

Technical paper

# Flame Atomic Absorption Spectrometric Determination of Iron, Magnesium, Strontium and Zinc in Human Teeth Using La + K Mixture

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## Abstract

Flame atomic absorption spectrometric determination of iron, magnesium, strontium and zinc in 44 deciduous human teeth collected from the people with different ages was carried out by using K + La chemical mixture. Optimum concentration and concentration ratio of K + La mixture on the absorbance values of analytes in the Bone Ash SRM 1400 (NIST) were studied. Detection limits (LOD) of analytes in the absence or presence of K + La mixture and its components were determined and compared. Detection limits in the presence of K + La mixture are  $11 \mu\text{g L}^{-1}$  for Fe,  $8.0 \mu\text{g L}^{-1}$  for Mg,  $6.3 \mu\text{g L}^{-1}$  for Sr and  $18 \mu\text{g L}^{-1}$  for Zn. With K + La mixture proposed and FAAS, Bone Ash SRM 1400 material was analyzed and the results found were in agreement with the certified values. The percent recoveries were increased up to 102% for analytes. La + K mixture proposed was applied to the determination of Fe, Mg, Sr and Zn in tooth samples.

**Keywords:** Tooth; FAAS; La + K mixture, metals

## 1. Introduction

Tooth, a part of skeleton, is a bio-indicator of great interest because it contains information on exposure to essential or toxic elements that become deposited in the tooth material.<sup>1,2</sup> The metals enter the human body through ingestion and inhalation. The intake via ingestion depends on food habits.<sup>3</sup> Iron, Mg, Sr and Zn essential elements occur naturally in most fresh vegetables, meats, grains and eggs.<sup>4</sup> Teeth can also accumulate such essential metals and can be taken into consideration when evaluating environmental pollution.<sup>5</sup> Determination of these metals in human teeth is important to evaluate the level of body supply with essential trace elements.<sup>2</sup> While these metals are essential, they can be toxic and leads to very harmful effects when taken in excess. Strontium deficiency affects bone strength and tooth resistance to caries. Strontium excess results in severe bone deformation. The Sr content of teeth indicates exactly the level of the element accumulation in bone.<sup>2</sup> It was shown that the zinc

concentration in teeth depended on its content in the diet and in the environment. The deficiency or increase of Zn concentration may be taken as indication of some illness.<sup>2,6</sup>

Various analytical methods have been applied for the determination of elements in tooth samples such as inductively coupled plasma-atomic emission spectrometry (ICP-AES) and ICP-MS.<sup>7</sup> Flame atomic absorption spectrometry (FAAS) is one of the most suitable techniques for the determination of analytes such as Fe, Mg, Sr and Zn in human teeth and bones, and biological samples due to its high sensitivity, specificity and simplicity.<sup>4,5,8,9</sup> However, interferences such as Ca, Na and P,<sup>7</sup> and high background signals in human tooth may occur in the direct determination of Fe, Mg, Sr and Zn.

The purposes of the present investigation were to develop a method for the determination of essential elements in tooth samples by FAAS using K, La and La + K mixture to reduce background signals and chemical interferences and to apply this method for the determination of

analytes in tooth samples. Lanthanum, releasing agent, was used to react with P in tooth samples to obtain free atoms of analyte elements such as Ca, Mg and Sr. Potassium was also used to prevent the ionizations of these analytes in samples.<sup>9,10</sup> Bone Ash SRM 1400 was analyzed in order to verify the accuracy of the method proposed. The concentrations of analytes in tooth samples may be undertaken in an effort to provide baseline data for healthy Turkey population.

## 2. Experimental

### 2.1. Instrumentation

A Hitachi Model 180/80 flame atomic absorption spectrometer (Japan) equipped with a Zeeman effect background corrector and automatic data processor was used to obtain all absorbance values of analytes throughout. Iron (248.2 nm wavelength), Mg (285.2 nm), Sr (460.7 nm) and Zn (213.8 nm) hollow cathode lamps were used as radiation sources. Slit widths and lamp currents used are 1.3 nm for Fe and Zn, 2.6 nm for Mg and Sr, and 10 mA for elements studied. Acetylene-air flame was used. Instrumental parameters and operating conditions for analytes were used as recommended by the manufacturer unless otherwise stated. All absorbance signals of analytes were carried out by integrated absorbance (5 s) mode throughout. Milestone Ethos Sel microwave oven (MLS Ethos 1600, Italy), equipped with temperature and pressure sensors, and Teflon digestion bombs and vessels, was used in order to dissolve the samples.

### 2.2. Materials, Reagents and Standards

All acids and reagents were of analytical-reagent grade. Ultra pure water (resistivity 18 M $\Omega$  cm) from an ultra pure water system (Nanopure Infinity, Barnstead, P/N-1161, Dubuque, USA) was used to prepare all solutions. 65% (w/w) nitric acid, 35% (w/w) H<sub>2</sub>O<sub>2</sub> and 37% (w/w) HCl from Merck (Darmstadt, Germany) were used to dissolve the samples and for dilution. Plastic bottles, Teflon vessels, and glassware materials were cleaned by soaking in 20% (v/v) HNO<sub>3</sub> for a day, rinsing three times with ultra pure water and drying. All solutions prepared were stored in high-density polypropylene bottles.

Potassium stock solution (30 g L<sup>-1</sup>) was prepared by dissolving 7.8 g KNO<sub>3</sub> (Merck) in 1% (v/v) HNO<sub>3</sub> and diluting to 100 mL with ultra pure water. Lanthanum stock solution (50 g L<sup>-1</sup>) was prepared by dissolving 8.84 g LaCl<sub>3</sub> (Merck) in 5% (v/v) HCl and diluting to 100 mL with ultra pure water.

Iron, Mg, Sr and Zn stock standard solutions (1.0 g L<sup>-1</sup>) (BDH chemicals, Poole, UK) were used and successively diluted to prepare working standard solutions using 0.2% (v/v) HNO<sub>3</sub>.

### 2.3. Collection of Samples

Forty – four deciduous teeth from 44 individuals, all of which required extraction for orthodontic reasons, were collected from the Turkish Atomic Energy Agency (TAEA) and Dental Faculty of Ankara University, Turkey. Whole tooth without filling was taken for analysis to reduce the risk of contamination from mechanical operation.<sup>11</sup> The age range of humans concerned was from 8 to 64. Twenty of the subjects were males and the others were females. Bone Ash SRM 1400 was used for the optimization of the method.

### 2.4. Decomposition of Samples

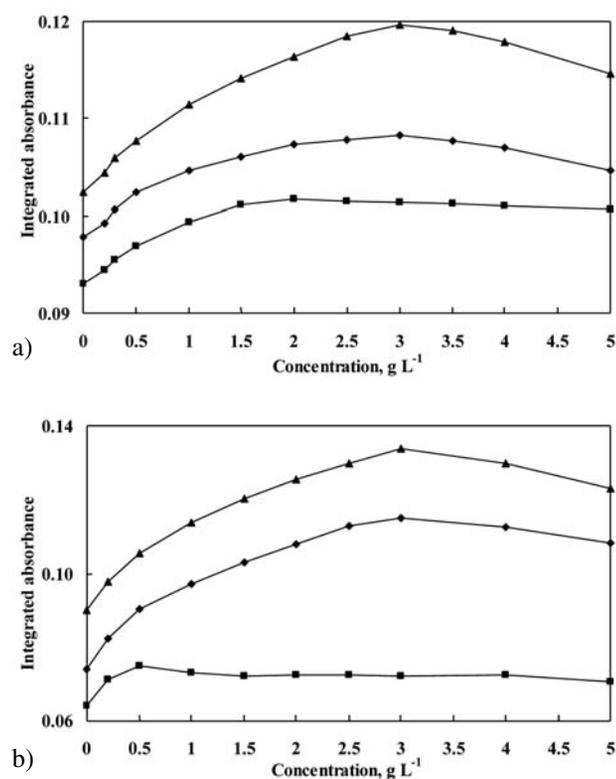
Tooth sample was put into a Teflon beaker and waited ten minutes in 5% (v/v) H<sub>2</sub>O<sub>2</sub> in order to clean outer surface of it. Sample was rinsed three times with ultra-pure water.<sup>12</sup> Samples were dried in an oven at 80 °C for 2 hours and weighed. After each tooth sample (the range of mass being from 0.6 to 2.4 g) or a portion of Bone Ash SRM 1400 (0.5–1.0 g) was accurately weighed into a Teflon digestion vessel with a cover, 3 mL of mixture of 65% (w/w) HNO<sub>3</sub> plus 30% (v/v) H<sub>2</sub>O<sub>2</sub> (2:1)<sup>13</sup> and 1 mL of H<sub>2</sub>O were added to each sample and it was left overnight at laboratory temperature in order to dissolve the sample without heating.<sup>4,11–14</sup> White suspended particles were observed above the solution. Samples were decomposed by using Milestone Ethos Sel microwave oven according to the procedures described in previous works.<sup>4,15–17</sup> The digestion vessel was closed and placed inside the microwave oven. Steps of microwave program applied were the heating from laboratory temperature to 140 °C for approximately 40 min and holding for 20 min (up to 700 W), and turned off the microwave and waiting for 20 min. After cooling to laboratory temperature, the vessel was opened and placed on a hot plate. Sample was gently heated at about 100 °C to evaporate nearly to 2 mL.<sup>11</sup> When a residue of sample material was remained, the decomposition procedure described was repeated to dissolve completely. The final solution was transferred to 10, 25 or 50 mL volumetric flasks by washing interior surface of digestion vessel with 2% (v/v) HNO<sub>3</sub> three times and the final acidity of solution was adjusted to 0.5% (v/v) HNO<sub>3</sub>. Blank solutions were prepared by using the procedure given above in order to observe the differences in dissolution of samples.

## 3. Results and Discussion

### 3.1. Modification in FAAS

Effects of concentration and concentration ratio of K, La and La + K modifier mixture on absorbance values of analytes in Bone Ash SRM 1400 solution were investigated. One mL of Bone Ash SRM 1400 solution contained

$26.4 \pm 1.08 \text{ mg L}^{-1}$  of Fe,  $2.39 \pm 0.05 \text{ mg L}^{-1}$  of Mg,  $36 \pm 1.1 \text{ mg L}^{-1}$  of Sr and  $7.24 \pm 0.12 \text{ mg L}^{-1}$  of Zn and appropriate concentrations of K ( $0\text{--}30 \text{ g L}^{-1}$ ), La ( $0\text{--}50 \text{ g L}^{-1}$ ) or K ( $0\text{--}30 \text{ g L}^{-1}$ ) + La ( $0\text{--}50 \text{ g L}^{-1}$ ) were added to each 10 mL volumetric flask and diluted to the mark with deionized water. While the concentration of analytes ( $2.64 \pm 0.11 \text{ mg L}^{-1}$  of Fe,  $0.24 \pm 0.01 \text{ mg L}^{-1}$  of Mg,  $3.60 \pm 0.11 \text{ mg L}^{-1}$  of Sr and  $0.72 \pm 0.01 \text{ mg L}^{-1}$  of Zn) in each solution was kept constant, the concentrations of K, La and K + La mixture solutions were varied as explained. These solutions were given into flame atomizer. Absorbance values of analytes versus concentration and concentration ratio of K, La and La + K mixture obtained by using integrated absorbance mode were plotted and they were shown in Figure 1 as an example. As can be seen in Figure 1,  $3.0 \text{ g L}^{-1}$  La,  $2.0 \text{ g L}^{-1}$  K and  $(3.0 \text{ g L}^{-1} \text{ La}) / (2.0 \text{ g L}^{-1} \text{ K})$  for Mg, and  $3.0 \text{ g L}^{-1}$  La,  $0.5 \text{ g L}^{-1}$  K and  $(3.0 \text{ g L}^{-1} \text{ La}) / (0.5 \text{ g L}^{-1} \text{ K})$  for Sr were found to be optimum concentration and concentration ratio of La, K and La + K mixture. No significant differences in absorbance values for Fe and Zn with various concentrations of La, K and La + K mixture were observed. Therefore, optimum mass ratios of La + K obtained for Mg were also used for the determination of Fe and Zn in samples. Standards, blanks, reference material and tooth samples were analyzed by using La + K mixture with mass ratios given above.



**Figure 1.** (a) Effect of concentration of La, K and La with fixed  $2 \text{ g L}^{-1}$  of K on absorbance values of Mg ( $0.24 \pm 0.01 \text{ mg L}^{-1}$ ), and (b) Effect of concentration of La, K and La with fixed  $0.5 \text{ g L}^{-1}$  of K on absorbance values of Sr ( $3.60 \pm 0.11 \text{ mg L}^{-1}$ ) in Bone Ash SRM 1400. La ( $\blacklozenge$ ), K ( $\blacksquare$ ), and La + K ( $\blacktriangle$ ).

### 3. 2. Analytical Characteristics

Calibration graph method was used for the determination of Fe, Mg, Sr and Zn in Bone Ash SRM 1400 and tooth samples in the presence of La + K mixture. Standard addition method was used for some samples. Linear ranges of calibration graphs for aqueous standard solutions of analytes are  $0\text{--}1.0 \text{ mg L}^{-1}$  for Mg and Zn, and  $0\text{--}6.0 \text{ mg L}^{-1}$  for Fe and Sr. Correlation coefficients ( $r$ ) of all calibration graphs and standard addition methods for the analytes studied were higher than 0.99.

Limit of detection (LOD, concentration of analyte related to the three times standard deviation of blank solution or minimum concentration of analyte in sample solution) is important for the sensitivity of the proposed method and it might be influenced by instrumental parameters.<sup>17,18</sup> The detection limits of analytes were determined with La, K, and La + K mixture and without from 20 consecutive measurements of a  $0.5\%$  (m/v) bone ash 1400 solution.<sup>19,20</sup> The LOD values found are given in Table 1. As can be seen, the lowest detection limits were obtained with La + K mixture. As a consequence, La + K mixture was recommended for the determination of Fe, Mg, Sr and Zn in tooth samples.

**Table 1.** Detection limits (LOD) of analytes in absence or presence of K, La and La + K mixture ( $3S_b$ ,  $n = 20$ )

| Element  | LOD ( $\mu\text{g L}^{-1}$ ) |     |     |    |
|--|------------------------------|-----|-----|----|
|  | Fe                           | Mg  | Sr  | Zn |
| No   | 27                           | 16  | 18  | 31 |
| $2.0 \text{ g L}^{-1} \text{ K}^a$                                   | 23                           | 13  | 11  | 19 |
| $3.0 \text{ g L}^{-1} \text{ La}$                                    | 18                           | 11  | 9.7 | 15 |
| $3.0 \text{ g L}^{-1} \text{ La} + 2.0 \text{ g L}^{-1} \text{ K}^a$ | 11                           | 8.0 | 6.3 | 18 |

<sup>a</sup>  $0.5 \text{ g L}^{-1}$  K for only Sr.

### 3. 3. Analysis

The Bone Ash SRM 1400 was analyzed without and with the optimum concentration ratio of La + K mixture proposed in order to validate the accuracy and performance of the method. It contains  $38.18 \pm 0.13\%$  Ca and  $17.91 \pm 0.10\%$  P as major elements. La + K mixture that provided higher absorbance values for analytes in Bone Ash SRM 1400 and tooth samples was used to prevent flame ionization of analytes and the effect of phosphate interferences. The Bone Ash SRM 1400 and a tooth solution chosen were analyzed using K + La mixture proposed for the method validation. A tooth sample solution dissolved in 50 mL volumetric flask was divided into two equal volumes in two 50 mL volumetric flasks in order to study recovery test. One mL of  $40 \mu\text{g mL}^{-1}$  Fe,  $5 \mu\text{g mL}^{-1}$  Mg,  $80 \mu\text{g mL}^{-1}$  Sr and  $10 \mu\text{g mL}^{-1}$  Zn standard aqueous solutions, respectively, were added into a flask and they were not added to the other flask. Both of them were diluted to

the mark again. The results of analytes found in Bone Ash SRM 1400 and the tooth solution by using parameters optimized are given in Table 2. They are presented as the average  $\pm$  confidence interval (11 degrees of freedom ( $n-1$ ) at 95% confidence level). As can be seen in Table 2, recoveries of analytes obtained with La + K are higher than the recoveries obtained without La + K mixture. The results of analytes obtained with La + K are in good

agreement with the Bone Ash SRM 1400 certified values. Percent recoveries of analytes obtained in samples are in range 97–102%. Standard deviations of results using La + K are lower than those in the absence of La + K.

The method with La + K mixture recommended was applied to the determination of Fe, Mg, Sr and Zn in tooth samples collected from people with different ages. The results found are also presented as average  $\pm$  confidence

**Table 2:** Recovery tests for analytes in Bone Ash SRM 1400 and a tooth sample solution without and with La + K mixture

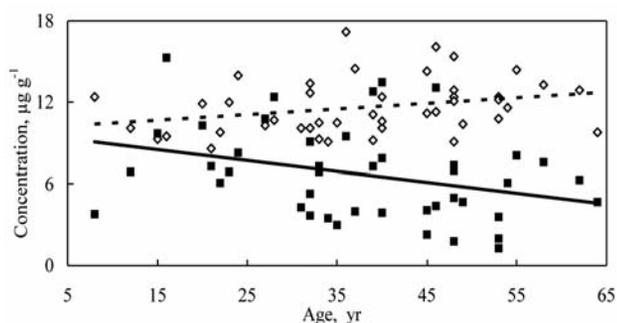
| Element   | Certified <sup>a</sup> /<br>Added <sup>b</sup> | Without La + K <sup>c</sup> | Recovery<br>(%) | With La + K <sup>c</sup> | Recovery<br>(%) |
|---|--|-----------------------------|-----------------|--------------------------|-----------------|
| Bone ash 1400, concentrations, $\mu\text{g g}^{-1}$         |  |                             |                 |                          |                 |
| Fe  | 660 $\pm$ 27                                   | 602 $\pm$ 32                | 91              | 664 $\pm$ 22             | 101             |
| Mg  | 6840 $\pm$ 130                                 | 6361 $\pm$ 163              | 93              | 6836 $\pm$ 124           | 100             |
| Sr  | 240 $\pm$ 7                                    | 216 $\pm$ 11                | 90              | 244 $\pm$ 6              | 102             |
| Zn  | 181 $\pm$ 3                                    | 170 $\pm$ 5                 | 94              | 177 $\pm$ 3              | 98              |
| Tooth sample solution, concentrations, $\mu\text{g L}^{-1}$ |  |                             |                 |                          |                 |
| Fe  | –  | 1.88 $\pm$ 0.09             | –               | 1.96 $\pm$ 0.04          | –               |
|   | 0.8  | 2.44 $\pm$ 0.14             | 91              | 2.81 $\pm$ 0.08          | 102             |
| Mg  | –  | 0.22 $\pm$ 0.01             | –               | 0.28 $\pm$ 0.01          | –               |
|   | 0.1  | 0.30 $\pm$ 0.02             | 94              | 0.38 $\pm$ 0.01          | 97              |
| Sr  | –  | 2.12 $\pm$ 0.11             | –               | 2.38 $\pm$ 0.06          | –               |
|   | 1.6  | 3.31 $\pm$ 0.18             | 89              | 4.02 $\pm$ 0.12          | 101             |
| Zn  | –  | 0.32 $\pm$ 0.02             | –               | 0.40 $\pm$ 0.01          | –               |
|   | 0.2  | 0.46 $\pm$ 0.04             | 89              | 0.60 $\pm$ 0.02          | 100             |

<sup>a</sup> Certified values for Bone Ash SRM 1400; <sup>b</sup> Added values for a tooth sample solution. <sup>c</sup> Mean of 12 replicate measurements with 95% confidence level,  $\bar{X} \pm (2.20x_s)/\sqrt{n}$

**Table 3.** Average ( $\pm$  S.D.) concentrations of metals in teeth of male and female donors in correlation with age

| Age range<br>(yr) | Tooth<br>No | Concentrations, $\bar{X} \pm$ S.D. (Min. value- Max. value) |                               |                                 |                             |
|-------------------|-------------|---|-------------------------------|---------------------------------|-----------------------------|
|                   |             | Fe ( $\mu\text{g g}^{-1}$ )                                 | Mg ( $\text{mg g}^{-1}$ )     | Sr ( $\mu\text{g g}^{-1}$ )     | Zn ( $\mu\text{g g}^{-1}$ ) |
| Male              |             |   |                               |                                 |                             |
| 10 – 20           | 2           | 7.9 $\pm$ 1.9<br>(6.0 – 9.8)                                | 5.9 $\pm$ 0.6<br>(5.3 – 6.5)  | 10.1 $\pm$ 1.0<br>(9.1 – 11.1)  | 182 $\pm$ 34<br>(148 – 216) |
| 21 – 30           | 2           | 7.6 $\pm$ 2.5<br>(5.1 – 10.0)                               | 6.2 $\pm$ 0.8<br>(5.4 – 7.0)  | 10.3 $\pm$ 1.0<br>(9.3 – 11.3)  | 179 $\pm$ 40<br>(139 – 219) |
| 31 – 40           | 7           | 7.4 $\pm$ 3.1<br>(3.7 – 12.8)                               | 6.4 $\pm$ 1.3<br>(5.2 – 8.9)  | 10.8 $\pm$ 2.1<br>(8.3 – 13.8)  | 175 $\pm$ 25<br>(144 – 224) |
| 41 – 50           | 4           | 6.7 $\pm$ 4.3<br>(4.1 – 13.1)                               | 6.6 $\pm$ 0.3<br>(6.4 – 6.9)  | 11.8 $\pm$ 1.3<br>(10.1 – 13.5) | 173 $\pm$ 24<br>(146 – 204) |
| 51 – 60           | 5           | 4.4 $\pm$ 3.2<br>(1.3 – 9.1)                                | 7.3 $\pm$ 0.7<br>(6.6 – 8.1)  | 12.7 $\pm$ 2.3<br>(10.2 – 15.4) | 179 $\pm$ 32<br>(156 – 234) |
| Female            |             |   |                               |                                 |                             |
| 8 – 20            | 3           | 9.8 $\pm$ 5.7<br>(3.8 – 15.3)                               | 5.7 $\pm$ 0.9<br>(5.0 – 6.7)  | 11.3 $\pm$ 1.6<br>(9.5 – 12.4)  | 206 $\pm$ 99<br>(139 – 320) |
| 21 – 30           | 4           | 9.6 $\pm$ 2.5<br>(6.9 – 12.4)                               | 6.1 $\pm$ 1.1<br>(5.1 – 7.2)  | 11.8 $\pm$ 1.7<br>(10.3 – 14.0) | 203 $\pm$ 21<br>(177 – 229) |
| 31 – 40           | 9           | 6.3 $\pm$ 3.7<br>(3.0 – 13.5)                               | 6.3 $\pm$ 0.4<br>(5.5 – 6.9)  | 12.2 $\pm$ 2.8<br>(8.3 – 17.2)  | 201 $\pm$ 49<br>(132 – 390) |
| 41 – 50           | 5           | 4.7 $\pm$ 2.5<br>(1.8 – 7.4)                                | 7.9 $\pm$ 0.6<br>(7.0 – 8.4)  | 12.8 $\pm$ 2.4<br>(9.1 – 15.4)  | 183 $\pm$ 49<br>(130 – 255) |
| 51 – 64           | 3           | 4.5 $\pm$ 2.1<br>(2.1 – 6.9)                                | 8.6 $\pm$ 1.0<br>(6.8 – 10.2) | 13.5 $\pm$ 2.6<br>(10.2 – 16.9) | 173 $\pm$ 42<br>(129 – 221) |

interval as given above. Average ( $\pm$  S.D.) concentrations of analytes in human teeth related to human age are given in Table 3. As can be seen, it was observed that Mg and Sr concentrations gradually increased with the ages of the male or female donors (for 8–64 age ranges) and iron and zinc concentration levels decreased in both males and females. Variation of elemental concentrations of Fe and Sr in the 44 tooth as a function of age of the tooth was shown in Figure 2 as an example. A trend towards increasing Sr concentration and decreasing Fe concentration with age was observed. Concentration range of zinc found is from 120 to 390  $\mu\text{g g}^{-1}$  ( $183 \pm 39 \mu\text{g g}^{-1}$  for males and  $212 \pm 90 \mu\text{g g}^{-1}$  for females) and they are in agreement with the  $293 \pm 34 \mu\text{g g}^{-1}$  at normal conditions.<sup>6</sup> No significant differences in metal levels were observed between men and women teeth at the same age group.



**Figure 2.** Variation in elemental concentration of Fe and Sr in the 44 teeth as a function of age. A trend towards decreasing Fe concentrations and increasing Sr concentrations with age, Sr ( $\diamond$ , ----), Fe ( $\blacksquare$ , —).

## 4. Conclusions

A method for the determination of Fe, Mg, Sr and Zn in human teeth of different populations in Ankara and Bone Ash SRM 1400 using FAAS with Zeeman background correction and La + K mixture was developed and verified. Optimum conditions and recovery studies have shown that the method with La + K mixture provides accurate results. Chemical interferences and ionization effects from the sample matrix are minimal by using La + K modifier mixture and nitric acid in dissolution of samples. Results obtained for Fe, Mg, Sr and Zn accumulation in human teeth show that a direct correlation observed between Mg and Sr concentrations with age, but inversely correlation observed between Fe and Zn concentrations with age, ignoring the sex of individuals. La + K mixture can be applied to the determination of analytes in human teeth after decomposition by nitric acid plus hydrogen peroxide and no chemical treatment is necessary. Teeth are available biological materials and they are good markers of exposure to environmental pollution and control.

## 5. Acknowledgments

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## Povzetek

S plamensko atomsko absorpcijsko spektrometrijo je bila v človeških zobeh različnih starosti proučevana vsebnost železa, magnezija, stroncija in cinka. Za analizo je bila uporabljena zmes K + La za katero so bile proučevane optimalne koncentracije in razmerje K + La ter določene meje detekcije. Te v prisotnosti K + La znašajo:  $11 \mu\text{g L}^{-1}$  za Fe,  $8.0 \mu\text{g L}^{-1}$  za Mg,  $6.3 \mu\text{g L}^{-1}$  za Sr in  $18 \mu\text{g L}^{-1}$  za Zn. Rezultati so se ujemali z vrednostmi standardov. Proučevana metoda z zmesjo K + La je bila predlagana za analizo omenjenih elementov v zobeh.