Original Research

The Effect of Hair Characteristics and Treatments on Mineral Composition of Scalp Hair

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Abstract

In the present work, the effects of hair type (weak/strong, greasy/normal/dry, presence of dandruff) and cosmetic treatments (using hair dryer, anti-dandruff shampoos) on hair mineral composition was discussed. The application of hair mineral analysis was exemplified by identification of exposure to elements from deodorants. Hair was sampled from 155 individuals who were asked to fill out a questionnaire. The elemental composition of hair was determined by ICP-OES (macroelements and major microelements) and ICP-MS (minor microelements, toxic elements, and other trace elements). Mercury was analyzed by AAS Mercury Analyzer. The results were elaborated statistically by Statistica 8.0. Statistical significance of differences was determined by U Mann-Whitney test (p<0.05, p<0.1). For elements, the content of which differed between the groups, the reference ranges were elaborated, as were values within the 10th and 90th percentiles. Healthy and strong hair not dried with a dryer contained statistically significantly more K and La and less Co and Ca than destroyed, weak, dried hair and hair with dandruff. In hair with dandruff, statistically higher levels of Hg were found. The results of hair mineral analysis showed that Zn and Se were deposited on hair cuticle exogenously from medicated anti-dandruff shampoo from active ingredients. This technique was used to assess dermal exposure from deodorants. Individuals who used stift, which contains the compound Zr as antiperspirant, contained 3 to 5 times higher levels of this element than those who used deo roll-on or spray, respectively.

Keywords: hair mineral analysis, hair characteristics, hair treatment, exposure to toxic metals

Introduction

Hair mineral analysis (HMA) is used in biomonitoring exposure from the environment, evaluation of systematic intoxication, determination of nutritional status of individuals and in diagnosis of various diseases [1], metabolic disorders [2], and also in the assessment of exposure at the workplace [3]. Recently, particular attention has been given to the supplementation of minerals to treat mineral imbalance. In order to elaborate on an efficient supplementation

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program, it is essential to determine the level of minerals in the body. There were some attempts to use hair as a diagnostic tool [2]. Although it can be used in epidemiological studies, some problems were encountered in diagnostics of individuals.

Hair is a metabolically inactive tissue and its composition reflects levels of trace elements that accumulate in the body [4]. Hair is synthesized and by determination of the composition of segments, it is possible to trace exposure history [5]. Hair mineral content depends on some individual characteristics. Literature reports that reference ranges should consider age, sex, hair-color, and smoking history [5].

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Gonzalez-Munoz et al. proved significant differences in the level of certain elements between genders [4]. Hong et al. showed that levels of Na, Cr, S, and Cd were positively correlated with age, and contents of Na, K, Cr, and Cd positively correlated with BMI (body mass index) [2]. Bormann de Souza et al. found that the level of Pb in hair of males was higher than in females [6].

Hair is an easy matrix to sample and store. It enables us to assess the status of many elements at a time and reflects the long-term elemental status of individuals [2]. Many papers discuss limitations of trace element determination in hair. However, literature still discusses the problems with the interpretation of the results and their correlation with composition of body fluids or internal organs [1]. Hair is susceptible to exogenous contamination. This interferes with elemental composition and makes it difficult to distinguish between the content of elements, which originate from an organism and were previously bioaccumulated, and the part, which comes from bound material (dust, sweat, sebaceous secretions, beauty treatments – shampoos, conditioners, dyes, permanent waves, bleaches, hair sprays, and pharmaceutical products) [3, 7, 8]. Therefore, various laboratories use their unique washing techniques, e.g. acetone, water, and acetone [7]. Washing methods do not remove all contamination and may remove some elements that originate from an organism. [8, 9]. This is associated with the type of washing agent and the time of contact [9].

Clinical utilization of HMA requires more fundamental studies in the area of various factors that influence the content of elements in hair. Therefore, the aim of the present work was to discuss the effect of hair characteristics (weak/strong, greasy/normal/dry, destroyed/healthy hair, the presence of dandruff) and also hair treatments (drying with dryer, using anti-dandruff shampoos), on their mineral composition. Normal reference ranges for various variables and elements were elaborated upon as values within the 10th and 90th percentiles. The applicative aspects of HMA were exemplified for determination of exposure to elements from different types of deodorants (deo roll-on, spray, stift, gel).

Experimental Procedures

Sampling and Preparation

The present research was carried out on hair sampled from 155 subjects, including 58 males and 97 females, average age 24±9 years. The population was rather uniform when considering age, and consisted mainly of students of biotechnology in the Department of Chemistry at Wrocław University of Technology. This means that the participants underwent similar environmental and occupational exposure. The population was considered as a typical group of students living in an urban area in Europe. The subjects were asked to fill out a questionnaire that included questions on the characteristics of hair (weak or strong; greasy, normal or dry; destroyed or healthy hair, the presence of dandruff) and also hair treatments (drying with dryer, using

anti-dandruff shampoos). Usefulness of HMA was evaluated by studying dermal exposure to elements from cosmetics – various types of deodorants (deo roll-on, spray, stift, or gel).

The participants cut 5 cm of hair from the nape of the neck with stainless steel scissors directly after four consecutive washings of their hair (15 minutes) with Johnson's Baby shampoo, and drying. The selection of the shampoo was determined by its composition – among the metal cations, only sodium was present. The hair was stored in paper envelopes and underwent digestion without additional washing steps. The goal was to elaborate easier analytical procedures without additional steps of extraction from hair with acids, organic solvents, etc., which on one hand are presented in the literature as the method which removes exogenous contamination, but on the other as the source of contamination.

Analytical Methods

The content of 34 elements in hair was determined by ICP-OES (macroelements) and ICP-MS (microelements and toxic elements), as described previously [10, 11]. The samples of hair (0.5 g) were solubilized with concentrated nitric acid (69% m/m) suprapur grade from Merck and digested in a Milestone Start D microwave oven (USA). After mineralization the samples were diluted with ultra pure water deionized in an Aquadem 50 L, WilhelmWerner GmbH, (Germany) and Millipore Simplicity UV (France) systems for 50 g. The samples were then analyzed directly by ICP-OES Vista MPX (Australia) with pneumatic nebulizer for the content of macroelements and after 10-times dilution, by ICP-MS Thermo Scientific (Germany) for the content of microelements, toxic elements and other trace elements. The contents of As, Co, Cd, Cr, Mn, Ni, Pb, and V were analyzed by ICP-MS. Mercury was determined by atomic absorption spectroscopy AMA-254 (Czech Republic). Hg in hair samples was analyzed directly, without mineralization. The remaining elements were detected by ICP-OES. The analytical process was controlled by NCS Reference Material - Human Hair NCS ZC81002 from China National Analysis Center. For the following elements, certified values (mg/kg) and uncertainty (mg/kg) were provided. All the measurements fit within the certified range. In parenthesis, recoveries (%) are reported: Zn $189\pm8 (97.9)$, Se $0.58\pm0.05 (91.4)$, Cr $4.77\pm0.38 (103)$, Mg 105±6 (95.2), Mn 2.94±0.20 (95.9), As 0.59±0.07 (89.8), Ca 1090 ± 72 (102), Fe 71.2 ± 6.6 (91.4), Cu 23.0 ± 1.4 (97.0), Sr 4.19±0.14 (97.6), Hg 2.16±0.21 (90.3), Na 266±12 (99.6), Pb 7.2±0.7 (93.1), Ni 3.17±0.40 (89.6), Cd 0.095±0.012 (91.6), Al 13.3±2.3 (97.7), and Co 0.135±0.008 (94.8). The analyses were carried out in a laboratory certified by ILAC-MRA and Polish Centre of Accreditation (No AB 696) according to ISO/IEC 17025. Quantification limits (LOQ), according to accreditation range are as follows (mg/kg): Al 0.2, B 1.0, Ca 0.5, Cd 0.001, Co 0.03, Cr 0.1, Cu 0.2, Hg 0.0003, K 2.5, Mg 0.025, Mn 0.025, Na 1.0, Ni 0.01, P 1.0, and Zn 0.05.

		No	(N=76)		Yes (N=79)				
Element	Mean	SD	Percentile – 10	Percentile – 90	Mean	SD	Percentile – 10	Percentile – 90	
Ala	15.5	16.4	4.00	38.8	8.40	5.40	3.60	15.1	
Caª	2052	1378	642	3973	2566	1500	1047	4678	
Coa	0.589	0.383	0.0310	0.962	0.723	0.325	0.0430	1.02	
Cr ^A	0.900	0.405	0.298	1.32	1.13	1.03	0.268	1.51	
Ka	245	587	32.3	547	77.3	115	22.5	133.	
Laª	0.507	0.344	0.152	0.995	0.645	0.397	0.211	1.06	
Mn ^A	1.21	0.991	0.481	2.82	0.954	0.696	0.459	2.00	
Pt ^a	0.169	0.410	0.000	0.744	0.445	0.897	0.000	1.71	
W ^A	1.91	1.98	0.000	4.37	2.49	2.21	0.000	5.65	
Zr ^a	0.656	0.998	0.0670	1.75	0.334	0.602	0.0370	0.694	

Table 1. The effect of hair drying (No – hair dryer is not used, Yes – hair dryer is used) on mineral content of hair – statistically significant differences, a) p<0.05, A) p<0.1, mg/kg.

Table 2. The differences in hair mineral composition of weak and strong hair, a) p<0.05, A) p<0.1, mg/kg.

		Wea	ak (N=56)		Strong (N=99)				
Element	Mean	SD	Percentile – 10	Percentile – 90	Mean	SD	Percentile – 10	Percentile – 90	
Al ^A	8.88	6.18	3.35	15.0	13.5	14.8	3.80	38.3	
Caª	2623	1499	814	4678	2140	1415	702	3888	
Coa	0.734	0.314	0.122	0.977	0.614	0.378	0.023	0.962	
K ^A	66.2	48.0	26.3	124	212	526	24.5	450	
Laª	0.691	0.368	0.169	1.21	0.513	0.369	0.158	0.945	

Statistical Methods

The results were treated statistically by Statistica ver. 8.0. The differences between the groups were investigated with U Mann-Whitney test and were found to be statistically significant at p<0.05 and 0.1. For the elements, for which the differences were found to be significant, reference ranges were determined, as values within the $10^{\rm th}$ and $90^{\rm th}$ percentiles.

Results and Discussion

In hair mineral analysis it is essential to digest the organic matter of hair. However, there are literature reports describing solubilization of hair samples without digestion of hair matrix. Hair can be also dissoluted in tetramethy-lammonium hydroxide [12, 13]. The dissolution and further dilution of the sample causes the concentration of elements in the analyzed solution to be usually very low. In the majority of cases, 100 times lower than in hair. This requires the use of sensitive analytical methods and instruments to determine the level of trace elements [14], such as ICP-OES with ultrasonic nebulizer or ICP-MS.

Previously, reference ranges for hair minerals (36 elements) in Polish students were elaborated upon [11]. The mean values together with standard deviation (SD), median, minimum and maximum content, variance, as well as the value of 10th and 90th percentiles (reference range) were reported. Also, the effect of gender and hair coloring on the reference range was discussed. In the present work, for the same population, the effect of the type of hair and hair treatments on hair mineral composition was investigated and exemplified for determination of dermal exposure to toxic elements from deodorants.

The data on the type of hair (weak/strong greasy/normal/dry and destroyed/damaged) were provided by the donors and were obtained on the basis of a questionnaire survey. Since this information is based on donor impressions, they should be treated as preliminary information on the effect of type of hair on their characteristics. In the available literature no studies on the effect of hair condition on their mineral content have been found.

In the present paper in Tables, only the results for which differences were statistically significant are presented. It is known that various physical factors (washing and drying) can cause hair damage [15]. The effect of hair drying on

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		Destro	oyed (N=53)		Healthy (N=102)				
Element	Mean	SD	Percentile – 10	Percentile – 90	Mean	SD	Percentile – 10	Percentile – 90	
Ala	8.63	5.47	3.70	14.6	13.5	14.7	3.76	33.8	
Bi ^A	0.106	0.263	0.000	0.349	0.279	0.589	0.000	1.06	
Ca ^A	2654	1576	1078	4678	2138	1370	726	3973	
Coa	0.749	0.268	0.164	0.961	0.609	0.392	0.023	0.965	
Cu ^A	27.3	40.7	8.90	43.7	18.7	15.1	9.10	31.2	
K ^A	64.7	47.4	26.7	124.	209	518.	26.1	394	
Laª	0.710	0.438	0.169	1.35	0.508	0.323	0.176	0.933	
Mn ^A	0.898	0.669	0.480	1.58	1.17	0.933	0.459	2.73	

Table 3. The effect of condition of hair (destroyed or healthy) on the content of elements, a) p<0.05, A) p<0.1, mg/kg.

Table 4. The mineral composition of greasy, normal and dry hair, a) p<0.05, A) p<0.1, mg/kg.

		Greas	sy (N=56)		Normal (N=74)				Dry (N=25)			
Element	Mean	SD	Percentile - 10	Percentile - 90	Mean	SD	Percentile - 10	Percentile - 90	Mean	SD	Percentile - 10	Percentile - 90
В	1.69 ^A	2.96	0.000	5.91	1.17	1.55	0.000	3.04	0.445 ^A	0.957	0.000	2.39
Ca	2601ª	1284	1190	4543	2039 ^a	1361	702	3994	2488	1951	902	6762
Cd	0.098ª	0.084	0.0540	0.179	0.0700ª	0.0290	0.0270	0.115	0.0780	0.0450	0.056	0.129
La	0.671ª	0.364	0.218	1.27	0.493ª	0.354	0.149	0.920	0.615	0.431	0.177	1.21
Li	0.104 ^A	0.082	0.000	0.208	0.0740 ^A	0.0760	0.000	0.188	0.0850	0.0690	0.000	0.165
P	149	40.0	102	188	142ª	37.0	110	178	171ª	73.0	122	255
W	2.78 ^A	2.17	0.150	5.65	1.97 ^A	2.06	0.000	4.45	1.63	1.90	0.000	3.06

mineral composition of hair is shown in Table 1, which reports the mean value together with SD, as well as the values of 10th and 90th percentiles, which represent normal ranges for a given variable. For this dependent variable, the population was divided into two groups consisting of similar numbers of individuals (76 of whom do not use a dryer and 79 of whom use dryer). Hair of individuals who declared the use of hair dryer contained more Pt (2.6 times), Co (63%), W (31%), Ca (25%), and Cr (25%) and less K (3.2 times), Zr (2 times), Al (1.8 times), and Mn (27%), than those who do not dry their hair.

Table 2 reports the elements for which statistically significant differences were found between weak and strong hair. Strong hair contained more K (3.2 times) and Al (1.5 times) and less La (35%), Ca (23%), and Co (20%) than weak hair.

In Table 3 the elements for which statistically significant differences were found between destroyed and healthy hair are shown. Healthy hair contained more K (3.2 times), Bi (2.6 times), Al (57%), and Mn (31%), and less Cu (46%), La (40%), Ca (24%), and Co (23%) than destroyed hair.

Table 4 presents the differences between elemental composition of greasy, normal, and dry hair. Greasy hair

contained more than normal hair of the following elements: Cd (40%), Li (40%), W (41%), Ca (28%), and La (36%). Greasy hair contained 3.8 times more B than dry hair. Dry hair contained 20% more P than normal hair.

In Table 5 the effect of using anti-dandruff shampoo on hair mineral content is reported. Hair of individuals who do not use such shampoos contained more Sr (52%) and less Se (2.3 times), Zr (72%), W (61%), and Zn (51%). In Table 6 the differences in the level of elements for individuals who have dandruff were reported. Hair of subjects who marked the presence of dandruff at present as compared to never, had higher content of Hg (42%) in their hair. Hair of individuals who have dandruff at present vs. had in the past contained more La (72%) and Ca (69%). Also, the difference in the content of Na (52%) in hair of individuals who never had dandruff as related to the past, was found.

Literature reports that dandruff affects half of the Indo-European population, in the studied population – one third [16]. Anti-dandruff shampoos contain as an active ingredient either selenium sulfide or zinc pyrithionate, which causes deposition of micro-fine crystals on hair [16]. This was reflected by the composition of hair treated by such shampoos.

		1	No (N=100)		Yes (N=55)				
Element	Mean	SD	Percentile – 10	Percentile – 90	Mean	SD	Percentile – 10	Percentile – 90	
Se ^A	3.43	3.34	0.0400	8.42	7.99	23.4	0.120	11.6	
Sra	7.73	8.84	1.26	17.9	5.10	5.01	1.11	9.46	
W ^a	1.81	1.57	0.000	3.74	2.92	2.72	0.000	6.64	
Zna	215	96.0	137	362	324	245	145	680	
Zr ^A	0.392	0.563	0.0440	0.907	0.673	1.16	0.0480	1.78	

Table 5. Use of anti-dandruff shampoo and the mineral composition of hair, a) p<0.05, A) p<0.1, mg/kg.

Table 6. Dandruff and hair mineral content, a) p<0.05, A) p<0.1, mg/kg.

	No (N=81)				At present (N=34)				In the past (N=40)			
Element	Mean	SD	Percentile - 10	Percentile - 90	Mean	SD	Percentile - 10	Percentile - 90	Mean	SD	Percentile - 10	Percentile - 90
Ca	2321	1496	767	4047	1679ª	985	708	2741	2841ª	1538	1022	5274
Hg	0.188 ^A	0.134	0.0630	0.358	0.267 ^A	0.209	0.0620	0.630	0.202	0.146	0.0670	0.426
La	0.566	0.386	0.163	1.03	0.424ª	0.290	0.169	0.881	0.730ª	0.375	0.295	1.34
Na	456ª	362	124	1026	376	266	93	710	299ª	149	122	503

In Table 7 the dermal exposure of elements from deodorants is reported. Hair of individuals who use spray contained 41% more Co as compared to deo roll-on. Subjects using deo roll-on contained more of the following elements as compared to those who used spray: Si (2.6 times), Mn (62%), and Mg (58%). Significant differences were found in the level of Zr, which is the component of antiperspirants. The level of this element was higher in hair of individuals, who used deodorant stift as compared with deo roll-on (2.65 times), and stift as compared to spray (5.4 times).

These results are consistent with the composition of deodorants, which contain antiperspirants such as aluminum zirconium tetrachlorohydrex glycine in an emollient base, and this ingredient can constitute up to 20% of commercially available deodorants [17]. It has been confirmed that humans are exposed through the skin, in particular to Zr [18]. Cullander et al. [18] measured the levels of metals in stratum corneum at the forearms. The authors detected elevated levels of Zr (50 ng/cm²) and Al (15 ng/cm²), which was related with the use of roll-on and stick antiperspirants. Ti, although present in these deodorants, was not accumulated. Dermal exposure occurred from aluminum zirconium tetrachlorohydrex. The content of Zr and Al in these cosmetic products was 10,000 mg/kg and 3,000 mg/kg, respectively [16].

Hair is exposed to factors that affect the composition of the surface of shaft (hair cuticle). Elements can be either adsorbed or leached [5]. Shampoos contain some active ingredients, eg. selenium sulfide as antifungal agent [5]. Zinc pyrithionate and selenium sulfide (a direct antimitotic effect on epidermal cells) are the components of medicated shampoos that treat seborrhea and dandruff. Using silicones (as dimethicone) causes increased luster, but on the other hand might affect the content of Si in hair [16]. Beside shampoos, also various conditioners contain various ions [16]. The effect of washing hair with various shampoos containing trace elements on hair mineral content was investigated by LeBlanc et al. [8]. The composition of hair was determined before and after washing with certain shampoo. Also, the concentrations of elements in shampoos were analyzed. The authors concluded that although various elements were present in shampoos, they were not deposited on the external surface of hair, with the exception of selenium sulfide. Elements present in the highest concentrations in shampoos include: Fe, B, Zn, Al, and Ni. Medicated shampoos contain very high levels of Al and Se (1000 times higher than regular shampoos; the form: selenium sulfide), Fe (50-100 times higher level), Zn (5-10 times higher; the levels can be 1000 times higher; the form: zinc pyrithionate) [8]. Among these elements, only Se was deposited on the external surface of hair [8].

Pharmaceutical preparations contain various minerals as excipients: TiO₂, ZnO (pigment and opacifier), aluminosilicates, smectites (emulsifiers, thickeners), kaolinite, zeolites [19]. Also, minerals are used as active ingredients of pharmaceutical preparations and cosmetics [19]. ZnO is used as antiseptic and disinfectant, dermatological and solar protector. TiO₂ is used as dermatological and solar protector.

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0.0190.174 5.067 2 216 **QD** 6 4.51^{abc} 0.037 2.23 234 252 Mean 1.095 06 -1.67 3.79 1.00 447 479 Percentile 0.413 0.490 01 -29.0 32.0 Percentile 0.199 0.537 <u>e</u> 0.511 1.25 176 161 **SD** 0.756 0.649 0.487 1.70 177 180 Mean 06 -2.03 0.921 3.84 268 1.71 216 Percentile 0.03200.460 0.442 42.0 21.0 01 -Percentile 0. Table 7. Effect of the type of deodorant on the content of elements in hair, abc) p<0.05, A) p<0.1, mg/kg. 0.710 1.356 0.373 1.44 106 128 **SD** 0.617 1.092° 0.960 108 131 Mean 0.412 1.020 06 -1.42 1.11 183 132 Percentile 0.772 01 -33.0 0.460 17.0 (N=53) 0.021 Percentile Spray 0.399 0.240 0.460 192 62.0 88.0 **SD** 0 0.814^{a} 0.859° 0.800^{a} 88.0^{A} 65.0^{a} Mean 0.888 06 -2.98 1.48 367 Percentile (09=N)0.0440 0.422 01 -38.0 0.470 27.0 Percentile Deo roll-on 0.475 0.377 1.04 127 227 **SD** 0.822^{a} 0.407^{a} 1.30^{a} 139^ 170 Mean Element Mgပ္ပ Mn \bar{z} Zr $S_{\underline{i}}$

06-

Percentile

01 -

Percentile

387 3.07 8.09

0.923

1.38

81.0

0.023

280

224

0.207

Concluding Remarks

Hair drying was found to increase the level of Pt, Co, W, Ca, and Cr. The possible explanation is that hair drying disturbs the structure of hair surface (squamous cells of hair cuticles are opened) and those elements become bound more easily by exogenous contamination from water or cosmetic agents [20]. On the other hand, hair of individuals who do not dry their hair with dryer contained more K, Zr, Al, and Mn. The content of these elements in hair dried by a dryer was lower, probably as a consequence of the destruction of the hair cuticle. Those elements could be eluted from hair. The analysis of statistical differences between weak and strong hair showed that strong hair contained more K and Al, and weak more La, Ca, and Co. Strong and not dried hair contained higher levels of K and Al and less Co and Ca. Healthy hair contained statistically higher levels of K, Bi, Al, and Mn. Destroyed hair - more Cu, La, Ca, and Co. If we assume that hair drying destroys hair, the level of the following elements will be consistent: healthy hair and not dried contained higher level of K, Al, Mn and lower Co and Ca. Consequently, not dried, healthy, and strong hair contained more K and Al and less Co and Ca than dried, destroyed, and weak hair. Those differences were statistically significant. Reassuming, in determination and interpretation of the results of hair mineral analysis (in particular the level of K, Al, Co, and Ca), individuals should be asked about the condition of their hair and whether they use a hair dryer, because this influences the level of those elements in hair.

Greasy hair contained more of the following elements: Cd, Li, W, Ca, La than normal hair. Greasy vs. dry contained more B and dry vs. normal – more P. Greasy hair vs. normal and dried contained more W and Ca, and destroyed – La, Ca, and weak – La, Ca. Similarly, more Ca and La in hair contained hair of subjects with dandruff. Consequently, destroyed, weak and greasy hair contained statistically significantly more Ca.

Using anti-dandruff shampoos caused hair to contain less Sr and more Se, Zr, W, and Zn. Zn and Zr were deposited from medicated anti-dandruff shampoos that contained the active ingredients selenium sulfide and zinc pyrithionate

Individuals who had dandruff at present vs. never, had more Hg in their hair. Those who had dandruff at present vs. in the past had more La and Ca in hair. Hair of individuals who never had dandruff vs. had in the past contained more Na.

Hair of subjects who used deodorants in spray vs. in deo roll-on contained more Co; in spray vs. deo roll-on – less Si, Mn; and Mg; in stift vs. deo roll-on: more Zr; stift vs. spray Zr. Compounds of zirconium are an active ingredient of antiperspirant: aluminum zirconium tetrachlorohydrex. It was found that Al from this compound used in deodorants was not absorbed, contrary to Zr – the level of which was 3-5 times higher.

HMA is useful in the assessment of exposure, but it is necessary to take into account some factors that influence the level of elements in hair, as well as their condition. In the present work, it was shown that some elements were deposited on hair from shampoos (Se and Zn) and originated probably from antiperspirants (Zr). The elements were eliminated from an organism to hair and was detected by hair mineral analysis.

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