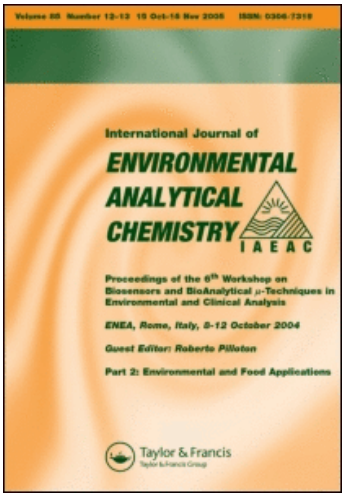

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Cadmium, lead, copper and manganese determination in human deciduous teeth by electrothermal atomic absorption spectrometry using a lanthanum, palladium and citric acid mixture as chemical modifier

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Cadmium, lead, copper and manganese were determined in human deciduous teeth and bone ash 1400 standard reference material by electrothermal atomic absorption spectrometry (ETAAS), using a lanthanum + palladium + citric acid (CA) modifier mixture. Optimum masses and mass ratios of La, La + Pd and La + Pd + CA modifiers for analytes in bone ash 1400 sample solution were investigated. Pyrolysis and atomization temperatures of analytes in a tooth sample solution were obtained with and without modifiers. The mixture of La + Pd + CA was found to be preferable for the determination of analytes in tooth samples and bone ash 1400, dissolved in a mixture of $HNO_3 + H_2O_2$. The detection limits and characteristic masses of analytes were obtained with or without modifiers based on integrated absorbance for tooth sample solution (2% m/v). The detection limits obtained with La + Pd + CA are 6,24,16 and 46 ng g^{-1} for Cd, Cu, Mn and Pb, respectively. Recovery tests for analytes in bone ash 1400 and a tooth solution with La and La + Pd + CA modifier mixture were studied and compared with certified and non certified values. The La + Pd + CA mixture was also applied to the determination of Cd, Pb, Cu and Mn in tooth samples.

Keywords: cadmium; lead; tooth; ETAAS; matrix modification

1. Introduction

Cadmium, lead, copper and manganese determination in human teeth are important for human health [1–6]. Teeth are used as biological markers of exposure to environmental pollution [3,7] and they are particularly well suited to evaluation of long-term exposure to trace elements such as Pb. The lead and cadmium content in teeth are mainly from environmental pollution and they are toxic even in low concentrations [3–13]. The copper and manganese content are related to diet and, although they are essential elements [7,13], an excess can lead to a wide variety of clinical effects [4,5]. Lead can produce deficits in psychological functions such as intelligence and learning ability in humans [4,9]. Cadmium can cause damage to all types of body cell such as the kidneys and the liver [3]. Copper and manganese have important roles in many enzymes [4]. The lack of copper is

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associated with Wilson's and Menke's diseases [4,14]. Bercovitz and Laufer [15,16] found a significant positive correlation between lead level and the donor's age in a non-occupationally exposed population. Therefore, determination of Cd, Pb, Mn and Cu levels accumulated in human teeth is important to evaluate both the degree of poisoning with toxic elements and the level of essential elements in teeth [7,10,13].

Electrothermal atomic absorption spectrometry (ETAAS) is one of the most suitable and popular methods for the determination of trace metals such as Cd and Pb in human teeth and bones due to its inherent high sensitivity, selectivity and low detection limits [3,4,9,12,16–19]. However, some interferences arising from anions and cations, such as Ca and P [12], high background signals and volatilization of analyte together with organic compounds may occur in the direct determination of analytes by ETAAS. Platform atomization, chemical matrix modification, integrated absorbance and a powerful background correction technique have been used to overcome these problems. Different permanent modifiers (W-Rh, W-Ir and W-Ru) [4,20,21] and other suitable modifiers, such as $NH_4H_2PO_4$ [1,9,10], La [19,22] and Pd and CA [23–25] have been used for determinations of elements such as Cd and Pb in various sample matrices in order to stabilize analytes to higher permissible pyrolysis temperatures and to reduce interference effects in sample matrix before atomization steps. La reacts with P in teeth and free atoms of analyte elements are obtained [19]. Pd is a commonly used modifier and CA is used as a reducing agent.

The objectives of this study were to develop a new method for the determination of Cd, Pb, Cu and Mn in bone ash 1400 and human teeth by ETAAS using La + Pd + CA modifier mixture proposed and to analyse real tooth samples. The effects of La + Pd + CA modifier mixture on analytes in sample solutions were investigated to reduce interferences from major elements such as Ca, Fe, Mg, P and F, and background in bone ash 1400 and tooth samples [12]. The proposed La + Pd + CA modifier mixture was applied to the determination of trace elements in tooth samples.

2. Experimental

2.1 *Instrumentation*

All Cd, Pb, Cu and Mn absorption measurements were carried out by a Hitachi Model 180/80 atomic absorption spectrometer (Japan), equipped with a graphite furnace (Hitachi 180/78), Zeeman-effect background corrector and an automatic data processing unit (180/205). Hitachi graphite platforms (P/N-190/6008) inserted into pyrolytic graphite coated graphite tubes (P/N-190/6007) were employed throughout the experiment. Lead (283.3 nm), Cd (228.8 nm), Mn (279.5 nm) and Cu (324.8 nm) (Hitachi) hollow cathode lamps were used as radiation sources. Slit width used for all lamps was 1.3 nm. Instrumental parameters and operating conditions for analytes were used as recommended by the manufacturer unless otherwise stated. A 20 μL volume of calibration or sample solution together with modifier solution was injected into the platform by an autosampler (P/N-170/126). Absorbance signals of analytes were carried out by the integrated absorbance (peak area) mode throughout. Argon (99.99% w/w) was used as the purge gas and interrupted during atomization. A Varian Model 9176 recorder was used in a 20 mV/FS span in order to obtain atomization and background signal profiles. The optimised graphite furnace temperature program and operating conditions for the determination of analytes are given in Table 1. A Milestone Ethos microwave oven

Table 1. Heating program for Cd, Pb, Mn and Cu determinations in tooth sample solutions with different modifiers.

Step	Temperature (°C)	Ramp (s)	Hold (s)	Ar flow rate (mL min^{-1})
Dry-1	50–120	30	–	250
Dry-2	120–200	20	10	250
Pyrolysis	200–Variable[a]	30	20	250
Atomization	Variable[b]	0	5	0
Cleaning	2650°C[c], 2800°C[d]	0	3	250

Notes: [a]See Table 2; [b]Optimum atomization temperatures of Cd, Pb, Mn and Cu found are 1500, 2000, 2500 and 2700°C, respectively; [c]2650°C for Pb and Cd; [d]2800°C for Mn and Cu.

(MLS Ethos 1600, Italy), equipped with temperature and pressure sensors, Teflon digestion bombs and vessels, was used to dissolve the samples.

2.2 *Materials, reagents and standards*

Ultrapure water (resistivity 18 MΩ cm) from an ultrapure water system (Nanopure Infinity, Barnstead, P/N-1161, Dubuque, USA) was used to prepare all solutions. All acids and reagents were of analytical grade. Nitric acid (65% w/w), H_2O_2 (35% w/w) and Triton X-100 (99.95% w/w) from Merck (Darmstadt, Germany) were used for dilution and to dissolve the samples. All solutions prepared were stored in high-density polypropylene bottles. Plastic bottles, autosampler cups, Teflon vessels, vials used for collecting samples and glassware materials were cleaned by soaking in HNO_3 (20% v/v) for a day, rinsing four times with ultra-pure water and drying. Autosampler washing solution containing HNO_3 (0.1% v/v) plus Triton X-100 (0.1% v/v) was used to avoid clogging of the autosampler sampling capillary tip and to improve dispersion of sample solution onto the platform [4,20].

Palladium stock solution (2.0 g L^{-1}) was prepared by dissolving 506 mg Pd $(NO_3)_2 \cdot 2H_2O$ (Merck) in HNO_3 (10% v/v) and diluting to 100 mL with ultra pure water after evaporation of acids. A La(III) solution (4.0 g L^{-1}) was prepared by diluting lanthanum chloride standard solution (BDH chemicals, Poole, UK). Citric acid (4% m/v) (Hydrous, granular, New York, USA) solution was prepared daily before use.

Analytical calibration solutions of standards were freshly prepared from the stock solutions of Cd, Pb, Cu and Mn (1.0 g L^{-1}) (BDH chemicals, Poole, UK) by suitable and serial dilution in HNO_3 (0.2% v/v) solution.

2.3 *Collection of samples*

Thirty seven deciduous teeth without fillings (all of which required extraction for orthodontic reasons) from 37 individuals with different ages were collected by the Turkish Atomic Energy Agency (TAEA) and the Dental Faculty of Ankara University, in Turkey, and were stored in refrigerators at 4°C until the time of analysis. The age range of humans concerned was from 8 to 54. Of the subjects, 17 were males and the others were females. Bone ash 1400 standard reference material (SRM) taken from National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) was used.

2.4 *Decomposition of samples*

A tooth sample was put into a Teflon beaker and left for 20 min in H_2O_2 (5% v/v) in order to clean its outer surface. The sample was rinsed several times with ultra-pure water [9,19]. Samples were dried in an oven at 80°C for 2 hours and weighed. After each tooth sample (the range of mass being from 0.37 to 1.74 g) or a portion of bone ash 1400 (0.5 to 1.5 g) was accurately weighed into a Teflon digestion vessel with a cover, a 3 ml mixture of HNO_3 (65% w/w) plus H_2O_2 (30% v/v) (2 : 1) and 1 mL of H_2O were added to each sample and left overnight at laboratory temperature in order to dissolve the sample without heating [4,9,19]. Prior dry ashing was found to be not necessary. White suspended particles were observed above the solution. Samples were decomposed by using a Milestone Ethos microwave oven according to the procedures described in a previous work [21]. Steps of the microwave program applied were the heating from laboratory temperature to 120°C in 10 min and waiting at this temperature for 10 min (up to 600 W); heating from 120°C to 140°C for 10 min and holding for 20 min (up to 800 W); and turning 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 [19]. When a residue of sample such as bone ash 1400 material remained, the decomposition procedure was repeated to dissolve it completely. The final solution was transferred to 10, 25 or 50 mL volumetric flasks by washing the inner surface of the digestion vessel with HNO_3 (2% v/v) three times and the final acidity of solution was adjusted to 0.5% (v/v) with HNO_3. Blank solutions (3 mL of a mixture of HNO_3 (65% w/w) plus H_2O_2 (30% v/v) (2 : 1) and 1 mL of H_2O) were placed into the two Teflon vessels and digested following the same procedure mentioned above.

2.5 *Analytical procedures*

Samples were diluted with nitric acid (0.1% v/v) plus Triton X-100 (0.1% v/v) until the absorbance signals of analytes were in the range of 0.07 to 0.25 absorbance units and they were used to obtain optimum parameters of modifiers for the ETAAS determinations. One mL of appropriate concentration of analyte in sample solution was mixed with 1 mL of modifier solution (2.0 g L^{-1} La or 0.4 g L^{-1} Pd or 2.0 g L^{-1} La + 0.4 g L^{-1} Pd or 2.0 g L^{-1} La + 0.4 g L^{-1} Pd + 10 g L^{-1} CA) and injected into the platform. The maximum absorbance values were found by changing the heating temperatures, ramp and hold times. The optimised heating temperature program is given in Table 1. Absorbance values versus mass or mass ratio of modifier curves obtained for analytes in bone ash 1400 sample solution are given in Figure 1, using the optimised heating temperature program given in Table 1 and the integrated absorbance mode. Pyrolysis and atomization temperature curves for the analytes in a tooth solution (5% m/v) were studied with or without the modifiers and are shown in Figure 2. The atomization and pyrolysis temperatures for the analytes are given in Tables 1 and 2. Standards, blanks, reference material and tooth samples were analysed using the conditions described in Tables 1 and 2.

3. Results and discussion

3.1 *Chemical modification in ETAAS*

The main purposes of using chemical modification in ETAAS are to stabilize the analyte elements to pyrolysis temperatures as high as possible by forming chemical compounds

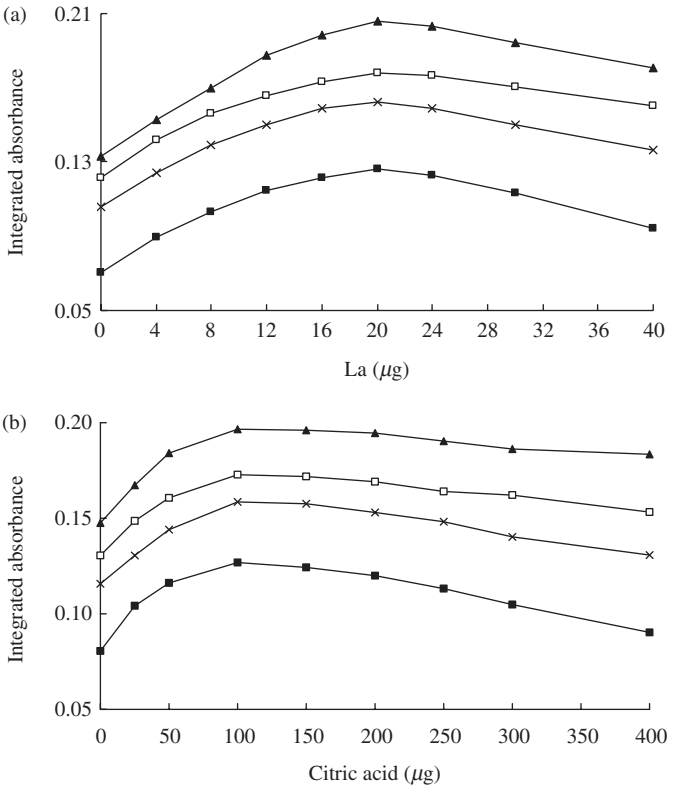

Figure 1. Effect of the mass of (a) La with fixed 4 μg of Pd (b) citric acid (CA) with fixed 20 μg of La + 4 μg of Pd in the La + Pd + CA modifier mixture on the absorbance values of Pb (□), Cd (■), Mn (▲) and Cu (×) in bone ash 1400 ($39.1 \pm 0.5\,\mu g\,L^{-1}$ Pb, $1.93\,\mu g\,L^{-1}$ Cd, $3.91\,\mu g\,L^{-1}$ Mn and $29.7\,\mu g\,L^{-1}$ Cu, respectively).

or intermetallic phases [26], to obtain best recoveries and to remove most of the matrix efficiently without loss of analyte mass. Optimization conditions of ETAAS with chemical modifiers were investigated in real sample solutions such as bone ash 1400 and human teeth for the determination of Cd, Pb, Cu and Mn.

The effect of masses and mass ratios of La, Pd, La + Pd and La + Pd + CA modifier mixture on analytes in bone ash 1400 solution ($39.1 \pm 0.5\,\mu g\,L^{-1}$ of Pb, $1.93\,\mu g\,L^{-1}$ of Cd, $3.91\,\mu g\,L^{-1}$ of Mn and $29.7\,\mu g\,L^{-1}$ of Cu, respectively) were studied and given in Figure 1. The mean of three absorbance measurements of analytes versus modifier mass was obtained by the integrated mode. The best results were found with a mixture of 20 μg of La, 4 μg of Pd, 100 μg of CA, 20 μg of La + 4 μg of Pd + 100 μg of CA in Triton X-100 (0.1% v/v) plus HNO_3 (0.1% v/v).

Pyrolysis and atomization temperature curves of analytes were investigated in a tooth sample solution randomly chosen. Pyrolysis temperatures, with or without the modifiers, were kept constant and atomization temperatures were varied. The mean of three absorbance measurements of Cd, Pb, Mn and Cu in the absence and presence of modifiers are shown in Figure 2. The atomization and pyrolysis temperatures are given in Tables 1 and 2. As can be seen in Table 1, atomization and cleaning temperatures of Cu and Mn are

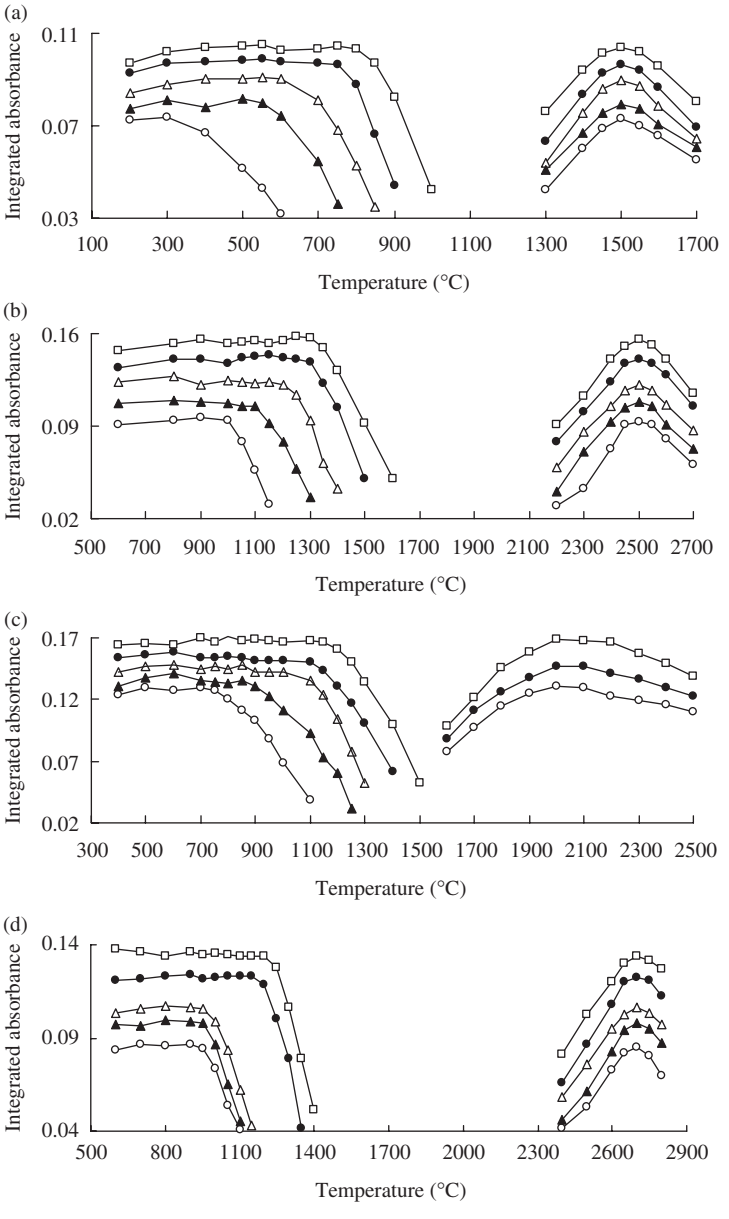

Figure 2. Pyrolysis and atomization curves for (a) Cd, (b) Mn, (c) Pb and (d) Cu in a tooth solution (Dilution factor is 20 mL g^{-1}) with and without the modifiers: without (○); 20 µg La (▲); 4 µg Pd (△); 20 µg La + 4 µg Pd (●); 20 µg La + 4 µg Pd + 100 µg CA (□).

higher, but they are recommended by the manufacturer. As can be seen in Table 2, pyrolysis temperatures of analytes obtained with La + Pd + CA are higher than those obtained with single or mixed modifiers or without the modifier. The temperatures obtained with La + Pd + CA are 50°C, higher than or equal to La + Pd, because CA may reduce the analytes and modifiers to their free reactive metals [23,24,26]. The pyrolysis

Table 2. Pyrolysis temperatures, detection limits and characteristic masses of analytes in a tooth sample solution obtained without and with the use of modifiers ($3S_b$, dilution factor of 50 mL g^{-1}).

Element	Without	La	Pd	La + CA	Pd + CA	La + Pd	La + Pd + CA
Pyrolysis temperatures (°C)							
Cd	300	550	600	650	700	750	800
Cu	900	950	950	1000	1000	1150	1200
Mn	1000	1100	1200	1150	1250	1300	1300
Pb	750	850	1000	900	1050	1100	1150
Detection limits (ng g^{-1})							
Cd	31	23	19	15	13	9	6
Cu	104	87	78	69	55	32	24
Mn	73	51	42	35	29	23	16
Pb	146	123	114	91	82	67	46
Characteristic masses (pg)							
Cd	3.3	2.8	2.8	2.6	2.5	2.3	2.1
Cu	34	29	28	26	24	21	18
Mn	9.4	8.3	7.7	7.1	6.8	6.4	6.1
Pb	35	32	31	30	27	25	21

temperatures of analytes in tooth sample solution with La + Pd + CA were compared with previous works [20,26–28]. The small differences observed with La + Pd + CA may be due to the differences of set and the actual temperatures, tubes and platforms used.

Atomization and background profiles of Cd, Pb, Cu and Mn in tooth sample solutions with and without the modifiers were comparatively studied in order to understand how the modifiers affect the atomization/background profiles of analytes [4,29]. The highest signal-to-background ratios of analytes were obtained with La + Pd + CA among the modifiers studied, and the lowest signal-to-background ratios were obtained without the use of a modifier. The peak times shifted to a later time were observed in the presence of La + Pd + CA [29]. Mean of blank signals obtained with La + Pd + CA were 0.0206 for Cd, 0.0102 for Mn, 0.0161 for Pb and 0.0088 for Cu, respectively.

3.2 *Analytical characteristics*

A calibration graph method was used for the determination of Cd, Pb, Cu and Mn in bone ash 1400 and tooth sample solutions in the presence or absence of modifiers. The calibration graphs constructed against aqueous standard solutions of analytes were linear up to 80 μg L^{-1} for Pb and Cu, 6 μg L^{-1} for Cd and 8 μg L^{-1} for Mn. Correlation coefficients (r) of calibration graphs for the analytes studied were 0.996 for Cd and Pb, 0.998 for Cu and Mn.

Limits of detection (LOD) and characteristic mass (m_o) are important for the sensitivity of the proposed method and they might be influenced by instrumental parameters [30]. The detection limits and characteristic masses of analytes were determined with and without the modifiers from 20 consecutive measurements of a tooth solution (2% m/v) [17]. The LOD and m_o values found are given in Table 2. As can be seen, the lowest detection limits and m_o values were obtained with La + Pd + CA. The results obtained for Cd, Pb, Cu and Mn were compared with previous works [4,9,20,21,31–33] and small differences observed in results are due to instrumental parameters and modifiers used.

Table 3. Recovery tests for analytes in bone ash 1400 and a tooth sample solution with La and La + Pd + CA modifier mixture.

Element	Certified[a]/ Added[b]	Found with La[c]	Recovery (%)	Found with La + Pd + CA[c]	Recovery (%)
Bone ash 1400, concentrations, $\mu g\,g^{-1}$					
Cd, $ng\,g^{-1}$	30	25.1 ± 2.5	84	29.1 ± 1.8	97
Pb	9.07 ± 0.12	7.82 ± 0.63	86	9.14 ± 0.24	101
Mn	17	15.1 ± 0.8	89	16.7 ± 0.4	98
Cu	2.3	2.01 ± 0.13	87	2.23 ± 0.08	97
Tooth sample solution, concentrations, $\mu g\,L^{-1}$					
Cd	–	1.9 ± 0.2		2.1 ± 0.2	–
	2.0	3.4 ± 0.3	87	3.9 ± 0.3	95
Pb	–	32.4 ± 2.1		33.5 ± 1.8	–
	20	48.1 ± 4.3	91	51.8 ± 2.8	97
Mn	–	2.3 ± 0.2		2.6 ± 0.1	–
	2.0	3.8 ± 0.3	88	4.5 ± 0.2	98
Cu	–	35.3 ± 1.8		37.2 ± 1.5	–
	20	49.9 ± 2.9	90	55.6 ± 2.2	97

Notes: [a]Certified and non-certified values for bone ash 1400; [b]Added values for a tooth sample solution; [c]Mean of seven replicate measurements with 95% confidence level, $\overline{X} \pm ts/\sqrt{n}$.

As a consequence, the La + Pd + CA modifier mixture in triton X-100 (0.1% v/v) plus nitric acid (0.1% v/v) used as diluent was recommended for the determination of Cd, Pb, Cu and Mn in tooth samples.

3.3 *Method validation and sample analysis*

The bone ash 1400 and a tooth solution were analysed using La and La + Pd + CA modifier 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 perform a recovery test. One mL of $2.0\,\mu g\,mL^{-1}$ Pb and Cu, $0.2\,\mu g\,mL^{-1}$ Cd and Mn standard aqueous solutions, respectively, were added to one of the flasks. Both flasks were diluted to the mark again. The contents of analytes found in bone ash 1400 and the tooth solution by using the optimised parameters are given in Table 3. The concentrations of Cd, Cu and Mn in bone ash 1400 given are non-certified.

Since two or more tooth samples were not taken from the same donor, recovery experiments were carried out by adding concentrations of aqueous solutions of each element to the dissolved tooth solution before the measurement in order to control differences and interferences such as viscosity of sample and aqueous solutions. The results are presented as the average ± confidence interval (6 degrees of freedom (n − 1) at 95% confidence level). As can be seen in Table 3, recoveries of analytes obtained with La + Pd + CA are higher than the recoveries obtained with La modifier. The results of analytes obtained with La + Pd + CA are in good agreement with the certified values, and recoveries are in the range of 97–101% [18,30]. Recoveries of analytes obtained in tooth solution with La + Pd + CA are also higher than 95%.

Whole tooth was used to reduce the risk of contamination from mechanical operation on the tooth and simplicity [19]. The concentrations of analytes found with La + Pd + CA

Table 4. Average (± SD) concentrations of metals in teeth of male and female donors in correlation with age.

Age range (yr)	Number of individuals	Concentrations, $\overline{X}$ ± SD (Minimum value–Maximum value)			
		Pb (μg g^{-1})	Cd (ng g^{-1})	Cu (μg g^{-1})	Mn (μg g^{-1})
Male					
21–30	2	1.66 ± 0.92 (1.01–2.61)	16.4 ± 7.21 (11.3–21.5)	0.99 ± 0.38 (0.72–1.26)	2.71 ± 1.46 (1.68–3.74)
31–40	6	2.74 ± 0.77 (1.74–3.83)	26.1 ± 17.5 (7.23–48.9)	0.71 ± 0.34 (0.36–1.31)	3.25 ± 1.73 (1.82–5.68)
41–50	4	4.52 ± 3.47 (1.86–10.7)	19.2 ± 12.3 (10.4–37.4)	0.59 ± 0.21 (0.35–0.85)	3.01 ± 1.15 (1.79–4.41)
51–54	5	6.39 ± 2.93 (3.45–10.1)	21.6 ± 8.22 (12.5–32.7)	1.31 ± 0.58 (0.64–1.86)	4.95 ± 2.81 (1.21–8.07)
Female					
8–20	3	1.04 ± 0.32 (0.71–1.34)	15.6 ± 2.7 (12.6 -18.0)	0.70 ± 0.55 (0.35–1.33)	2.06 ± 1.02 (0.89–2.74)
21–30	3	1.74 ± 1.03 (0.92–2.90)	17.5 ± 5.26 (11.8–23.2)	0.87 ± 0.47 (0.49–1.40)	4.32 ± 2.31 (1.74–6.17)
31–40	9	2.44 ± 1.65 (0.52–5.53)	25.3 ± 14.6 (12.0–49.7)	0.80 ± 0.40 (0.39–1.58)	2.73 ± 1.50 (0.81–5.71)
41–50	5	7.37 ± 2.71 (3.00–10.4)	28.3 ± 13.9 (12.4–42.3)	0.74 ± 0.24 (0.47–1.01)	4.06 ± 1.81 (2.17–6.10)

in tooth samples are also presented in the same way as described above. Average (± SD) concentrations of Pb, Cd, Cu and Mn in human teeth related to human age are given in Table 4. As can be seen, lead concentration increased gradually with the age of the donors (for 8–40 age range) and there were no significant differences in lead levels up to 40 years of both males and females. As a result, both sexes were exposed to the environmental pollution and accumulation of lead in the tooth increased with the donor's age. No significant differences in metal levels were observed between men's and women's teeth in any particular age group.

4. Conclusions

A method with La + Pd + CA modifier mixture for the Cd, Pb, Cu and Mn determination in human teeth and bone ash 1400 using ETAAS has been developed and verified. Recovery studies have shown that the method provides accurate results. The La + Pd + CA can be applied to the direct determination of analytes in human teeth after decomposition by nitric acid plus hydrogen peroxide. Teeth are available biological materials and they are good markers of exposure to environmental pollution and control. The number of samples processed in this study is not enough to reach statistical conclusions on the correlations between metal levels and age or sex. Therefore, a deeper study applying this method to a higher and well selected and planned collection of samples will provide useful information.

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