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Determination of chosen elements in hair samples collected from students of Technical University of Łódź

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For many years hair analysis has drawn attention as a non-invasive method and has been particularly used for assessing exposure especially to toxic metals in occupational and environmental health surveys. This study was conducted to explore potential applications of hair analysis in determination of the affect of workplace on the content of elements in the investigated material. Hair samples were collected from two groups of students in Łódź. The concentrations of elements in hair were determined by ICP-MS and ICP-OES methods. Also, we compared the results with literature data and examined factors influencing metal content.

1. INTRODUCTION

Monitoring with biological media can provide an estimation of the total dose absorbed and make an important approach to qualify health risk, as well as to evaluate the impact of environmental pollution. For the last thirty years hair as a clinical sample has played a major role in the assessment of community and occupational toxic trace elements exposure and in the study of nutrional and bodily status of several essential metals [1-4], for diagnosis of diseases or in forensic science (e.g. heavy metal poisoning diagnosis [5] or drugs detection in organism [6]).

The use of human hair as a biomarker has received a great deal of attention mainly because metal content in hair may reflect past changes in metabolism and in environmental exposure. As a result, nowadays we can observe a current trend towards making use of hair as a tool of choice for monitoring health state of our organism. That kind of investigation can deliver us information about lack of elements or, on the other hand, can signal any undue accumulation. In contrast to blood or urine measurements, which do not correspond to long-term exposure, the concentration of most trace elements are higher in hair, as a rule, by even one order of magnitude [2, 3, 4, 7]. Moreover, hair samples can be collected quickly, non-invasively and no special storage/transportation conditions are needed [2, 4, 7]. However, there exist a number of studies in which hair anomalies were not consistent with nutrional status and clinical symptoms and did not correlate with other biological media such as blood or urine, thus the usefulness of hair application is questioned [8-11].

We should also take into account the limitations of studies involving hair analysis. The main disadvantage includes difficulty in differentiating between endogenous and exogenous deposition, resulting in large variations in element's range. Some of the inconsistencies can be attributed to discords in sample collection and preparation procedures. Therefore, it seems to be essential to remove surface contaminants, dust and attached dirt from hair sample by proper washing [1, 2, 7, 12, 13]. A review of the literature shows that many washing procedures to remove exogenous contamination have been proposed [4, 7, 13, 14]. However, a number of studies have used the International Atomic Energy Agency (IAEA) involving sequential washing with acetone and water, as a matter of routine [7, 13, 14]. However, some authors questioned the use of a method recommended by IAEA as not sufficient enough to remove exogenous contaminants [13, 15, 16]. Also many others factors affect the trace metals content in hair such as geographical location, lifestyle, dietary habits (nutrional requirements) or ethnic origin. The metal content may also depend on age, sex, hair colour, smoking habits, etc., which can make hair analysis even more complicated [1, 2, 4, 7, 12, 13].

Summing up, one of the basic reasons why till now hair analysis has been the object of divergent opinions is difficulty of establishing normal and reference values in human hair due to variations of hair compositions. The best solution to overcome this problem seems to be testing sufficiently large population groups considering the broad spectrum of factors influencing metal content [7, 17, 18]. Exemplary in the study of Senofonte et al., 2000 hair samples collected from youngsters (3–15 years of age) living in several urban areas of Rome were analyzed in order to determine the content of elements with the aim of assessing reference values [2]. The assessment of reference values for elements in human biological tissues, fluids is also given by Caroli et al., 1994 [19]. It is also recommendable to supplement this examination with analysis of other biological media, like the above mentioned blood or urine [7].

A number of studies reported to use the hair analysis to receive information connected with environmental and occupational exposure. Bath et al., 1982 studied the potential differences in elements content among urban subjects and individuals living in industrial areas [20]. The paper of Nowak, 1998 [21] reports the contents and relationship of elements in human hair for a non-industrialized population of Poland. In the work of Chojnacka et al., 2005 inter-element interactions in human hair sampled from population group living in urbanized and industrialized region of south-west Poland were investigated [22]. Hair samples were also used in a preliminary assessment of heavy metals exposure of the human population living near an abandoned cupric pyrite mine [23]. Takayuchi et al., 1986 compared the element concentrations in hair of inhabitants of heavy–metal polluted areas with those of normal individuals [24]. Cadmium content in hair and kidney obtained from corpses in the Gdańsk region was determined by Hać et al., 1998 [25]. The. lead exposure and associated risk factors in urban children in the Silesia region were investigated by Jarosińska et al., 2004 [26]. The aim of the study of Özden et al., 2007 [27] was to asses the content of Cd and Pb in hair of school children exposed to smoking and heavy traffic as well as some industrial emissions. The exposure to toxic metals of children in rural and industrial areas of Poland was examined by Chopicka et al., 1995 [28].

2. EXPERIMENTAL

In this study an attempt has been made to investigate the differences in concentrations of elements in hair samples collected from two groups of students of the Technical University of Łódź. The first group consists of students from the Chemistry Department occupationally exposed to heavy metals and also platinum-group elements (PGEs). They deal with adoption of car catalytic converters and preparation of vehicle exhaust catalysts, in addition to some other applications (e.g. industry). There is a need to study possible health risk related to aspects of PGEs and bioaccumulation of these elements in living organisms, because it is well known that platinum group elements are associated with allergenic potential, increased hair loss, asthma and other serious problems. The second group of hair samples comes from students theoretically non-exposed to chemical reagents from the Mechanic Department. Students were asked to answer a questionnaire employed in the sampling campaign including information about factors which may affect the elements content as: sex, hair colour, smoking habits, characterization of possibly treated and dyed hair and many others. That detailed form enabled us to conduct statistical analysis in order to assess differences between measured concentrations of metals (check whether the content of one metal in hair is correlated with the concentration of another metal). Another aim of the study is to compare the obtained results with the value limits for metals in hair in the Łódź region [18] and with the trace element content in reference man [17]. The procedure adopted in this work connected with preparation of samples is based on the following steps: collection of approximately 200 mg of human hair from nape of the neck using stainless steel scissors (only first 3–4 cm closest to the scalp were used), washing separately with acetone, then with water and again with acetone (according to the International Atomic Energy Agency recommended procedure) and finally digestion in nitric acid with microwave system Milestone MLS-1200 MEGA. Uncoloured hair were requested, but not got in all cases. The content of chosen metals was measured with the inductively coupled plasma atomic emission spectrometry (ICP-OES) IRIS-AP, Thermo Jarrell Ash (emission lines were as follows: Ca 315.887 nm, Na 589.592 nm, Fe 259.940 nm, Mg 280.270 nm, Mn 259.373 nm, Sr 407.771 nm, Zn 213.856 nm) and inductively coupled plasma mass spectrometry (ICP-MS), X-Series, Thermo Electron Corporation (^{197}Au, ^{59}Co, ^{111}Cd, ^{53}Cr, ^{65}Cu, ^{208}Pb, ^{105}Pd, ^{195}Pt, ^{95}Mo) techniques. We have also investigated so called toxic proportions (ratio of content of defined two element) especially of Ca/Pb, Zn/Cd and Fe/Pb as well as other proportions of Ca/Mg, Ca/Na, Ca/Fe, Fe/Co, Na/Mg or Ca/Sr, for instance. Scanning electron microscopy Hitachi S-4700 was used to assess visually the effect of dyed hair and washed hair in contrast to respectively untreated and unwashed hair contaminated by dust participles and exogenous elements.

3. RESULTS AND DISCUSSION

In total, 75 students participated in this research. The collected samples came from boys and girls attending Technical University of Łódź aged 22–27. It is hard to compare the results for two departments because of lack of well-defined statutory limits. In our case we refer to average levels of elements in hair samples reported only for Łódź region [18], which is the best solution for us because it includes the so-called local conditions. In order to verify the accuracy of the method and the results Certificate Reference Material of Human Hair NCS ZC 81002b (Beijing, China, approved by China National Analysis Center for Iron and Steel) was used and a good agreement was achieved (Table 1).

The study generally showed the higher concentration of measured elements in hair collected from the Chemistry Department, which can be attributed to occupational chemical exposition (Table 2). On the other hand, for students from the Mechanic Department we have noticed values of metal proportions closer to recommendable values and advisable ratios of selected metals. The results of metals determination in hair in relation to sex, smoking habits, hair colour are presented in Figure 1. In general as expected we did not notice abnormally high differences between male and female. However, in literature significantly higher female hair concentrations have been reported especially for Cu, Co, Ni, Mn, and coming from jewellery made of such metals as Ag, Au [1]. It should be noticed that in our case we observed higher concentrations of lead in man's hair as well as for smokers.

Tab. 1. Results from the measurements of certificate material [mg/kg] (U – uncertainty).

ELEMENT	CERTIFICATE VALUE ± U [mg/kg]	OBTAINED RESULT± RSD [mg/kg]	RECOVERY [%]
Ca	1537 ± 68	1604 ± 60	104.3
Cd	0.072 ± 0.01	0.063 ± 0.008	87.55
Co	0.153 ± 0.015	0.165 ± 0.011	107.8
Cr	8.74 ± 0.97	9.54 ± 0.62	109.1
Cu	33.6 ± 2.3	35.6 ± 1.3	106
Fe	160 ± 16	175 ± 15	109.4
Mg	248 ± 14	250 ± 10	100.8
Mn	3.83 ± 0.39	3.61 ± 0.12	94.25
Mo	1.06 ± 0.12	1.06 ± 0.09	100
Na	445 ± 40	410 ± 20	92.16
Pb	3.83 ± 0.18	3.94 ± 0.09	102.9
Sr	8.17 ± 0.69	8.81 ±0.3	107.8
Zn	191 ± 16	200 ± 8	104.7

That positive association could be attributed to the fact that women are more likely to be exposed to passive smoking. No statistical differences were found for other elements between these two groups. When we take into consideration hair colour we can state that the highest concentrations of Sr and Pb were reported for blond hair, Mn, Co and Cr for dark blond and Mo and Ba for dark hair respectively, which is generally in agreement with the previously published literature data [12]. A linear regression model was used to check if there exists a relation between investigated metals in hair samples. However, calculated correlation coefficients (not presented in this paper) were below value ± 0.3 (not even close to 1/-1), so we could not state any significant linear correspondence. The papers on inter-element interactions in hair are rather limited. Chojnacka et al., 2005 [22] studied the inter-element interactions by the calculation of correlation coefficients. Several statistically significant dependences were observed: mainly between the elements of environmental concern, which probably suggest their common origin: from polluted environment. Also, a group of elements, mutually positively correlated was distinguished (Ca, Mg, Mn and Sr) that were antagonistic to another group (Pt and W). This was probably due to similar chemical nature of these elements (Ca, Mg, Mn and Sr). Rodushkin et al., 2000 [29] reported synergistic interactions between Hf–Zr, Pb–K, Cs–Rb, Sr–Mg, Nb–Th, K–Na and Tl–Fe. The authors also found no significant correlations for essential elements (S, P, Zn, Cu and Se) and potentially toxic (Cd, Pb, As and Tl).

Tab. 2. Recommendable content of elements and advisable proportions (ratio) between content of metals in hair (concentration of element/concentration of element'), based on researches in the Łódź region [18].

	Recommendable value	Chemistry department	Mechanic department
Ca[mg/kg]	300	350	240
Co [mg/kg]	0.05	0.28	0.04
Cd [mg/kg]	0.3	0.18	0.08
Cr [mg/kg]	0.8	2.23	0.55
Cu [mg/kg]	12.5	19.9	15.7
Fe [mg/kg]	17	10	5.8
Mg [mg/kg]	26	20	18
Mn [mg/kg]	1.5	0.72	0.56
Mo [mg/kg]	0.04	1.78	0.08
Na [mg/kg]	240	120	90
Pb [mg/kg]	4	0.87	1.78
Sr [mg/kg]	3.2	6.08	3.30
Zn [mg/kg]	165	186	65
Ca/Mg [-]	7	17.5	13.3
Ca/Na [-]	2.6	2.9	2.7
Ca/Fe [-]	25	35	41
Ca/Sr [-]	131	58	73
Na/Mg [-]	4	6	5
Fe/Co [-]	440	55	72
Fe/Cu [-]	0.9	0.5	0.4
Cu/Mo [-]	625	11.2	196
Zn/Cu [-]	8	9.3	4.1
Ca/Pb [-]	84	402	135
Fe/Pb [-]	4.4	11.5	3.2
Zn/Cd [-]	500	1033	812

Average content of metals is generally in agreement with reevaluated concentrations of selected trace elements for the 70 kg Reference Man (Table 3) [17]. Only in some cases there are some minor differences e.g. for Cr in data previously reported. The biggest variations are noted for Mo concentrations in hair taken from students of the Chemistry Department, where those values covering up to one order of magnitude could be suspicious. It could be attributed to preparation of catalysts in the Chemistry Department based especially on molybdenum. The obtained results are also within a ranges given by Chojnacka et al., 2005 [22]; Miekeley et al., 1998 [8]; Nowak, 1998 [21] and Rodushkin et al., 2000 [29] with the same two exceptions for Cr and Mo content. Normal concentration ranges for Cr are 0.78÷1.0 [mg/kg] (with a mean for Poland 0.6 [mg/kg]) and for Mo 0.21÷0.44 [mg/kg].

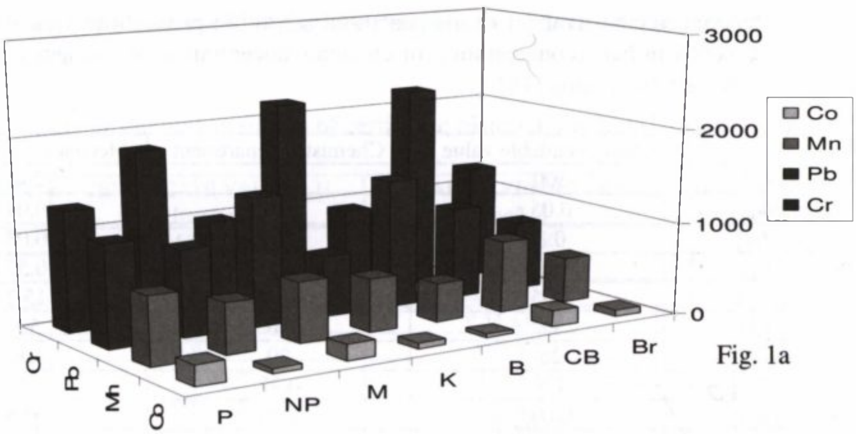

Fig. 1a

	P	NP	M	K	B	CB	Br
Co	218,3795	51,38167	171,3871	61,83982	37,57591	172,0588	70,625
Mn	732,2553	547,4494	657,0896	578,8761	421,4027	776,922	495,0125
Pb	1102,842	944,1861	1390,817	669,0193	1387,067	993,164	751,5813
Cr	1296,242	1830,709	996,9417	2182,693	935,8545	2195,936	1240,575

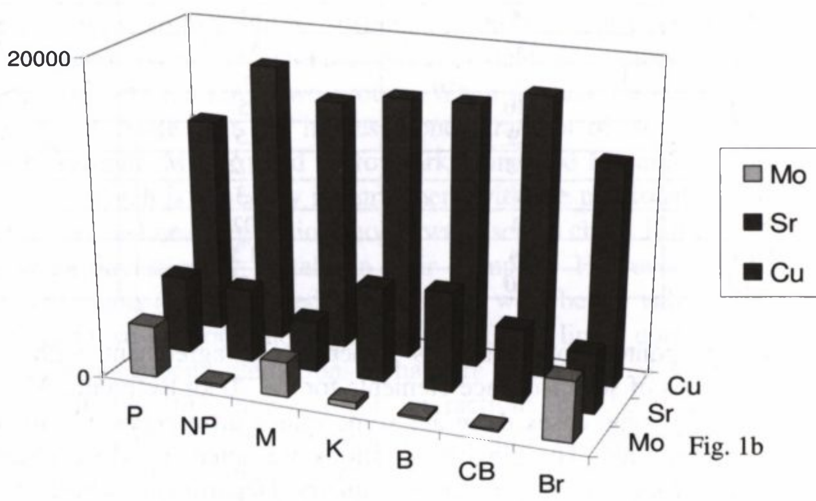

Fig. 1b

	P	NP	M	K	B	CB	Br
Mo	3280,072	95,75091	2295,974	370,6346	127,3773	101,6868	3846,114
Sr	4914,579	4697,333	3277,458	6061,786	6376,818	4679,32	3828,813
Cu	13864,26	17838,39	16018,42	16701,64	16782,63	17709,52	14046,31

Fig. 1. Average results of selected metals with regard to different factors: sex, smoking habits, hair color, which can affect metal concentrations; P – smokers, NP – non-smokers, M – man, K – woman, B – blond hair, CB – dark blond, Br – dark [μg/kg].

Tab. 3. Reference elemental concentration in adult human hair in relation to average content of elements in investigated hair samples collected from two departments [17].

Element	Reference man	Investigated hair samples
Cd [μg/kg]	250–1000	86.5
Co [μg/kg]	50–300	191.5
Cr [μg/kg]	300–1200	1597
Cu[mg/kg]	15–25	15.7
Fe [mg/kg]	30–60	7.9
Pb [mg/kg]	2–20	9.5
Mn [μg/kg]	500–1500	660
Mo [μg/kg]	50–200	1135
Zn [mg/kg]	150–250	143

Unfortunately the physiological role of the PGEs as well as knowledge about possible influence of even low doses connected with the exposure to these elements are still lacking and not well-documented. For these reasons in this research we make an attempt to assess possible risk to these metals and compare them with the published results. Preliminary biomonitoring data for platinum and palladium concentrations found in the literature were much higher than those obtained in our study (Tables 4 and 5).

Tab. 4. Literature data for Pt and Pd concentrations determined in hair samples collected from workers exposed to platinum-group elements, DL-detection limit [30].

Investigated group	Pd in hair [μg/g]	Pt in hair [μg/g]
Preparation of solutions for coating	0.78	0.24
Coating of supports for catalytic converters	1.82	2.55
Adsorption on the carrier	3.25	0.15
Recovery of PGEs from exhausted catalytic converters	5.18	0.22
Administrative service	0.09	0.04
Control group	< DL	0.001

Tab. 5. The comparison of bioaccumulation of platinum group elements in hair from Mechanic and Chemistry Departments [μg/kg].

Department	Pd	Pt	Au
Chemistry Department	27.17	11.22	176.64
Mechanic Department	16.82	8.66	106.01

The described levels of investigated elements in the literature vary between the type of process and production area [30]. The lowest results were observed for two groups: internal personnel employed in administrative services and for external controls (workers not employed in the plant under investigation). In our study the concentrations of platinum group elements were higher in the case of students from the Chemistry Department but they were comparable with the reference groups mentioned before. That may be caused by longer exposition time in contrast to students working occasionally with PGEs. The SEM technique enables us to compare the visible changes between surfaces of undyed (without any chemical treatment) and dyed hair, which may affect the content of metals. Figure 2 indicates relevant changes connected with dying of hair.

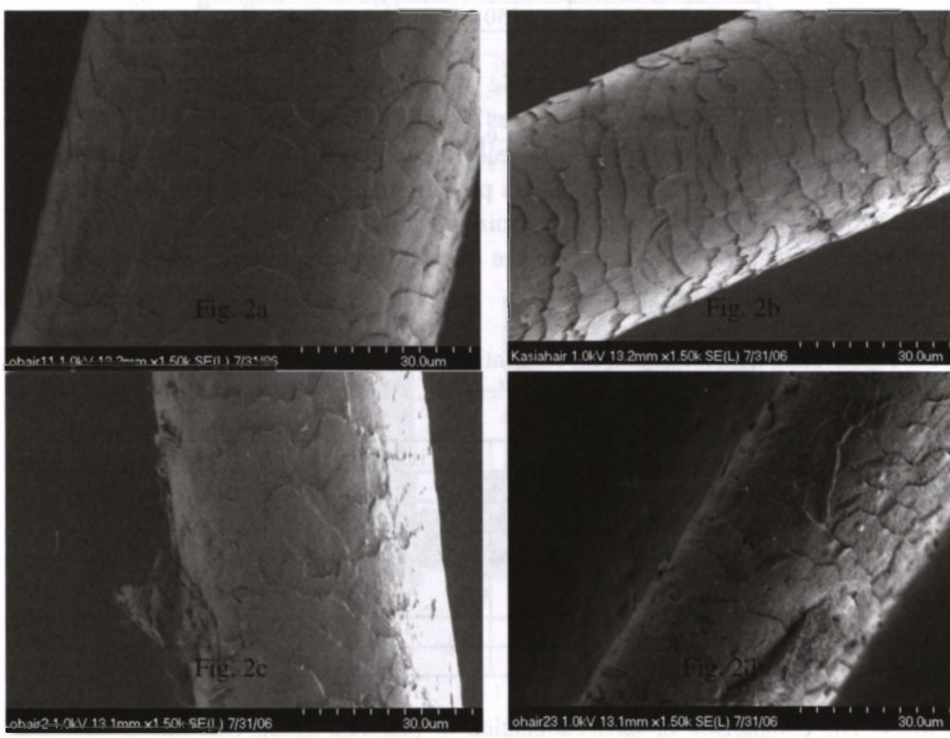

Fig. 2. Exemplary scanning electron micrograph of hair surfaces, respectively natural (Figures 2a, b) and dyed (Figures 2c, d).

The damage caused by chemical treatment is clearly seen. In the case of dyed hair we can easily observed significant cuticle lifting and cracks, fissures and breaking away of material from the surface in contrast to untreated hair. Without doubt many more studies are needed to establish the influence of chemical treatment on the composition and surface changes of hair. The obtained results suggest that hair samples can be potentially used as indicators of occupational exposure to metals. It was also found that the hair content varied with sex, smoking habits and hair colour. Therefore, reference values for the element content should be established taking into account factors, which can influence the composition of hair and make this analysis more reliable. These results indicate need to monitor and control level of toxic elements in biological media in order to start preventing some possible activities.

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