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ORIGINAL PAPER

As AND Pb AND THEIR POTENTIAL SOURCE IN THE HAIR OF RESIDENTS OF CRACOW*

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Abstract

The hair content of arsenic and lead of residents of Cracow, an agglomeration with nearly one -million population, was determined and compared to those of inhabitants of Lubasz (a village with around 3,200 population in central Poland). The sample material (composed of scalp hair samples) was collected in July and August of 2014. The residents who agreed to participate in the test had to be over eighteen years old and in the case of Cracow have been living there for at least two years. The maximum values reached 0.278 mg kg^{-1} arsenic and 31.7 mg kg^{-1} lead, and those of lead were determined in the samples collected in Lubasz. The highest mean content of arsenic (0.077 mg kg^{-1}) was obtained for residents of Dębniki, a district of Cracow. The impact of the quality of the environment in which the tested persons lived as well as other selected factors that can affect their hair arsenic and lead content (for example: gender, age, state of health, work place, type of food, smoking, drinking) was assessed. The results have confirmed the general knowledge that a proper diet and a clean, unaffected environment are basic factors minimizing the adverse impact of arsenic and lead on human beings. The factors whose influence on the hair content of arsenic and lead were considered are: the place of residence, occupational exposure, sex, age, diet, alcohol consumption and hair colour. Due to the small number of samples that have been examined this is a pilot study.

Keywords: environmental contamination, potential harmful elements, factors.

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INTRODUCTION

Hair, a natural formation of epidermis, has several functions. The protective ones, because it covers sensitive fragments of the skin; regulatory ones because it controls body temperature; and also an excretory role because it helps remove some toxic elements from the organism (MICHALAK et al. 2014). Hair is used as research material for assessing the degree of environmental contamination and for detecting, mainly in humans, the processes of environment-related poisoning even prior to the appearance of symptoms of a disease (obviously, the authors omit forensic issues). The elemental analysis of hair has numerous advantages. Hair collecting is less invasive than taking blood or urine samples, while its storage and transport are simpler and safer due to the lack of an exposure risk. Determination of the elemental content of hair reflects the current exposure of an organism, but it can also be indicative of the accumulation of elements over a longer time, weeks or even a few years, depending on the length of hair (WANG et al. 2009). The hair concentration of elements is usually from 10 to 100 higher than the respective values of blood or urine (WIECHUŁA et al. 2012). In addition, hair maintains constant chemical composition for a long time owing to its keratin shell, which limits the loss of internal compounds and prevents penetration of chemicals from the outside. Furthermore, the external contaminants deposited on the surface of hair are easily removable by simple washing, which makes the results of a properly conducted analysis sound and reproducible (GROMYSZ-KAŁKOWSKA et al. 2002). However, comparison and interpretation of results can be difficult because of the absence of well-established standards of hair analysis and the scarcity of data pertaining to normal ranges of trace elements of hair (HAMBIDGE 1982).

Pollutants, including potentially harmful elements (PHE), enter the human body *via* the respiratory system, the digestive system and the skin. The elements inhaled with the air, swallowed with food and penetrating the skin eventually enter blood. The blood which nourishes hair roots provides both nutrients and elements undesirable for and even toxic to the organism. The PHE in hair can be of an endogenous (from blood), semi-endogenous (absorbed through the skin) and/or exogenous origin (from the surrounding environment) (WIERCIŃSKI, ZABOROWSKA 1996). There are two mechanisms engaged in the process of absorption of toxicants trough the skin. The first is the trans-epidermal transport (through the epidermis) and the other one is trans-follicular transport, i.e., *via* the hair-sebaceous system. The cells of sebaceous glands and of the walls of hair follicles are relatively easily permeable, and can therefore absorb metals. Keratin that builds hair is a protein from the group of scleroproteins, which contains amino acids such as leucine, cysteine, glutamic acid and arginine. Cysteine contains a thiol group, which willingly binds PHE. They become permanently fixed within hair due to hydrolysis of the thioester bond that forms between the thiol and carboxyl groups of a fatty shell of hair (WIECHUŁA et al. 2012).

The study was conducted in order to determine As and Pb hair concentrations of adults living in various districts of Cracow, an agglomeration with a population of nearly one million. The material for comparison was collected from adults living in Lubasz, a village with around 3,200 inhabitants located in central Poland. An attempt was also made to establish possible sources of these two elements, taking into consideration various factors, for instance a diet of the respondents or their occupational exposure to As and Pb. The project started in July of 2014 and ended in September of 2015.

MATERIAL AND METHODS

Two distinctly different locations were chosen to carry out investigations: Cracow and Lubasz (Figure 1). They differ mainly in their population size, industrialization level and the lifestyle of inhabitants.

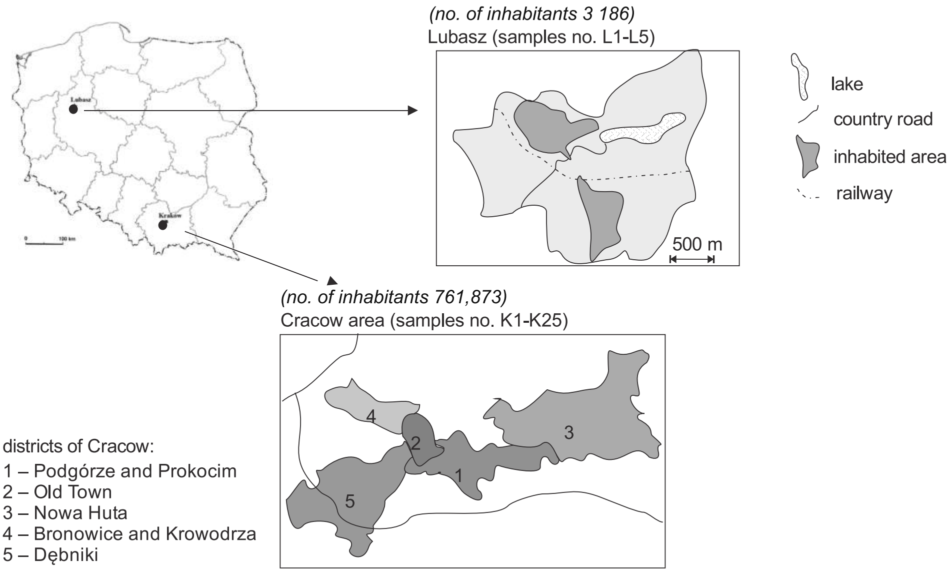

Fig. 1. Sampling areas

With its population of 761,873, Cracow is the second largest town in Poland (GUS 2015), and it is divided into 18 districts. There are two industrial plants considered especially noxious for the environment due to the air pollution they generate (Report 2015*a*): the ArcelorMittal Poland steel plant and the Kraków Heat and Power Generating Plant. The Cracow industrial region is dominated by the electric machinery, iron/steel and food industries. Its area is also substantially burdened by communication emissions caused by high intensity road traffic estimated at about 150,000 cars a day (www.

gazetakrakowska). Pollutants which contribute most to the deterioration of air quality are particulate matter PM10 and PM2.5, nitrogen dioxide and benzo(a)pyrene (Report 2015*b*). Green areas are an important environmental element of Cracow, although they occupy only 10.8% of the city area, including 4.38% of forested land. Together with river valleys, they form crucial ventilating corridors of the town, which is located mostly within the Vistula River valley.

Lubasz is a village with a population of 3,168, situated on the border of the Great Poland Lake District and the Toruń-Eberswalde ice marginal valley, and the administrative seat of a commune (lubasz.pl). The village itself is surrounded by gently undulating fields and meadows. There are two industrial plants (production of electric cells and metal-plastic furniture), which emit mainly dusts, SO_2 and CO_2. A more serious thread is the low-stack emission resulting from burning mainly coal muds of inferior quality, bituminous coal and coke in home furnaces and boiler houses. The most significant linear source of pollution is the voivodeship (regional) road No. 182. The intensity of traffic in Lubasz is less than 5,000 vehicles per day and includes mainly light vehicles: private cars and van transporters (91-92%).

The analyzed material was composed of scalp hair samples, which were collected from 25 residents of Cracow (basic set, samples nos K1-K25) and from 5 residents of Lubasz (control set, samples nos L1-L5). The basic set samples represent five districts of Cracow: Podgórze & Prokocim, Stare Miasto (Old Town, i.e. the city central part), Nowa Huta, Bronowice & Krowodrza and Dębniki. The control samples were collected in Górczyn, the southern part of the Lubasz village, located south of the railway line. The village was chosen because of the small number of possible sources of As- and Pb-bearing pollutants. Contrary to Cracow, there are no problems with air ventilation in its area, while large patches of forests prevent transport of contaminants from outside. The residents of the village frequently consume rural, often home-grown products.

The sample material was collected in July and August of 2014. Due to the small number of samples that have been examined (n = 30), this is a pilot study. The residents who agreed to take part in the test had to be over 18 years old and in the case of Cracow have been living there for at least two years. Their hair had to be natural and not dyed. Samples weighing approximately 4 g were collected from the scalp. People subjected to the study also filled in a questionnaire, providing the following information: their gender, age, name of a district and duration of residence in Cracow or Lubasz, hair colouring habits in the past, place they spend most time during a normal day, journeys made, exposure to deleterious factors at a work place. Further questions referred to cooking and eating habits: whether one cooks at home or eats outside; what is the major origin of basic foodstuff (supermarkets or rural-grown household products), whether one consumes higher amounts of meat, fish, coffee and tea with lemon juice. A description of one's lifestyle followed: living under constant stress, being overworked, smoking, drinking alcoholic beverages, and frequently falling ill. The survey also asked about hair shampooing, and if so to specify a cosmetic brand mark.

All the hair samples were washed with mild shampoo *Johnson's Baby* in order to remove surface contaminants such as sweat, sebum, cosmetics, dust and other dirt. After washing, hair samples were rinsed with distilled water and dried to air-dry conditions at room temperature for 24 hours. Next, each sample was rinsed with acetone, then thrice with deionized water and once again with acetone. Each time the contact with the solutions was 10 minutes. Finally, the samples were dried in a laboratory dryer at the temp. 50°C (Samanta et al. 2004). The next step was digestion of hair (weight of each sample was 0.5 g) in 5 ml 65% HNO_3 for 2 h using a DigiPREP HT mineralizer at 130°C temp. The concentrations of As and Pb were determined using atomic absorption spectrometry with a Thermo Scientific iCE 3000 analyzer (Eneji et al. 2012), in the Laboratory of Trace Analyses AGH at University of Science and Technology (certificate of accreditation PCA no. AB1050 at the detection limits of As equal $1 \cdot 10^{-3}$ mg dm^{-3}, and Pb equal $1 \cdot 10^{-4}$ mg dm^{-3}). Statistical calculations and data presentation were performed with Statistica ver. 10 and Excel applications. The significance of correlations was determined with the use of Student's *t*-test, significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

The hair As content of the residents of Cracow ranged from 0.005 to 0.279 mg kg^{-1} (Table 1). Its highest mean (0.077 mg kg^{-1}) was found in hair from the residents of Dębniki, whereas the lowest one (0.019 mg kg^{-1}) was determined in hair of the inhabitants of Podgórze, part of the housing estate Prokocim.

The mean As hair content (0.045 mg kg^{-1}) of the Cracow population (the basic set) is significantly higher than the mean hair As content (0.032 mg kg^{-1}) of the Lubasz control set. However, a comparison of the calculated means and medians of the hair As content of both populations gave an insignificant difference. The median of the basic set is 0.025 mg kg^{-1}, while that of the control set is 0.028 mg kg^{-1}, which indicates a large scatter of the test results. The largest scatter, measured also by the standard deviation (SD), is in samples K16-K25 (Figure 2) collected in the districts Dębniki (SD = 0.113 mg kg^{-1}) and Bronowice & Krowodrza (SD = 0.081 mg kg^{-1}). Both locations are characterized by the highest car traffic and the density of urban development. The smallest scatter of the hair As values is for the residents of Lubasz (control set) and the residents of Podgórze & Prokocim districts of Cracow (for both sample sets SD is 0.009 mg kg^{-1}).

The background of the hair As was calculated according to the formula proposed by Reimann and Garret (2005). Its upper limit, equal to the median plus its two absolute deviation values, is 0.073 mg kg^{-1} and was exceeded in

Table 1

As and Pb content (mg kg^{-1}) in the analyzed hair samples of residents of Cracow and Lubasz

Location (symbol of samples)			As	Pb
Cracow	Podgórze & Prokocim (*K1 – K5*)	min. – max.	0.01 – 0.035	1.93 – 12.30
		Av ± SD	0.019 ± 0.009	6.15 ± 4.13
		Me	0.015	6.53
	Old Town (*K6 –K10*)	min. –max.	0.02 – 0.051	0.07 – 4.72
		Av ± SD	0.028 ± 0.015	1.82 ± 2.25
		Me	0.025	0.39
	Nowa Huta (*K11 – K15*)	min. – max.	0.021 – 0.056	1.50 – 6.76
		Av ± SD	0.033 ± 0.016	3.10 ± 2.11
		Me	0.030	2.34
	Bronowice & Krowodrza (*K16 – K20*)	min. – max.	0.0051 – 0.206	0.13 – 9.49
		Av ± SD	0.068 ± 0.081	3.59 ± 4.08
		Me	0.041	1.26
	Dębniki (*K21 – K25*)	min. – max.	0.015 – 0.278	3.15 – 16.80
		Av ± SD	0.077 ± 0.113	7.45 ± 5.55
		Me	0.035	6.94
	for all samples from Cracow	min. – max.	0.005 – 0.279	0.07 – 16.80
		Av ± SD	0.045 ± 0.061	4.43 ± 4.02
		Me	0.025	3.15
Lubasz (*L1 – L5*)		min. – max.	0.020 – 0.042	4.67 – 31.70
		Av ± SD	0.032 ± 0.009	11.61 ± 11.29
		Me	0.035	7.33
For all samples Lubasz and Cracow		min. –max.	0.005 – 0.278	0.07 – 31.70
		Av ± SD	0.043 ± 0.056	5.62 ± 6.13
		Me ± 2MAD	0.028 ± 0.045	4.22 ± 7.95

Av – average, SD – standard deviation, Me – median, MAD – median absolute deviation

two samples (K18 and K25) obtained from a 27-year-old woman (0.021 mg kg^{-1} As), a resident of Bronowice who declared working in low temperatures (16°C), and a 47-year-old male (0.278 mg kg^{-1} As) from Dębniki, exposed to high exhaust emissions and noise.

The Pb hair content of the population of Cracow ranges from 0.07 to 16.8 mg kg^{-1}. The highest mean content was found in hair of the residents of Dębniki (7.45 mg kg^{-1}) and the lowest one in hair of people living in the Old Town (1.84 mg kg^{-1}).

The Pb hair content exceeding the upper limit of the background equal to 12.17 mg kg^{-1} Pb was found in two samples (L1 and K24). The first one

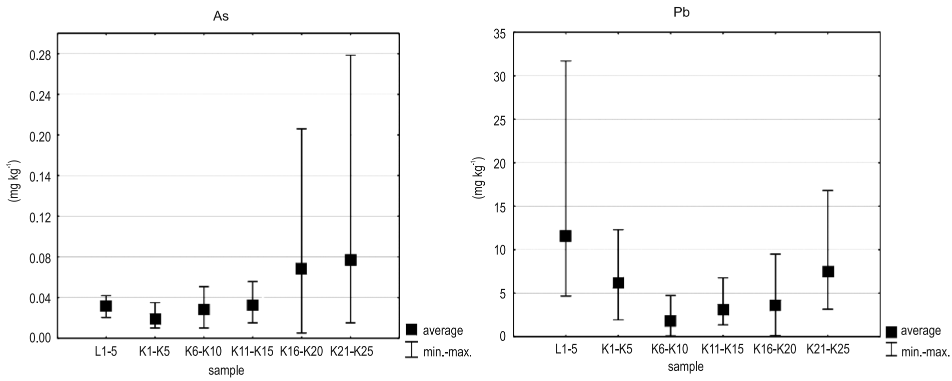

Fig. 2. Scatters of As and Pb hair content of the respondents living in Cracow (K1-K25) and Lubasz (L1-L5)

(L1) containing 31.7 mg kg^{-1} Pb was collected from a non-smoking, 37-year -old man, who lives in Lubasz. He declared frequent contacts with metal scrap and used car parts. The second respondent (K24) was a 29-year-old woman, a resident of Dębniki in Cracow; she is a regular smoker and her diet is rich in meat.

The Pb hair content results are scattered more than those of As (Figure 2). The highest scatter is shown by the control set population (SD = 11.29 mg kg^{-1}, mean 11.61 mg kg^{-1}); a significantly lower scatter characterizes the basic set population (SD = 4.43 mg kg^{-1}, mean 4.02 mg kg^{-1}).

Impact of selected factors on the hair content of As and Pb

The group of 30 persons tested consists of 22 men (73%) and 8 women (27%). The mean concentrations of the hair As of both sexes are similar and amount to 0.042 mg kg^{-1} for men and 0.045 mg kg^{-1} for women (Table 2). In contrast, the mean content of the hair Pb of men differs by 1.37 mg kg^{-1} (30%) from that of women (5.99 and 4.62 mg kg^{-1}, respectively). The scope of the investigations was insufficient to draw any sound explanations on the gender-age Pb distributions and concentrations.

The gender difference was also found by Bartoń et al. (1998), who analyzed hair samples of children from southern Poland and found that the Pb content of hair from boys was almost 230% higher (3.27 mg kg^{-1}, n = 35) than that from girls (1.44 mg kg^{-1}, n = 29). A similar gender distinction was also observed among school-age children in Sardinia: the mean content of the hair Pb of 41 boys from a town of Sant'Antioco was 6.71 mg kg^{-1}, while that of 67 girls was 4.99 mg kg^{-1} (Floris et al. 2007).

Another factor affecting the metabolism of the PHE is the age. Children and seniors are more sensitive to toxic elements than middle-aged persons because children have not yet developed adequate levels of enzymes, whereas in certain seniors the systems and organs (such as the liver) do not always

Table 2

The influence of factors on the mean content of As and Pb in the hair of analyzed populations

Factor		*n*	As	Pb
			(mg kg^{-1})	
Gender	male	22	0.042	**5.99**
	female	8	0.045	4.62
Age (years old)	18-30	13	0.043	4.41
	31-40	7	0.028	**6.81**
	>40	9	**0.055**	6.32
Occupational exposure	yes	12	**0.067**	**7.15**
	no	18	0.026	4.60
Frequent consumption of ecoproducts	yes	12	0.028	**7.51**
	no	18	**0.053**	4.36
Frequent consumption of meat	yes	23	**0.047**	**6.02**
	no	7	0.027	4.32
Frequent consumption of fish	yes	11	0.047	**8.04**
	no	19	0.040	4.22
Frequent coffee drinking	yes	18	0.036	5.03
	no	12	0.053	6.51
Frequent drinking of tea with lemon	yes	14	0.026	5.42
	no	16	**0.057**	5.80
Frequent alcohol drinking	yes	5	0.034	**10.7**
	no	25	0.044	4.61
Frequent smoking	yes	7	0.030	4.24
	no	23	**0.047**	6.04
Dark hair	yes	6	0.030	**9.61**
	no	16	0.047	5.60

n – number of samples, bolded – significant differences at $P \leq 0.05$

function properly. Accordingly, the content of some PHE in human tissues usually increases with age. Bearing this in mind, the population tested was divided into three age groups: of 18-30, 30-40 and above 40 years old persons. The group of 18-30 year-olds consists of 13 persons and their hair mean content is: 0.043 mg As kg^{-1} and 4.41 mg Pb kg^{-1}. The group of 31-40 year-olds consists of seven people and their mean content is 0.028 mg kg^{-1} As and 6.81 mg kg^{-1} Pb. The group of over 40 year-olds consists of 9 persons and their mean content is 0.055 mg kg^{-1} As and 6.32 mg kg^{-1} Pb. The highest mean content of the hair As is in the oldest age group and the lowest in the middle-aged (31-40 years) group, whereas the mean content of the hair Pb of the two older groups differs only by approximately 8% (6.81 and 6.32 mg kg^{-1}, respectively) but is significantly higher than the mean content of the youngest group (4.41 mg kg^{-1}). Thus, Pb follows the trend of concentrating in hairs with age. Similar trends were observed in Japanese, whose hair content of toxic metals was analyzed. The highest concentrations of As, exceeding 0.09 mg kg^{-1} for men and 0.06 mg kg^{-1} for women, were in the oldest persons (aged above 50 years) and the lowest, i.e. from 0.025 to 0.065 mg kg^{-1}, were found in hair of persons aged 16-39 years (Hiroshi et al. 2005).

The persons parting in the current test were asked whether they felt exposed to harmful factors at work. The question did not specify the type of exposure, therefore not everyone who replied positively must have had a real contact with sources of As and Pb. Although feelings are not scientifically an independent and objective measure, it appeared that the mean hair content of As and Pb is higher by 150% and 64%, respectively, within the group of people "exposed" in comparison to the group of persons who did not report working in deleterious or harmful conditions.

A proper diet is a very important health factor for humans as it has also some influence on the quantity of various elements, including toxic ones, that enter or are removed from an organism (Hall 2002, Guha Mazumder 2003, Kmiecik et al. 2009). The assessment of the hair As and Pb content of the respondents who often consume products from supermarkets has not confirmed that people buying food in large stores or eating fast food absorb higher doses of all toxic elements than those who consume food of mostly rural origin. Although their mean As hair content was significantly lower than that of persons who declared eating ecoproducts (0.03 *versus* 0.05 mg kg^{-1}), the Pb mean content was distinctly opposite (7.51 *versus* 4.36 mg kg^{-1}).

Meat can be a probable source of Pb: the hair of persons frequently eating meat contains an average of 6.02 mg kg^{-1} Pb, whereas an average of those who denied frequent consumption of meat is distinctly lower and equals 4.32 mg kg^{-1}. Also Chojnacka et al. (2014) noticed that a meat-rich diet affects the quantity of Pb in human organisms. The same trend has been established with respect to fish meat, which means that fish can also be a source of some PHE. The group of people rarely eating fish had a mean hair content of Pb equal 4.22 mg kg^{-1}, while the mean Pb concentration in the hair of people frequently consuming fish was nearly twice as high, reaching 8.04 mg kg^{-1}. On the other hand, there were no differences of As between the groups of fish eaters and non-eaters (Table 2).

Three questions concerned beverages used by respondents. Drinking much coffee did not turn out to be a factor which correlated with an increase of the hair content of As and Pb. The study of Chojnacka et al. (2014) gave a similar result as the difference between drinkers and non-drinkers was statistically insignificant. According to Chojnacka et al. (2012), an additional source of As and Pb can be tea spiked with lemon, but our survey has not confirmed this supposition. The mean hair content of Pb was comparable in both groups of respondents, and the level of As was even higher in the group only occasionally drinking tea with lemon (Table 2). Concerning alcoholic beverages, the persons drinking them frequently had their mean hair content of Pb (10.7 mg kg^{-1}) approximately 230% higher than the group of persons with the opposite habit (4.61 mg kg^{-1}). Alcoholic drinks may contain certain amounts of Pb and, what is worse, drinking alcohol intensifies the toxic effect of Pb. In the case of As, its hair content of frequent alcohol drinkers was found to be approximately 45% lower (it might indicate a flushing effect of alcohol but such a supposition should be carefully verified).

Another factor – cigarette smoking – undoubtedly has a negative influence on the human health, which is generally acknowledged and has been proven in various medical tests. Both As and Pb are released during smoking, but our results did not show increased content of these elements in the group of smokers (Table 2).

The respondents were also asked about the state of their health and the presence of diagnosed serious or chronic diseases (as only two positive answers were given, they have not been entered into Table 2 and are discussed below). The first person (sample K3) indicated diabetes; an exposure to Pb and As can cause an increase in the illness rate as does cadmium in diabetics (Grzebinoga, Marchewka 2009). In our test, the As hair concentration (0.015 mg kg^{-1}) was clearly lower (three times) than the mean As concentration of all the samples (0.043 mg kg^{-1}), while that of Pb (6.53 mg kg^{-1}) was 20% higher than the mean (5.62 mg kg^{-1}). The other person (sample K7) declared suffering from glaucoma, osteoporosis and ventricular arrhythmias, but no increases in his hair content of As and Pb were recorded.

The last issue considered was the natural hair colour and its possible link to the hair content of As and Pb. Kozłowski and Nowak (1998) reported that black-haired men have usually higher Pb content of their hair. In our group, six out of 16 men were black-haired. Their mean hair concentration of Pb amounts to 9.61 mg kg^{-1}, which is 72% higher than that of the other men. A similar relationship was noticed by Srogi (2004) in Silesia: a higher hair content of Pb was found in the hair of dark-haired persons than in people with lighter hair colours. No such relationship of the As content has been found.

CONCLUSIONS

The results have confirmed the general knowledge that a proper diet and a clean, unaffected environment are basic factors minimizing the adverse impact of As and Pb on human beings.

Referring to specific experimental and comparative issues, the following conclusions have been put forward:

1. The factors whose influence on the hair content of As and Pb were

considered are: the place of residence, occupational exposure, sex, age, diet, alcohol consumption and hair colour.

2. Surprisingly, the inhabitants of the village Lubasz have higher mean Pb and As content of their hair than the residents of Cracow, although there are more contamination sources in the large city. Except for the low statistics of our test or the type of occupational contact with pollutants in Lubasz, no other explanations can be given at present.

3. Of the five Cracow districts considered, Dębniki is an area where hair samples with the highest mean concentrations of both As and Pb were collected.

4. As the current investigations should be considered as a pilot study, establishing in which districts of Cracow the population is most endangered by heavy metals would require more intensive sampling and collecting more research material.

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