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Analysis of Some Metals in Human Hair by the AAS Method

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Abstract: The essential and toxic elements are contained in drinking water, food and the air - in the entire general human surrounding. Considering the effects of these elements on human health, the recommended/allowed levels of their intake into the organism are defined by the national and international regulations. Those levels are an important indicator of the state an organism is, which is determined by different biological samples of human origin. In this work, the determination of the concentrations of metals was performed on human hair samples of the people living on the area of Kiseljak. The metal levels that were established in the hair were of those essential metals (copper, zinc, calcium, magnesium, iron) and of two toxic metals (chromium and cadmium). The human hair sampling was carried out during the period of November 2014. - February 2015. The group of responders was male and female donors of various ages, (2-66 years old). By the examination of the results, the specifications that were taken into consideration were the following: age and gender of the hair donor, chemical treatment of the hair, smoking habits of the donors. The technique that was applied for determining the concentration of the heavy metals in the samples was the atomic absorption spectrometry (AAS). The final results showed a normal and in some cases increased, concentration of essential metals. The content of Cr and Cd in all analyzed samples was below the limit of quantification of used technique.

INTRODUCTION

Heavy metals is a term that covers a group of elements with similar chemical properties. Some of them, including copper, iron, zinc, play an important role in human organism and are called essential metals, while others are not known as being useful for our health, or more precisely they are toxic. High concentrations of heavy metals may cause health problems (Puntaric et. al., 2012). People may commercial products of different purposes. Depending on their type and chemical properties, elements differ in their mobility in the environment and their toxic effect on plants, animals and humans. Although certain inorganic types also have different chemical properties, major differences are achieved by creation of metal-carbon links, or by creation of organometallic compounds (Bošnir, 2005). Hair, just like the fat tissue, is the organism's storage of toxic and other matters, and the longer the hair

is, the longer the period over which the analysis can determine the organism's status. Hair analysis is very important because it indicates the actual status of organism and the actual nutritional status, as well as the quantity of stored and accumulated toxins, all of which can be reliably determined only by the hair mineral analysis (Chojnacka et al., 2005). It is an analysis whose results do not vary day to day and are not subject to multiple changes like the blood count, or blood or urine tests (Chojnacka et al., 2005). Lack or increased concentration of essential trace elements in hair indicates serious problems in the physiology of human organism (Dombovari et al., 1998; (Dombovari et al., 1999). Thus, for example, a low concentration of bioelements such as Zn, Fe, Ca in human hair is a typical indicator of deficiency diseases, metabolic and physiological disorders (Katz et al., 1988). In addition to that, human hair is a very attractive biological material in terms of sampling, transporting and storage, and also because it provides information on the concentration of certain trace elements that are found in hair in much larger concentrations than in other biological samples which makes the analysis easier (Zhunk et al., 1995). For all the reasons described above, determination of contents (concentration) of trace elements in hair has been a continual activity in the field of biomedical and environmental studies over the past three decades (Arnold, Sachs, 1994), (Ciszewski et al., 1997).

EXPERIMENTAL

The experimental section of this paper is devoted to analyzing the contents of elements Cu, Zn, Ca, Mg, Fe, Cr and Cd in human hair using the method of atomic absorption spectrometry (AAS), flame technique. Hair samples were taken from 34 individuals, 14 male and 20 female. Determination of trace heavy metal concentration was performed on healthy population taking into consideration specifications such as gender, age, smoking habits and chemical treatment of hair.

Preparation of samples for analysis - Previously washed samples of human hair were cut into 0.5-1 cm long portions which were then weighed three times to within 1,0 ± 0,1 g. Acid digestion of samples was performed with concentrated HNO_3 adding 1 mL 30% H_2O_2. The solution was filtered and the contents quantitatively transferred to a 50 mL volumetric flask. Distilled water was added until the volumetric flasks were filled to the mark. These prepared solutions were used for determination of metals using the method of atomic absorption spectrometry, flame technique.

Determination of metal contents in hair samples - Contents of metals (essential and non-essential) were determined using the method of atomic absorption spectrometry - flame technique (FAAS, Spectra AA-10, Varian). The instrument was previously calibrated with standard solutions of the tested metals. Standard solutions of the metals, 1000 mg/L, are original standards of the Merck Company (Germany). Concentrations were determined using the calibration curve method.

Analytical quality control - All used reagents had the analytical grade of purity (Merck, Germany). Repeatability of results was checked by performing three tests for each sample and calculating the value of standard deviation. Given the lack of the certified reference material (CRM), analytical recovery was determined for the entire analytical procedure for all analyzed metals by adding standard metal solutions. Method accuracy was confirmed based on satisfactory recovery factor values (93.2 – 105.8%).

RESULTS AND DISCUSSION

In this paper the concentrations of copper, zinc, chrome, cadmium, calcium, magnesium and iron in 34 hair samples classified by gender, age, smoking habits and chemical treatment of hair, was determined. The respondent group consisted of male and female subjects of different age (2-66 years of age), but with the same place of residence, Kiseljak.

Table 1. Data on hair samples and abbreviated codes of samples used for analysis

Sample code	Sex	Age	Color	Smoker	Chemically treated
A	F	44	black	YES	NO
B	M	45	black	YES	YES
C	M	8	brown	NO	NO
Ć	M	14	brown	NO	NO
Č	M	60	gray	YES	NO
D	F	56	brown	YES	YES
Đ	F	33	brown	YES	YES
DŽ	M	15	brown	NO	NO
E	M	18	black	NO	NO
F	F	18	brown	NO	NO
G	F	60	gray	NO	YES
H	F	26	blond	NO	YES
I	M	2	blond	NO	NO
J	F	9	brown	NO	NO
K	M	47	black	NO	NO
L	M	25	black	NO	NO
LJ	M	41	brown	YES	NO
M	M	63	gray	YES	NO
N	M	11	brown	NO	NO
NJ	F	48	blond	YES	YES
O	F	23	brown	NO	YES
P	F	44	brown	YES	YES
R	F	41	red	YES	YES
S	F	35	blond	YES	YES
Š	F	45	blond	YES	YES
T	F	4	blond	NO	NO
U	F	27	brown	YES	NO
V	M	2	brown	NO	NO
Z	F	50	black	YES	YES
Ž	F	65	brown	YES	YES
X	M	66	gray	NO	NO
Y	F	60	brown	NO	YES
Q	F	14	brown	NO	NO
q	F	63	red	YES	YES

In all respondents the contents of toxic metals Cr and Cd was below the lower limit of quantification. Contents of the analyzed essential metals did not significantly deviate from normal values (Biolab Medical Unit, 2010) of the analyzed metals in healthy individuals and coincide with reference data from other international studies (Baranowska et al., 2004; Sokolowska et al., 2007).

Human hair is approximately 80 % protein and 15 % water, with smaller amounts of lipid and inorganic substances, and its composition also includes copper, zinc, iron and other elements (Wilson, 2007). Normal values, or intervals of concentration of some metals in hair, expressed in μg/g of hair are given in Table 2 (Biolab Medical Unit, 2010).

Table 2. Normal element concentrations in human hair (Biolab Medical Unit, 2010)

Element	Concentration (μg/g)	Element	Concentration (μg/g)
Ca	200 - 2800	Mn	0.2 – 2.00
Cr	0.10 – 1.50	Mg	60 – 160
Co	0.01 – 0.20	P	100 - 200
Cu	10 – 100	K	50 – 300
Fe	5.0 -30	Se	0.40 – 2.00
Na	50 - 1000	Zn	160 - 240
Al	< 50.0	As	< 1.00
Cd	< 0.10	Pb	< 2.00

In this work, mass concentration of seven metals was determined. Obtained results for the content of copper, zinc, calcium, magnesium and iron in hair samples will be presented in charts as mean values of concentrations. In addition to the presentation of total results, the results of analysis of these metals were compared based on gender, age, smoking habits and chemical treatment of hair. Contents of chromium and cadmium were below the limit of quantification of the used method in all samples.

Copper content in human hair samples - Concentration of copper in hair samples was in the range between 6.20 and 25.02 μg/g. Therefore, as the results show, most of the tested hair samples contain copper in concentrations which are, in most cases, slightly below the lower optimal concentration of copper, but within the range of normal values. Comparison of the results with the results of other published papers shows that the measured concentrations of copper are similar to those obtained in researches conducted by other authors in Bosnia and Herzegovina (Hajdar, 2014), as well as researches conducted in other European countries (Sokolowska et al., 2007; Biolab Medical Unit, 2010).
Copper content in the hair of female population ranged between 7.67 and 25.02 μg/g, while that in male population was between 6.20 and 21.12 μg/g. There are no pronounced gender-related differences in copper content.

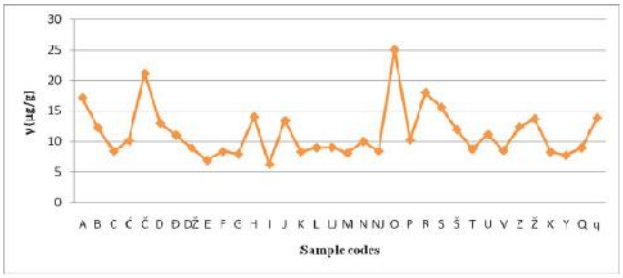

Figure 1. Mass concentration of copper in human hair samples

As can be seen from Figure 2, copper content increases with age until the age of 30 when it reaches its maximum value and then begins to slightly decrease with age.

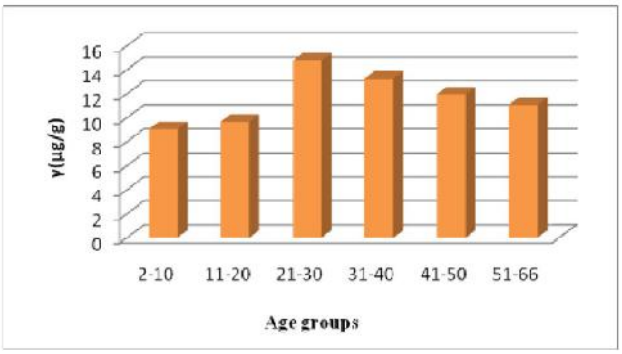

Figure 2. Comparison of age-related copper concentrations in hair

During the sampling, data related to smoking habits were collected from hair donors. Mean values of Cu concentrations in hair samples of smokers was 12.90 μg/g, and in non-smokers' hair 9.90 μg/g. However, it cannot be generally stated that copper content in all hair samples of smokers is higher than the content in non-smokers. As regards the copper content in chemically treated and untreated hair, content in chemically treated hair ranged between 7.67 and 17.98 μg/g, and in chemically untreated between 6.20 and 21.12 μg/g. A slightly higher mean value of copper concentration in chemically treated hair was obtained and it was 12.97 μg/g, while the content in untreated hair was 10.00 μg/g.

Zinc content in human hair samples - Obtained values of zinc concentration ranged between 30.40 and 214.55 μg/g and all of them fall within the normal range of zinc concentrations (Biolab Medical Unit, 2010) in hair samples and most are close to the optimum concentration values (The Agency for Toxic Substances Disease Registry, 2003). Similar results were also obtained in other publications (Mahmutović, 2012; Hajdar, 2014).
Lower content of zinc was found in nine samples. Those are samples taken from the youngest and the oldest individuals. There are numerous reasons for less-than-optimal zinc concentrations, such as diet, stress, menstrual

cycle, internal infections and numerous other factors (Freeman, 1999).

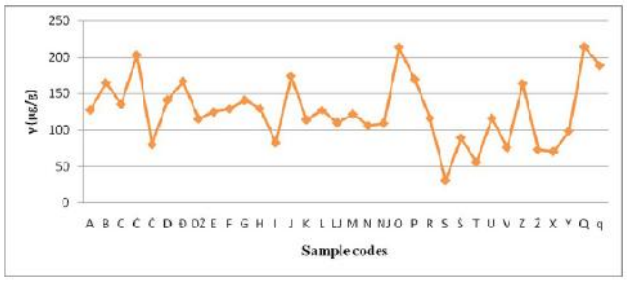

Figure 3. Mass concentration of zinc in human hair samples

Mean value of zinc concentration in male population is 111.32 µg/g, and in female population 138.58 µg/g, which indicates that zinc concentration in female hair is slightly higher.

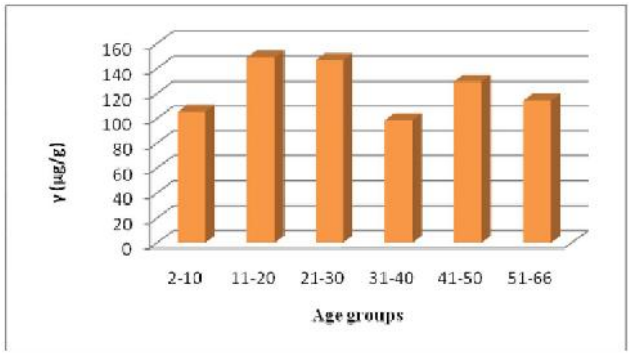

Figure 4. Comparison of age-related zinc concentrations in hair

As shown in Figure 4, obtained results do not indicate a regular age-related change in zinc concentration, more precisely, the data does not allow for reliable linking of zinc concentrations and respondents' age. Based on the mean values of Zn concentrations in samples of smokers, which was 123.0 µg/g, and in samples of non-smokers, which was 128 µg/g, it can be concluded that there are no pronounced differences in zinc content. A slightly higher zinc concentration of 133.11 µg/g was found in chemically treated hair in comparison to mean values of zinc concentration found in untreated hair which was 120.16 µg/g.

Calcium content in human hair samples - As shown in Figure 5, calcium concentrations in hair samples ranged between 99.04 and 14882.63 µg/g. It can be concluded that the values of calcium concentration obtained in most of the samples are within the range of normal values. Mean values obtained in several samples deviate from the normal concentration, but only in female hair samples, while in one sample the mean value of calcium concentration is below the lower limit of normal values and it is the sample taken from a male child aged 2 years. Increased calcium in blood can occur due to increased discharge of calcium from bones, increased absorption from the digestive system or due to decreased discharge in kidneys *(Phyllis, 2006*).

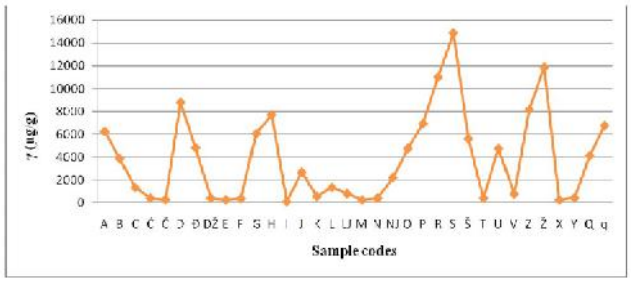

Figure 5. Mass concentration of calcium in hair samples

Mean value of Ca concentration in hair samples of smokers is 6080.48 µg/g, and the value in non-smokers' hair is 1798.33 µg/g. It can be concluded that higher calcium concentration was found in hair samples taken from smokers which can be explained by the fact that calcium is an integral component of tobacco (Rehak, 2013). Mean value of calcium concentration in chemically treated hair was 6928.93 µg/g, and mean value in chemically untreated hair was 1353.88 µg/g. Mean value of calcium concentration in male respondents is 795.05 µg/g, while mean value in female respondents is higher and above the limit of normal values and was 6224.83 µg/g. Results for age-related calcium content are shown in Figure 6.

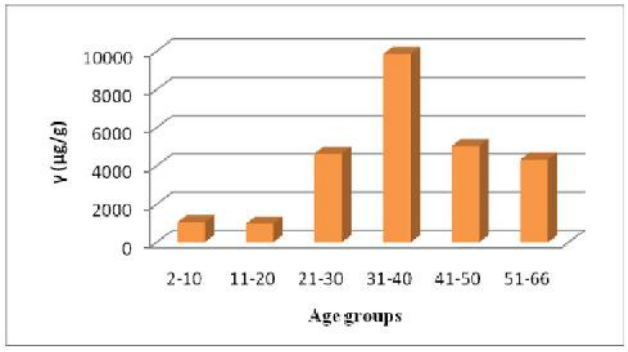

Figure 6. Comparison of age-related calcium concentration in hair

Calcium content changed with age, more precisely it reaches its peak at the age between 31 and 40 years, when it slowly begins to decrease, according to the literature (*Pereira, 2004*). The lowest calcium concentration in hair is found in age group 11 - 20 years because in this period our bones need a lot of calcium, which they obtain from the organism, in order to reach their maximum density before the age of 30 years, when the bones slowly start to lose calcium (Pereira, 2004), which is confirmed by the obtained results.

Magnesium content in human hair samples - Magnesium content in hair samples ranged between 2.11 and 382.34 μg/g. Obtained values of magnesium concentration are within the concentrations that are considered normal. Lower than normal magnesium values were found mainly in male respondents. Mean value of magnesium concentration in male hair was 47.43 μg/g, and in female hair was 135.44 μg/g. Mean values of magnesium concentration are shown in Figure 7.

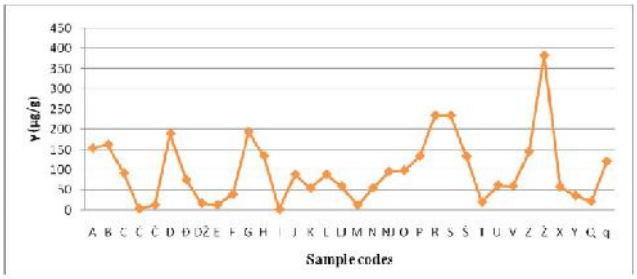

Figure 7. Mass concentration of magnesium in hair samples

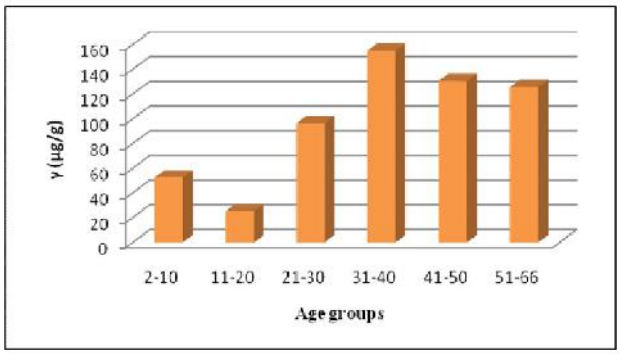

Figure 8. Comparison of age-related magnesium concentration in hair

Obtained results indicate a regular change in Mg concentration with age. Age group 11 - 20 years was the one with the lowest magnesium concentration, but this is explained by the fact that our bones need a lot of magnesium during childhood and at young age, which they then take from the organism to reach their maximum density at about the age of thirty years, when the bones slowly start to lose magnesium (Pereira et al., 2004), which is confirmed by the obtained results.

If we look at the mean value of Mg concentrations found in hair samples obtained from smokers, which was 138.03 μg/g, and those obtained from non-smokers, which was 59.81 μg/g, then we can conclude that higher magnesium concentration was found in hair samples taken from smokers, which can be explained by the fact that tobacco contains magnesium which participates in photosynthesis as an integral part of chlorophyll to a lower degree, and to a higher degree was found in the form of other compounds (Rehak, 2013).

Higher mean value of magnesium concentration of 158.06 μg/g was found in chemically treated hair when compared to the mean value of chemically untreated hair, which was 47.97 μg/g.

Iron content in human hair samples - Results of iron content in hair samples are shown in Figure 9. Iron concentrations in 34 analyzed samples range between 18.04 and 310.30 μg/g. If we compare the obtained results with the results of other experimental measurements, we can see that the found iron concentrations are similar to the values of concentrations obtained in other publications (Fleming, 2001).

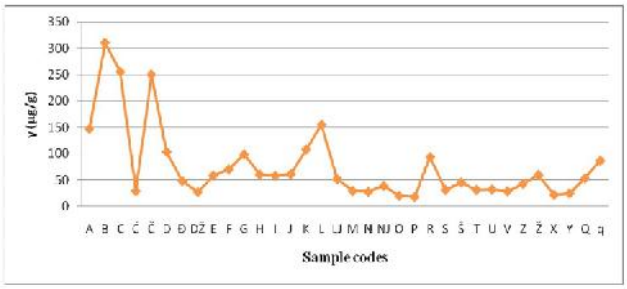

Figure 9. Mass concentration of iron in hair samples

Iron content in the hair of male population ranged between 22.17 and 310.30 μg/g, and the mean value of concentrations was 96.32 μg/g. Iron content in female population ranged between 18.04 and 147.53 μg/g, and the mean value of concentration was lower as expected and was 59.78 μg/g. As shown in Figure 10, it is not possible to link the iron content in hair with the age of respondents.

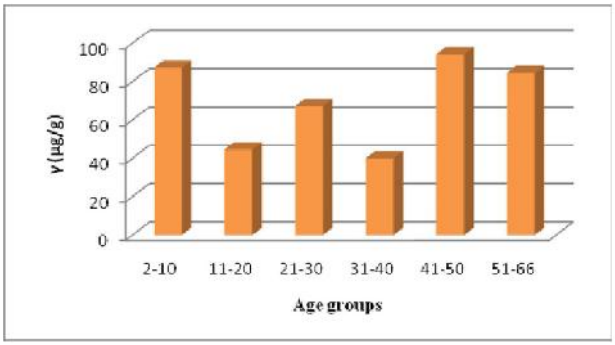

Figure 10. Comparison of age-related iron concentration in hair

Mean Fe concentration in hair samples obtained from smokers were slightly higher and was 86.87 μg/g, while in non-smokers were 66.18 μg/g. Thus, in both cases we have close values. Somewhat higher mean value of iron concentration at 78.88 μg/g was found in chemically untreated hair compared with the mean value of chemically treated hair which was at 72.17 μg/g.

Analysis of concentration of some metals in hair indicated age and gender-related differences in concentrations. In a

Polish research conducted on large groups of respondents (600 to 2,500 respondents out of the total of 3,349 tested individuals), analysis of biometals and toxic metals (Ca, Mg, Zn, Cu, Fe, Pb, Cd) was performed in several groups of different age, or different gender (men and women). Results of the research have indicated differences in the concentration of some of the mentioned metals, or their fluctuations in different periods of life, especially in female respondents (same as in our work) (Baranowska et al., 2004; Sokolowska et al., 2007). The mentioned results almost entirely coincide with the results obtained in this paper, in terms of the stated tested parameters.

CONCLUSIONS

Content of copper, zinc, calcium, magnesium and iron, chrome and cadmium in human hair samples was determined using the method of atomic absorption spectrometry, flame technique. In most of the cases, obtained concentrations were within the range of values that are considered normal. Chromium and cadmium contents were below the limit of quantification of the used method in all samples.

There are no age-related differences in copper content in hair. Higher concentrations of zinc, calcium and magnesium in hair were found in female individuals, and as expected, mean values of iron concentrations in hair were found in male respondents.

Copper content increases with age until the age of 30, when it reaches its maximum value and then begins to slightly decrease with age. Obtained results indicate a regular age-related change in calcium and magnesium content. Age group 11 - 20 years is the one with the lowest magnesium concentration, but this is explained by the fact that our bones need a lot of magnesium during childhood and at young age, which they then take from the organism to reach their maximum density at about the age of thirty years, when the bones slowly start to lose magnesium, which is confirmed by the obtained results. It is not possible to link the zinc and magnesium content in hair with the age of respondents.

Higher mean concentrations of copper, calcium, magnesium and iron were found in smokers. However, it cannot be generally stated that content of these metals in hair samples of smokers is higher than the content in non-smokers. Higher content of calcium and magnesium in hair could be explained by the fact that calcium and magnesium are contained in tobacco leaves. Zinc concentration in smokers' hair is slightly lower than that in non-smokers' hair.

Mean values of copper, zinc, calcium and magnesium concentrations are higher in individuals whose hair has been chemically treated that in those with untreated hair. Slightly higher mean value of iron concentration was found in chemically untreated hair.

The mass concentration of most of analyzed metals are over the normal concentration of metals in human hair (Biolab MedicalUnit, 2010).

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Summary/Sažetak

Esencijalni i toksični elementi se nalaze u hrani, vodi za piće i zraku - u cjelokupnoj ljudskoj, općoj i radnoj okolini. Preporučene ili dopuštene razine njihovog unosa u organizam, vezano za efekte na zdravlje, regulisane su nacionalnim i međunarodnim propisima i pokazateljima. Sadržaj teških metala je važan indikator stanja organizma i određuje se u različitim biološkim uzorcima humanog porijekla. U ovom radu određivane su koncentracije metala u ljudskoj kosi osoba koje žive na području općine Kiseljak. Određivana je koncentracija esencijalnih metala (Cu, Zn, Caj, Mg i Fe) i dva toksična metala (Cd i Cr). Uzorkovanje kose vršeno je u periodu od novembra 2014. godine do februara 2015. godine sa ciljem utvrđivanja razlike u koncentracijama tragova elemenata u ljudskoj kosi kod donatora. Grupa ispitanika sačinjena je od osoba muškog i ženskog spola različitih starosnih skupina (2-66 godina), a u obzir je uzeta starosna dob, spol, da li je kosa hemijski tretirana ili ne, da li je donator pušač ili ne. Za određivanje koncentracije ispitivanih teških metala primijenjena je instrumentalna metoda analize - atomska apsorpciona spektrometrija (AAS), plamena tehnika. Sadržaj esencijalnih metala je u granicama normalnih vrijednosti za većinu ispitivanih uzoraka, uz izvjesna odstupanja koja su bliska području normalnih koncentracija. Sadržaj Cd i Cr u svim uzorcima je bio ispod donje granice kvantifikacije korištene metode.