

Trace Element Profiles in Single Strands of Human Hair Determined by HR-ICP-MS

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Abstract Trace element analysis of human hair has the potential to reveal retrospective information about an individual's nutritional status and exposure. As trace elements are incorporated into the hair during the growth process, longitudinal segments of the hair may reflect the body burden during the growth period. We have evaluated the potential of human hair to indicate exposure or nutritional status over time by analysing trace element profiles in single strands of human hair. The hair strands from five healthy and occupationally unexposed subjects were cut into 1-cm long segments starting from the scalp. By using high-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS), we achieved profiles of 12 elements in single strands of human hair, namely, Ag, As, Au, Cd, Cu, Hg, Fe, Pb, Se, Sr, U and Zn. We have shown that trace element analysis along single strands of human hair can yield information about essential and toxic elements, and for some elements, can be correlated with seasonal changes in diet and exposure. The information obtained from the trace element profiles of human hair in this study substantiates the potential of hair as a biomarker.

Keywords Trace elements · Hair · Single strands · Longitudinal profile · HR-ICP-MS · Nutritional status · Selenium · Strontium · Mercury

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Introduction

Hair has a unique potential to reveal retrospective information about the nutritional status and exposure of subjects [1]. Hair grows approximately 1 cm a month. Trace elements are incorporated into hair during the growth process and reflect the composition of trace elements in blood plasma at the time of formation [2, 3]. Hair grows approximately 1 cm a month, and trace element composition in hair reflects blood levels at the time the hair was generated. Blood and urine analysis on the other hand, reflects the trace element status only at the time the sample was obtained. Important information in several historical or forensic cases has been obtained from hair analyses [4]. Hair has also been reported to be a valuable indicator of environmental pollution [5–7].

In addition to its potential as a biomarker, the analysis of hair samples has several advantages. The primary component of hair is the protein keratin, which makes it stable and robust. At the same time, hair is easily collected and does not require any special storage or preservation. Also, many trace elements accumulate in hair at concentrations at least ten times higher than in blood serum or urine [8].

Until now, trace element analysis in hair has mainly been performed by bulk analysis after total digestion of multistrand hair samples. By examining the distribution profile of trace elements along hair strands, it is possible to trace the intake and/or exposure history of individuals. Giovanoli-Jakubczak and Berg analysed Hg in bundles of hair cut into 1 cm segments as early as in 1974 [7]. Since then, more sophisticated methods have been developed that makes it possible to determine trace element concentrations along single strands of human hair. Because about 10% of scalp hair may be in a resting phase at any point in time, it is desirable to analyse a single strand for accurate estimation [9]. Dombovári et al. studied the cross-sectional and longitudinal distribution of Zn, K, Ca, Fe and Cl in single hair strands using micro-PIXE [10]. Stadlbauer et al. have analysed single hairs from root to tip by laser ablation ICP-DRCMS [11]. A study by Sela et al. determined Zn, Fe, Cu, Cr, Pb and U in single strands of human hair by LA-ICP-SFMS [1]. They also compared results obtained by LA-ICP-SFMS with those measured by ICP-QMS in digested hair samples and found that these agreed well. In the present study we have analysed 1-cm long segments of single hair strands from nonexposed subjects by high-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS). A similar study has been reported by Rodushkin et al.; however, they analysed 2-cm long segments at different distances from the scalp and thus did not get a profile of the whole hair [12].

Studies of trace elements in hair have led to many discussions and opinions about analytical difficulties [13]. Much controversy exists regarding the use of hair samples as an indicator for nutritional status and environmental exposure. Hair is exposed to exogenous contaminants such as atmospheric pollutants, water, sweat and cosmetics [14]. As a result, trace elements in hair can be of both endogenous (internal) and exogenous (external) origin. To reduce the problem of surface contamination, different washing procedures have been proposed and used [14–17]. Because no standardised washing procedure for hair has been widely adopted, results produced from various laboratories are not easily compared or reproduced. There are also difficulties in establishing normal or reference values of hair due to the natural variance of hair composition [18].

In the present work, an analytical method for determining trace elements in single strands of human hair by HR-ICP-MS has been developed. The hair strands were cut into 1-cm long segments starting from the scalp. A profile of 12 elements was achieved by analysing these small segments. The total concentration of 37 trace elements in bundles of hair was also determined. We have shown that in spite of the potential problems with

exogenous contamination, important information can be obtained from the trace element profiles of human hair. This substantiates the potential of human hair as a biomarker, even for subjects without any occupational exposure.

Materials and Methods

Sample Collection

Hair samples were collected from five individuals, three women and two men. All hair strands had a length of 24–36 cm starting from the scalp. The hair strands were cut at the top of the neck for three of the subjects. Because of religious causes, this was not possible for two of the participants. Hair samples from these two were collected from natural hair loss during brushing of the hair. All participants had washed their hair at the day of the sampling. Information about age, sex, diet, smoking habits and hair dye was obtained from each subject (Table 1). The five donors are very different in origin, diet and gender. These have all been intentionally selected to cover as many factors as possible in the present study.

Washing Procedure

To find a suitable washing procedure, six washing solutions were tested: deionized water, acetone (Merck), methanol (Lab-Scan), EDTA (1%, KEBO Lab), Triton-X (1%, Sigma) and a household dishwashing liquid (Prilian perfect, Ecolab, Norway). Hair samples for this purpose were collected from one person and from the same spot on the scalp. Single strands of hair were weighed and thereafter soaked in 50 ml of the selected washing solution for 30 min at room temperature. Four replicates were prepared for each detergent. After washing, the samples were rinsed with deionized water to remove remains of the wash solutions, and subsequently dried at 40°C overnight.

Sample Preparation for Trace Element Analysis

Hair strands were washed before preparation by employing the same washing procedure as described above. The household dishwashing liquid was selected for further analyses. Hair strands were cut into 1-cm long segments starting from the scalp. Disposable plastic gloves and fresh filter paper was employed during the cutting of the hair strands. Each segment was then prepared separately for trace element analysis. Samples were added concentrated HNO₃ (0.125 ml, Scanpure, Scanlab) and ultrapure water (0.5 ml, Q-option, Elga) and

Table 1 Characteristics of Subjects

Subject number	Age	Sex	Diet	Smoker	Origin	Hair dye
1	28	F	Vegetarian	N	India	N
2	31	F	Omnivorous	N	Norway	Y
3	35	F	Omnivorous	Y	Norway	Y
4	31	M	Omnivorous	N	Norway	N
5	32	M	Vegetarian	N	India	N

M male, *F* female, *Y* yes, *N* no, *Smoker* has smoked the last 5 years, Hair dye: has dyed/bleached the hair several times during the last 3 years

digested using a high performance microwave reactor (UltraClave, Milestone, Germany). After digestion, samples were decanted into 14-ml tubes suitable for HR-ICP-MS analysis (Falcon, cat. no. 352059) and diluted with ultrapure water (2.375 ml) to achieve a final acid concentration of 0.6 M.

For the determination of total trace element content in hair, bundles of hair were weighed (50–300 mg) and washed with Prilian perfect. Samples were then added 2.5 ml HNO₃ (Scanpure) and 2.5 ml ultrapure water and digested using the UltraClave. After digestion, samples were diluted to 50 ml to achieve a final acid concentration of 0.6 M. Because of limited quantities, hair from three persons was used for this purpose.

Trace Element Analysis

HR-ICP-MS analyses were performed using a Thermo Finnigan model Element 2 instrument (Bremen, Germany). The radio frequency power was set at 1,400 W. The samples were introduced using a SC-FAST flow injection analysis system (ESI, USA) with a peristaltic pump (1 ml/min). The instrument was equipped with a concentric Meinhardt nebulizer connected to a Scott PFA spray chamber, platinum skimmer and interface cones and a demountable torch of quartz with a guard electrode. The nebulizer argon gas flow rate was adjusted to give a stable signal with maximum intensity for the nuclides ⁷Li, ¹¹⁵In and ²³⁸U. The instrument was calibrated using 0.6 M HNO₃ solutions of multielement standards. Calibration curves using five different concentrations were made using these standards. To check for instrumental drift, one of these multielement standards with known metal concentrations was analysed for every ten samples. Certified multielement aqueous standard solutions (SPS-SW1 and SPS-SW-2, Spectrapure, Norway) were analysed at the beginning and end of each analytical sequence as a quality control of the instrument.

Analytical Quality Control

The accuracy of the method was verified by analysing the certified reference material Human Hair (GBW09101, China). This reference material is a homogenized powder of hair from several donors and has not been treated with a washing procedure. The concentrations found were within 90–115% of the certified values for all trace elements, except for Pb (130%), Al (120%) and Sb (125%). The results are shown in Table 2 together with the detection limits. To assess possible contamination during sample preparation, blank samples of ultrapure water were prepared using the same procedure as for the samples. Because the level of trace elements is very low in the analysed hair segments, results are limited by blank levels or detection limits.

Results and Discussion

Analysis of trace elements along the strands of human hair offers the opportunity to collect time-related information about an individual's nutritional status or exposure [13]. We have analysed hair strands of five healthy and occupationally unexposed individuals to investigate the feasibility of this type of study by HR-ICP-MS. Trace element profiles of selected elements are given in Figs. 1, 2 and 3.

The analysis of 1-cm long segments of single strands of human hair requires a very high analytical sensitivity and precision. Table 2 shows the detection limits of the instrument together with the results from the analytical quality control. Because the individual hair

Table 2 Concentrations of Trace Elements Determined in the Certified Reference Material Human Hair (GBW09101, China) and Analytical Detection Limits

Element	Average values	Certified values	DL	IDL
Ag	0.37	(0.35)	0.001	0.002
Al	16.0	13.3	0.60	0.2
As	0.68	0.59	0.00	0.03
Ba	6.65	(5.41)	0.01	0.01
Ca	1096	1090	7	10
Cd	0.093	0.095	0.002	0.002
Co	0.147	0.135	0.001	0.004
Cr	5.50	4.77	0.032	0.005
Cu	25	23	0.07	0.02
Fe	78.9	71.2	0.79	0.02
Hg	2.53	2.16	0.001	0.001
K	9.3	(11.8)	1.5	5.0
La	0.014	(0.014)	0.002	0.002
Mg	109	105	0.31	0.35
Mn	3.13	2.94	0.014	0.006
Mo	0.65	(0.58)	0.004	0.020
Na	302	266	3	10
Ni	3.62	3.17	0.03	0.01
P	171	(184)	0.2	0.4
Pb	9.3	7.2	0.034	0.002
S	55,350	(46,900)	5	10
Sb	0.26	(0.21)	0.001	0.003
Sc	0.00333	(0.00287)	0.000	0.004
Se	0.67	0.58	0.002	0.25
Sr	5.01	4.19	0.005	0.025
V	0.077	(0.069)	0.002	0.003
Zn	219	189	0.35	0.03

Concentrations are given in $\mu\text{g/g}$ hair

IDL Instrumental detection limit, DL detection limit given as $3 \times \text{SD}$ of the blank samples.

Non-certified values given by the producer are given in brackets

segments are so small (about 0.05 mg), the concentrations in the digested solutions are very low, so the results are limited by the instrumental detection limits or by the contamination of the samples. We were able to achieve profiles of 12 elements by this method, namely, Ag, As, Au, Cd, Cu, Hg, Fe, Pb, Se, Sr, U and Zn.

Hair grows at a rate of approximately 1 cm per month, and it grows in phases. It grows in the anagen phase and rests in the telogen phase [2]. About 10% of the scalp hair is in the resting phase at any point in time, and it lasts for approximately 3 months [19]. There is a possibility of differences in trace element profiles between strands when single strands of hair is analysed. An example of the reproducibility of the method is shown in Fig. 1 where the profile of Sr in different hair strands from a female subject is shown. The figure shows that the profiles of different hair strands are similar when the levels are above the detection limit ($R^2=0.89$ and 0.92). In many of the elements, the levels are barely above the detection limits because of the small sample size. For such low levels, acceptable reproducibility is difficult to achieve.

An important issue regarding trace element analysis of hair is the potential for contamination from external sources. The usual way to try to correct for this problem is to

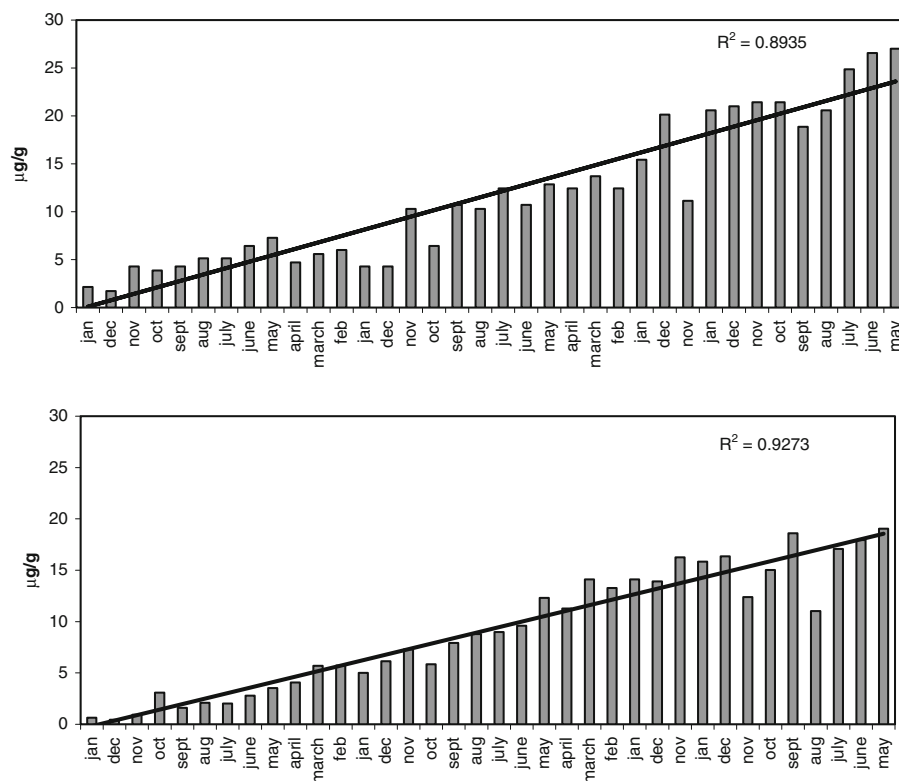


Fig. 1 The concentration profile of Sr along hair strands of a woman originally from India (subject 1). The figures indicate the reproducibility of the method. The *upper figure* gives the concentration of Sr along a single hair strand. The *lower figure* shows the average concentration of Sr in two hair strands. Concentrations are given in microgram per gram of hair

apply a washing procedure. The purpose of hair washing is to remove oily and greasy material as well as dust and other particulate matter from the hair surface [17]. However, when a washing procedure is used, it is difficult to determine if elements are also removed from the interior of the hair. Many studies have addressed hair washing procedures, but the results are very dissimilar [14–17, 20, 21]. We performed a simple test of six different detergents' ability to remove trace elements from the hair. Two organic solvents (acetone, methanol), a polar detergent (ultrapure water), a nonionic detergent (Triton-X), a complexing agent (EDTA) and a household dishwashing liquid were tested. A household dishwashing liquid was selected for the further analyses. It was the detergent that overall removed the highest amount of trace elements. A procedure applied by many is washing with acetone and water as recommended by the IAEA [22]. A brief test indicated that the washing procedure used in this study was just as effective as the IAEA procedure, except that it removed less Ca, Na and K. These elements were however not of interest in the present study. The various elements have different properties and binding abilities, and finding a uniform washing procedure for the purpose of broad-range multielement analysis seems difficult [20]. The issue of washing procedures is clearly a complex one, and a detailed investigation of the effects of different washing procedures is beyond the scope of the present study. However, it is imperative to note that difficulties in differentiation

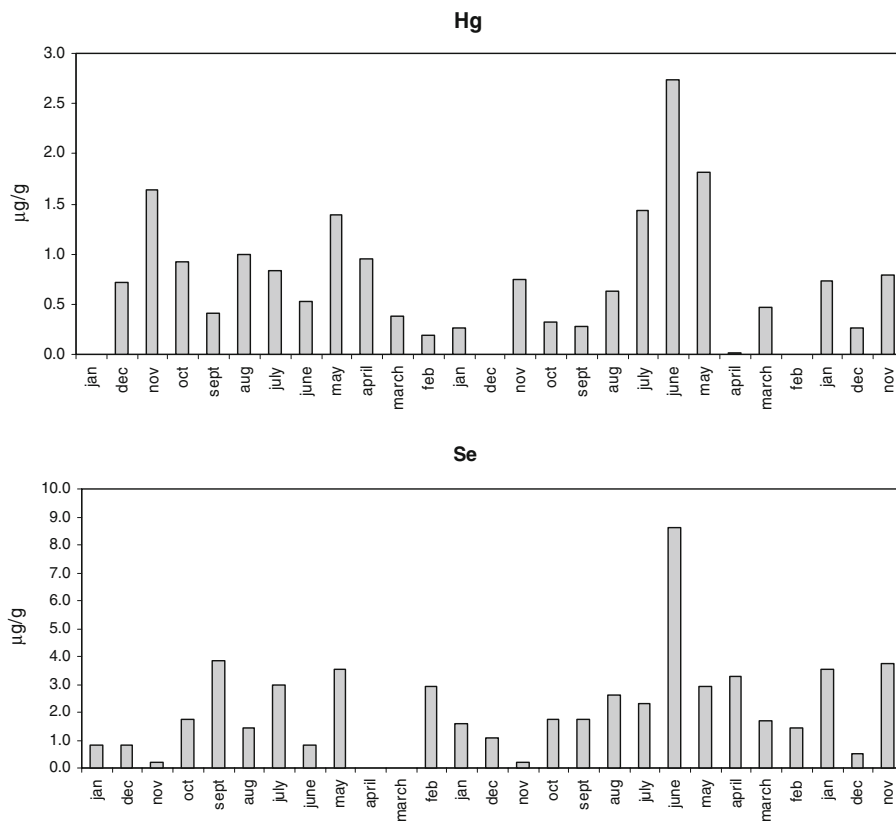


Fig. 2 The concentrations of Hg (*upper figure*) and Se (*lower figure*) along a single hair strand from subject 6. Concentrations are given in microgram per gram of hair

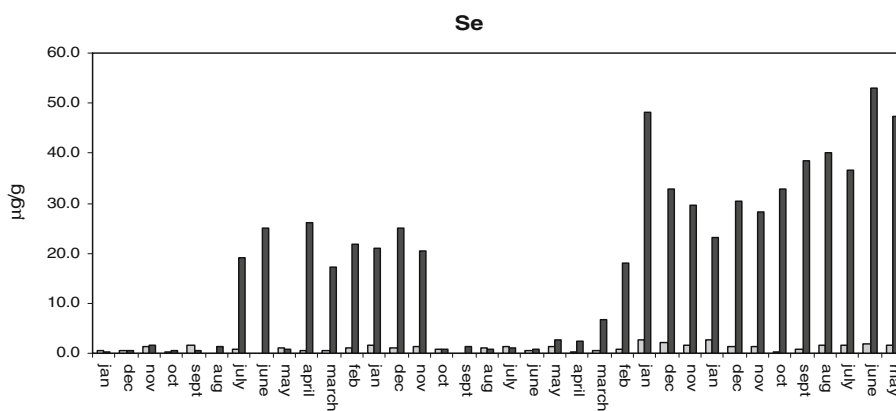


Fig. 3 The concentration profile of Se along the hair strands from a married couple (subjects 1 and 5). *Open bars* indicate the woman, and the *filled bars* indicate the man. Concentrations are given in microgram per gram of hair

between exogenous and endogenous contents in hair are inherent problems in the interpretation of trace element data in hair.

In addition to the problems in differentiating between the exogenous and endogenous origins of elements, there are other difficulties in the interpretation of data on trace elements in hair. The amount of trace elements in hair is a function of many factors such as hair colour and length as well as age, sex, diet and smoking. It is therefore difficult to establish reference values for trace elements in human hair [18, 23]. The elemental content of hair also varies with geographical region [13]. Shampoo, water quality, hair cosmetics and hair treatments might also alter trace element status of hair [24, 25]. Because of the lack of reference values of trace element concentrations in human hair, we have also analysed total trace element content in hair. Table 3 shows the average concentration of 37 trace elements in bundles of hair from three persons. Results from two other studies are also given for the purpose of comparison.

Two of the subjects in this study are married and have the same ethnical origin and diet (subjects 1 and 5, Table 1). The man always wears a turban outside the house. This makes his hair minimally exposed to outdoor contamination, offering an excellent opportunity to evaluate the significance of external contamination. The profile of Pb, a typical outdoor contaminant, showed no clear differences between these two individuals. Their profiles of Cu and Zn were also very similar. This indicates that the washing procedure is satisfactory and that external contamination is a minor problem in this study.

The key aspect addressed in this study is whether the results actually can provide information about exposure, intake or nutritional status. Figure 2 shows the concentration profiles of Hg and Se from a male subject (subject 6). The variations of both elements coincide, and they are seasonal. It is well known that both Hg and Se accumulates in fish and other seafood [26, 27]. By interviewing the subject, we found that he is an eager angler and that the variations coincide with his seasonal fishing habits. The period from April to October is characterized by seasonal fishing and a subsequent higher intake of fish. In April/May, he fishes sea trout, in August mackerel, and in September/October, he fishes a lot of herring. In these periods, we can clearly observe an increased level of Hg and Se coinciding with a higher intake of fish [28, 29]. These observations are in agreement with a study by Yoshinaga et al. (1993), who measured Hg in 5-mm long segments of a single hair strand by flow-injection ICP-MS. The subject in this study did not usually consume fish, and his concentration profile of Hg reflected a higher consumption of fish during a stay in Japan. Cortes Toro et al. also reported that Hg levels in hair is an indicator of Hg body burden [22].

Some trace elements deposited externally tend to incorporate into the hair. Figure 1 shows the concentration profile of Sr in a woman originally from India (subject 1). The analysed hair covers a period of approximately 3 years. The woman has lived in Norway for the last 3 years. Before this, she lived in India. It seems that she was exposed to Sr more than 3 years ago, when she was living in India and that cessation of the exposure upon moving to Norway is the cause of the gradual decline in Sr concentrations shown in the figure. We suspect that a high concentration of Sr in the water is the source of the Sr exposure. In a study of trace element concentrations in groundwater in India, the level of Sr was 1,300 $\mu\text{g/l}$ (average of ten sites) [30]. In comparison, the average level of Sr in Norwegian drinking water is 27 $\mu\text{g/l}$ [31].

The concentration profiles of Se in hair from the married couple (subjects 1 and 5, Table 1) is shown in Fig. 3. Because of problems with dandruff, the man periodically uses a shampoo containing Se. This is most certainly the explanation of the differences in concentration between the two. The results are also in agreement with a study by Gordus

Table 3 Trace Element Concentrations in bundles of Human Hair (average of three persons)

Element	This study		Rodushkin [12]	Chojnacka [23]
	Average	SD		
Ag	0.195	0.065	0.231	0.395
Al	8.6	2.4	8.2	14.1
As	0.007	0.003	0.085	0.044
Au	0.01	0.01	0.03	0.049
B	0.29	0.16	0.67	2.041
Be	0.0014	0.001	0.0013	0.055
Cd	0.041	0.012	0.058	0.114
Ce	0.014	0.006	0.039	
Co	0.036	0.024	0.013	0.034
Cs	0.00078	0.00020	0.00067	
Cu	76.5	10.3	25.0	12.4
Fe	13.3	6.7	9.6	15.0
Ga	0.0026	0.0005	0.0025	
Hg	0.235	0.038	0.261	0.500
Ir	0.0001	0.0000		
K	38	16	81	210
La	0.030	0.018	0.035	
Li	0.058	0.061	0.017	
Mn	0.36	0.26	0.56	0.60
Mo	0.028	0.008	0.059	0.017
P	198	47	133	132
Pb	1.00	0.36	0.96	1.06
Pr	0.0028	0.0007		
Rb	0.039	0.015	0.093	
S	58522	5138	47700	
Sb	0.018	0.009	0.022	0.455
Sc	0.0019	0.0007	0.0014	
Se	0.39	0.09	0.83	0.68
Sm	0.0014	0.0003		
Tb	0.0003	0.0000		
Th	0.0008	0.0003	0.0013	
Tl	0.00017	0.00008	0.00061	
U	0.059	0.027	0.057	
V	0.020	0.008	0.027	0.092
W	0.0100	0.0069	0.0053	0.002
Y	0.014	0.004	0.023	
Zn	190	22	142	157

Results are given in microgram per gram. Results from two other studies are also given [12, 23]

[32] who found that the use of scalp medications containing selenium led to Se hair levels which were 20–40 times normal levels.

There is a general need to develop biomarkers of nutritional status and environmental exposure [33]. In this study, we have presented a method for trace-element analysis along single strands of human hair. We have shown that this method can provide important information about intake, nutritional status and exposure of essential as well as toxic trace elements, in spite of potential problems with exogenous contamination of the hair strands.

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