	42.40±6.29	33.99±9.14	0.0101*
Mn	24.04±4.23	29.84±9.81	0.0325*
Fe	5.85±1.12	1.22±0.36	0.0010*
Cu	270±44	234±59	0.0044*
Zn	1383±254	1303±318	0.1049
Sr	82.09±17.05	106±28	0.0095*
Mo	2.31±0.19	3.29±0.99	0.0136
Ag	55.91±10.41	26.07±6.84	0.0045*
Cd	1.98±0.27	6.70±1.18	0.0019*
Ba	18.94±2.05	34.60±8.43	0.0047*
Pb	29.23±4.13	77±21.2	0.0031*
Bi	35.98±3.47	12.55±3.67	0.0037*
U	0.91±0.38	0.97±0.28	0.3270

*Note: *$p \leq 0.05$*

We found a statistically significant difference in the concentrations of Al, Mg, Sr, Cd, Ba and Pb between the pulps of healthy and carious teeth. The concentrations were lower in healthy teeth pulps than carious teeth pulps. On the other hand, we found that the concentrations of Cr, Fe, Cu, Ag and Bi also showed statistically significant differences in the pulps of carious teeth compared to those of healthy teeth. However, there was no statistically significant difference in the concentrations of Na, Zn and U between primary and permanent teeth. Moreover, after normalising the mean concentrations of trace elements to that of Ca, we found that the concentrations of Al, Cr, Mn, Co, Fe, Ni, Cu, Zn, Sr, Mo, Ag, Bi and U are lower in the pulps of carious teeth than in those of healthy teeth, and the mean concentrations of Mg, Cd, Ba and Pb were higher in the pulps of carious teeth than in those of healthy teeth (Fig. 3).

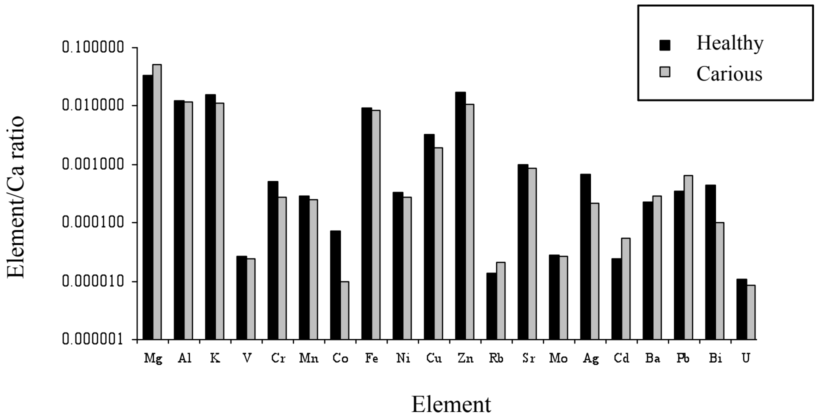

Figure 3: Comparison of the trace element/Ca ratio in teeth pulps.

The information on the concentrations of trace elements present in the pulps of teeth is scarce and contradictory.[18–20] Carvalho *et al.*[19] measured the concentrations of Mn, Fe, Cu, Zn and Pb in the pulps of restored and healthy teeth using a synchrotron microprobe. They found that the concentrations of Mn, Fe, Cu, Zn and Pb in the pulps of restored teeth were 5±3, 16±6, 43±20, 570±120 and 43±25, respectively. In contrast, the concentrations of Mn, Fe, Cu, Zn and Pb in pulps from healthy teeth were 1.7±1.2, 2±111, 2.3±1.4, 286±50 and 46±15, respectively. Also, the concentration of Zn in teeth pulp from miners and fishermen was found to be 541 ppm and 583 ppm, respectively[18], and in pulps from healthy and restored teeth the concentrations were 286 ppm and 570 ppm, respectively[19]; these concentrations of Zn are within the normal range (218–520 ppm) for healthy teeth.[20] In our study, we found that the concentrations of Zn measured in the pulps of healthy and carious teeth were 138±3254 and 1303±318 ppm, respectively.

As shown in Table 5 Mg is one of the most abundant elements in the pulp of carious teeth. The mean concentrations of Mg in the pulps of healthy and carious teeth were 2733±589 and 6285±1981 ppm, respectively. The element important for the functions of nerves and muscles, as well as for the regulation of blood pressure is Na.[21] An important ion that is found in the intracellular fluid of cells is K; along with Na, K is important for the maintenance of electrical potential in the nervous system and, therefore, for the efficient functioning of nerves and muscles. The mean concentrations of Na in healthy and carious permanent teeth were 1793±342 and 1385±705 ppm, respectively. The concentrations of Cd in the pulps of healthy and carious teeth were 1.98±0.27 and 6.70±1.18 ppm, respectively. Shearer *et al.*[22] showed that rats exposed to Cd during the neonatal period developed severe dental caries and that this effect was not prevented by adding F^- to the drinking water.

4. CONCLUSION

Here, we analysed the concentrations of 16 trace elements in whole permanent teeth and pulps. The concentrations of Mg, Cd, Pb and Ba were higher in permanent teeth compared to primary teeth and higher in carious teeth pulps compared to healthy teeth pulps. Moreover, the ratios of the concentrations of Mg, Cd, Pb and Ba to those of Ca were higher in pulps from carious teeth than in those from healthy teeth. According to these observations, we conclude that the trace elements Mg, Cd, Pb and Ba are positively associated with caries.

5. ACKNOWLEDGEMENT

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