

# Zinc and Lead Concentrations in the Pubic Hair of Women Living in Areas with Different Contamination Degrees

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## Abstract

Our paper contains analytical results of zinc and lead concentrations in the pubic hair of women living in areas with different contamination degrees. Women living in the area of Knurów, Silesia (a high degree of industrialization) and female residents of the area of Łańcut (Subcarpathian region), where the level of urbanization is much smaller, constituted the respondent group. Overall, the hair of 159 women aged 22 up to 75 was tested. Washed hair was mineralized by a microwave method with HNO<sub>3</sub>. Metal concentrations were determined using the AAS technique and SpectrAA 880 as well as Spectra 880Z spectrophotometers by the Varian Company. It has been found that the average lead concentration in the pubic hair of the women from the Subcarpathian region was significantly higher than the concentration of this element in the group of women from Silesia, which was probably related to the influence of low emissions in the area. Zinc concentrations in the pubic hair of the women in both treatment groups did not differ. The concentration of lead in the pubic hair of the women from the Subcarpathian region increased with age.

**Keywords:** pubic hair, lead, zinc, pollution

## Introduction

Hair is a biological material that allows visualization of long-term exposure, among others, to metals. As research material, it has many advantages, such as simple and non-invasive sampling, ease of transportation and storage, and stability resulting from the fact that hair undergoes destructive processes more slowly than other tissues [1]. Apart from scalp hair, researchers use hair from other parts of the body, e.g. pubic or armpit hair. Unlike scalp hair, pubic and armpit hair does not undergo frequent cosmetic treatments, which can damage the outer keratin layer, and is not direct-

ly exposed to environmental contamination. The scatter of elements in pubic hair is lower than that in scalp hair, but their concentrations correlate strongly with the concentrations in the latter [2, 3]. The possibility for contaminating pubic hair with metals present in urine, indicated by some analyses of drug concentrations in pubic hair, does not apply herein since their concentrations in urine are considerably lower than in hair [4].

Heavy metals, which have their source in the environment, are permanently bonded on the surface of hair as a result of thioester bond hydrolysis. These bonds are formed between the free thiol groups of hair layer proteins and carboxyl groups of thin fatty shell, whose main component is 18-methyleicosanoic acid (18-MEA). This layer

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is an ultimate hair protection against external influences, reducing friction and giving the hair surface a hydrophobic character, which in normal conditions protects hair against a reaction with metal ions [5, 6]. Since the process of elements incorporation in hair fiber is closely associated with its growth cycle, the analysis of element concentrations in hair gives the possibility for retrospective monitoring of nutritional status and the size of environmental exposure. In the case of diffusion of chemical substances, such as drugs or medication, studying the distribution of concentration of drugs and medication on hair length allows the time exposure to a given agent to be approximately determined [7]. Therefore, hair is successfully used in environmental studies to compare the contamination degrees of different areas or to detect excessively contaminated sites, and to monitor occupational exposure to heavy metals [8-12].

Lead is one of the most widespread environmental pollutants that poses a risk to human health. Danger of its adverse effects on a human organism is associated with its long half-life, displacement of necessary metals from connections with structural and enzymatic proteins, and the ability to accumulate in the body [13].

Zinc, in turn, is essential for proper functioning of many enzymes and development of the organism. However, despite the fact that it belongs to trace bioelements and symptoms of its excess are extremely rare, concentrations beyond necessary amounts for its biological functions can damage many biochemical processes [14].

The aim of our study was to evaluate concentrations of zinc and lead in women's pubic hair from areas with different degrees of industrialization. The criterion for the selection of elements was based on the fact that lead is common in the environment and important as the most hazardous environmental poison – besides cadmium and mercury. As far as zinc is concerned, it interacts with lead and its concentrations in the environment and human body significantly affect the extent of lead absorption and, consequently, toxicity.

### Experimental Procedures

The tests were carried out with the approval of the Bioethics Committee of the Medical University of Silesia in Katowice L.dz.NN-013-75/I/02 and women from whom the material was obtained.

The material for testing was pubic hair taken from women living in Knurów (Silesia) and Łańcut (Subcarpathian region), two areas with different degrees of industrialization.

According to the data released by the Central Statistical Office, only 4% of the total area of the Subcarpathian region is developed (76.3 thousand ha), while in the Silesia region, with a total area of 1,233.3 thousand hectares, the grounds occupy 141.3 thousand ha, which corresponds to 11% of the total area of Silesia [15]. Taking into account the area percentage of the regions constituting urban areas, the

city of Knurów can be regarded as situated in a higher degree of industrialization area in relation to the city and environs of Łańcut.

A confidential questionnaire was conducted among all the women, including questions about age, place of residence, living conditions, professional exposure to metals, cigarette smoking, past and current diseases, and medications. Women who were chronically ill, occupationally exposed to metals, taking medications or supplements that contained zinc, and smoking cigarettes were excluded from the study.

Finally, the study included 159 women aged 22-75. The average age was  $45.4 \pm 12.1$ . The group of Knurów residents contained 72 people. The age of women in this group was 22-75, with an average of  $47.5 \pm 13.3$ . The second group – the residents of Łańcut and nearby villages – included 87 women aged 22 -70, with an average of  $43.7 \pm 10.7$ .

The women from both groups were divided into five age groups. The first group included women aged 22-30, the second from 31 up to 39, the third from 40 up to 48, the fourth from 49 up to 57, the fifth from 58 up to 77.

The collected pubic hair was prepared following a procedure established by the International Atomic Energy Agency [16]. The hair was washed with deionized water and pure acetone. The hair samples were rinsed with acetone, then three times with water followed by acetone again. Each time the contact time with the solutions was about 10 min. After being dried at 105°C to constant mass, uniform weight (about 200 mg) of hair samples was digested in a microwave, using 2 cm<sup>3</sup> of concentrated spectrally pure HNO<sub>3</sub>. After digestion, the acid was vaporized out of the samples, 0.5 cm<sup>3</sup> of HNO<sub>3</sub> and 10 cm<sup>3</sup> of water was added and then the content was transferred into 25 cm<sup>3</sup> measuring flasks and filled to volume.

Apart from the pubic hair, digestion was simultaneously carried out on CRM 397 reference material (Trace elements in human hair, Community Bureau of Reference). The results from six runs were as follows: Zncertified 205 µg/g, Zn<sub>assayed</sub> 199 µg/g, Pb<sub>certified</sub> 33.0 µg/g, and Pb<sub>assayed</sub> 31.5 µg/g.

The concentrations of metals in the pubic hair and reference material samples were assayed using flame (Zn) and flameless (Pb) atomic absorption spectrometry, using Varian SpectrAA 880 and SpectrAA 880Z spectrophotometers. The concentrations of the elements in the pubic hair were determined following commonly acknowledged measurement procedures.

To analyze the results obtained in the study, computer programs Statistica 9 and Microsoft Excel were used. The Shapiro-Wilk test was conducted, which proved that the distributions of Pb and Zn results in particular groups were not normal. The data sets did not contain any outliers (Grubbs test). The significance of differences between the groups was analyzed using Mann-Whitney's U test and ANOVA rank Kruskal-Wallis. The study on the interaction between zinc and lead concentrations in hair was based on Spearman's rank test.

Table 1. Statistical characteristics of lead and zinc concentration in pubic hair of women from the Silesian and Subcarpathian regions [ $\mu\text{g/g}$ ].

Parameter	Subcarpathia region	Silesian region
N	87	72
Pb		
Mean	1.18	0.91
SD	0.95	0.88
Median	0.9	0.7
Min	0.08	0.06
Max	5.58	5.07
Zn		
Mean	186.77	204.71
SD	83.82	128.78
Median	165.2	165.36
Min	116.8	102.76
Max	786.8	867.84
Zn/Pb ratio		
Mean	303.09	577.58
SD	311.51	911.91
Median	200.58	237.64
Min	29.75	48.63
Max	1820.24	5455.51

## Results

Lead concentration in the pubic hair of women in the Łańcut group (and its surroundings in the Subcarpathian region) ranged from 0.08  $\mu\text{g/g}$  up to 5.58  $\mu\text{g/g}$ , whereas the women from Knurów showed 0.06  $\mu\text{g/g}$  up to 5.07  $\mu\text{g/g}$  (Table 1). The median of lead in the pubic hair of the women from the Subcarpathian region was 0.90  $\mu\text{g/g}$ , while

the median calculated for the groups coming from Silesia was 0.70  $\mu\text{g/g}$  (Table 1). This difference was statistically significant (Mann-Whitney's U test,  $p = 0.03$ ).

Zinc level assayed in the pubic hair of the Łańcut women and contained between 116.8  $\mu\text{g/g}$  and 786.8  $\mu\text{g/g}$ , whereas the women from Knurów measured between 102.76  $\mu\text{g/g}$  and 867.84  $\mu\text{g/g}$ . The medians of zinc concentration in the pubic hair of both groups of women were similar, reaching 165.2  $\mu\text{g/g}$  for the test group from the Subcarpathian region and 165.36  $\mu\text{g/g}$  for the test group from the Silesian region. This difference was not statistically significant (Mann-Whitney's U test,  $p = 0.33$ ).

Table 1 shows ratio values of zinc and lead concentrations in both groups. The average value of the Zn/Pb ratio calculated for the hair in the group from the Silesian region was higher than the values obtained for the group from the Subcarpathian region, but this difference was not statistically significant (Mann-Whitney's U test,  $p > 0.05$ ).

The median concentrations of lead and zinc in different age groups are summarized in Table 2. The largest median lead concentration in hair was found in the third group (aged 40-48) and the fourth group (aged 49-57) from the Subcarpathian region (1.07  $\mu\text{g/g}$  and 1.06  $\mu\text{g/g}$ ). As to the lowest levels, they were observed in younger women – the age group between 31 and 39 years of age from the Subcarpathian region (0.56  $\mu\text{g/g}$ ) and women between 22 and 30 years of age from Silesia (0.48  $\mu\text{g/g}$ ). An increase of lead concentration in hair with age was observed for the women from the Subcarpathian region. This dependence was described by a statistically significant correlation coefficient  $r = 0.28$  (Fig. 1). No such relation was observed in the group of women from Silesia.

The median zinc concentration in the hair of the Łańcut women was the highest in the first age group (198.80  $\mu\text{g/g}$ ). In the group from Silesia, the largest zinc concentration was found in the hair of women between 40 and 48 years of age. The lowest zinc concentration was in the fourth group (49-57) of women from the Subcarpathian region – 159.30  $\mu\text{g/g}$  and the group of women aged 22-30 from Silesia. Differences in zinc and lead concentrations in hair in different age groups were not statistically significant (ANOVA rank Kruskal-Wallis,  $p > 0.05$ ).

Among the subjects from Silesian, the highest Zn/Pb ratio was found for the youngest age group (22-30), while

Table 2. Median zinc and lead concentrations and the value of the Zn/Pb ratio in pubic hair of women from the Subcarpathian and Silesia regions in different age groups [ $\mu\text{g/g}$ ].

Age group	Subcarpathia region				Silesia region			
	N	Pb [ $\mu\text{g/g}$ ]	Zn [ $\mu\text{g/g}$ ]	Zn/Pb ratio	N	Pb [ $\mu\text{g/g}$ ]	Zn [ $\mu\text{g/g}$ ]	Zn/Pb ratio
22-30 years	11	0.65	198.80	228.22	11	0.48	155.59	525.19
31-39 years	18	0.56	169.05	299.15	6	0.64	157.74	325.69
40-48 years	32	1.07	161.20	164.58	25	0.78	173.90	247.28
49-57 years	19	1.06	159.30	150.27	17	0.78	161.10	228.01
58-75 years	7	0.88	165.90	168.61	13	0.61	167.12	296.24

the lowest for the fourth group (49-57), Table 2. Meanwhile, in the group from Subcarpathia the highest median ratio of Zn/Pb was calculated for the second group, and the lowest for the fourth group (49-57). Differences in the Zn/Pb ratio in the hair of the women of different age groups were not statistically significant (ANOVA rank Kruskal-Wallis,  $p > 0.05$ ). A statistically significant negative correlation ( $r = -0.29$ ) between the Zn/Pb ratio and the age of women from Subcarpathia was observed (Fig. 2).

In the groups of women from both Subcarpathia and Silesia there was a weak correlation between the concentrations of lead and zinc. The correlation coefficient based on Spearman's rank test was  $-0.18$  in the Subcarpathian group, while in the group from Silesia it was  $-0.08$ .

### Discussion of Results

The elemental composition of hair is affected by many factors. Therefore, to avoid the impact of such treatments as coloring or perm, as well as the effects of using large quantities of cosmetics, pubic hair was used in the test. All the women declared themselves to be non-smokers and living in good conditions, which to some extent affected their diet and the fact of smoking [17-19]. However, due to the fact that most of the literature data on lead and zinc concentration in hair refers to scalp hair, the results were compared with those available in many studies.

The reference employed herein was research by Wilhelm et al., [3] which proved that metal concentrations in head and pubic hair were similar and exhibited significant and positive correlations.

Zinc concentration in hair given by different authors varies within wide limits [2, 6, 20-23]. Average zinc concentration in the hair of all the women was  $194.89 \pm 106.57$   $\mu\text{g/g}$  and was higher than the value obtained by Srogi [21] ( $146.32 \pm 14.93$   $\mu\text{g/g}$ ) and lower than the average value obtained by Chojnacka et al. [20] ( $242 \pm 154$   $\mu\text{g/g}$ ), and Dongarra et al. [22] ( $210.8 \pm 54.1$   $\mu\text{g/g}$ ). A similar zinc concentration in hair was assayed by Wiechula et al. [2] and Lukasiak et al. [23].

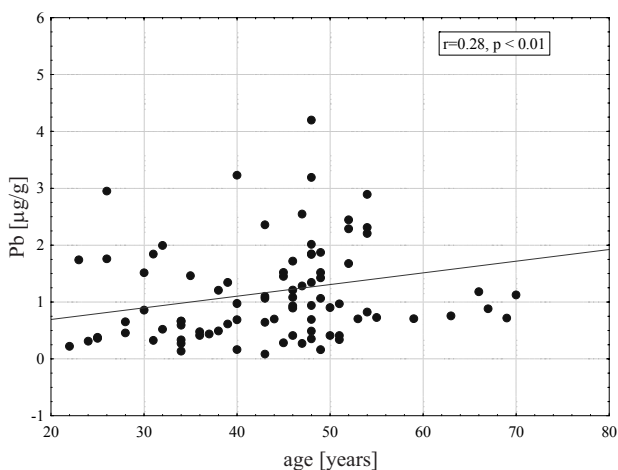


Fig. 1. Dependence between lead concentrations in hair and age of women from Subcarpathia.

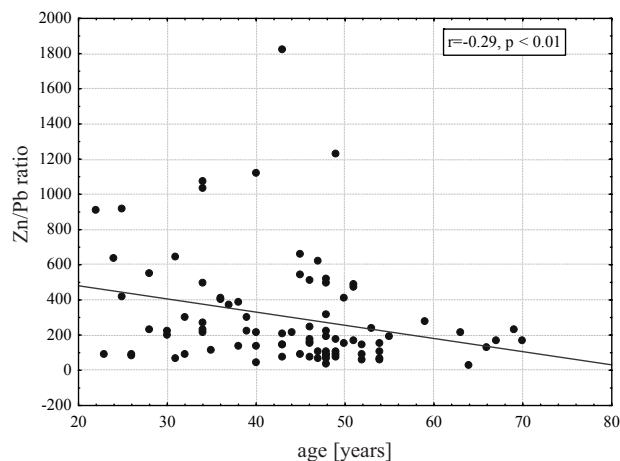


Fig. 2. Dependence between age and the Zn/Pb ratio in pubic hair of Subcarpathian women.

There was no significant difference in zinc concentration in the hair of Subcarpathian and Silesian women. The median levels of zinc were similar and amounted to  $165.2$   $\mu\text{g/g}$  for the group from Subcarpathia and  $165.36$   $\mu\text{g/g}$  for the group from Silesia. In contrast, other authors observed significant differences in zinc concentrations in the hair of people living in areas with different degrees of contamination [21].

The average lead concentration in the whole study group reached  $1.06 \pm 0.93$   $\mu\text{g/g}$  and was lower than the results obtained by Chojnacka et al. [20] ( $3.03 \pm 1.48$   $\mu\text{g/g}$ ) and Srogi [21] ( $3.94$   $\mu\text{g/g}$ ) and Kozielc et al. [24] ( $10.22$   $\mu\text{g/g}$ ), but very similar to the results of Dongarra et al. [22] ( $1.03 \pm 0.80$   $\mu\text{g/g}$ ).

Various authors indicate the possibility of using lead concentrations in hair as an indicator of environmental pollution. For example, studies by Sanna et al. [8] on a group of teenagers aged 10-15 from three cities of Sardinia, with different lead exposure, have shown that its concentration was higher in the young people from areas with a greater degree of industrialization. Comparable results were obtained by Özden et al. [9] while studying the impact of smoking and proximity to major transportation hubs on lead concentration in the hair of the youth. Also, the studies by Trojanowski et al. [10] show that lead concentration in hair was higher in people living in larger cities compared to those from rural areas.

The higher lead concentration in the hair of the Subcarpathian women ( $0.90$   $\mu\text{g/g}$ ), compared with the hair of Silesian inhabitants ( $0.70$   $\mu\text{g/g}$ ), may be associated with the influence of the so-called 'low emissions'. This term denotes primarily emissions from transportation routes, as well as local coal-fired boiler furnaces and individual households. Analysis of the data on air pollution in Poland shows that in 2009 particulate emissions from plants particularly harmful to the environment amounted to 2,000 tons in the Subcarpathian region and 11,700 tons in the Silesian region [15]. This data also indicates that in Subcarpathia individual heating of detached houses and buildings, where coal is the most commonly used fuel char-



acterized by poor heating characteristics and high pollution, was a significant source of particulate emissions. In addition, the low awareness of environmental pollution results in burning different types of waste, which may contain heavy metals such as lead [25, 26]. It is also worth remembering that the Jędrzychowice-Korczowa transit route (road E40) connecting Western Europe with the Ukraine runs through Łańcut. This is a route with very heavy traffic. Determination of emissions from sources characteristic of low emissions is difficult to estimate and can vary significantly. Therefore, we cannot exclude low emissions in the places of residence of the Subcarpathian women – a significant source of lead and directly translated into an increase of lead concentrations in hair. In Silesia, more than 50% of the total particulate pollution was produced by large power plants [27]. Emissions in these plants is subjected to continuous monitoring.

Apart from the level of essential and toxic metals, the appropriate balance between them is equally important to the proper functioning of the body [2]. Many studies indicate that in the future, the interpretation of mineral composition analysis of hair may be based on investigations into proportions between different elements [20]. In the case of lead and zinc, the right ratio can be extremely important, since these elements compete with each other in their physiological and metabolic activities [28].

The average values of the Zn/Pb ratio in the pubic hair of Subcarpathian and Silesian women were 303.09 and 577.58, respectively. They were much higher than those obtained among women who dye hair (122) and do not use dye (103) in the study by Chojnacka et al. [20], and also than the values obtained in the study by Afridi et al. [29] in all age groups of healthy women and those hospitalized after myocardial infarction. The average Zn/Pb ratio calculated for the group from Silesia was higher than the value in the group from Subcarpathia.

Many metals can accumulate in hair over many years of exposure [21]. Hair accumulates a lot of arsenic, mercury, copper and – most importantly – zinc and lead [17]. This is due both to taking in metals through ingestion and deposition of metal particles from atmosphere on hair and the possibility of their gradual infiltration into the fiber [17]. This may be the reason for a gradual increase of lead concentration with age observed in studies by many authors [10, 17]. By examining the dependence between age and the level of marked metals in the hair from both groups of women, it was found that among women from the Subcarpathian region the level of lead increased with age, but the concentration of zinc did not change. A similar lack of relationship between zinc concentrations in hair and age was shown in the previous studies [2]. Meanwhile, Właźlak et al. [30], in an experiment conducted on a group of perimenopausal women aged 42-58, showed that levels of zinc in hair decrease with age, which can cause symptoms such as skin diseases, loss of appetite, brittleness of hair and nails, and increased susceptibility to infection [30]. However, in the group from Silesia zinc levels rose with age and lead concentrations in hair decreased. This may be related to the interaction between these two metals, as it is known that a

deficit of zinc in the body increases the absorption of lead [31, 32], which is connected with the competition of those ions for bonds in the intestine wall. The interaction between zinc and lead, as well as other divalent indispensable elements, results from their similar chemical properties and affinity with similar proteins, including metallothionein. One should also bear in mind that lead competes with those metals for the binding location in enzymes, in which they act as cofactors, e.g. with zinc in porphobilinogen synthase or delta-aminolevulinic acid dehydratase [33].

The female group from the Subcarpathian region demonstrated a lower zinc concentration in the hair of over 30-year-olds than the youngest group. The lack of protective function of zinc resulted in an increasing lead concentration in hair with age and decreasing Zn/Pb ratio. Zinc supplementation can limit lead absorption and its toxicity.

## Conclusions

1. The average concentration of lead in the pubic hair of women from the Subcarpathian region was significantly higher than the concentrations of this element in the group of women from Silesia, which was probably related to the influence of low emissions.
2. Zinc concentrations in pubic hair of the women in both treatment groups did not differ significantly and was within the reference ranges presented in literature.
3. Lead concentration increased with age in the pubic hair of the women from the Subcarpathian region. The dependences between age and zinc concentrations in the group from the Subcarpathian region, and between age and the concentrations of lead and zinc in the group from Silesia, were not statistically significant.

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