Biol Trace Elem Res (2016) 173:47–54
DOI 10.1007/s12011-016-0643-1

Age-Dependent Changes in Pb Concentration in Human Teeth

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Received: 29 October 2015 / Accepted: 9 February 2016 / Published online: 18 February 2016

Abstract The result of exposure to Pb is its accumulation in mineralized tissues. In human body, they constitute a reservoir of approx. 90 % of the Pb reserve. The conducted research aimed at determining the accumulation of Pb in calcified tissues of permanent teeth. The concentration of Pb in 390 samples of teeth taken from a selected group of Polish people was determined using the AAS method. Average concentration of Pb in teeth amounted to 14.3 ± 8.18 μg/g, range of changes: 2.21–54.8 μgPb/g. Accumulation of Pb in human body was determined based on changes in Pb concentration in teeth of subjects aged 13–84 years. It was found that in calcified tissues of teeth, the increase in concentration of Pb that occurs with age is a statistically significant process ($p = 0.02$, the ANOVA Kruskal–Wallis test). It was determined that the annual increase in concentration of Pb in tissues of teeth is approx. 0.1 μg/g. Moreover, a different course of changes in Pb concentration in tissues of teeth in people born in different years was observed. The level of Pb concentration in teeth of the oldest subjects (>60 years) decreased for those born in the 1930s compared to those in the 1950s. Teeth from younger persons (<60 years) were characterized by an increasing level of Pb concentration. The analysis of changes of Pb indicates that for low exposure, a relatively greater accumulation of Pb concentration in calcified tissues of teeth can occur.

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Keywords Teeth · Pb accumulation · Age · Environmental exposure

Introduction

The main source of lead in the environment is anthropogenic activity. It exceeds the natural lead cycle several times [1]. Between 1930 and 2010, the total lead emission was 173.8 Mt [2]. The consequence of a versatile use of Pb was its significantly increased concentration in all elements of the environment: air, water, soil. Reports on toxicity of Pb resulted in actions aimed at restricting its use and reducing its concentration in the environment. For example, Pb concentration in paint and gasoline has been drastically reduced [3]. In the 20th century, tetraethyl lead was commonly used as an anti-knock agent in gasoline. The final years of that century brought restrictions on its use. In Europe, including Poland, gasoline with the addition of tetraethyl lead was eliminated from automotive cars in 2005; however, an intercontinental source of pollution with tetraethyl lead is still aviation fuel [4, 5]. Due to the use of leaded gasoline, the highest concentrations of organic lead compounds were recorded in soils near roadways and airports [6, 7]. These pollutants are classified as extremely resistant to biodegradation [8]. What is more, literature reports show a long-range migration of lead compounds [9, 10].

Despite the fact that the greatest threat of Pb compound poisoning can be found in the industrial sector, environmental sources are an easily available Pb reserve for living organisms. Efficiency absorbed into the human body by inhalation and ingestion may fluctuate depending on various factors. For example, the amount of Pb delivered into the human body of an adult by ingestion (approx. 28 mg/day^{-1}) can vary depending on dietary preferences [3].

Toxicity of Pb and its compounds to living organisms is unquestionable. It has a toxic effect on the functioning of most tissues and organs in the human body [3]. It also contributes to neurological–behavioral disorders and reduces intellectual development [11–13].

To assess the population's exposure to Pb, it is more important to use biomarkers rather than environmental monitoring [14]. Circulation of Pb in the human body leads to its accumulation in bone tissue. It is believed that the total amount of Pb in bones and teeth of adults is >90 %, and in children, >70 % [3]. Pb may be partially released from bone tissue, e.g. during the processes of its reconstruction [15]. This way bone tissue as a Pb reservoir in the body can become its potential and additional source of exposure.

The subject matter of the conducted research was to determine the concentration of Pb in calcified tissues of permanent teeth. Based on changes in Pb concentration in teeth of people aged 13–84 years, the process of accumulation of Pb in calcified tissues was determined. The research was conducted over 14 years covering the period from 1998 to 2012. Results of the conducted research were to indicate the course of changes in the level of Pb concentration in permanent teeth of adults over the given time. The research was also aimed at answering the question whether and how the reduction of potential sources of pollution was reflected in changes of Pb concentration in mineralized tissues of teeth which are the main area of accumulation of this element in human body.

Materials and Methods

The object of the research was 390 permanent teeth taken from women and men aged 13–84 years (mean = 46.6). The average age of women ($n = 208$) was 47.4 years, and men ($n = 182$), 45.7 years. Subjects included in the research were divided into age groups. The following age groups were distinguished: 1 (up to 29 years of age), 2 (30–39 years of age), 3 (40–49 years of age), 4 (50–59 years of age), 5 (over 60 years of age (Table 1). The teeth were taken from people living in the southern industrial region of Poland (Silesian Province) (Fig. 1). The teeth taken to the research were collected over the years 1998–2012. The teeth were collected in a dentist's office during an extraction procedure carried out for medical indications. The teeth taken for the research were postoperative residues. Indications for teeth extractions were caries and its complications, as well as orthodontic or prosthetic indications. Based on a medical certificate, it was stated that due to the course of caries, the tooth crown loss amounted to 20–50 %.

The patients had expressed written consent to allow conducting the research on determining the content of Pb removed from their teeth. One tooth was taken to the research from each patient. The taken teeth were provided with a questionnaire containing questions about gender, age, place of residence, and health status. When determining the health status, applied pharmacological treatments were taken into account. The research did not include teeth taken from patients suffering from metabolic diseases, and using hormonal substitution, as well as those who had required drug treatments for medical indications in other diseases for more than three months.

The research included posterior teeth (premolars and molars). Root canal-treated teeth and those with dental fillings were not included in the research. After removal, the teeth were cleaned off soft tissue, then washed and rinsed with distilled water.

After drying to constant weight (80 °C/24 h), the teeth were crushed in a porcelain mortar until a homogeneous sample was received. Samples prepared this way were stored in containers (plastic). The research included approx. 0.5 g sample weight which was mineralized wet (spectrally pure nitric acid— Merck, Germany; microwave digester Magnum II—ERTEC-Poland) [16].

Pb concentration in the samples of teeth was determined using the flame AAS method (AAS—3, Carl Zeiss Jena). Validation of designations was checked using reference material (NIST 1486 Bone Meal; concentration in certified material: 1.335 μgPb/g; measured: $n = 10$, 1.245–1.476 μgPb/g, mean 1.358 ± 0.07).

Statistical analysis of results was made using Statistica 10 (StatSoft). Due to the Pb concentration in teeth samples that

Table 1 Statistical characteristics and Pb concentration in the teeth of the subjects

	Age of subjects (years)	Number of subjects	Regression equation, (x—years of the subjects)	Coefficient of determination (R^2)	Statistical significance (p)	Median (μg/g)
All	13–84	390	$Pb = 9.5062 + 0.1103*x$	0.0756	0.0000	12.9
Age group						
1	<29	57	$Pb = 12.6525 - 0.0446*x$	0.0385	0.1433	10.7
2	30–39	68	$Pb = 11.8393 + 0.0381*x$	0.0208	0.2409	14.8
3	40–49	86	$Pb = 7.2287 + 0.1899*x$	0.0139	0.2804	13.0
4	50–59	104	$Pb = 18.5896 - 0.0713*x$	0.0165	0.1934	12.2
5	>60	75	$Pb = -10.0632 + 0.4031*x$	0.3319	0.0000	14.3

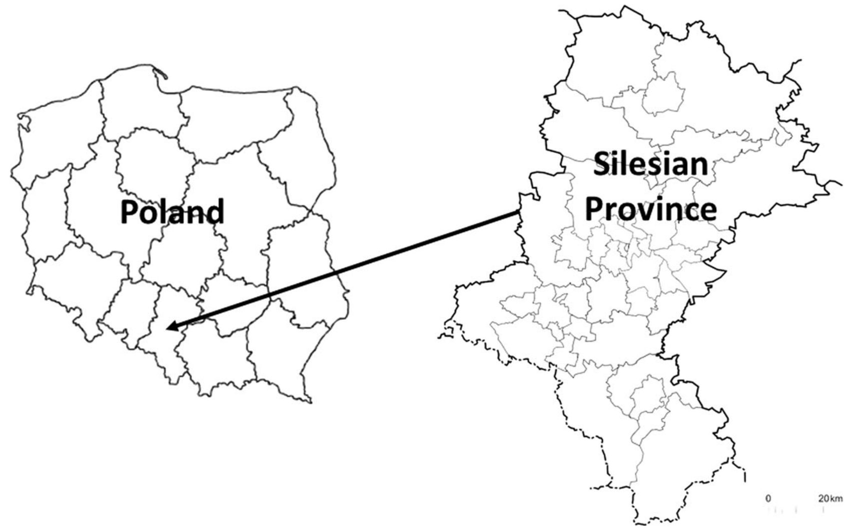


Fig. 1 Place of residence of people whose teeth were taken for research

differed from a normal distribution, non-parametric tests were used for calculations. When assessing differences between the groups (the Mann–Whitney U test for two samples and the ANOVA Kruskal–Wallis test by ranks for multiple samples), the level of significance amounting to $p \leq 0.05$ was statistically significant.

Results

The median determining the average concentration of Pb in tested teeth samples was 12.9 μg/g (Table 1). In the teeth of subjects from the distinguished age groups, statistically significant differences in Pb concentration in teeth were found ($p = 0.02$, the ANOVA Kruskal–Wallis test). The correlation coefficient that defines the coexistence of Pb and age in permanent teeth also pointed to the existence of a statistically significant dependence between these variables ($p = 0.02$, Spearman's R test). The statistical analysis of changes in Pb concentration as a function of age of the studied population was based on a scatterplot (Fig. 2). The regression calculation was used to statistically describe the studied population. For dependencies of changes in Pb concentration in the function of age, Table 1 shows regression equations defining the accumulation of Pb in calcified tissues of teeth. For the general population, the regression equation was as follows: $Pb = 9.50620 + 0.1103 * x$. The estimated increase in concentration of Pb in teeth during the year was approximately 0.1 μg/g. For each age group, the accumulation of Pb in teeth occurred for those aged 30–49 years (second and third age group), and for the oldest subjects (over 60 years of age). The decrease in Pb concentration in the age function was observed in the teeth of the youngest, i.e., 13–29 years, and persons aged 50–59 years (Table 1). In the light of the research results, the difference in Pb concentration in the teeth of men and women was not statistically significant ($p = 0.056$, the Mann–Whitney U test). However, Pb concentration was greater in the teeth of men (15.1 μg/g) compared to the teeth of women (13.6 μg/g) (Table 1). The linear dependence that illustrates the changes between Pb concentration in teeth and the age of the subjects pointed to a greater degree of accumulation of Pb in the teeth of men compared to women (Fig. 2). The highest average Pb concentration was found in the teeth of the oldest men, i.e., after 60 years of age (median 15.6 μgPb/g, AM 18.2 μgPb/g) (Table 2). A comparable level of Pb concentration for men and women was observed in the teeth of middle-aged people, i.e., in the second age group (30–39 years of age—median 14.8 μgPb/g). The lowest Pb concentration, respectively median: 9.5 μgPb/g (women's teeth) and 10.8 μgPb/g (men's teeth) was found in the teeth of the youngest subjects (i.e., up to 29 years of age).

To determine whether the period of years over which human body was subject to contamination has an impact on Pb concentration in mineralized tissues of teeth, the assessment of Pb concentration was made in teeth taken for research in the years 1998–2012. Figure 3 illustrates the way Pb average concentration changed in the teeth of the subjects from the distinguished age groups born in different years. It was found that in the teeth of the youngest (up to 29 years of age), the level of Pb concentration was lower for those born before the year 1970 compared to those who were born later, i.e., in the 1980s and 1990s. A similar dependence was observed for the second, third, and fourth age groups. A different tendency to changes in concentrations of Pb in teeth was observed for subjects from the fifth age group. The oldest subjects in the study, who were born in the years 1920–1930, had a higher Pb concentration in the teeth than people born in the 1940s. The teeth of those subjects showed a gradual decline in Pb concentration in the function of the year of birth (Fig. 3).

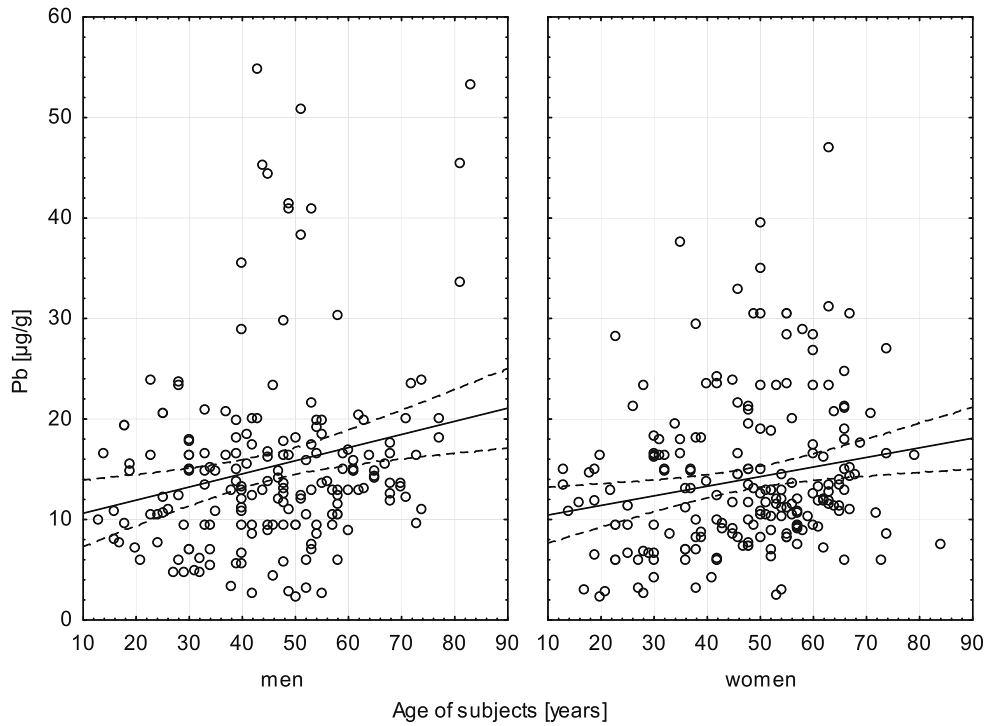


Fig. 2 Relationship between Pb concentration and age of the subjects in teeth of men and women

Discussion

In the light of literature reports, Pb concentration in teeth is highly diverse. Average Pb concentration in calcified tissues of teeth range from approx. 6.0 µg/g [17–19] to approx. 30 µg/g [20]. The average Pb concentration in calcified tissues of teeth determined in the conducted research was 12.9 µg/g (Table 1).

Table 2 Concentration of Pb in teeth from men and women, (µg/g)

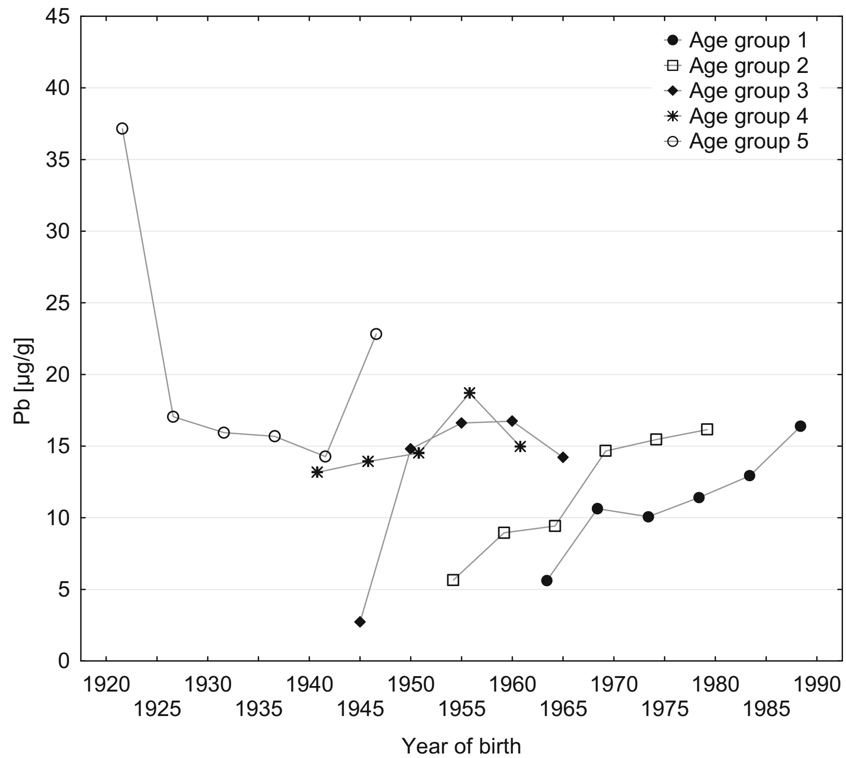
	Age of subjects (years)	AM	SD	Median
Women				
All	13–84	13.6	7.3	12.2
Age group				
1	<29	10.4	6.5	9.5
2	30–39	13.5	7.0	14.8
3	40–49	14.0	7.2	12.3
4	50 –59	14.5	7.9	11.7
5	>60	16.1	8.2	13.3
Men				
All	13–83	15.1	9.1	13.6
Age group				
1	<29	12.9	5.9	10.8
2	30–39	12.8	5.2	14.8
3	40–49	16.9	11.8	13.1
4	50–59	14.9	9.6	12.9
5	>60	18.2	9.5	15.6

AM arithmetic mean, *SD* standard deviation

A variety of factors has influence on Pb concentration in teeth. One factor is the participation of the process of caries. For deciduous teeth, a higher Pb concentration was found in healthy teeth compared to carious teeth. For permanent teeth, the research results indicated an inverse dependence or occurrence of dependencies without characteristics of statistical significance [20–22]. Due to the fact that in the light of literature reports, the influence of the presence of caries on Pb concentration in mineralized tissues of teeth is inconclusive, permanent carious teeth were analyzed in the conducted research.

Another factor that influences Pb concentration in teeth is the degree of exposure [23]. Accumulation of Pb in teeth expressed in increasing concentrations of this element in the function of age is a relatively well-described process [3, 19, 24]. The results of our research have confirmed that with the increasing age of the subjects, a higher Pb concentration in calcified tissues of teeth was observed (Fig. 2). The process of accumulation has been confirmed by the statistically significant value of the correlation coefficient (Spearman's R test, $p<0.05$). A statistically significant dependence between the age of subjects and Pb concentration in teeth was also shown in the results by Kumagai et al. [24]. Therefore, the subjects' age is an important criterion for differentiating the population's exposure to Pb. The resulting average Pb concentration in the teeth of the studied population (mean: age = 46 years, 12.9 µgPb/g) was higher than for the Japanese population of a comparable age [24] (mean: age = 49 years, 4 µgPb/g). Differences in Pb concentration in teeth of residents of Poland and Japan illustrate different environmental exposure of the studied populations [24]. The difference between Pb

Fig. 3 Changes in average concentrations of Pb in teeth of people from different age group born in different years



concentrations in teeth occurring within a continental range can also be differentiated in a smaller area. The results of separate original research suggest a level of Pb in the teeth taken in 2005–2006 from a different population of the area (Silesian Province, Poland) (11.3 μPb/g) [25] that is comparable to the one found in the current research. However, a lower Pb content in teeth among residents of other European countries was observed which ranged from approximately 6.0 μg/g (teeth of people living in Germany and Italy) [17–19] to 8.6 μg/g (teeth of people living in Austria) [19, 26]. The above data relate to populations of younger people who have low Pb concentration in teeth compared to the elderly (Fig. 2). Referring the average Pb concentration in teeth of the youngest subjects (10 μgPb/g) to literature data [17–19, 26] confirms the higher Pb concentration in teeth of the subjects. The higher Pb concentration in hard tissues of the Polish population that has been found in the research is the result of the observed higher concentration of Pb in the environment in Poland than in the neighboring countries [27].

The conducted long-term air quality analysis indicates a significant reduction in Pb pollution. For example, over the years 1990–2012, Pb concentration in dust with a particle diameter up to 10 μm (PM10) in the area of residence of the subjects was reduced from 0.2 to 0.06 μgPb/g [28]. Literature research provides information that in the years 1970–1980, higher Pb concentration in teeth was observed than what is observed now [29–31]. The results of the conducted research on determination of Pb concentration in teeth, taking into account the year of birth of the subjects (Fig. 3), indicated that for the oldest subjects (fifth age group, age > 60 years), the levels of Pb concentration in teeth decreased in the function of their year of birth. For this research group, Pb concentration in teeth was the highest in subjects born in the 1920s and 1930s and decreased reaching the lowest value in people born in the 1940s (Fig. 3). A converse course had the curve that illustrated the changes in the average concentrations of Pb in teeth of the youngest subjects (under 60 years of age) (Fig. 3).

Changes in Pb concentration in teeth of the youngest subjects (under 60 years of age) were characterized by an increasing average concentration of Pb in teeth. In all patients aged below 60, the later birth year generated higher Pb concentration in teeth. Increasing Pb concentration in teeth is observed in spite of the reduced Pb content in the environment [2, 28]. In the light of the research results, we can see that the year of birth from which we observe the progressive increase in Pb concentration in teeth is the year 1945.

The calculated value of the regression coefficient (Table 1) defining an increase in concentration of Pb in teeth in the function of age of the subjects was lower than for the population from Kuwait ($y = 1.2x + 17.6$, where $y = \text{Pb}$, $x = \text{year}$) [19]. The compared populations are characterized by a similar age bracket and almost twice the difference in Pb concentration in teeth. The regression equations indicate a varied process of accumulation of Pb in calcified teeth tissues [19]. The observed higher average Pb concentration in teeth tissues of the subjects led to a lower accumulation of Pb. Therefore, people who are less influenced by the exposure may experience a relatively larger accumulation of Pb in calcified tissues of teeth. A confirmation of this observation is the direction of changes mapped out by the course chart that connects the average concentration of Pb in teeth of Polish people born in different years (Fig. 3). Mean Pb concentration in teeth of the youngest subjects who are least exposed to pollution was higher for those born in the 1990s compared to previous years.

The determined high Pb concentration in teeth of men compared to women, respectively 13.6 and 12.2 μgPb/g (Table 2), is a reflection of a tendency observed in other authors' research [20, 21, 24, 32]. It has been observed that varied Pb concentration in teeth of men and women concerned mainly the youngest subjects (first age group) (Table 2). Among these subjects, the high Pb concentration in the bodies of boys compared to girls is explained by different lifestyle habits and hygiene [33, 34]. The small exposure of girls compared to boys was manifested by reduced levels of Pb concentration in teeth under 30 years of age (first age group) (Table 2). In the teeth of men and women from the second age group (30–39 years), the level of Pb concentration was identical (median 14.8 μgPb/g). A similar level of Pb concentration that occurred in the teeth of men and women aged 30–49 years is the result of an increased accumulation of Pb in tissues of women. The teeth of women aged 30–39 years showed the greatest Pb concentration (median 14.8 μgPb/g). The teeth taken from women in this group showed almost two times the average Pb concentration compared to the teeth of younger subjects (Table 2). The increased accumulation of Pb in the teeth of women aged 30–49 may reflect additional exposure to Pb associated with the use of cosmetic products. It has been shown that the use of make-up cosmetics (mainly eye liner and lipstick) can be a potential source of exposure to Pb [35]. Analyzing Pb concentration in particular samples of teeth, it was observed that in middle-aged men (i.e., 40–60 years), Pb concentration often reached maximum values that exceeded 40 μgPb/g compared to samples of the teeth taken from women (Fig. 2). Higher Pb concentration in the teeth of working-age men (>40 years) compared to the rest of the subjects may indicate their further professional exposure to this metal. This fact is especially important since the area where the subjects lived in the previous years was full of industrial plants dealing with processing and emission of Pb [36].

Demineralization changes that occur in the menopausal age in women can lead to an increase in the concentration of toxic metals including Pb [37, 38]. Elemental exchange between Ca and Pb can occur in the structure of mineralized tissues [39]. In the research, no statistically significant differences between the average Pb concentration in the teeth of men and women above 50 years of age were observed. Moreover, the difference in concentration of Pb in the teeth taken from women aged 50–59 years and the youngest women was the smallest (respective median, 11.7 and 9.5 μg/g) (Table 2). The results of the research do not show that in the period of demineralization, changes of hormonal origin teeth tissues showed an increased accumulation of Pb. In the light of the results obtained, it can be concluded that hydroxyapatite of teeth is characterized by a stable elemental structure compared to bone tissue. In contrast, the observed decrease in the average concentration of Pb in calcified tissues of teeth of both women and men aged 40–59 years may be the result of the influence of dietary factors. Literature research indicates that a type of diet can influence the elemental composition of calcified tissues of teeth [39, 40]. For example, consumption of soft drinks and sour-tasting food can lead to elemental mobilization, including Pb, from the structure of hydroxyapatite [41]. The decrease in the concentration of elements in teeth under the influence of nutrients is associated with the time of their reaction. In the conducted research, a low Pb concentration in the teeth of both women and men aged 40–59 years was observed compared to the concentration of this element in the teeth of younger (30–39 years) and older subjects (>60 years) (Table 2). The decreased average Pb concentration in the teeth of mainly 50-year-old women indicated that the reaction of dietary and hormonal factors may be particularly important for the structure of calcified tissues of teeth.

Conclusion

In the light of the results obtained, it was observed that, with age, there is accumulation of Pb in calcified tissues of teeth. This dependence was statistically significant ($p < 0.05$). It was shown that in the teeth of men and women, the course of the process of Pb accumulation is varied and can be influenced by different factors. However, higher Pb concentration in the teeth of men rather than women was not statistically significant.

The long-term assessment of changes in Pb concentration in teeth showed that the year of birth has influence on the process of accumulation of Pb in calcified tissues of teeth. For the oldest subjects, i.e., above >60 years of age, the earlier year of birth was associated with a higher Pb concentration in their teeth. Different research results were obtained for younger people (13–59 years). The direction of changes in average Pb concentration showed that people born in earlier years were characterized by lower levels of Pb concentration in calcified tissues of teeth. Later, years of birth of the subjects translated into higher Pb concentration in teeth. A worrying phenomenon is the fact that despite the confirmed reduction of Pb content in the air [28, 36], a comparable level of Pb concentration in calcified tissues of teeth is observed. The analysis of changes of Pb indicates that for low exposure, a relatively greater accumulation of Pb concentration in calcified tissues of teeth can occur.

Acknowledgments This work was financed by the Medical University of Silesia in Katowice (contract No. KNW-1-027/N/5/0)

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