Determination of trace element levels in human scalp hair in occupationally exposed subjects by XRF

Y. Dede,^{1*} H. N. Erten,¹ A. Zararsiz,² N. Efe²

¹ *Bilkent University, Department of Chemistry, Ankara, Turkey*

² *Ankara Nuclear Research and Training Center, Besevler, Ankara, Turkey*

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Trace element levels in hair of individuals living in urban areas were determined by energy dispersive XRF. Two groups of subjects were investigated, the first group was assumed to be from a healthy environment, the other one was exposed to a high level of contamination due to working conditions. The results were compared to data reported in the literature. The concentrations of Ca, Fe, Cu, Zn and Pb in the scalp hair were determined and the correlation between hair trace element levels and environmental effects was discussed. The results given by the second group show that environmental exposure effects hair trace element levels which are related to body trace element concentrations.

Introduction

X-ray fluorescence technique is widely applied for the analysis of inorganic constituents in light and heavy matrices, such as biological and geological samples. However, some problem arises for the quantitative analyses due to interelement and/or matrix effects.^{1–3}

During the past three decades the investigation of trace element concentrations in human scalp hair has become increasingly popular for monitoring environmental exposure,^{4–6} evaluating systematic intoxication,^{7,8} assessing nutritional status⁹ and diagnosing diseases.¹⁰ The low level of essential bioelements such as Zn, Ca, Fe, Cu etc. in human hair is found to be typical of deficiency diseases,¹¹ metabolic disturbances¹² and physiological disorders.¹³ LAKER¹⁴ discussed the advantages of hair analyses for the investigation of trace element levels over other materials such as blood and urine because it is easier to collect and store hair samples than other materials and the trace elements are present in higher concentrations in hair, especially in comparison to those in blood samples.

It is known that during the growth phase of hair the follicles of the scalp produce hair at a rate of 0.2–0.5 mm/day. The growth rate and the trace element levels vary in function of age, race, gender, anatomical location of the hair strand on scalp, season, nutritional status, geographical habitation, medicine intake, color and length of the hair strand and many other factors. With the follicle activity the trace elements in the body participate in the production of the strand and when the hair fiber is close to the surface, it begins to be keratinized. As a result, a sealed, natural and permanent container is formed for the endogeneous trace elements. Therefore, it is generally accepted that trace element concentrations of hair reflect the trace element levels in

the body up to a time equal to the moment the fiber started to grow. In this way, the hair analysis provides data for both the current and near past status of the subject.

In this study we have determined hair trace element levels of two groups of subjects in Ankara, Turkey, the first has been assumed to be in normal and healthy conditions and was taken as reference group, and the other to be in contact with environmental exposure due to working conditions.

Both groups are made up of males living in metropolitan area and no member of the healthy group smoked and used hair dyes. On the other hand, the second group of workers were exposed to a high rate of environmental contamination; exhaust gases, metal dusts, some chemicals used in the industry, dyes etc. Strict safety precautions have not been applied by the involved workers.

Experimental

Scalp hair has been divided into five categories according to the different concentrations of elements investigated. Approximately 1 g of hair was cut from the closest distance to the scalp with stainless steel scissors. From the head of each individual not less than 100 strands (distributed over the head) were collected. Hair samples were taken from a total of 33 people living in urban area. Nineteen subjects were assumed to be healthy and 14 of them were workers of automobile industry having high risk of environmental contamination. Samples were washed according to the IAEA standard procedure;¹⁵ first with acetone, then thrice with distilled water and finally with acetone again. In every wash, samples were left in the solvents for ten minutes.

* E-mail: c120577@metu.edu.tr

Washed samples were dried for 12 hours at 60 °C and ashed at 500 °C for 3 hours in the form of black ash and kept in clean, dry glass vials till the day of analyses. The sample pellets were made under a pressure of 10 tons/inch² with a composition of 75 mg ash and 175 mg cellulose. All samples were prepared in intermediate thickness.

Sample analyses were carried out using an energy dispersive X-ray fluorescence spectrometer as described in a previous study.¹⁶ The system employs an annular ¹⁰⁹Cd (370 MBq) radioisotope source for excitation of samples and the resulting photons are detected by a Si(Li) detector having a resolution of 180 eV at 5.9 keV coupled to a multichannel analyzer and computer as described in Reference 17.

Standard samples with K, Ca, Fe, Ni, Cu, Zn, Sr, and Pb were prepared from their pure chemical compounds following qualitative analyses of the hair samples. The errors resulting from weighing small quantities of the compounds were minimized by adding the required volume of solutions to pure cellulose (Whatman CF11). The standards were then dried, homogenized and compressed into pellets under the same pressure and in the same form as the sample pellets.

X-ray fluorescence analysis of samples was accomplished by the direct comparison method during which photons of 22–25 keV energy from ¹⁰⁹Cd source were used. The net peak intensities were calculated by the software AXIL.

Results and discussion

The sensitivity of the analysis of trace elements in biological samples depends on the mass density of the fluorescent element in the sample, matrix composition, fluorescent X-ray lines, geometry and design of the detection system, and excitation energy. For estimation of sensitivity and accuracy of a broad range of trace element, detection limits are calculated in similar matrices.

In the present study the minimum detection limits (MDL) of some trace elements of interest were calculated for ¹⁰⁹Cd excitation, and listed in the first column of Table 1. The results are for samples in ashed form. Ashing caused a mass loss of about 87%. The detection limits given in the second column of Table 1 is not only the sensitivity of the device alone but includes the results obtained from the ashed samples. Results below the MDL for ashed hair were excluded and the values reported in Tables 3 and 4 are above the MDL. The excluded results were abbreviated as Bdl; standing for “below detection limit”.

Table 1. MDL for elements of interest in ashed and dry hair

Element	MDL for ashed hair, ppm	MDL for dry hair, ppm
Ca	113	14.1
Fe	5.86	0.73
Cu	3.21	0.40
Zn	2.34	0.30
Pb	2.29	0.30

Table 2. Comparison of the measured concentrations with the certified values of NBS standard 1577 (Bovine Liver)

Element	Certified value, ppm	Measured value, ppm, (n = 5)	Accuracy, %
Ca	116 ± 4	128 ± 11	10
Fe	184 ± 12	189 ± 10	2.7
Cu	160 ± 10	152 ± 9	5.2
Zn	127 ± 9	121 ± 7	4.9

n – Number of measurements.

The accuracy of the method was verified by the standard Bovine Liver (NBS 1577), the results are given in Table 2.

Tables 3 and 4 give the trace element levels determined by XRF in the healthy group and the workers group, respectively.

The arithmetic ($\bar{X}$) and geometric (GM) mean values were calculated with the data above the detection limit. Since the second group is expected to have some abnormalities due to external effects, Students *t*-test was performed on the results in Table 4 by using the values of Table 3 as references. The confidence intervals for elements calculated from the 95% *t*-values are given below for dry hair in ppm: Ca (170–750); Fe (14.1–43.9); Cu (6.99–29.59); Zn (3.13–325).

Since the healthy population did not have any significant contamination of Pb, the Pb levels of auto workshop workers in Lahor (5.6 ppm)¹⁸ and in Mineralab (0–30 ppm)¹⁹ were taken for comparison values.

The hair trace element levels of the workers group are compared with the confidence intervals obtained from the healthy group in Table 5.

It can be seen from Table 4 that all subjects having elevated Ca levels also have elevated Fe levels. This relation may be due to the working environment contamination (exhaust gases, metal dusts, some chemicals used in the industry, dyes, etc.), the metabolism of these elements or to the high consumption of Ca and Fe containing foods such as milk products. The very smooth relation expressed graphically in Fig. 1 may support the metabolic effect and high intake of Ca and Fe dominating in the current situation.

Table 3. Trace element concentrations (in ppm) for the healthy urban group in Ankara, determined by XRF spectrometry

Sample No.	Age	Sex	Smoker	Ca	Fe	Cu	Zn	Pb
1	29	M	no	301 ± 17	21.6 ± 4.6	9.12 ± 3.02	456 ± 21	Bdl
2	21	M	no	479 ± 22	27.3 ± 5.1	19.9 ± 3.15	156 ± 13	Bdl
3	20	M	no	183 ± 14	28.3 ± 5.2	Bdl	121 ± 14	Bdl
4	17	M	no	248 ± 16	13.5 ± 3.6	9.41 ± 3.07	135 ± 10	Bdl
5	17	M	no	507 ± 23	39.6 ± 6.3	15.6 ± 3.9	201 ± 11	Bdl
6	12	M	no	337 ± 18	31.0 ± 5.6	15.5 ± 3.9	104 ± 10	Bdl
7	21	M	no	421 ± 21	31.8 ± 5.5	18.7 ± 4.2	129 ± 11	Bdl
8	17	M	no	374 ± 19	23.9 ± 4.7	17.2 ± 4.1	106 ± 10	Bdl
9	20	M	no	379 ± 19	23.7 ± 4.8	19.2 ± 4.3	168 ± 13	Bdl
10	20	M	no	370 ± 18	24.5 ± 4.9	18.6 ± 4.2	213 ± 15	Bdl
11	20	M	no	657 ± 26	25.1 ± 5.0	Bdl	116 ± 11	Bdl
12	19	M	no	599 ± 24	37.7 ± 6.1	22.7 ± 4.5	157 ± 13	Bdl
13	16	M	no	532 ± 23	32.9 ± 5.7	25.2 ± 5.0	150 ± 12	Bdl
14	17	M	no	408 ± 20	20.4 ± 4.5	19.5 ± 4.3	184 ± 14	Bd
15	18	M	no	446 ± 21	34.3 ± 5.9	15.9 ± 4.0	230 ± 15	Bdl
16	21	M	no	652 ± 26	30.9 ± 5.6	26.5 ± 5.1	133 ± 12	Bdl
17	18	M	no	530 ± 23	32.2 ± 5.7	29.3 ± 5.3	198 ± 14	Bdl
18	20	M	no	761 ± 28	47.2 ± 6.8	19.6 ± 4.3	95.3 ± 9.8	Bdl
$\bar{X}$				458	29.0	18.2	164	
GM				434	28.0	17.3	161	
std				136	7.11	5.35	76.7	
Range				183–761	13.5–47.2	9.12–29.3	68.9–456	
95% confidence interval				170–750	14.1–43.9	6.99–29.5	3.13–325	

*Bdl – Below detection limit.

Table 4. Trace element concentrations (in ppm) for the group working in an industrial site in Ankara, determined by XRF spectrometry

Sample No.	Age	Sex	Pollution interval (years)	Smoker	Ca	Fe	Cu	Zn	Pb
19	16	M	2	no	372 ± 19	19.7 ± 4.4	8.17 ± 2.86	129 ± 11	1.36 ± 1.17
20	20	M	2	no	944 ± 31	121 ± 11	8.65 ± 2.94	175 ± 13	2.06 ± 1.44
21	37	M	21	no	530 ± 23	15.3 ± 3.9	7.47 ± 2.73	112 ± 11	2.54 ± 1.59
22	37	M	21	no	273 ± 16	7.70 ± 2.81	9.05 ± 3.01	170 ± 13	1.15 ± 1.07
23	32	M	21	no	583 ± 25	40.9 ± 6.4	20.7 ± 4.6	183 ± 14	3.38 ± 1.84
24	23	M	8	no	407 ± 20	27.2 ± 5.2	13.7 ± 3.7	172 ± 13	5.19 ± 2.28
25	40	M	2	no	355 ± 19	7.61 ± 2.76	11.9 ± 3.5	209 ± 15	1.49 ± 1.22
26	35	M	23	yes	1122 ± 34	318 ± 18	33.6 ± 5.8	184 ± 14	13.1 ± 3.6
27	17	M	4	yes	1298 ± 36	214 ± 15	17.9 ± 4.2	187 ± 14	7.02 ± 2.65
28	19	M	7	yes	2603 ± 51	841 ± 29	19.1 ± 4.4	213 ± 15	40.5 ± 6.4
29	15	M	3	no	593 ± 24	145 ± 12	12.1 ± 3.5	150 ± 12	6.14 ± 2.48
30	20	M	10	yes	822 ± 29	43.1 ± 6.57	11.1 ± 3.3	184 ± 14	2.61 ± 1.62
31	27	M	10	yes	244 ± 16	191 ± 14	8.76 ± 2.96	100 ± 10	17.1 ± 4.1
32	16	M	2	yes	1058 ± 33	205 ± 14	20.6 ± 4.5	309 ± 18	79.6 ± 8.9

Table 5. Comparison of hair trace element levels in the workers group with those in the healthy group

Element	Number of depleted results	Number of results in the 95% confidence interval	Number of elevated results
Ca	—	8	6
Fe	2	4	8
Cu	—	13	1
Zn	—	14	—
Pb*	—	8	6
Pb**	—	12	2

* When the Pb levels of auto workshop workers in Lahor is considered as the normal range.¹⁸

** When the Pb levels reported by Mineralab is considered as the normal range.¹⁹

Fig. 1. Correlation between Fe and Ca levels in the hair of individuals belonging to the workers group: $r = 0.98$, $Y = 0.43 X - 266$

Table 6. Normal concentrations and ranges of trace elements in human hair published in the literature (in ppm)

Year	Author	K	Ca	Fe	Co	Ni	Cu	Zn	Sr	Pb
	MineraLab. ¹⁹	75–180	200–600	20–50	0.20–1.0	1.0–2.0	13–35	160–240	—	20–30
	Doctor's ²⁰	42–430	204–712	21–50	—	1.4	17–67	104–288	—	15
1969	YURACHEK et al. ²¹	—	210	8.72	—	—	17.7	236	—	—
1982	WEIESENER ²²	—	—	—	—	—	32.3	211	—	—
1984	MATSUBARA ²³	17	410	10	—	—	9	150	—	—
1985	SUZUKI ²⁴	36	640	210	0.099	1.73	16.1	164	3.5	2.73
1986	COETZEE et al. ²⁶	—	—	72.5	13.5	2.7	22	186	—	—
1997	CHO et al. ²⁷	—	—	—	—	—	22.8	163	—	—
1997	ALI et al. ¹²	—	487	312	—	14.4	21.7	154	—	17.4
1997	CHLOPICKA et al. ⁴	—	—	—	—	—	—	—	—	0.91
1997	PONZETTA et al. ²⁸	—	1811	242	—	6.4	29	228	12.2	15.7
1997	NOWAK et al. ⁶	—	—	24.1	1.1	4.6	—	105	—	18.3
1999	This work	—	458	29.0	—	—	18.2	164	—	—

The normal Zn levels in every individual is a support of the importance of Zn for metabolism.

We may conclude that smokers have higher Fe and Pb levels than non smokers. This is probably due to the normal mechanism of smoking effecting the inhalation of evaporated metals.

Hair levels may reflect body trace element levels but since the normal ranges are quite large, interpretation of results is somewhat uncertain. Thus forming a control group for each study is very beneficial. The very wide ranges of trace element distributions found in the literature for different populations are presented in Table 6.

Conclusions

It is clear that the trace element levels of occupationally exposed workers are affected by the working conditions and lack of safety precautions.

The levels of Ca and Fe in contaminated workers show a positive correlation. It may be the pollution effect, the metabolic effect or the result of food intake rich in Ca and Fe. The effect of smoking can be seen on Fe and Pb levels. The healthy group provided satisfactory data for comparison and since the two groups are from the same urban area, the confusions occurring when the normal ranges of other populations are used, are averaged out as most studies show the geographical habitation as a factor influencing trace element levels.

Variations are not only a result of external contamination. The feeding habits, degree of exposure, diseases with the possibility of effecting trace element levels, medicine intake and all other factors should be defined more clearly to find the real causes of fluctuating levels.

In order to let the workers live in a healthier environment, working conditions may need improvement and effective safety precautions should be taken.

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