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APPLICATION OF ATOMIC ABSORPTION SPECTROMETRY IN THE ELEMENTS AND HEAVY METALS DETERMINATION IN BIOLOGICAL MEDIA – HUMAN HAIR

ABSTRACT *Hair analysis is used in assessment of the body mineral status, and environmental or occupational exposures. This study was undertaken to compare the elements concentrations [calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), iron (Fe), lead (Pb), and cadmium (Cd)] in hair from 995 persons taken in the years 2007-2010. The measurements were performed by atomic absorption spectrometry (AAS). The average values for concentrations were as follows (in $\mu\text{g/g}$): Ca – 414, Mg – 19, Zn – 160, Cu – 13, Fe – 10, Pb – 1.0, Cd – 0.07. The contents of Ca, Mg, Zn, Cu were statistically higher in the hair of females in comparison with males and the concentrations of Pb and Cd were lower. Significant correlations were found to exist between age of volunteers and the amounts of Ca, Mg, Zn, Fe and Pb. Both in case of correlations between age and elements contents and the correlating metals pairs, the variations between females and males hair were stated.*

Keywords: *atomic absorption spectrometry, hair, gender, elements, human health*

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PROCEEDINGS OF ELECTROTECHNICAL INSTITUTE, Issue 255, 2012

1. INTRODUCTION

Hair is a biological medium more often used in medical diagnostics as a marker of elements levels in the body. The advantages of hair analysis have been already described and discussed in the literature [2, 16]. It is also known that the content of elements in hair is affected by many factors e.g., sex, age, natural and occupational environment, nutritional habits (including diet supplementation), addictions, state of health, stress, and medications used. In human body some glands as thyroid, parathyroid, adrenal cortex, pancreas, and ovaries control the processes of elements accumulation and excretion. An elemental profile of hair is used in the assessment of deficiency or excess of elements in the body. Hair is employed as a biomarker of environmental and occupational exposure to essential and trace elements [1, 2, 9, 16, 18, 30, 31]. Differential contents of elements, mainly heavy metals, were observed in hair of people living in different geographic regions [1, 9, 16, 31] and working in an environment contaminated with toxic metals [18, 30]. Elemental hair composition was also examined in patients with various disorders e.g., renal failure [5, 7], some types of cancer [1, 13, 21, 22, 32], cerebral palsy [15], diabetes mellitus [12], endocrinologic pathologies [19], and drugs addiction [3]. In hair of patients the researchers have found increased accumulation or smaller amounts in comparison to the control subjects of such elements as Ag, Al, Ca, Cd, Cr, Cu, Co, Fe, K, Li, Mg, Mn, Na, Ni, P, Pb, Sb, Se, Sr and Zn [1, 3, 5, 7, 12, 13, 15, 19, 21, 22, 32]. Research works over the content of elements in human hair have been conducted since the 60s of last century [23]. Procedures for hair sampling, washing and preparation for instrumental analysis [2, 16] and the usefulness of the analytical methods such as ICP-AES (inductively coupled plasma atomic emission spectroscopy), ICP-MS (inductively coupled plasma mass spectrometry), AAS (atomic absorption spectrometry), and NAA (neutron activation analysis) in the assessment of the elemental composition of hair have been tested and standardized. In AAS the amount of light at the resonant wavelength which is absorbed as the radiation passes through the atoms at the ground state is measured. Now research works are continued on the quantitative proportions and cross-correlations between elements in hair and in body fluids (serum, blood, urine) as well as in the other tissues [5, 7, 8, 14, 17, 26, 27, 29]. Moreover, basing on the populational studies, the reference ranges for the elements concentrations in hair have been elaborated [10, 11, 20, 28] which is helpful for physicians, biochemists and analysts/chemists in assessing their amounts in the body, disturbances in the elements metabolism, or human environmental and occupational exposure.

The main purpose of this study was to evaluate the distribution of elements in hair, and their interactions in dependence on gender, as well as to compare obtained results with earlier data. Quantitative analysis was performed with the use of AAS method.

2. MATERIALS AND METHODS

Our studies have been conducted in the years 2007 to 2010. Samples of hair collected from 995 Polish volunteers: 483 women (age 1 to 95 years) and 512 men (age 0.5 to 83 years) were taken from several points of the head in amount of about 0.5 g. The hair samples were washed with acetone, redistilled and deionized water, dried and weighted (approximately 0.25 g). Next, the hair specimens were mineralized in the mixture of ultra-quality acids: HNO₃/HClO₄ (3:1, v/v). The mineralizates transferred to volumetric flasks (25 mL) were made up to the mark with deionized water.

The Ca, Mg, Zn, Cu, Fe, Pb and Cd contents were determined by AAS method. The concentrations of Ca, Mg, Zn, Cu and Fe were measured using the flame technique (FAAS) and the determinations of Pb and Cd amounts were performed in the graphite furnace (GFAAS). In this study measurements were performed at the following analytical lines (λ): Ca – 422.7 nm, Mg – 285.2 nm, Zn – 213.9 nm, Cu – 324.7 nm, Fe – 248.3 nm, Pb – 217.0 nm and Cd – 228.8 nm.

Instrumental parameters, analytical characterization of the procedures used during the measurements and the results of the reference material (NCS ZC 81002, human hair) analysis, which were in a good agreement with the certified values, were already described in our earlier works [7, 8]. The results obtained in this study were statistically elaborated using the software package Statistica (version 9.1).

3. RESULTS AND DISCUSSION

The medians values for the selected elements concentrations (Ca, Mg, Zn, Cu and Fe) in female and male hair during the period of time under the study are illustrated in Figure 1. Number of females and males participating in the study were the following: 2007 – 133 and 187; 2008 – 141 and 121; 2009 – 132 and 129; 2010 – 77 and 75.

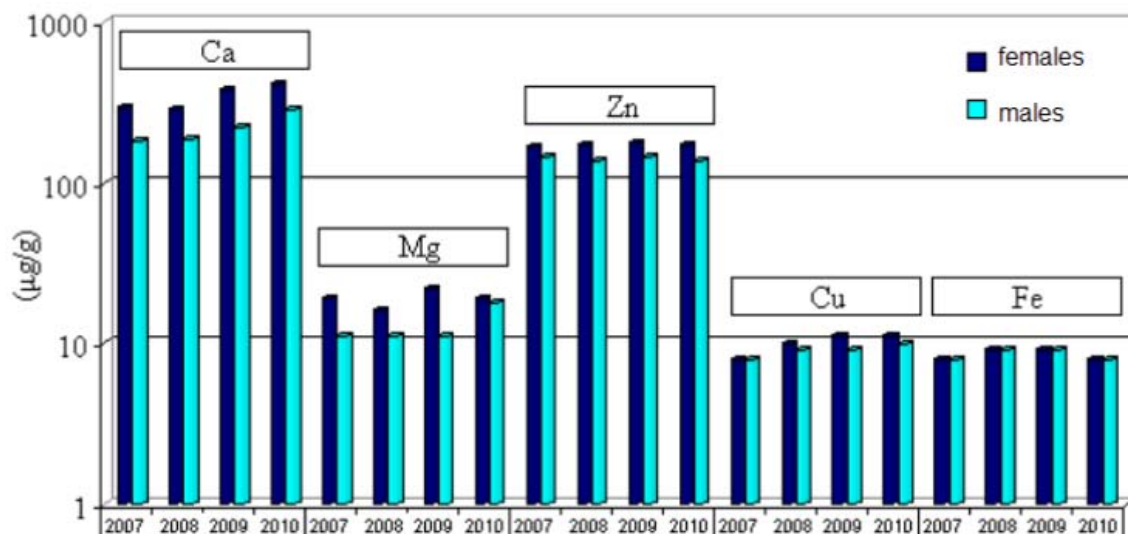


Fig. 1. Distribution of Ca, Mg, Zn, Cu and Fe in hair of females and males (medians values, years: 2007-2010), ($\mu\text{g/g}$)

Contents of elements in hair in the subsequent years of the study (2007, 2008, 2009 and 2010; medians, in $\mu\text{g/g}$) were as follows: Ca – 213, 229, 280, 342; Mg – 13, 14, 15, 17; Zn – 155, 158, 157, 166; Cu – 10, 10, 10, 10; Fe – 8, 9, 10, 8; Pb – 0.7, 0.5, 0.7, 0.6 and Cd – 0.05, 0.05, 0.05, 0.05, respectively. As for the concentrations of Pb in the hair of females and males, medians values were as follows: 0.7/0.8, 0.4/0.6, 0.6/0.9 and 0.6/0.8 $\mu\text{g/g}$ in 2010. In case of Cd, there were no differences in the hair of both sexes. We stated the statistically significant differences between concentrations of Ca in hair in years: 2007 and 2009, 2007 and 2010, 2008 and 2010, for Fe – 2007 and 2010, 2008 and 2010, and for Pb – 2007 and 2008, 2008 and 2009 (test ANOVA).

The ranges of the calculated ratios: Ca/Mg, Ca/Pb, Mg/Pb and Zn/Cd for the values of the 10th and 90th percentile in hair were as follows: (all population) 19-23, 480-482, 21-25, 1420-2962; (females) 19-30, 565-906, 30, 1540-5000; (males) 18-20, 224-300, 11-17 and 1340-2090, respectively.

The estimated elements contents in hair of our tested population are comparable to the results presented by other research workers and cover the concentration ranges given by the authors as a reference or permissible (in case of toxic metals) [1, 3, 10, 11, 19, 20, 28, 29, 31]. Over past years an increase in mean concentrations of Ca and Mg in hair have been observed. Minor changes have taken place in case of Zn, Fe and Pb. Furthermore, significant changes in the content of Ca, Fe and Pb in the last few years have been stated. The contents of Cu and Cd did not change considerably.

The Ca, Mg, Zn, Cu, Fe, Pb and Cd contents in terms of means, medians, standard deviations (SD), ranges, and values for the 10th and 90th percentiles are given in Table 1.

TABLE 1

Basic statistical parameters for selected elements contents in hair of Polish females and males collected in the years 2007-2010, ($\mu\text{g/g}$)

	Ca	Mg	Zn	Cu	Fe	Pb	Cd
Total subjects (n = 995)							
Mean	414	19	160	13	10	1.0	0.07
Median	248	14	158	10	8	0.7	0.05
SD ^{a)}	524	18	85	13	12	1.3	0.18
Range	20 4746	2 255	19 1154	3 181	3 273	0.1 17.8	0.05 3.6
Percentiles 10th, 90th	96 820	5 36	71 237	7 18	6 14	0.2 1.7	0.05 0.08
Females (n = 483)							
Mean	550	23	173	14	10	0.7	0.06
Median	331	19	172	11	8	0.6	0.05
SD	651	21	76	11	14	0.6	0.17
Range	33 4746	2 255	19 613	3 103	3 273	0.1 4.7	0.05 3.6
Percentiles 10th, 90th	113 1269	6 42	77 250	7 22	6 14	0.2 1.4	0.05 0.05
Males (n = 512)							
Mean	286 ^{b)}	15 ^{b)}	149 ^{b)}	13 ^{b)}	10	1.2 ^{b)}	0.08 ^{b)}
Median	199	12	143	9	8	0.8	0.05
SD	308	13	92	15	10	1.7	0.20
Range	20 3244	2 104	19 1154	6 181	4 134	0.1 17.8	0.05 3.2
Percentiles 10th, 90th	90 555	5 28	67 209	7 16	6 14	0.3 2.5	0.05 0.10

a) Standard deviation;

b) Statistically significant differences between elements contents in hair of females and males (the Kolmogorov-Smirnov test, $p < 0.05$)

The mean values for Ca, Mg, Zn, Cu, and Fe contents in Polish females/males hair collected in the years 1985 -1991 were as follows (in $\mu\text{g/g}$): 260/259, 25/23, 163/153, 13/13, and 17/17, respectively [23], and in the years 1991 - 2006 (females/males, medians, in $\mu\text{g/g}$): 391/225, 27/17, 167/154, 10/10, 14/14, Pb – 0.7/1.3, and Cd – 0.11/0.13 [24]. Taking into account the results obtained in this study (years 2007-2010) we observed that the Cd level was considerably lower than in the years 1991-2006. Lower concentration was also observed for Fe, but with a tendency to increase like for Ca and Mg. The Zn and Pb median values were comparable and the content of Cu appeared to be the most stable.

In the study conducted in 2007-2010, contents of Ca, Mg, Zn, Cu were statistically significantly higher in hair of Polish females compared to males, while in male hair higher amounts of Cd and Pb were stated. A comparable amount of Fe was found. Such relationships between the content of these elements were observed during separated 4 years of research. Similarly, higher amounts of Ca, Mg and Zn in hair of females than those for males were reported by other authors [14, 27, 28]. Differences in levels of Ca, Mg and Zn in female and male hair can be the result of different functioning of endocrine glands, hormones regulating the bone metabolism activity (parathyroid hormone PTH and calcitonin), and an increased Zn urine excretion by men. Higher Pb levels in males hair probably may result from the occupational exposure and cigarette smoking [6]. Variations in elemental composition of female and male hair may also come from the differential permeability of intestinal barrier in both sexes. Up to now, little is known about sex and age related changes in the hair amino acids profile. Such relationships were described mainly in case of tryptophan and serine [4, 25], but perhaps the amount of elements in hair is also a function of changing cysteine level i.e., chelating amino acid in this human tissue.

To maintain homeostasis in the body, in regard to the antagonistic and synergistic interactions between elements, it is also important to keep the correct quantitative ratios between them. For example, improper balance between Ca and Mg contents may be the cause of the Ca deposition in soft tissues, while an excessive amount of heavy metals in comparison to bioelements limits bioavailability of essential elements. High proportion between Ca and Mg and, on the other hand, a low amounts of other elements (Mg, Zn, Cu and Fe) may be a symptom of improper nutrition and impaired bioavailability of Ca. Low Mg levels may be accompanied by high concentration of stress hormones. A high Ca/Fe ratio may indicate stomach disorders and a low one – liver disturbances. In this case, it may be also observed the high ratio of Zn/Cu. Antagonistic relationships between Pb and Ca and Mg, and also between Cd and Zn are already known. Comparing the ratios between these elements (average values), we can notice differences in the elemental composition of females and males hair i.e., Ca/Mg – 24/19, Ca/Pb – 786/238, Mg/Pb – 33/12.5 and Zn/Cd – 2883/1863. A greater amount of bioelements and, on the other hand, a smaller content of heavy metals in tissues reduces their toxicity.

Results of correlation analysis are listed in Tables 2, 3 and 4. Correlations were calculated by the Spearman rank correlation test. Bold values are significant at $p < 0.05$.

Our study showed a positive and statistically significant correlation between age and content of elements in the subgroup of females for Ca, Mg, Zn, and negative for Fe and Pb (Table 2).

TABLE 2

Spearman correlation coefficients between age of participants and elements contents in hair

	Ca	Mg	Zn	Cu	Fe	Pb	Cd
Total subjects	0.46	0.53	0.48	0.09	-0.35	-0.24	-0.04
Females	0.34	0.42	0.39	0.04	-0.35	-0.24	-0.01
Males	0.49	0.57	0.50	0.07	-0.36	-0.18	-0.01

In the subgroup of males the relationships were similar. However, we must mention that the considerably lower contents of Ca, Mg and Zn were observed in hair of small children and elderly persons (over 60 years of age) than in other age categories. The distribution of all selected elements in different age groups will be the subject of further publications. In hair of Korean preschool children significant positive correlation between age and Ca and Zn was stated [20].

TABLE 3

Spearman correlation coefficient matrix for selected elements in total hair samples

	Mg	Zn	Cu	Fe	Pb	Cd
Ca	0.84	0.59	0.28	-0.22	-0.12	-0.04
Mg		0.61	0.27	-0.24	-0.19	-0.05
Zn			0.10	-0.33	-0.28	-0.09
Cu				0.05	0.12	0.04
Fe					0.27	0.13
Pb						0.33

TABLE 4

Spearman correlation coefficient matrix for selected elements in hair samples of females (above diagonal) and males (below diagonal)

	Ca	Mg	Zn	Cu	Fe	Pb	Cd
Ca		0.84	0.54	0.31	-0.19	-0.05	0.05
Mg	0.81		0.56	0.34	-0.22	-0.13	0.03
Zn	0.54	0.58		0.07	-0.28	-0.23	-0.01
Cu	0.17	0.14	0.06		0.02	0.12	0.03
Fe	-0.26	-0.27	-0.39	0.10		0.17	0.07
Pb	-0.05	-0.13	-0.25	0.22	0.37		0.23
Cd	-0.01	-0.02	-0.12	0.09	0.19	0.38	

In this study we found differences between the pairs of correlating elements in females and males hair. The same observations were made by other authors [14]. Such differences were also noted in hair of patients in comparison with control subjects [15, 21, 22]. Significant correlations were found, for example, between Ca – Mg, Fe – Cd, Zn – Cd, Ca – Cd, Fe – Pb, Fe – Zn, Ca – Zn and Ca – Fe [15, 16, 21, 22] which is in agreement with our data.

4. CONCLUSIONS

Results regarding to the elements contents in human hair presented in this paper are based on a large group of participants, which allows for a greater accuracy of their interpretation. We observed in hair the tendency to an increase in Ca, Mg and Zn contents, while the Cd amount was decreasing during the studied period. Slight changes in the concentrations were found for Zn, Fe, Cu and Pb. The data obtained in this study are in agreement with those available in the literature. Our findings confirmed the differences in some elements contents in females and males hair reported also by other authors. The amounts of Ca, Mg, Zn and Cu were higher in hair of females than those for males, while the amounts of Pb and Cd were lower. Moreover, we have also stated significant correlation between the age of participants and the concentrations of elements excluding Cu and Cd. Other factors differentiating females and males hair were the ratios of bioelements and toxic metals. Only in case of Ca – Mg, Ca – Zn and Mg – Zn pairs the correlations occurred strong, and the other relationships between metals concentrations, despite the fact that they were statistically significant, were medium or weak. We have also seen elements concentrations in hair changing with age and these observations will be presented in next publications. The applied spectroscopic method (atomic absorption spectrometry) can be used in monitoring of selected elements content in human hair.

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ZASTOSOWANIE SPEKTROMETRII ABSORPCJI
ATOMOWEJ W BADANIACH ZAWARTOŚCI
PIERWIASTKÓW I METALI CIĘŻKICH
W PRÓBKACH BIOLOGICZNYCH – WŁOSACH LUDZKICH

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STRESZCZENIE *Włosy, krew i mocz są wykorzystywane w ocenie stanu pierwiastkowego organizmu oraz środowiskowej i zawodowej na nie ekspozycji. Niniejsza praca została podjęta w celu określenia zawartości pierwiastków i metali ciężkich we włosach kobiet i mężczyzn (995 osób). Włosy pobierano w latach 2007-2010. Oznaczono zawartość takich pierwiastków, jak: Ca, Mg, Zn, Cu, Fe, Pb i Cd. Średnie stężenie pierwiastków ($\mu\text{g/g}$) w badanych próbkach włosów było następujące: Ca – 414, Mg – 19, Zn – 160, Cu – 13, Fe – 10, Pb – 1.0, Cd – 0.07. W badaniach zastosowano metodę spektrometrii absorpcji atomowej. Stwierdzono istotne różnice w zawartości pierwiastków we włosach kobiet i mężczyzn, a także znamienne korelacje między zawartością pierwiastków i wiekiem badanych osób.*

Słowa kluczowe: *atomowa spektrometria absorpcyjna, włosy, płeć, pierwiastki, zdrowie człowieka*

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IEI, Warszawa 2012. Nakład 180 egz. Ark. wyd. 30. Ark. druk. 22,19. Pap. off. K1.III. 80 g.

Oddano do druku w kwietniu 2012 r. Druk ukończono w maju 2012 r.

Redakcja – Wydawnictwo Instytutu Elektrotechniki

Indeks nr 37656
