Polish Journal of Environmental Studies Vol. 14, No. 1 (2005), 35-40

Original Research

Level and Relationship of Elements in Scalp Hair of Males: Effect of Air Pollution and Smoking Habits

R. Gryboś[1]*, P. Zagrodzki[2,3], M. Krośniak[2], Ł. Łagan[4], J. Szklarzewicz[1], J. Gołaś[4], W. Przybylski[1]

[1]Faculty of Chemistry, Jagiellonian University, 30-060 Kraków, Ingardena 3, Poland
[2]Department of Food Chemistry and Nutrition, Collegium Medicum, Jagiellonian University, 30-688 Kraków, Medyczna 9, Poland
[3]H.Niewodniczański Institute of Nuclear Physics, 31-342 Kraków, Radzikowskiego 152, Poland
[4]AGH University of Science and Technology, Department of Environmental Protection, 30-059 Kraków, al. Mickiewicza 30, Poland

Received: 22 December, 2003
Accepted: 25 June, 2004

Abstract

Concentrations of Cd, Cr, Cu, Mn, Mo, Ni, Pb, Se, Tl and Zn were analyzed by ICP-MS in the scalp hair of male subjects from an urban area, three different quarters of Krakow, Poland, and from a rural area as control, which were assumed to differ in ambient pollution by metals. A questionnaire on personal data, nutritional habits, socio-economic, occupation and health status was completed by the subjects. Significant differences ($p < 0.05$) were found in the levels of Cd, Cu, Mn and Ni between the locations. Using statistical methods of principal components analysis, the relationships were found between metals in the scalp hair as follows: Mn-Ni, Cr-Tl and Cd-Pb. Two separated clusters of smokers were revealed in the principal components, space suggesting other factors like environmental contamination could confound the values of parameters and relationships between them.

Keywords: metals in scalp hair; ICP-MS; multielemental analysis; cluster analysis; principal components analysis

Introduction

The degree of contamination of the organism by metals depends on their levels in the environment as well as on the effectiveness of all the ways in which they are transported and absorbed by the organism. Exposure of an organism to metals can be monitored by determining their concentrations in urine, blood, nails and hair [1,2]. The hair is a good biological indicator because of the simplicity of sampling and storage. Hair analysis usually leads to higher concentrations of analytical solutions than those in biological fluids. Moreover, there is a correlation between blood or other tissue levels of some metals and those seen in hair [3]. However, hair reflects to a certain extent the total uptake rather than a current level of the metal in the organism. There are two main ways of accumulation of metals in the hair:

i) endogenous (from circulating blood),
ii) exogenous (from ambient air, soil, dust, shampoo, etc.).

A clear distinction between both ways cannot always be made.

Age, sex, site of growth and colour of hair are factors affecting the level of endogenous metals. Younger subjects usually have higher metal levels in hair than older ones [4,5]. Females usually have higher levels than males [6,7]. Levels of iron, copper, nickel and zinc were higher in red hair compared with brown, whereas mercury was lower [8]. Site of growth (scalp or pubis) significantly influenced the concentration of several elements [9].

*Corresponding author; e-mail: grybos@chemia.uj.edu.pl

Exogenous metals can be adsorbed and/or bounded to hair. Adsorbed metals can be removed to some extent by several washing procedures depending on the metal and the procedure applied [10, 11]. On the other hand, washing is likely to extract to some degree the endogenous elements [12]. Thus, the strict regime of washing time and other conditions must be employed to minimize variation in the extraction rate.

A relationship has been established between the mean levels of metals in scalp hair and environmental exposure [5,13] and health status [1,14].

This paper describes the effects of behavioural and environmental factors on element levels in the scalp hair of males living in three different quarters of Kraków as well as the relationships between elements in the scalp hair.

Materials and Methods

Samples

Forty samples of the scalp hair of males aged 30-50 were collected in three different quarters of Kraków which were assumed to differ in ambient pollution by metals (related to fallout of dust containing the metals). Seventeen samples came from a relatively low-polluted region of Limanowa (control group). Limanowa is located in a rural area, about 50 km from Kraków; the regional authorities use this location as control area for measurement of environmental pollution. The choice of males guaranteed that the effect of cosmetic treatment of hair on the representatives of data was minimized. The males were requested to complete a questionnaire on personal data, socio-economic status, nutritional habits, occupation and health status after giving permission for the study.

Samples of scalp hair app. 0.4 g were taken from at least six spots on the head. The hair was cut by surgical scissors just above the skin and only the 4 cm of hair measured from the cutting point were taken for analysis. The samples were stored in polyethylene foil envelopes.

Analysis

The samples were washed according to the procedure recommended by the Advisory Group of the International Atomic Energy Agency (IAEA, Report on the Second Research Co-ordinated Meeting, Neuherberg, Germany, Sept. 2, 1985, Apx. 1/3, p2.) to remove surface dirt and grease and dried at 70^{o}C. After cooling to room temperature in a desiccator, about 300 mg of hair from each sample were weighed into Teflon Vessels (CEM Corp., Mattews, USA) and 5 ml of 65% HNO_3 (Suprapur, Merck) was added. The digestion of samples were carried out in CEM MDS 2000 microwave oven at parameters given in Table 1. After cooling, the samples were quantitatively transferred into 50 ml flasks, then 1 ml of a 10 μg/ml rhodium solution was added as an internal standard for ICP-MS measurements, and filled to the mark with deionized water (18.2 MΩ/cm). Calibration of the spectrometer was carried out using Merck IV Multielemental standard solutions (7 different concentrations of elements in the range 0-100 μg/ l).

Table 1. Digestion parameters of hair samples (CEM MDS 2000 microwave oven, 600W).

Step	Power (%)	Maximum pressure (psi)	Time (min)
I	80	50	10
II	100	100	11
III	100	110	12

Element concentrations were determined using ICP-MS spectrometer (HP 4500). The instrumental parameters used are given in Table 2.

The accuracy and precision of method was tested by measuring the content of elements in certified human hair reference material GBW 09101 from the Shanghai Institute of Nuclear Research. The reference material was prepared for analysis in the same way as the samples. The results were in agreement with the reference values (within 95-103%) for all metals tested.

Statistical Approach

The normality of the parameter distribution was checked by the Kolmogorov-Smirnov and chi-square tests. The mean descriptive statistics of all parameters were calculated for the whole study group. Median values and their confidence intervals were calculated for different regions. To compare the different regions, the Kruskal-Wallis test was applied. Partial correlation coeffi-

Table 2. ICP-MS operating conditions.

Parameters	Conditions
RF power	1350 W
Plasma gas flow	16 l/min
Auxiliary gas flow	1 l/min
Carrier gas flow	1.37 l/min
Sample uptake rate (analysis)	0.2 ml/min
Sample uptake rate (uptake)	2.0 ml/min
Nebulizer	Babington (v-groove)
Rinse time	45 s
Sample uptake time	30 s
Stabilization time	45 s
Number of points/peak	3
Integration time/point	100 ms
Number of replicates	3
Acquisition time/replicate	31.5
Total acquisition time	94.5 s (3 reps)
Total analysis time/sample	3 min 35 s
Isotopes	$^{111,114}Cd$, ^{52}Cr, $^{63,65}Cu$, ^{55}Mn, ^{98}Mo, ^{60}Ni, $^{207,208}Pb$, $^{77,80}Se$, $^{203,205}Tl$, ^{66}Zn

cients (PCC) were calculated for all parameters. Principal components analysis (PCA) was used in order to describe the correlation structure in the data set and to reveal possible influence of other factors (domicile, cigarette smoking, alcohol drinking) on the scatter of individual cases in the new coordinate system, obtained through the reduction of dimensionality of the original set of parameters (Karhunen-Loeve projection). Before use of this method, the parameters were transformed into logarithms and standardized to give the variables equal weight. Principal components were rotated using the Varimax method. The Ward's minimum-variance method was used to achieve the separated clusters grouping parameters of PCA model. Similarities in cluster analysis were calculated from Manhattan distance.

The probability level of $p < 0.05$ was considered statistically significant. Statistical analyses were carried out using the commercially available statistical package STATISTICA (StatSoft, Tulsa, USA).

Sampling Region

Kraków is located in the southern part of Poland in the valley of the Vistula River. The main sources of air pollution are the T. Sendzimir Steelworks and Elektrociepłownia Kraków S.A. (combined heat and

Table 3. Main descriptive statistics of element content in male scalp hair in the whole study group (μg/kg dry wt.).

Element	Mean	Median	Minimum	Maximum	SD
Cd	83.3	51.0	0.05	339.5	74.9
Cr	91.6	79.4	37.2	247.3	43.8
Cu	10,289	8,543	4,559	56,048	8,726
Mn	268.9	213.2	70.9	951.6	190.2
Mo	29.9	26.0	1.1	183.3	28.0
Ni	1,675	1,602	210	4,193	893
Pb	900	535	51	5,697	1,101
Se	49.4	41.6	11.5	242.7	51.9
Tl	6.96	6.04	3.95	19.80	2.57
Zn	80,006	77,095	30,617	166,437	25,654

Table 4. Median values of element content in scalp hair from different regions (μg/kg dry wt.) and limits for medians (in brackets).

	Region			
Element	B (n=12)	C (n=14)	M (n=14)	L (n=17)
Cd	79.6 (34.5; 130.7)	24.4 (3.4; 79.1)	125.2 (27.9; 183.1)	46.1 (27.0; 130.2)
Cr	82.4 (64.9; 89.3)	80.9 (68.8; 179.9)	74.5 (63.7; 138.8)	66.2 (59.5; 85.8)
Cu	9,428 (8,443; 10,181)	8,542 (5,440; 12,493)	8,079 (6,545; 10,923)	7,462 (5,978; 10,046)
Mn	294.2 (176.9; 574.2)	213.5 (125.7; 316.2)	165.3 (118.0; 288.6)	168.5 (118.3; 284.1)
Mo	21.1 (14.2; 37.3)	25.5 (8.3; 43.0)	29.1 (16.7; 45.7)	19.4 (13.0; 32.6)
Ni	2,148 (1,676; 2,378)	1,891 (1,647; 2,689)	1,191 (658; 2,412)	914 (679; 1,143)
Pb	710 (519; 2,295)	206 (117; 846)	585 (198; 1,329)	382 (140; 1,460)
Se	46.9 (11.5; 91.2)	57.0 (0.01; 131.9)	28.0 (0.01; 60.8)	38.2 (6.61; 57.3)
Tl	5.96 (5.03; 6.05)	6.09 (5.74; 8.34)	6.45 (5.23; 8.92)	6.02 (5.82; 7.18)
Zn	83,190 (58,148; 95,601)	77,581 (56,611; 93,443)	76,472 (51,007; 91,199)	76,568 (70,334; 92,346)

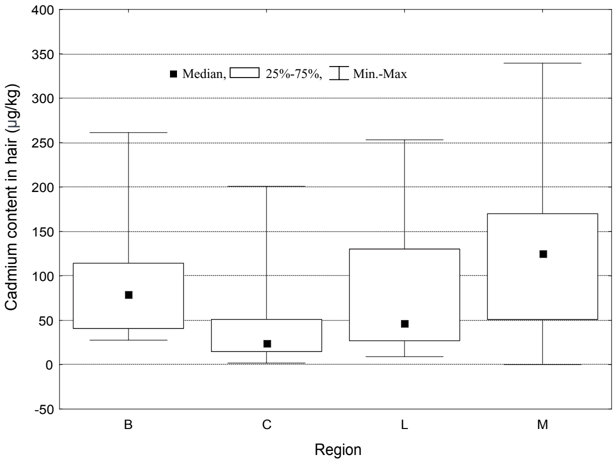

Fig. 1. Box-plot for cadmium content in hair in different regions.

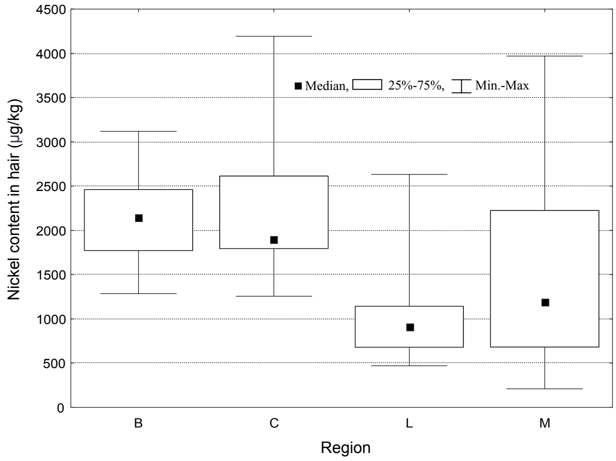

Fig. 2. Box-plot for nickel content in hair in different regions.

power plant). Wind blows mainly along the west-east axis with superiority of west direction. Thus air pollution (quality) is significantly affected by the external sources located along this axis. The highly industrialized region of Silesia spreads 30 km to the west from Kraków, the Skawina S.A. Power Plant is located 15 km to the south-west and industrial Tarnów region 70 km to the east. Three study area in Kraków were selected: Czerwony Prądnik (C), Nowy Bieżanów (B) and Mogiła (M), the district nearest the large steel factory T. Sendzimir, as well as a relatively non-polluted rural region Limanowa (for comparison). The annual averages of the suspended dust in air levels in 2001 were as follows: 25 (L), 26 (B), 27.3 (C) and 28.4 µg/m^3 (M) [15].

Results and Discussion

The main descriptive statistics of metal concentrations in scalp hair are summarized in Tables 3 and 4. Figures 1 and 2 show the examples of box-plots for Cd and Ni. Our results are in general agreement with values reported by other authors [5, 16].

Because most parameters had nongaussian distribution, the Kruskal-Wallis test was applied to compare results obtained for different groups. There were statistically significant differences in the levels of Cd, Cu, Mn, and Ni between the locations (Table 5). Among them, the Ni level was the most differentiating parameter. No statistically significant differences were observed for Cr, Mo, Se, Tl, Pb, and Zn elements. In accordance to our expectations the lowest (or near lowest) concentrations of most of the elements were found in samples from Limanowa. It was proved that this agricultural region was the least polluted among regions under investigations. The limited data (e.g. the concentration of suspended dust in air) available on ambient air pollution, arranged in ascending order, generally correspond with the most frequent sequence of the study areas arranged concerning the growing metal concentration in scalp hairs. This sequence can be summarized as follows: B > C > M$\geq^*$ L for Cr, Cu, Mn, Ni and Zn ($^*\geq$ means that the difference between M and L in respect of median values of some metals was lower than 3%). This suggests that apart from domicile, some additional factors of environmental stress, like ev-

Table 5. Significant differences in element content in scalp hair for investigated regions (p-values are given in brackets).

	B	C	L	M
B	-	Cd (0.008)	Cu (0.042) Ni (0.000)	Mn (0.027) Ni (0.027)
C	Cd (0.008)	-	Ni (0.000)	Cd (0.024)

Table 6. Partial correlation coefficients for original parameters (p<0.05).

	Mo	Ni	Pb	Tl
Cd	0.296	-	0.551	-
Cr	0.331	-	-	0.328
Mn	-	0.314	-0.278	-

Table 7. Eigenvalues of the correlation matrix of the original parameters and percent of variance accounted for by the PCA model.

	I principal component	II principal component	III principal component
Eigenvalue	2.359	1.649	1.306
Variance (%)	26.2	18.3	14.5

Table 8. Principal component loadings and communalities of original parameters.

Parameter	I principal component	II principal component	III principal component	Communality
Cr	0.260	0.571	**0.497**	0.330
Mn	0.259	**0.718**	-0.061	0.267
Ni	-0.232	**0.818**	0.048	0.290
Zn	-0.525	-0.040	0.058	0.153
Se	0.055	0.328	-0.731	0.159
Mo	0.523	0.113	0.460	0.228
Cd	**0.807**	-0.042	0.217	0.451
Tl	-0.076	0.300	**0.627**	0.211
Pb	**0.842**	0.071	-0.181	0.442

ery day route through overcrowded and polluted streets of Kraków to places of employment, and periods of time spent there might confound the metal concentrations in hair of inhabitants living in particular districts.

The results of PCC and PCA are summarized in Tables 6-8 and in Figure 3. Only the first three statistically significant components, which represented most of the total variance of the data set, are shown. The first component is loaded mainly by Cd and Pb. Mn and Ni appeared to be correlated with the second component. The association of the third component was positive with two elements (Cr, Tl). This correlation pattern was confirmed by PCC (Table 6) and cluster analysis (Fig. 4).

A correlation between toxic elements Cd and Pb agrees with the findings of other authors and indicates a common external source of pollution [7, 17]. The inverse relationship between the pair Cd-Pb and Zn points to the possible competition between these elements in human hair. As Mn and Ni were uncorrelated with above metals, an independent origin (or mechanism) can be suggested regarding the presence of Mn and Ni in hair.

Neither domicile nor alcohol consumption exerted any "separating" effect on subjects (results not shown). However, two separate groups of smokers were apparent from the scatter of individuals in K-L projection (Fig. 5). To improve visual clarity and to separate clusters of smokers by straight lines, co-ordinates of all points in K-L pro-

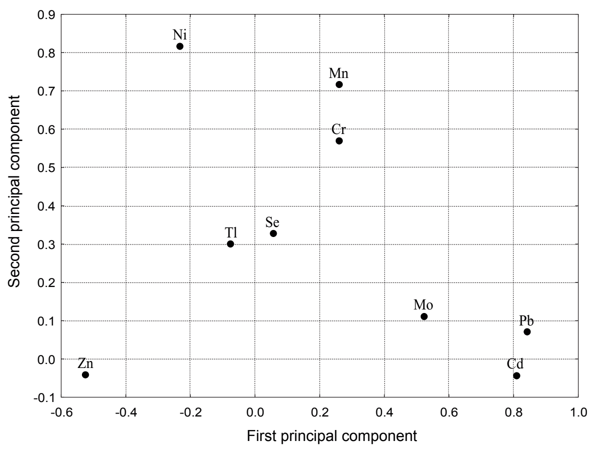

Fig. 3. Principal components projection of the nine original parameters. Principal components are Varimax rotated.

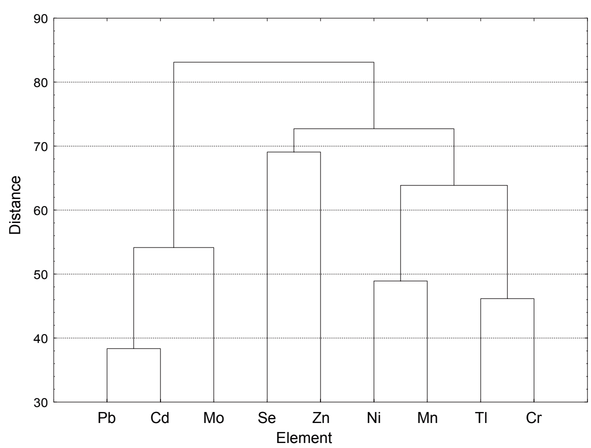

Fig. 4. Dendrogram for parameters of PCA model. Manhattan distance and Wards' minimum-variance method were applied.

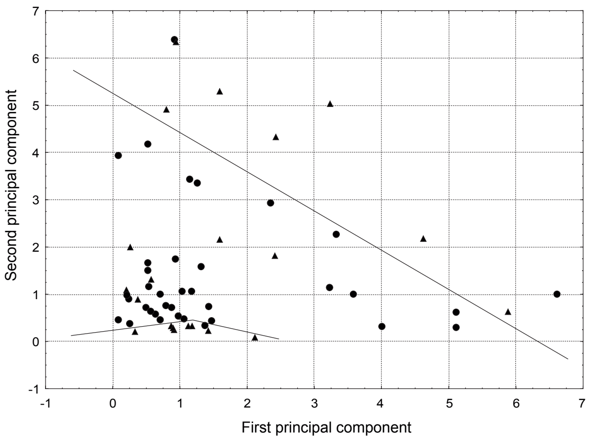

Fig. 5. Karhunem-Loeve projection of the whole study group. Scores were transformed by means of exponential function. The border lines between smoking and non-smoking subjects are depicted (▲ smoking subjects, ● non-smoking subjects).

jection were transformed by means of exponential function. The first group consisted of inhabitants of different districts of Kraków and one subject from Limanowa (3B, 2C, 3M, L), while the second group was almost homogenous gathering subjects mainly from Limanowa (6L, M). These two clusters of smokers were differently located in the principal components space, i.e. they exhibited different values of parameters and relations between them.

Our results were obtained for a relatively small group of subjects, and this fact precludes the assessment of normal ranges and firm general conclusions. Further studies need to be carried out in order to identify the greater number of analytical data in this area.

Acknowledgements

This study was partially sponsored by The Kościuszko Foundation, Inc., An American Center for Polish Culture and the AGH University Grant no.11.11.150.84. The authors wish to thank students Anna Maria Kostuch and Marta Mówińska, who took part in sample preparation.

References

1. NAGINIENE R., ABDRACHMANOVAS O., KREGZDYTE R., RYSELIS S. Investigation of heavy metals in people with alopecia. Trace Elem Electrolytes **19**, 87, **2002.**
2. YOO Y.C., LEE S.K., YANG J.Y., IN S.W., KIMA K.W., CHUNG K.H., CHUNG M.G., CHOUNG S.Y. Organ distribution of heavy metals in autopsy material from normal Korean. J Health Sci. **48**, 186, **2002.**
3. VAHTER M., BERGLUND M., AKESSON A., LIDEN C. Metals and women's health. Environ Res **88**, 145, **2002.**
4. KOWAL N.E., JOHNSON D.E., KRAEMER D.F., PAHREN D.F. Normal levels of cadmium in diet, urine, blood, and tissues of inhabitants of the United States. J Toxicol Environ Health **5**, 995, **1979.**
5. CAROLI S., SENOFONTE O., VIOLANTE N., FORNARELLI L., POWAR A. Assessment of reference values for elements in hair of urban normal subjects. Microchem J. **46**, 174, **1992**.
6. RYAN D.E., HOLYBECNER J., STUART D.E. Trace elements in scalp hair of persons with multiple sclerosis and of normal individuals. Clin Chem. **24**, 1996, **1978.**
7. WIBOWO A.A.E., HERBER R.F.M., DAS H.A , ROELEVELD N., ZIELHUIS R.L. Levels of metals in hair of young children as an indicator of environmental pollution. Environ Res. **40**, 346, **1986.**
8. SKYPECK H.H., JOSEPH B.J. Chemical Toxicology and Clinical Chemistry of Metals, edited by Brown SS, Savory J, London Academic Press, London, pp. 159-163, **1983.**
9. MERZENICH H., HARTWIG A., AHRENS W., BEYERSMANN D., SCHLEPEGRELL R., SCHOLZE M., TIMM J., JOCKEL K.H. Biomonitoring on carcinogenic metals and oxidative DNA damage in a cross-sectional study. Cancer Epidemiol Biomarkers Prev. **10**, 515, **2001.**
10. CASE C.P., ELLIS L., TURNER J.C., FAIRMAN B. Development of a routine method for the determination of trace metals in whole blood by magnetic sector inductively coupled plasma mass spectrometry with particular relevance to patients with total hip and knee arthroplasty. Clin Chem. **47**, 275, **2001**.
11. MAURICE J.F., WIBETOE G., SJASTAD K.E. Longitudinal distribution of thallium in human scalp hair determined by isotope dilution electrothermal vaporization inductively coupled plasma mass spectrometry. J Anal At Spectrom **17**, 485, **2002**.
12. TAYLOR A. Usefulness of measurements of trace elements in hair. Ann Clin Biochem **23**, 364, **1986.**
13. SEIFERT B., BECKER K., HELM D., KRAUSE C., SCHULZ C., SEIWERT M. The German Environmental Survey 1990/1992: reference dust, drinking water and indoor air. J Expo Anal Environ Epidemiol **10**, 552, **2000.**
14. MIEKELEY N., FORTEAS L.M.D., da SILVEIRA C.L.P., LIMA M.B. Elemental anomalies in hair as indicators of endocrinologic pathologies and deficiencies in calcium and bone metabolism. J Trace Elem Med Biol. **15**, 46, **2001.**
15. Report WIOŚ Cracow. Poland, unpublished data, **2001**.
16. RODUSHKIN I., AXELSSON M.D. Application of double focusing sector field ICP-MS for multielemental characterization of human hair and nails. Part II. A study of the inhabitants of northern Sweden. Sci Total Environ. **262**, 21, **2000.**
17. NOWAK B. Contents and relationship of elements in human hair for a non-industrialised population in Poland. Sci Total Environ. **209**, 59, **1998.**