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Full Length Research Paper

The effects of a chemically polluted environment on patients' teeth in Pancevo, Serbia

Cenic-Milosevic Desank, Mileusnic Ivan*, Pejanovic Djordje, Ristic Tamara, Jakovljevic Ankica, Popovic Milica, Melih Irena and Kolak Veljko

[1]Faculty of Stomatology Pancevo, Institute of Stomatology, Zarka Zrenjanina 179, 26000 Pancevo, Serbia.

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Modern urban areas have recently become increasingly polluted. Heavy metals are one group of industrial pollutants which have no biological functions and accumulate in various parts of human bodies. The principal hypothesis of this study was that the pollution of the environment by certain heavy metals, that is, lead, cadmium, nickel and mercury, caused long-lasting changes in teeth, through deposition in dental hard tissues. Therefore, the aim was to compare their concentration in saliva, dental calculus and teeth extracted from inhabitants of Pancevo and Belgrade belonging to 5 age groups. Cadmium and mercury could not be detected in saliva from both cities, while lead contents remained equal. Cadmium, nickel and mercury could not be detected in dental calculus, while lead was present in all groups from both cities significantly more in the older groups from Pancevo. The concentration of lead in extracted teeth in all groups from Pancevo was significantly ($p<0.05$) higher than in group I from Belgrade, while when other groups from Pancevo were compared to their counterparts from Belgrade the significance was even higher ($p<0.001$). The concentration of nickel in extracted teeth in group I from Belgrade was significantly higher than in group I from Pancevo, while in group IV from Pancevo it was significantly higher than in group IV from Belgrade. The concentration of mercury in extracted teeth in groups III, IV and V from Pancevo was significantly higher than in corresponding groups from Belgrade. The correlation for patients from Pancevo – the number of extracted teeth and lead concentration in extracted teeth is statistically significant. Heavy metals from our environment, particularly lead, accumulated in hard dental tissues. The concentration of lead increased rapidly in older patients from Pancevo and Belgrade, which means that the deposition of lead is age-dependent. One possible cause of tooth loss in patients from Pancevo could be long-term environmental exposure to lead.

Key words: Heavy-metal deposition, environmental lead, hard dental tissues, saliva.

INTRODUCTION

Industrialisation, along with the development of large urban centres, has led to widespread contamination of air, water and soil. The main causes of pollution are production and use of energy, industrial chemicals and increased agricultural activity. As a result, all biological organisms, including humans, live in a chemically polluted environment.

Pancevo is an industrial centre 15 km from the capital city of Belgrade. Its main polluters have always been an oil refinery, a petrochemical plant and a factory which produces artificial fertilizers. Pancevo was one of the most polluted urban areas in Serbia at the end of the 20th century, with an increase in the level of pollution in 1999.

The Institute for Public Health in Pancevo has monitored concentrations of SO_2, NO_2, NH_3 and soot in the air in the ten-year period from 1991 to 2001. The results showed that concentrations of soot and NH_3 were high above the legal limit during this period.

In 2004, a comparative pilot study was conducted to determine the differences between heavy-metals concentrations in the saliva of Pancevo schoolchildren

*Corresponding author. E-mail: imileusnic@gmail.com. Tel: (381) 13 351 292. Fax: (381) 13 366 582.

Table 1. Mean values of ages of patients from Pancevo and Belgrade.

Experimental groups	Mean age	
Variable	Pancevo	Belgrade
	Mean ± SE[a]	Mean ± SE
I (20 – 30)	27.20 ± 2.80	28.10 ± 3.45
II (31 – 40)	36.40 ± 4.20	34.32 ± 4.10
III (41 – 50)	45.30 ± 3.70	46.25 ± 3.70
IV (51 – 60)	56.60 ± 5.20	55.80 ± 4.60
V (over 60)	69.50 ± 7.25	72.50 ± 5.80

[a] SE= standard error.

and from the neighbouring city of Belgrade (Cenic, 2004). The concentrations of heavy metals in stimulated saliva obtained from child volunteers were measured. The results of this study showed that the mean concentrations of lead, cadmium, copper and aluminium were statistically significantly higher in the saliva of school children from Pancevo than from Belgrade. Although Belgrade is a large city of almost 2 million inhabitants the pollution in Pancevo, as measured through the content of heavy metals in saliva, proved to be higher than in Belgrade.

Environmental lead pollution is a global problem. Lead is one of the most important and widely distributed pollutants in the environment (Frank et al., 1989, Cleymaet et al., 1991, 1991a) and a great part of this pollution comes from vehicle exhaust fumes through the combustion of leaded petrol. This, and other human activities such as the extensive use of lead in industry, has resulted in the redistribution of lead in the environment and, hence, the contamination of air, water and food. Consequently, the levels of lead content in blood, saliva and other human organs (Karahalil et al., 2007) are significantly increased. The levels of lead in blood and saliva reflect recent exposure. Long-term deposition of lead is much greater in skeletal tissues than in soft tissues (Hu et al., 1998). Heavy metals, that is lead and cadmium, have no known physiological functions and are toxic even in low concentrations (Alomary et al., 2006).

It has been demonstrated in a number of studies (Fosse and Justesen,1978l; Van Wyk and Grobler, 1983; Haavikko et al., 1984; Anttila and Anttila, 1987; Anttila, 1986, 1987, 1987a; Frank et al., 1988, 1990; Heilmann et al., 1990; Cleymaet et al., 1991, 1991a, 1991b, 1991c, 1991d, Hernandez-Guerrero et al. 2004, Gomes et al., 2004) that hard dental tissues have the capacity to accumulate lead and other heavy metals from the environment.

This paper presents one part of the results from a study supported by The Ministry of Science and Technological Development, Republic of Serbia (Project Number 21045), conducted between April 2008 and December 2010, entitled: ''The Effects of a Chemically Polluted Environment on Oral tissues and Teeth of Patients from Pancevo, Serbia''.

The principal hypothesis of this study was that the pollution of the environment by certain heavy metals, that is, lead, cadmium, nickel and mercury, caused long-lasting changes in teeth, through deposition in dental hard tissues. Therefore, the aim of this study was to compare the concentration of heavy metals in saliva, dental calculus and teeth extracted from inhabitants of Pancevo and Belgrade belonging to different age groups.

MATERIALS AND METHODS

This study was undertaken on 160 female and 160 male patients. The volunteers were selected from patients who visited the Institute of Stomatology at the faculty of Stomatology, Pancevo, Serbia. The primary criterion for inclusion and subsequent sample collection was that these patients had been living in either Pancevo or Belgrade for a period of at least 15 years prior to the study beginning. They had to be in good general health with no signs of disease or medication use. The volunteers were then divided into five separate groups for each city, with 32 volunteers in each group (Table 1).

The study proposal was submitted to the Research Ethics Committee (Approval Protocol No. 1323/1-2008, according to Resolution sections 3, 7, and 8 of the National Commission of Ethics in Research). Patients had to sign an informed consent form prior to the inclusion in the study. Additionally, signed permission for collection of samples of saliva, dental calculus and extracted teeth (only if extraction was necessary as a therapeutic procedure) had to be obtained from each participant in the study. Patients were also asked to fill out a questionnaire in which many aspects of their lives were recorded, especially those pertaining to any previous diseases and/or exposure to a polluted environment.

Clinical examination

All patients were submitted to a thorough clinical extra oral and intraoral examination and the results were recorded in two separate categories: mucous membranes and teeth.

Mucous membranes were evaluated for any signs of ulcerations and white lesions, especially leukoplakia and lichen planus. The number of missing teeth was recorded and any teeth to be extracted were evaluated for the presence of fillings, regardless of the material used.

Collection of samples

Saliva

Prior to the collection of saliva, the patients relaxed in a chair for a few minutes. The collection of unstimulated saliva was done without any masticatory or gustatory stimulus. Collection from all patients was carried out at the same time of day and tongue, jaw, cheek and lip movements were avoided during the collection period. The patients were instructed to refrain from eating, drinking, smoking, and oral hygiene procedures for at least 90 minutes prior to the collection of samples. They were instructed to allow saliva to passively drain from the lower lip into a plastic cup. Samples collected in this way were used to determine the pH value of saliva and heavy-metal content.

Dental calculus

Supragingival dental calculus was collected from the patients using hand instruments, that is scalers in standard fashion. The working end of the instrument was carefully placed apically to the calculus deposit and special attention was paid not to cause any unnecessary bleeding from the adjacent gingival. The instrument was activated in a coronal direction with one firm motion and the dislodged calculus was placed in a plastic container.

Extracted teeth

Following a detailed examination, all teeth to be extracted were carefully evaluated for the presence of fillings or caries. The final decision for tooth extraction was reached following careful consideration of periodontal status and restorative possibilities. In many cases the cause for extraction was either subsequent orthodontic therapy or progressive periodontal disease. The weight of each sample was at least 0.5 g which is a cut-off value for valid chemical analysis.

Chemical analysis

The determination of saliva pH was done by a pH-meter (C 830 - Multi Parameter Analyser, Consort, and Turnhout, Belgium). All chemical analyses were done in an independent laboratory, Department of Ecotoxicology at The Institute of Public Health, Pancevo.

The collected samples of dental calculus and extracted teeth were stored in ultra-filtered deionised water. Before analysis all soft tissue remnants and surface stains were removed. Drying of samples to a constant mass was done under laboratory conditions for 48 h at 80℃. Dried samples were then finely ground to grains under 1 mm in diameter. Samples of saliva were initially prepared by ultrasonic emulsification. Subsequent sample preparation was the same for saliva, dental calculus and extracted teeth. For further voltammetric analysis all organic substances must be removed from the sample. This was done by UV digestion (MILESTONE SK-10, Milestone, and Sorisole, Italy). Batches of 0.5 g of the samples were diluted for 30 min using 7 ml of 65% HNO_3 and 1 ml of 30% H_2O_2 at 200℃. After cooling to room temperature, the digested samples were transferred directly to the appropriate vessel for further analyses. Solid samples (teeth and calculus) and saliva were analyzed in the same way.

The concentrations of heavy metals in the final digested solution of samples were determined by the PS control system for voltammeter 797 VA Computrace (Metrohm, Herisau, Switzerland).

This method was chosen because it can distinguish between different oxidation states of metal ions as well as between free and bound metal ions, which provides important information regarding the bioavailability and toxicity of heavy metals. Validation of the voltammeter was done using the GLP Wizard of the machine. Chemical analyses were done using Cd, Pb, Ni and Hg ion standard. The decisive parameters for the validation of the measuring instrument are the accuracy and the scatter of the result. Both values are calculated automatically by the internal software of the 797 VA Computrace. Electronic validation: current at -200 mV: measured values -2 µA: tolerances from -1.6 to -2.4 µA; current at +200 mV: measured values +2 µA: tolerances from +1.6 to +2.4 µA. Peak voltage: measured values: -497 mV; tolerances: from -450 mV to +450 mV. Chemical validation: measuring: 20 ml H_2O +0.5 ml KCl +100 µl Cd, Pb, Ni or Hg standards. Electrolyte: c (KCl) = 3 mol/L. Standard: β(Cd) = 1 g/L, β(Pb) = 1 g/L, β(Ni) = 1 g/L, β(Hg) = 1 g/L. Accuracy was 0.95 - 1.05 g/L and scatter was ±3%.

Sensitivity of the method was achieved to the level of: Cd – 0.02-0.025 µg/m^3, Ni – 0.5-50 ng/m^3, Pb - 0.0005 - 2.5 µg/m^3 and Hg – 0.0075 - 2.5 µg/m^3.

Reagents used for the voltammetric determination of cadmium and lead were: NaOH, suprapure, w (NaOH) = 30%, acetic acid, suprapure, w (CH_3COOH) = 100%, KCl, suprapure, Cd stock solution-β(Cd) = 1 g/L (commercially available), Pb stock solution-β(Pb) = 1 g/L (commercially available). Solutions used: KCl-acetate: c(KCl) = 1.5 mol/L, c(CH_3COONa)=0.5 mol/L, 55.9 g KCl +25 ml NaOH+ 14.2 ml CH_3COOH filled up to 500 ml with high purity water. Standard solutions β(Cd) = 0.1 mg/L, β(Pb) = 0.5 mg/L. Reagents used for the voltammetric determination of nickel were: ammoniac solution, suprapure, w(NH3) = 25%, hydrochloric acid, suprapure, w(HCl) = 30%, dimethylglyoxim disodium salt octahydrate, puriss.p.a., CAS 75006-64-3; Ni stock solution β(Ni) = 1 g/L (commercially available). Solutions used :NH_4Cl buffer pH 9.5, c(NH_4Cl) = 1 mol/L, c(NH_3) = 2 mol/L, 112.5 ml NH_3 +53 ml HCl, filled up to 500 ml with high purity water. DMG solution: c(Na_2DMG) = 0.1 mol/L, dissolve 0.304 g dimethylglyoxim disodium salt in 10 ml ultrapure water, standard solution - β(Ni) = 1 mg/L.

All diluted solutions for Cd, Ni and Pb were prepared using c(HNO_3) = 0.014 mol/L. Reagents used for the voltammetric determination of mercury were: sulfuric acid, suprapure, w(H_2SO4) = 96%, CAS 7664-93-9, ethylenediaminetetraacetic acid disodium salt dihydrate, for analysis, Na $_2C_{10}H_{14}N$ $_2O_8$ ~ $2H_2O$, CAS 6381-92-6, sodium chloride, suprapure, NaCl, CAS 7647-14-5, Hg standard stock solution β(Hg) = 1 g/L (commercially available), nitric acid suprapure, w(HNO_3) = 65%, ultrapure water type 1.

Solutions used: c(H2SO4) = 2 mol/L, c(Na_2EDTA) = 0.02 mol/L, c(NaCl) = 0.05 mol/L. (0.372 g Na_2EDTA and 0.146g NaCl are dissolved in approx. 40 ml ultrapure water and 5.56 ml H_2SO4. After cooling down to room temperature ultrapure water was added up to 50 ml. β(Hg) = 1 mg/L /0.1 ml Hg standard (1 g/L) and 0.1 ml HNO_3 (65%) are made up to 100 ml with ultrapure water. Detection limits for the equipment used in this study, as stated by the manufacturer, were as follows: Cd-0.02 µg/g, Pb-0.02 µg/g, Ni-0.02 µg/g and Hg-0,2 µg/g. Detection limits for atomic spectroscopy for the same metals are: Cd-0.45, Pb-0.2, Ni-0.02 and Hg-0.4 µg/g.

Statistical significance was calculated by Student's t-test and its modification by Bonferroni (Sylvan, 1980).

The processor CORR from the SAS package, version 6.4 (SAS Institute, 1989), was used to estimate correlations between trait pairs (extracted teeth and concentration of heavy metals) within locality. Correlations were computed as Pearson product-moment correlations.

RESULTS

All results were statistically processed and shown in tables. Each investigated parameter is represented for both sexes, because there were no sex-related

Table 2. Total number and mean values of extracted teeth from Pancevo and Belgrade.

Experimental groups	Extracted teeth			
Variable	Pancevo		Belgrade	
	Total	Mean ± SE[a]	Total	Mean ± SE
I (20 – 30)	72	2.25 ± 0.75	44	1.37 ± 0.63
II (31 – 40)	166	5.18 ± 1.20	233	7.25 ± 1.42
III (41 – 50)	332	10.37 ± 2.12	169	5.28 ± 1.80
IV (51 – 60)	428	13.37 ± 3.45	68	2.12 ± 0.61
V (over 60)	452	14.12 ± 3.85	158	4.93 ± 1.48

[a]SE=standard error.

Table 3. Mean values of saliva pH from Pancevo and Belgrade.

Experimental groups	Saliva pH	
Variable	Pancevo	Belgrade
	Mean ± SE[a]	Mean ± SE
I (20 – 30)	6.21 ± 0.62	7.10 ± 0.40
II (31 – 40)	6.28 ± 0.60	6.90 ± 0.10
III (41 – 50)	6.35 ± 0.45	7.20 ± 0.45
IV (51 – 60)	6.66 ± 0.66	6.90 ± 0.25
V (over 60)	6.33 ± 0.67	6.70 ± 0.35

[a]SE= standard error.

Table 4. Concentrations of lead and nickel in saliva from Pancevo and Belgrade.

Experimental group	Lead		Nickel	
Variable	Pancevo	Belgrade	Pancevo	Belgrade
	Mean ± SE[a]	Mean ± SE	Mean ± SE	Mean ± SE
I (20 – 30)	0.02	0.06 ± 0.01	/[b]	/
II (31 – 40)	0.02	0.02	/	/
III (41 – 50)	0.02	0.03 ± 0.01	0.21 ± 0.10	/
IV (51 – 60)	0.02	0.02	/	/
V (over 60)	0.02	0.02	0.16 ± 0.07	0.02

[a] SE= standard error; [b] /=undetectable (below 0.02 µg/g).

differences in the results which are presented, therefore, by mean value and statistical significance, separately marked. The mean values of the number of extracted teeth in all five age groups, from Pancevo and from Belgrade are given in Table 2.

It was clear that the number of extracted teeth was statistically significantly ($p<0.001$) higher in groups III, IV and V from Pancevo than in groups III, IV and V from Belgrade. The number of extracted teeth in group I from Pancevo was higher (no statistical significance) than in group I from Belgrade, whereas the number of extracted teeth was higher (no statistical significance) in group II from Belgrade than in group II from Pancevo.

The mean values of saliva pH in all five groups from Pancevo and Belgrade are given in Table 3.

The saliva pH was statistically significantly ($p<0.05$) higher in groups I and III from Belgrade than in groups I and III from Pancevo. In all other groups, that is II, IV and V from Belgrade, saliva pH was higher than in groups II, IV and V from Pancevo, but without statistical significance.

The mean values of lead and nickel concentrations in Belgrade are given in Table 4. Concentrations of lead and nickel are presented in µg/g. The levels of cadmium

Table 5. Concentrations of lead in dental calculus.

Experimental group	Concentration of lead	
Variable	Pancevo	Belgrade
	Mean ± SE[a]	Mean ± SE
I (20 – 30)	0.17 ± 0.12	/[b]
II (31 – 40)	0.24 ± 0.16	/
III (41 – 50)	0.99 ± 0.21	/
IV (51 – 60)	4.37 ± 0.78	/
V (over 60)	2.01 ± 0.54	/

[a] SE= standard error; [b] /=undetectable (below 0.02 µg/g).

Table 6. Mean values of lead, nickel and mercury concentrations in extracted teeth.

Experimental group	Lead		Nickel		Mercury	
Variable	Pancevo	Belgrade	Pancevo	Belgrade	Pancevo	Belgrade
	Mean ± SE[a]	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
I (20 – 30)	1.57 ± 0.35	0.61 ± 0.14	/[b]	2.20 ± 0.46	/	/
II (31 – 40)	4.48 ± 1.12	0.39 ± 0.09	/	/	/	/
III (41 – 50)	4.60 ± 0.78	0.80 ± 0.22	/	/	2.78 ± 0.27	/
IV (51 – 60)	11.10 ± 2.43	4.15 ± 1.76	2.17 ± 0.87	/	5.14 ± 1.25	/
V (over 60)	26.61 ± 3.89	4.86 ± 2.00	/	/	1.66 ± 0.57	/

[a]SE= standard error; [b] /=undetectable (below 0.02 µg/g).

and mercury in all patients from Pancevo and Belgrade were smaller than 0.02 µg/g, which is a threshold value of detection.

The results showed that there was no statistically significant difference in the concentration of lead in saliva between patients from Pancevo and from Belgrade. Notably, the mean concentration of lead in saliva in group I from Belgrade was higher than in group I from Pancevo. At the same time, the concentration of nickel in saliva could only be detected in groups III and V from Pancevo and in group V from Belgrade.

The mean values of lead concentration in dental calculus in all five age groups from Pancevo and Belgrade are given in Table 5. Concentrations of lead are presented in µg/g. Although levels of 4 heavy metals, that is, lead (Pb), cadmium (Cd), nickel (Ni) and mercury (Hg) were sought in dental calculus, the levels of nickel, cadmium and mercury in all patients from Pancevo and from Belgrade were lower than the threshold level of 0.02µg/g. Results obtained for the concentration of lead in dental calculus indicated that it was statistically significantly ($p<0.001$) higher in groups III, IV and V from Pancevo, than in groups III, IV and V from Belgrade. In groups I and II from Pancevo, the concentration of lead was higher than in groups I and II from Belgrade, but without statistical significance.

The mean concentrations of lead, nickel and mercury in extracted teeth in all five groups from Pancevo and from Belgrade are given in Table 6. Concentrations of lead, nickel and mercury are presented in µg/g. The levels of cadmium in all patients from Pancevo and from Belgrade never reached the threshold of 0.02 µg/g. The concentration of lead in extracted teeth in all groups from Pancevo was statistically significantly ($p<0.05$) higher than in all groups from Belgrade. At the same time, the concentration of nickel in extracted teeth in group I from Belgrade was statistically significantly ($p<0.001$) higher than in group I from Pancevo, while the level of nickel in extracted teeth in group IV from Pancevo was statistically significantly ($p<0.001$) higher than in group IV from Belgrade. The concentration of mercury in extracted teeth in groups III, IV and V from Pancevo was statistically significantly ($p<0.001$) higher than in groups III, IV and V from Belgrade. When the measured levels of heavy metals did not reach the threshold values for the method used, they were marked as 'undetectable' in the corresponding tables.

Correlation was done separately for patients from Pancevo and from Belgrade, with two variables – the number of extracted teeth from 32 patients in each of the five groups, and the concentration of lead in 8 teeth from each of the five groups. For patients from Belgrade correlation is negative and without statistical significance. However, the correlation for patients from Pancevo – the number of extracted teeth ($32 \times 5 = 160$) and lead concentration in extracted teeth ($8 \times 5 = 40$) – is statistically significant ($p<0.001$).

DISCUSSION

It was noticed during this study that patients from Pancevo had fewer teeth than patients from Belgrade.

Patients from both cities had similar oral hygiene habits and visited dentists at approximately the same intervals. Therefore, one possible cause of tooth loss in Pancevo patients, aged over 40 years, could be long-term exposure to a polluted environment. Another probable consequence of the chemically polluted environment in Pancevo could be the elevated acidity of saliva found in samples collected from patients living in Pancevo. The results obtained for saliva pH indicate that saliva from this group had a lower pH than from patients living in Belgrade.

The concentration of lead in saliva collected from the youngest group of patients, that is, groups I, was higher in group I from Belgrade than in group I from Pancevo, which indicates that the pollution of the environment by this heavy metal is now higher in Belgrade than in Pancevo. These results confirmed findings of other authors, like Karahalil et al. (2007) who suggested that the most common method of monitoring lead toxicity is to measure its levels in blood or saliva. However, the content of lead in blood or saliva is a marker of recent exposure which can return to normal even after excessive initial exposure. The mean biological life of lead in blood or saliva is only about 30 days after which most of it accumulates in bone and hard dental tissues.

Study results for the concentration of nickel in saliva in groups III (40 to 50 years of age) and V (over 60 years of age) from Pancevo and in group V from Belgrade suggest that this may be the consequence of the presence of removable partial dentures. Removable partial dentures are made from metal alloys which contain certain amounts of nickel, which can dissipate within the oral environment.

The content of lead in dental calculus in all groups of patients from Pancevo was higher than in all groups of patients from Belgrade, where values below 0.02 µg/g were detected. Since the concentration of lead in saliva taken from patients living in Pancevo was not above threshold value this finding suggests and confirms that the content of lead in saliva reflects recent exposure, while its deposition either in skeletal tissues or in this case dental calculus is the result of a protracted period of exposure.

Teeth are not a uniform mass of calcified tissues and it has been well established that lead is not homogeneously distributed within a fully developed tooth, with lead levels in dentine being significantly higher than in enamel (Grobler, 2000). Furthermore, Arora et al. (2004) presented the spatial distribution of lead in the roots of human primary teeth while other authors measured the lead content in whole teeth (Rabinowitz, 1995). By analysis of the study results obtained for the concentration of lead in saliva and in extracted teeth it is obvious that lead in saliva or blood did not have any effect on the concentration of lead in hard dental tissues.

Research by this study showed that the concentration of lead in extracted teeth in all groups of patients from Pancevo was higher compared to patients from Belgrade. Additionally, the concentration of lead increased rapidly for older patients from both locations, which means that the concentration of lead is age-dependent. The same trend was also found for the concentration of lead in dental calculus. Other studies showed that the concentrations of lead and cadmium are age-dependent too. Baranowski et al. (2004) and Nowak and Chmielnicka (2000) found a positive correlation between age and lead in human teeth.

The high level of nickel in extracted teeth from young patients (group I) from Belgrade is probably the consequence of fixed orthodontic appliance therapy, while the high level of nickel in extracted teeth of older patients (group IV) from Pancevo is probably the consequence of the presence of removable partial dentures. It should be noted that there was a statistically significant ($p<0.001$) correlation between the number of extracted teeth and the concentration of lead found in patients living in Pancevo, whereas the same correlation in patients from Belgrade was of no statistical significance. This evidence suggests that the possible cause of tooth loss in patients from Pancevo could be long-term environmental exposure to lead.

In this study a consistent relationship was demonstrated between long-term environmental heavy-metal (particularly lead) exposure and its incorporation into hard dental tissues. This is similar to the results of Costa de Almeida et al. (2007), although, they took samples from children *in vivo* and using different methods. They measured the content of lead in the surface enamel of deciduous teeth sampled *in vivo* from children living in uncontaminated and in lead-contaminated areas of Brazil. It had been shown previously that heavy metals can be incorporated into dental tissues if there is exposure during the process of dentinogenesis (Gdula-Argasinska et al., 2004).

Therefore, unlike bone, in which the mineral phase is subject to turnover, once formed, teeth provide a permanent, cumulative and relatively stable record of environmental exposure (Tvinnereim et al., 1997).

In this Pancevo study, the concentration of heavy metals in each extracted tooth was measured. Further studies are planned to analyze the bone structure of patients whose teeth show a high concentration of heavy metals, particularly lead.

Conclusion

Lead from our environment accumulated in dental hard tissues. The concentration of lead increased rapidly in older patients from Pancevo and Belgrade, which means that the deposition of lead is age-dependent. According to the correlation between the number of extracted teeth and the concentration of lead found in patients living in Pancevo, one possible cause of tooth loss in patients from Pancevo could be long-term environmental exposure to lead.

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