Original Research

Human Hair as a Biomarker in Assessing Exposure to Toxic Metals

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Abstract

This study discusses the content of different elements including toxic metals (Ba, Cd, Pb, Sr) in hair of adult subjects exposed to a wide spectrum of pollutants. Scalp hair samples were collected from inhabitants of the rural outskirts of Gdańsk, located in the north of Poland (from citizens living near a phosphate fertilizer waste disposal place) and from the donors who were employed in two factories in southwestern Poland (coal mine in Zabrze and lead manufacturing company in Oława) characterized as urbanized and highly industrialized areas (exposed groups). The results were compared with an average element composition of the hair of students attending the Technical University of Łódź (control group), people who were assumed not to have any direct contact with these elements, and with literature data. The determination of elements was performed with ICP-MS and ICP-OES methods. A questionnaire involving personal data information about such things as sex, age or smoking habits was completed by the volunteers. Our results revealed significant differences in concentrations of metals for studied groups and suggest that the populations we studied are exposed to toxic metals. The most probable exposure pathways seemed to be workplace and environmental pollution. This work was also undertaken to study inter-element interactions which were analyzed by the evaluation of correlation coefficients between a pair of two metals. But, with a few exceptions, no essential relation has been established.

Keywords: exposure, hair analysis, ICP-MS, ICP-OES, toxic metals

Introduction

The use of human hair as an excellent tool to assess changes in our bodies has received a great deal of attention for a few decades and become successful in different applications [1], including criminal investigations such as detection of doping relevant substances [2] or drugs and their metabolites [3, 4]. Complementary information concerning monitoring of exposure to a broad spectrum of pollutants can be provided by means of hair analysis. Determination of toxic substances in human media results from general interest to evaluate the degree of impact mainly of work place (occupational exposure), criminal activity or environmental conditions on health status of individuals as well as population groups [1].

Trace metal analysis on hair material presents several advantages when compared with other body tissue or fluids [5], while it was found that hair level of some elements, toxic in particular, is strongly correlated with many disorders. Hair analysis is useful, especially in reflecting the long-term history of individual exposure. We are able to trace back changes in hair composition in time. Hair study is a non-invasive method of investigation. Moreover, it can deliver valuable information about our state of health, the application of certain drugs and diagnosis of some diseases [6].

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On the other hand, hair analysis also presents a few limitations. Difficulties arise due to the lack of well defined reference concentration ranges. It is a consequence of existing huge differences in levels of elements depending on factors of sex, age, region, ethnicity, hair color, dietary habits and problems with removal of exogenous contaminants from the environment. Other incorporation sources like cosmetics or hair beauty treatment processes (e.g. bleaching, dyeing or permanent waving) contribute as well to an increase in total element content and can additionally complicate correct interpretation of the results [1, 7-10]. The level of metals can also be affected by seasonal variations or synergistic and antagonistic effect, for instance [9]. Recently, attention has been aimed at simultaneous investigations of human hair and nails. Nails are particularly recommendable due to the fact that this biological material is less exposed to external contamination, and they are mainly applied when we do not possess a sufficient quantity of hair for analysis [10-12].

Currently, numerous papers are focused on the choice of the most suitable analytical method, sensitive enough to quantify reliably the content of different elements in this biological medium even at very low, ultratrace levels. The neutron activation technique of hair investigation still remains one of the most universally used. However, in some cases atomic absorption spectrometry is more preferable, especially in the case of determination of such elements as Pb, Cd or Ni. Current techniques including plasma (ICP-AES and ICP-MS), X-ray fluorescence and proton-induced X-ray emission are more and more commonly applied for multi-element studies [13]. Recently, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) – an alternative technique for wet-analysis that does not require any special sample preparation, has been employed with success for the quantitative measurement of essential elements (Zn, Fe and Cu) and toxic elements (Cr, Pb and U) on single hair strands [14], and has been used as a tool to monitor the concentration of Pt along the hair from a patient who had been treated with cisplatin as cytostatic drug in cancer therapy [15].

The aim of the present study was to determine the content of chosen toxic elements in human hair of the population groups of different localities living in rural or urbanized and industrialized regions of Northern, Central and Southern Poland, respectively, and exposed to various factors, as well as to check if there exists a correlation between exposure source and element hair composition. This paper also discusses the relationship between concentration of elements in the scalp hair samples of different regions. Additionally, dependence of factors affecting the level of metals such as sex, smoking habits or age was examined. The obtained results were compared with the average content of various populations (e.g. students' group) and on the basis of literature data. Moreover, correlation coefficients among investigated metals in order to identify a possible existence of metal relations in hair samples were also calculated.

Materials and Methods

All hair material was collected during 2006 in different communities of central, northern and southern Poland. We studied 109 samples collected from adult donors (39 men and 70 women) between 18 and 84 years of age. The main criterion was to choose volunteers that spend the notable part of the day in the same place of work or life. Uncolored hair material was requested, but not received in all cases. Hair samples from the scalp were used in the assessment of heavy metals occupational exposure of people working in two industrial companies: lead-works in Oława city (Silesia region, one of the biggest producers of lead oxide in Poland, 22 samples) and the "Makoszowy" coal mine (Zabrze city, Silesia region, 18 samples) and from people who are environmentally exposed due to a phosphate fertilizer waste disposal site in Wislinka, near Gdańsk (49 samples). For comparison, the content of toxic metals was also determined in students' hair samples from the Technical University of Łódź (20 samples). The discussed population was treated as a control group where the occupational and environmental exposure potential was considered negligible. After the collection of approximately 200 mg of human hair from the nape of the neck using stainless steel scissors (only the first 3-4 cm closest to the scalp were used), hair samples were washed separately with acetone, then with water and again with acetone (according to the International Atomic Energy Agencies recommended procedure [13, 16]) to remove external contaminations, with an exception of hair collected from the people living near the of waste disposal place. In that case, in order to evaluate the influence of possible phosphogypsum dump dusting, we did not wash the studied material. Phosphogypsum stockpiled in Wislinka is a waste product with the main component $CaSO_4 \cdot 2H_2O$ (95-98% dry mass). However, it also contains a variety of impurities such as high fluorine content (0.3-1.8%), trace elements such as: Cd, As, Mn, Pb, Ni, Cu, and radioactive metals including Sr, Th, U, Po, Ra. All samples after the washing procedure were dried and stored in plastic bags at room temperature [7].

All the reagents used were of trace analytical pure grade (Merck). The glassware was first washed with detergent and then left to soak for approximately 24h in 2% nitric acid. Before standards preparation, the whole glass was washed with high-purity water from Millipore and dried afterwards. The procedure adopted for sample digestion was based on the commonly used method in literature data [16-18]. Each sample (approximately 0.2 g) was placed in closed Teflon vessels with 5 ml concentrated nitric acid and digested in a microwave oven system Milestone MLS-1200 MEGA. The digestion conditions programme applied and reagent used were assorted to obtain a complete mineralization of samples. Before the vials opening, the Teflon vessels were placed for 45 min in a freezer. After digestion, samples were diluted to a final volume with demineralized Milli-Q water. Certificate reference material was digested with the same procedure as each sample. High pure Millipore water was used for every dilution.

Table 1. Results from the measurements of certificate material [mg kg^{-1}].

Element	Certificate Value ± U [mg/kg]	Obtained Result ± RSD [mg/kg]	Recovery [%]
Ba	11.10 ± 1.30	11.74 ± 0.09	105.8
Cd	0.072 ± 0.010	0.063 ± 0.008	87.55
Pb	3.83 ± 0.18	3.94 ± 0.09	102.9
Sr	8.17 ± 0.69	8.81 ±0.30	107.8

U-uncertainty

The content of chosen toxic metals was measured with inductively coupled plasma atomic emission spectrometry, ICP-OES, Thermo Jarrell Ash (emission lines were as follows: Ba 493.409 nm, Sr 407.771 nm) and inductively coupled plasma mass spectrometry, ICP-MS, X-Series, Thermo Electron Corporation (the following isotopes were selected: ^{111}Cd, ^{208}Pb). The analytical process and accuracy of the measurements were controlled by the use of Certificate Reference Material of Human Hair NCS ZC 81002 from China National Analysis Center and a good agreement in the measured values with the certificate ones was achieved. Table 1 presents the obtained recovery values for the determination of CRM. Three replicates were performed for each sample and the relative standard deviation did not exceed 5% in all cases. Additionally, inter element correlation coefficients (r) were calculated using Pearson's test to investigate the possible relationship among concentrations of analyzed metals determined in scalp hair. It was assumed that the correlation will be statistically significant at $p < 0.05$. The descriptive statistics for hair elements data included: average concentration, median, range (min.-max.), and standard deviation (SD).

This study was conducted to establish concentrations of selected toxic metals in hair samples for occupationally and environmentally exposed populations and to identify differences in metal concentrations of all investigated subgroups, as well as inter-element relations. The results were compared with trace element content in hair of reference man [8] and with literature data. A special attempt was made to demonstrate the possibility of potential hair application as a biological indicator, which enables an identification of source of exposure. This work also emphasizes the significance of the SEM method as a complementary and useful technique for assessing environmental pollution.

Table 2. Recommendable content of elements and advisable proportions between metals in hair and reference elemental concentration in adult human hair in relation to average content and ranges of elements in investigated hair samples [8, 19].

Element	Ba [mg kg^{-1}]	Cd [mg kg^{-1}]	Sr [mg kg^{-1}]	Pb [mg kg^{-1}]
Reference man [8]	0.4-2	0.25-1.0	-	2-20
Recomendable value [19]	1.5	0.3	3.2	4.0
ŁÓDŹ				
Average	0.724	0.172	1.700	0.360
Median	0,77	0.130	1.245	0.313
(Min.-Max.)	(0.159-1.121)	(0.040-0.867)	(0.660-3.737)	(0.160-1.001)
SD	0.254	0.192	1.215	0.200
OLAWA				
Average	21.27	0.726	6.652	8.638
Median	23.46	0.106	3.451	1.024
(Min.-Max.)	(1.154-60.92)	(0.019-4.128)	(0.517-45.570)	(0.205-49.11)
SD	15.55	1.170	10.37	15.69
ZABRZE				
Average	20.31	0.191	4.469	4.307
Median	11.76	0.122	2.600	1.640
(Min.-Max.)	(0.129-76.21)	(0.035-0.870)	(0.432-21.51)	(0.728-59.06)
SD	23.05	0.201	5.526	12.26
GDAŃSK				
Average	1.313	0.232	8.869	1.998
Median	0.960	0.132	2.528	1.194
(Min.-Max.)	(0.294-3.889)	(0.018-1.723)	(0.433-42.73)	(0.036-9.546)
SD	0.908	0.295	11.88	2.232

Results and Discussion

The main descriptive statistics of element concentration in human hair are presented in Table 2 and Fig 1. Table 2 summarizes mean value, median, range and standard deviation of elemental analytical results of scalp human hair samples taken from groups of subjects unexposed and occupationally and environmentally exposed to toxic metals. Due the fact that the content of metals in hair is not constant and depends on numerous factors, the studied population was divided into four groups based on the place of work and residence.

When comparing the element content in human hair of exposed people (Oława-lead manufacturing company, Zabrze-coal mine and Gdańsk-phosphogypsum waste disposal place) and student group (reference group), considerable differences are clearly seen. Generally, the lowest average values are observed for control group of students, as we suspected. For each element the results are comparable or even lower than recommendable values [19] (concentrations of various metals proposed by laboratory of trace elements in human hair based on the quantitative hair analysis of people living in the Łódź region) and concentration ranges established for reference mean [8] (ranges of concentrations of elements quoted by Iyegar on the basis of different physiological data for a 70 kg, 170 cm man with western living habits). The dispersion of results for each determined metal in hair for a group of students is the lowest (25-75% of all results are gathered in considerably small ranges) when compared with box-plots for other populations (Fig. 1). It was proved that students living in this urban area are the least exposed to analyzed metals among people from regions under investigation. The largest differences between mean values for studied populations and reference values found in the literature data were recorded for Ba concentrations in human hair material. The observed contents of this metal for individual subjects were sometimes even over 30 times higher than recommended levels [8, 19], mainly recorded for samples collected from two occupationally and environmentally exposed groups from the highly industrialized Silesia region. These dissimilarities may be partly attributed to differences in the level of air pollution between studied regions (south, central and north Poland). Relatively smaller variations in the level of metals were found for Sr, Cd and Pb, respectively. The analysis of box-plots for each region revealed the following tendencies: the highest exposure for Ba seems to be present in volunteers from Olawa and Zabrze, for Cd from Oława, for Sr

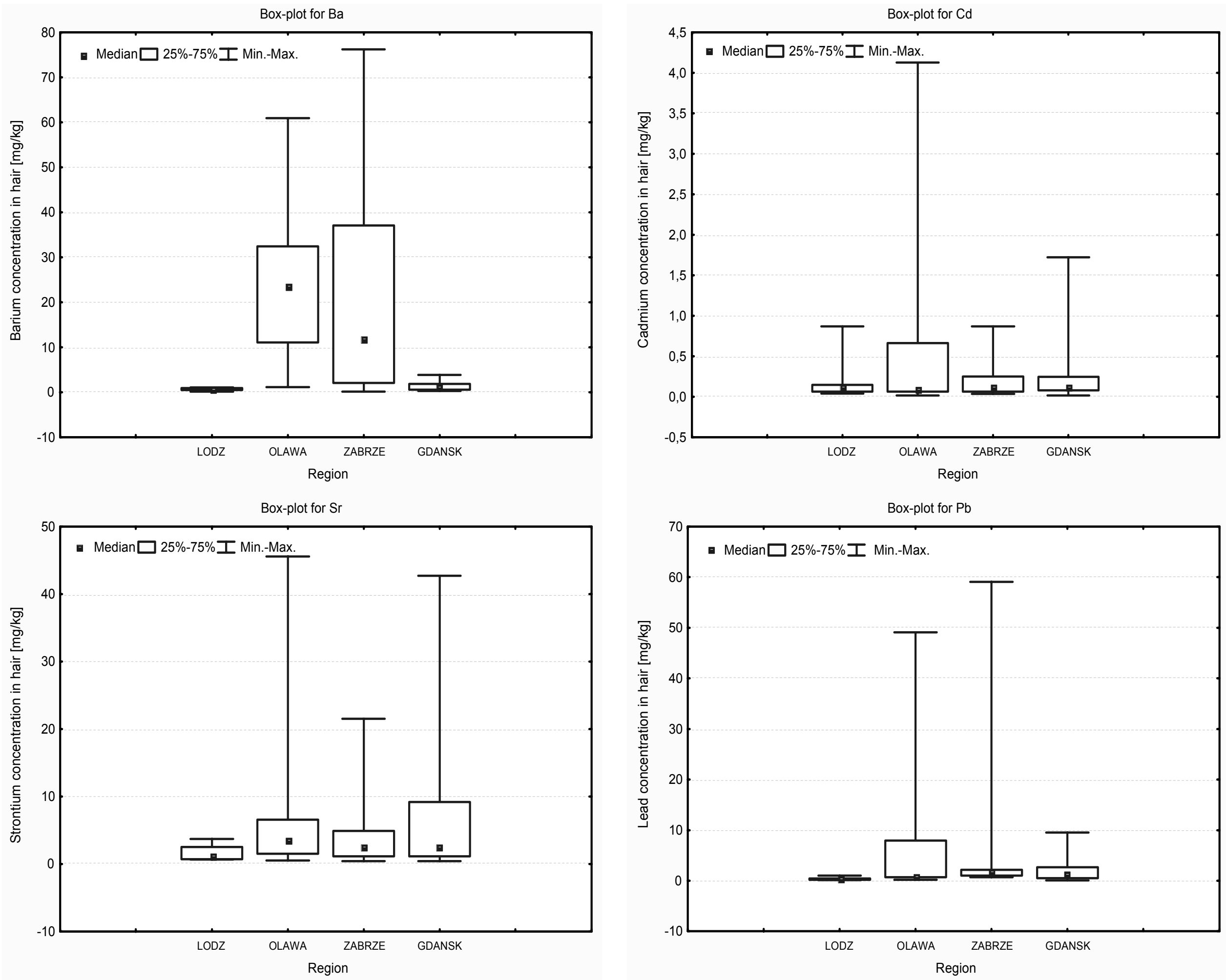

Fig. 1. Box-plot for Ba, Cd, Sr and Pb, respectively, in hair of people living and working in various places (Łódź, Oława, Zabrze, Gdańsk).

Table 3. Reference values for human hair [20], element concentrations in hair for 5 [21] and 83 [9, 18] subjects living in an urban area in southwestern Poland (Wrocław city), for 266 subjects living in a non-industrialized region of southern Poland (Silesian Beskid Śląski) [5], in 62 hair samples of volunteers living in industrial and polluted cites with numerous factories: Katowice, Gliwice, Pyskowice and Tychy (Silesia) [22], in 22 citizens living near the phosphatic fertilizer plant in Gdańsk (northern Poland) [23]; for 114 subjects from an urban population group living in northeastern Sweden [10]; in 45 samples of non-occupationally exposed healthy individuals [24] and for 1091 subjects of Rio de Janeiro [20] (mg kg^{-1}).

Element	Reference value [20]	Wrocław [21]	Wrocław [9]	Southern Poland [5]	Southern Poland [22]	Northern Poland [23]	Sweden [10]	Reference range [24]	Rio de Janeiro [20]
Ba	0.3-3.5	2.89	2.025	-	-	2.5-2.8	0.64	0.05-1.58	6.9
Cd	< 1.0	0.173	0.114	0.61	-	-	0.058	0.004-0.17	0.59
Sr	1.0-7.6	8.187	2.882	-	7.50	-	1.20	0.17-4.63	5.1
Pb	< 6.0	1.75	1.046	4.99	11.6	-	0.960	0.13-4.57	12.5

from Gdańsk and Oława, and for lead from Oława. Therefore, we considered the urbanized and industrialized Oława as the most polluted among areas investigated in this work where the level of metals is probably more related to local conditions than. personal abilities. This is not surprising since the Silesia region is recognized as one of the most highly contaminated regions in Poland and Central Europe.

The average elemental composition of hair for studied populations was compared with the mean composition or ranges of metal content of hair from populations living in various areas of Poland and the world. Table 3 shows results for element concentration of analyzed metals reported in a few literature studies. The data presented in this table refers to calculated so-called "normal" or reference intervals established by Miekeley et al., 1998 [20]; and to determined element concentrations in hair of people living in an urban and industrialized area of southwestern Poland (Wrocław city, Lower Silesia) of 5 [21] and 83 [9, 18] subjects; in 266 inhabitants of a non-industrialized region of southern Poland (Silesian Beskid Śląski, agricultural and forest mountain area) [5], in 62 hair samples of volunteers living in industrial and polluted cites with numerous factories: Katowice, Gliwice, Pyskowice and Tychy (Silesia) [22]; in 22 citizens living near the phosphatic fertilizer plant in Gdańsk (North Poland) [23]; in 114 samples of hair material collected from an urban population group living in northeastern Sweden without known occupational exposure to metals [10]; in 45 samples of non-occupationally exposed healthy individuals [24]; and in 1091 subjects of Rio de Janeiro [20]. When comparing the results obtained for the control group of students with other literature data concerning element concentrations in hair of subjects living in different urban and non-industrialized regions, the mean concentrations of discussed elements established in this study seem to be much lower than values published elsewhere (Table 3). In the case of exposed groups, average concentrations were in general higher than recommendable ranges [19, 20], or those assessed for reference [8]. As an example, the range of barium concentration (2.5-2.8 mg kg^{-1}) evaluated for the 22 samples taken from the most exposed districts of Gdańsk in the vicinity of a phosphatic fertilizer plant [23] do not correspond to the results gathered for the Oława and Zabrze studied populations. Simultaneously, these mentioned values are significantly higher when compared with levels determined in hair material from inhabitants of the rural outskirts of Gdańsk living near the phosphate fertilizer waste disposal place and sometimes almost a few times lower than the level of Ba in hair of exposed subjects from the Silesia region where the metallurgical industry and high air pollution are present [22]. In discussed work [23], essential differences between citizens of the factory neighborhood and the control group were confirmed (e.g. for S or Mg), but it was hard to uniquivocally confirm the effect of the phosphatic factory on these divergences.

The average contents and dispersion of the results for cadmium in hair are not very different between analyzed groups, and the values are very close to the levels considered as reference. Therefore, we can state after detailed analysis of values for individuals and confrontation of the obtained results with data reported in the literature that exposure connected with levels of this metal turned out to be the least dangerous among all investigated metals. Generally, Cd levels were within the ranges given by Chatt et al, 1988 [25] elaborated for healthy people and recognized as normal concentration values (0.23-0.27 mg kg^{-1}). Although we should take into account the fact that in several cases content of cadmium for some subjects exceeded the recommendable values or oscillate around the upper limit range for this metal as seen from Fig. 1 (especially for inhabitants of the rural outskirts of Gdańsk, living in the closest neighborhood of the phosphate fertilizer dump and employees of lead manufacturing company in Oława working as mechanics or laboratory assistants). Our results were comparable with average cadmium concentrations (0.35 mg kg^{-1} Cd) determined in hair material of 67 people who died between 1996 and 1997 in the Gdańsk region [26] and lower (with the exception of the Oława population) than the mean concentration established in 624 hair samples for the environmentally exposed population: Katowice and Sosnowiec (0.56 mg kg^{-1} Cd) [27]. For example, a much higher cadmium concentration for occupationally exposed people ranging to 90 mg kg^{-1} Cd was reported in hair in workers of a metallurgic plant in Russia [28]. The results of another study [29] also showed elevated cadmium concentrations in hair collected from donors involved in the manufacture of lead-acid batteries (values up to 1.93 mg kg^{-1}), nickel-cadmium batteries (values up to 40 mg kg^{-1}), and gas vending station workers (values up to 3.3 mg kg^{-1}). However, it is well known that cadmium content is strongly dependent on the applied washing procedure [7, 30]. It was found that considerable differences in Cd levels are observed after employment of washing procedure with acetone and water [31]. As a consequence, we could not exclude that in our study some loss of Cd took place during the washing of hair samples, which influenced the final concentration of this toxic metal and understated the recorded values.

As seen from Table 2 and Fig. 1 in the case of other metals (Ba, Sr, Pb), a few times abnormal concentrations were noticed. These considerably high values were detected mainly for persons exposed to direct contact with. chemicals in their work or residence place. It was found that the level of these elements for some single subjects was even over one hundred times higher than the discussed earlier reference values. The highest concentration of toxic metals for Oława workers (lead oxide producer) was stated for people employed in a laboratory. For instance, in the hair of one of the investigated donors (laboratory assistant), concentrations ranging up to 10, 48, 34 [mg kg^{-1}] were recorded for Sr, Pb and Ba, respectively. The highest metal content in hair samples collected from people working in a coal mine in Zabrze was observed particularly for metalworkers (e.g. 76 mg kg^{-1} of Ba) and electricians (over 59 mg kg^{-1} for Pb). Some of the obtained values for Pb seem to be pretty high when compare with data reported in the literature. The recommended reference range of lead determined in hair samples of healthy persons is 3-4 mg kg^{-1} [25]. Only mean concentrations of subjects from Łódź and Gdańsk were below this level and treated as normal. Similarly, hair lead levels (2.5 mg kg^{-1}) given by Rodrigues et al. [32] for 280 adults between 18 and 60 years of age from 3 different Brazilian states are in good accordance with those just mentioned as reference content [25]. A study performed in Lithuania revealed significant differences in lead content in human hair of people with various exposure levels to this metal. The determined mean concentration of lead in hair of workers of a ceramic plant occupationally exposed to Pb (7.6 mg kg^{-1}) was relatively higher than for workers not exposed to Pb (3.2 mg kg^{-1}), and randomly selected controls (2.6 mg kg^{-1}) [33]. The average Pb concentration in hair taken from corpses of deceased persons who had died suddenly from the Gdańsk region (5.2 mg kg^{-1}) was higher than measured in our study for the population living in Wislinka, Łódź or Zabrze [34], but very similar to the levels found in other Polish cities as Katowice and Sosnowiec (5.7 mg kg^{-1}) [27]. In the study of Baranowska et al., the concentration of lead in hair samples of volunteers living in the Silesia region [22] was 11.6 mg kg^{-1} and exceed mean values estimated in our work. Values given for other populations in permanent contact with a polluted workplace were as follows: up to 288 mg kg^{-1} for donors employed in factory manufacturing lead-acid batteries, up to 2.3 mg kg^{-1} for subjects working with nickel-cadmium batteries, and up to 11.4 mg kg^{-1} for gas vending station workers. The author also suggests that some kind of competition between the absorption of Cd and Pb by humans might exist [29], and high levels of lead correspond to low levels of cadmium, which is rather not true in our case (see positive correlation coefficients for this pair of metals).

In general, the values of strontium concentration were almost in the middle of the limit ranges found in the literature data, except a few cases. However, the levels of strontium established in hair varied greatly. The mean concentration of Sr evaluated in hair of healthy adults living in Brazil [32] was quite low (1.6 mg kg^{-1}), while the range of divergence was 1-12 mg kg^{-1}. In the study of Baranowska et al. [22], some higher concentration was observed in the hair samples of inhabitants of Katowice, Gliwice, Pyskowice and Tychy (average value was 7.50 mg kg^{-1} with range 1-18 mg kg^{-1}). This is consistent with the results obtained by Chojnacka et al. [21] for the population living in an urban area of southwestern Poland (mean was 8.187 mg kg^{-1} and the range 0.0004-25.4 mg kg^{-1}). Our data reported for the populations of Oława and Zabrze (Silesia region) are in agreement with those findings [21, 22]. The influence of the environment was demonstrated the most in the example of donors form northern Poland. As expected, the highest concentration of Sr appeared in the hair of subjects from the outskirts of Gdańsk. This fact may be connected with effect of dusting, which in consequence strongly affects mainly Sr content in hair. The levels of Sr, in particular, determined in hair of individuals were much higher than those reported in other literature data but for washed hair material. Therefore, the results gathered for this group should be considered separately due to omitting the step of washing in sample preparation. It is well known that adsorbed metals can be removed to some extent after washing, thus it is hard to assess the degree of exogenous contamination to total element composition.

Sr is one of the main impurities of phosphogypsum by-product. Stockpiled phosphate waste in the dump in Wislinka, near Gdańsk, can contain even up to 1% of Sr [35]. Despite this toxic symptoms of Sr overdosing have not been reported, and the intravenous administration of high doses of Sr may induce hypocalcaemia as a result of increased renal excretion of Ca. Administered Sr is almost exclusively deposited in bone [36].

Based on results of this work, it was proven that the composition of hair samples taken from Gdańsk citizens was strongly influenced by the existence of phosphate waste disposal place, substantially causing an increase of metal contribution in biological media. We observed a similar tendency for each of the determined metals. In general, the concentration of elements (particularly Sr) decreased with increasing distance from the waste disposal site. Some selected diagrams illustrating these relations for chosen individuals are presented in Figs. 2-4. The elevated concentrations of studied elements in hair samples of volunteers from Wislinka may result from exposure to these metals connected with the presence of large phosphate industry

Table 4. Statistical analysis of the hair element-to-element Pearson's correlation coefficients in studied groups (p<0.05).

Element/ Element	Łódź	Oława	Zabrze	Gdańsk
Ba/Cd	0.16	0.30	-0.07	0.31
Ba/Pb	-0.04	0.36	-0.18	0.21
Ba/Sr	0.14	***0.55***	0.04	0.11
Cd/Pb	***0.72***	***0.58***	***0.46***	***0.43***
Cd/Sr	-0.12	-0.18	0.29	-0.25
Pb/Sr	0.02	-0.11	-0.06	-0.32

waste disposal sites and the penetration of toxic substances from dumps into adjacent environments and humans, e.g. by caring dust particles containing mentioned impurities by wind and in consequence their inhalation and/or adsorption. However, it should be emphasized that despite the samples not being washed, the recorded values for studied metals were comparable in most cases with values typically reported in the literature for non-exposed or urban populations. The main danger seems to be connected with heightened Sr levels, which may turned out to be negligible after washing.

A Pearson's regression model was used to confirm if there is a possible existence of a relationship among determined elements in hair samples for the following groups: students, people working in a coal mine, lead manufacturing company and inhabitants of Gdańsk. In Table 4 the lists of the metal-to-metal correlation coefficients matrix are gathered.

Generally we can state that no strong and unambiguous statistically significant correspondence was found because calculated correlation coefficients differ considerably from value ± 1. Most likely it is caused by the fact that the total content of investigated elements is affected by the combination of many other sources contributing to the final element levels than only the exposure itself, e.g. dietary habits (not included in this paper), which makes hair analysis very complicated and impedes distinguishing the existence of inter-element dependences. The strongest relation was noticed for the pair of metals Pb-Cd. This statistically significant positive correlation (r= 0.43-0.72, p<0.05) indicates a common external source of these elements. This fact implies that these two elements are definitely to the most extent associated with environmental or occupational expo-

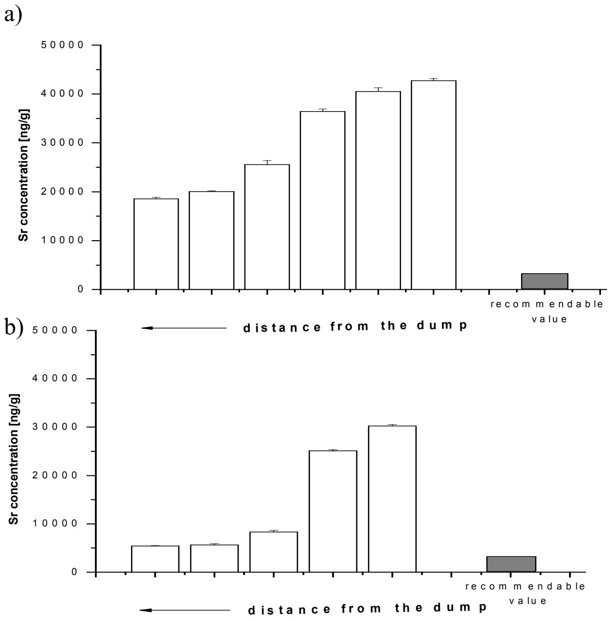

Fig. 2. Diagram of the strontium content in hair samples collected from 300 to 800 m (a) and from 1,500 to 1,700 m away (b) from a phosphate fertilizer waste disposal site [μg kg^{-1}].

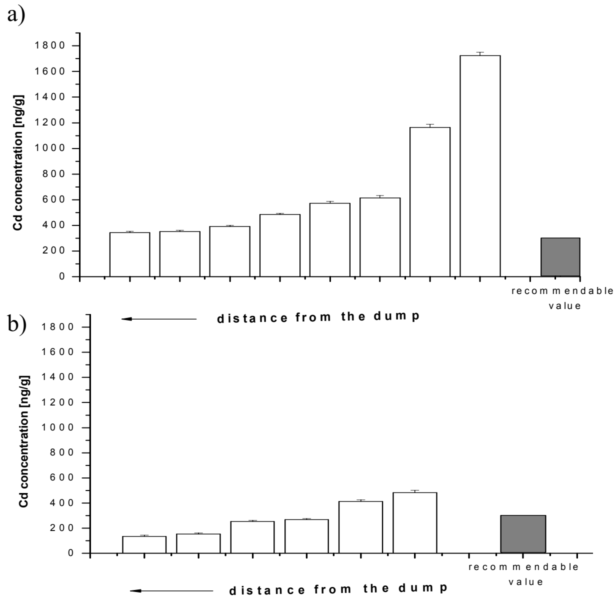

Fig. 3. Diagram of the cadmium content in hair samples collected from 300 to 800 m (a) and from 1,500 to 1,700 m away (b) from a phosphate fertilizer waste disposal site [μg kg^{-1}],

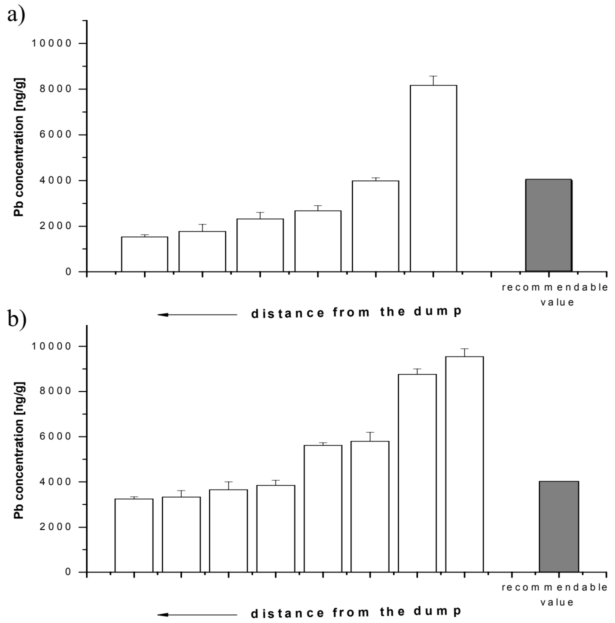

Fig. 4. Diagram of the lead content in hair samples collected from 300 to 800 m (a) and from 1,500 to 1,700 m away (b) from a phosphate fertilizer waste disposal site [μg kg^{-1}].

sure than regulated by other factors such as age or sex. This finding is in agreement with previous papers dealing with some other populations [5, 37, 38]. The example of element-to-element correlation between Cd and Pb content in hair of donors from Oława is illustrated in Fig. 5. The analysis of this diagram led to the conclusion that the strongest interaction among the pair of metals is observed in low concentration ranges, for higher concentrations the linear relation is negligible. Moreover, some other statistically significant dependence was shown in hair of citizens of Oława. Strontium, which is well known for its industrial uses presents positive and strong interaction ($r=0.55$, $p<0.05$) with barium. A weak negative Pb/Sr correlation in hair ($r= -0.32$, $p< 0.05$) was found only in material collected from Gdańsk. Speculating, this dependence may suggest that Sr mainly comes from the dusting process while the hair content of Pb originates from other important sources of this element, e.g. emissions from power plants, fabrics or traffic (many subjects work in the town centre).

It should be taken into consideration that the interrelation between toxic and essential elements are of a complex nature and may result in disorders of processes controlled by these metals when there is no confirmed epexegesis how the level of hair elements can change with parameters of internal and/or external origin [37].

We have also evaluated the significance of factors like age, smoking habits and sex, which can influence metal content. However, not all of the potential sources of affecting factors were considered. We did not consider exposure from other parameters such as dietary habits. Due to the fact that not all samples were washed, we decided to deliberate the differences in content of elements in relation to sex, age and smoking preferences in two groups (A and B). Fig. 6 shows the concentration of elements in washed samples (group A, collected from people living in Łódź, Zabrze, Oława) and unwashed material (group B, taken from citizens of Gdańsk) in relation to age. The studied populations were divided into three age subgroups: 18-25 years (I), 25-45 years (II) and over 46 years (III) of age. In the case of group A, the lead content increases dramatically with age while Ba and Cd present the same tendency in metal accumulation – the levels were found to be lowest in the age 18-25 interval, then the concentrations appeared to increase with age, reaching a maximum for the 25-45 year-old range followed by a slight drop in the oldest group. The Sr content seems to be quite comparable for three age intervals. In group B no statistically significant age-dependence was stated for Cd, Pb, and Ba. The age-content of Sr relation was similar to that observed for group A. There are many investigations concerning analysis of factors affecting the level of endogenous elements; nevertheless, there is no agreement about their direct impact. In general, it is assumed that hair of younger subjects contains higher amount of metals than the older ones [38]. However, in the study of Nowak, 1998 [5] higher content of Cd and Pb was detected for healthy subjects in the 31-80-year-old interval than the 1-30-year interval, contrary to published results [10] where no age variation was noticed for these metals. Baranowska et al. reported that the content of Cd in hair increases consequently with age, and for Sr the correlation was reversed, whereas Pb was accumulated in the highest extent in the middle age (36-60) [22]. No such pattern has been concluded in the work of Chojnacka et al. [18]. The study reveled that the level of Cd does not fluctuate with age, hair of children (<15 years) seems to accumulate Pb the most, and young people between 15 and 25 years old have a tendency to contain the highest levels of Sr and older ones (20-35 years) of Ba.

The changes in the mean concentrations of washed hair (taken from people living in Łódź, Zabrze, Oława) and unwashed (received from inhabitants of Gdańsk) as a function of sex and smoking habits are illustrated in Fig. 7.

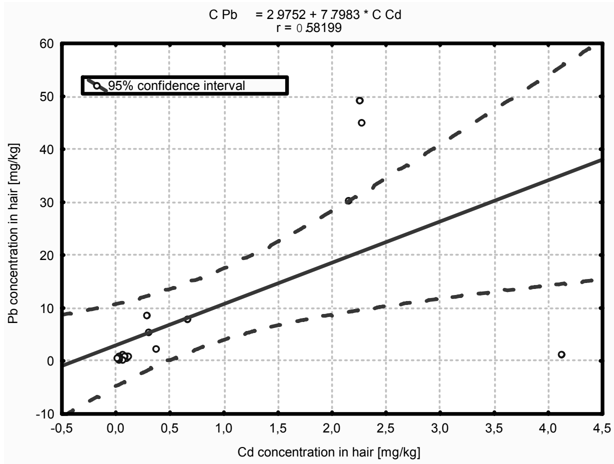

Fig. 5. Example of correlation between concentration of Cd and Pb in hair samples collected from Oława company.

a)

b)

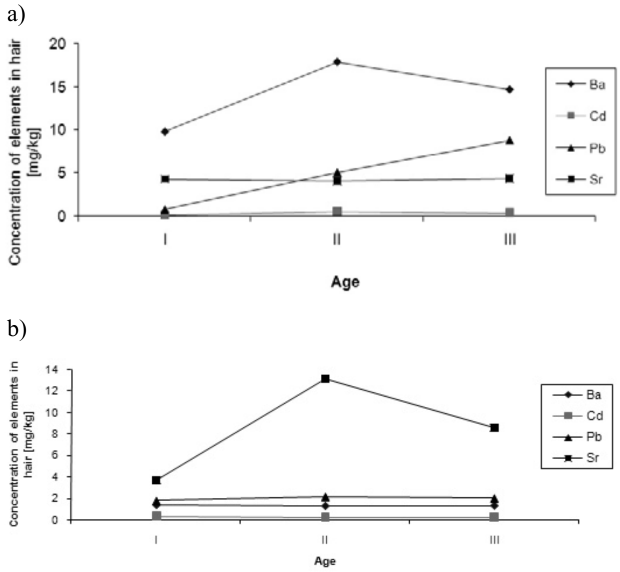

Fig. 6. Concentration of elements in washed hair samples collected from people living in Łódź, Oława and Zabrze for under 25 years (I, N=23), 25-45 years (II, N=23) and over 46 years (III, N=14) of age [mg/kg] (a), and in unwashed hair samples collected from people living in Gdańsk for under 25 years (I, N=31), 25-45 years (II, N=7) and over 46 years (III, N=11) of age [mg kg^{-1}] (b).

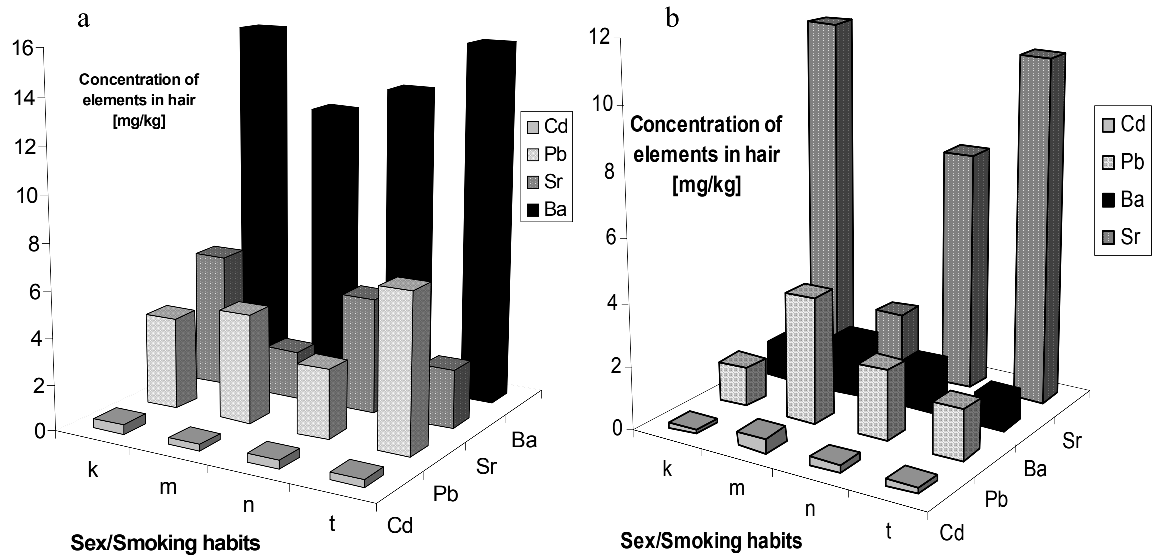

Fig. 7. Concentration of elements in washed hair samples collected from people living in Łódź, Oława and Zabrze of female (k, N=34), male (m, N=26), non-smokers (n, N=40) and smokers (t, N=20) [mg/kg] (a), and in unwashed hair samples collected from people living in Gdańsk of female (k, N=36), male (m, N=13), non-smokers (n, N=31) and smokers (t, N=18) [mg kg^{-1}] (b).

In female hair of the first group, the level of Ba and Sr was considerably higher when compared with male hair, Cd concentration does not change notably with sex, and the Pb content was slightly lower. The differences in metal levels connected with the sex of the volunteers from Gdańsk were more distinct in contrary to the previous group. Substantially higher contents of Pb, Cd, and Ba, and dramatically lower Sr are found males. Some studies suggest that females accumulate higher levels of elements most commonly [38]. Fundamentally lower male hair levels have been previously reported for Ba [10, 18] and Sr [10, 18, 21, 22]. In other literature studies in females and males the content of Pb [22], Ba [21] and Cd [18, 21] was approximately the same, whereas higher Pb [5, 18, 21] and Cd [5, 22] was described elsewhere.

Also, people having various smoking preferences were distinguished. It was found that the level of Cd does not differ significantly for non-smokers and smokers. Similar results were published by Chojnacka et al. [18]. However, in another study [10], over five times higher cadmium concentrations were detected in hair of smokers compared to non-smokers. The level of Pb and Ba within group A (washed hair) was higher for smokers than non-smokers in contrast to group B. The study of Chojnacka et al. [18] revealed a higher concentration of Ba in non-smokers and lead in smokers hair, while in other works [10] no influence of smoking habits on Pb levels was shown. In contrast to unwashed hair material from Gdańsk, the Sr concentration was lower in samples collected from non-smoking donors living in both Łódź, Zabrze and Oława, which stays in agreement with some literature data [18]. Probably this reverse trend in Sr accumulation for Group B is a result of outdoor smoking. Most examined smokers were over 50 years old (many of them were retired), spending notable parts of the day outside and in consequence being more exposed to dusting from the phosphate dump, which may led to extension adsorption of strontium.

All of these observed fluctuations between groupa A and B may be a result of the differences in sample preparation stage and relatively small number of subjects taking part in this study. This enables us to form some general conclusions.

SEM images present significant changes connected with exposure to toxic metals that damage the structure of the investigated samples (Fig. 8). Also, chosen students' hair was examined in order to provide a standard for comparison. Relevant differences are seen on the surface of hair collected from the subjects exposed to occupational and environmental pollution in contrast to hair coming from the students. This control hair structure (students') is very smooth without any structure breaks. Based on visible observations, we are able to order hair structure from the least to the most destroyed and that in general stays in agreement with the degree of toxic metals accumulation in hair. In the case of exposed hair samples taken from the people working in a coal mine, the hair structure seems to be the least affected while for sample collected from employees of the Oława company some losses of rough structure in hair are noticed. Undoubtedly the most degraded hair material was collected from inhabitants of Wislinka. Serious damages in hair structure presented in terms of breaking away the material from the surface with some attached exogenous particles possible as a result of dusting were observed. This could be attributed to the fact that many of the volunteers from Gdańsk (the average age was the highest from all discussed populations and was 50 years old) have lived in the vicinity of phosphate dump from the beginning of its creation (almost 40 years), so the exposure time to toxic substances is the longest from all examined groups.

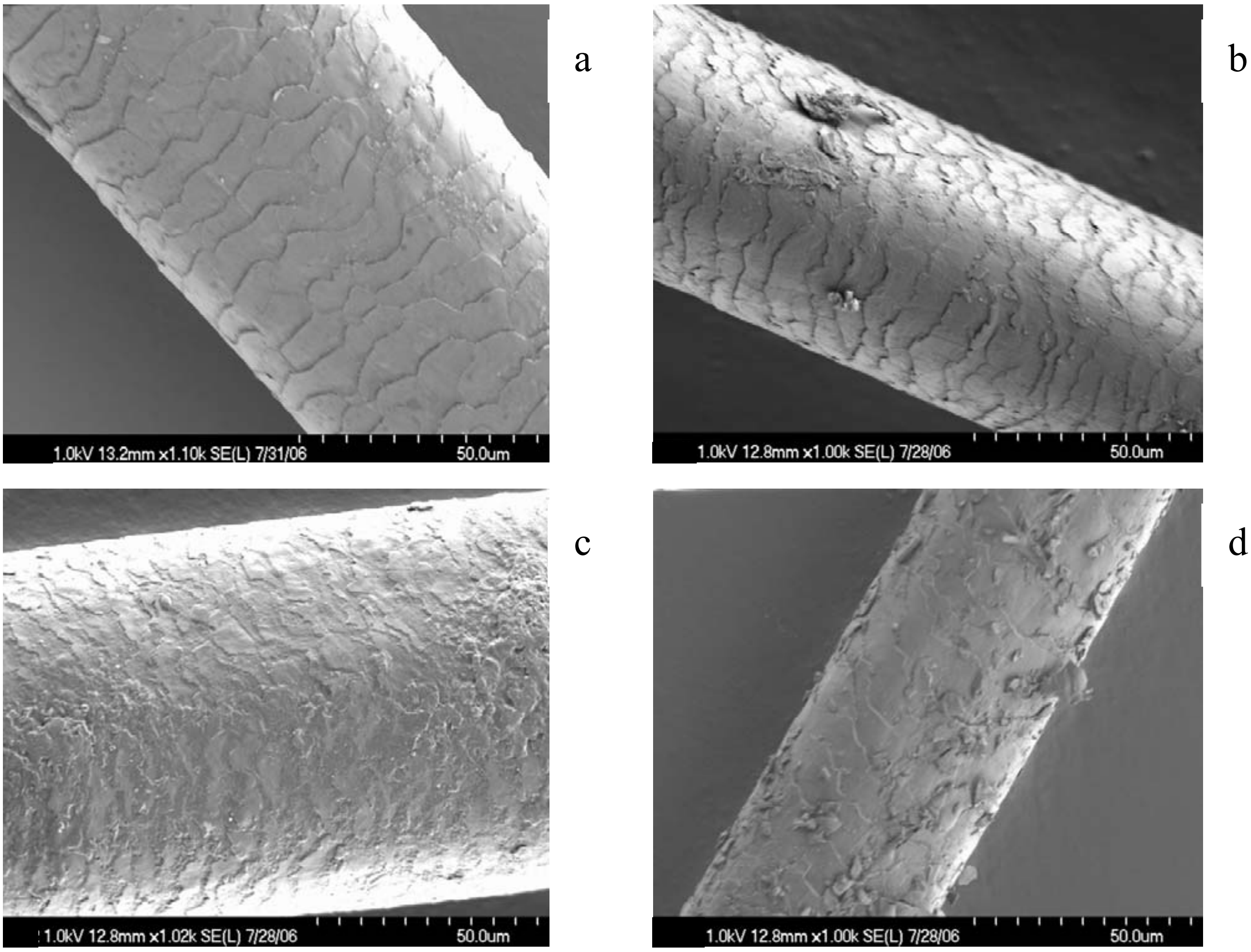

Fig. 8. Examples of scanning electron micrographs of hair surfaces, respectively collected from students (a, x 1,000), from subjects working in the "Makoszowy" coal mine in Zabrze (b, x 1,000), in lead manufacturing company in Oława (c, x 1,000), and from inhabitants of Wislinka (d, x 1,000).

Conclusions

Our research proved that hair samples can be potentially used as a reacting and accumulating indicator of occupational and/or environmental exposure. Most likely, it can be supposed that the source of exposure and various mechanisms of incorporation of toxic metals into the human body are the main factors responsible for huge differences in metal concentrations among the four studied population groups. However, there is a necessity to elaborate one standardized methodology of human hair material treatment, sample preparation (including washing) and to evaluate reference values, which will take into consideration main parameters affecting elemental composition of hair. That kind of approach can make hair analysis more reliable and can create a standard for comparison with other studies. Moreover, more detailed investigations concerning the analysis of relationships between the levels of dangerous substances and elements in hair scalp material and other human media are required in order to establish the potential routes of element accumulation and their behaviour in human organism. Our study revealed as well that SEM images can also be very helpful in the determination of accumulation tendency. It was noticed that the more toxic elements that were adsorbed and accumulated by hair, the larger area of hair structure that was degraded. However, the accumulation of toxic elements in hair might partially be dependent on other factors connected with sex, age, smoking habits and should be more deeply analyzed using a sophisticated statistical approach in future research.

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