Determination of the distribution of trace elements in human hair as a function of the position on the head by SRXRF AND TXRF†

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On reviewing the data in the literature it is obvious that the differences in the concentrations of certain special elements in human hair for a various number of people are too large to be used as standards or deviations from standards for determining the trace-elemental composition of hair. New questions have arisen with the publication of non-compatible data. What is the distribution of elements on the donor's head area? What is the character of this distribution? Is the distribution function identical for all elements? Hair samples were taken from five points on the heads of six people. The hair samples were analysed using the method of X-ray fluorescence excited by synchrotron radiation (SRXRF), and the results were compared with those from a similar sample analysed using the method of total reflection X-ray fluorescence (TXRF). Elements which show a constant concentration all over the head are identified.

Keywords: SRXRF analysis; TXRF analysis; trace elements; human hair analysis.

1. Introduction

The total technogenic load of an organism can be determined from the heavy metal concentration in human or animal samples (blood, urine, hair, bone, teeth, breast milk). Human hair from the head is one of the most easily accessible samples. As a diagnostic material, hair differs from the other materials in that its sampling is simple and almost non-destructive. Hair carries information on the composition of microelements at the time before sampling. Examination of this composition is currently widely applied, as illustrated in numerous papers and books (*e.g.* Hambidge & Am, 1982; Bos *et al.*, 1985; Zhuk & Kist, 1990; Steindel & Howanitz, 2001; Spyrou *et al.*, 2002). The results of chemical analyses of hair have raised many doubts and scepticism. The data obtained by different authors differ over a wide range (Steindel & Howanitz, 2001). Currently, information is given of the inclusion of trace elements in the hair matrix. Five sources of microelements are known for human hair: matrix, skin fat, sweat, epidermis and exogenic sources (Bos *et al.*, 1985). The aim of many papers examining the elemental composition of hair is a correlation to the composition of trace elements in other tissues (Attar Khudre *et al.*, 1990; Zhuk & Kist, 1990). Kruse-Jarres (2000) concludes that 'confidence in the accuracy of hair analyses and their interpretation for appropriate clinical application is still markedly lacking. Studies have shown that there is no correlation between trace-element concentrations in hair and those in blood or other relevant organs such as liver, muscle or bone. Thus, hair analyses are unsuitable for the detection of clinically relevant deficiencies of essential trace elements in human organisms'. The basic problem of clinical examinations is the lack of distinct knowledge of a standard trace-element concentration (Hambidge & Am, 1982). On reviewing the data in the literature it is obvious that the differences in concentrations of certain special elements in human hair for a various number of people (different nationality, age, pathology) are too large to be used as standards or deviations from standards for the trace-elemental composition of hair. For example, for Mn a range of 0.02–6.14 mg kg^{-1} is given for a group of students (n = 20), and Cu shows a range of 2.3–44.1 mg kg^{-1}. Furthermore, for people with breast cancer (n = 28) the ranges are 0.1–1.5 mg kg^{-1} for Mn and 9–62 mg kg^{-1} for Cu (Spyrou *et al.*, 2002).

The literature data are mostly derived from analytical methods such as ICP-MS or AAS. As claimed by Bass *et al.* (2001), ICP-MS is the most valuable technique for ultratrace and multielemental analysis of human hair, which is confirmed by the analytical studies.

The arguments given above show that such analyses are generally performed in order to obtain precise results for the concentration of trace elements in human hair. Furthermore, the correlation of the trace-elemental composition in hair and in other organs seems to be of interest (Kruse-Jarres, 2000). Usually in the references the sampling point of the analysed hair is not given; therefore no results are available for the distribution of the elements over the area on the head. The present paper aims to supply answers to these open points. It is important to differentiate the elements from the point of constant or not-constant concentration all over the area.

For the analysis, X-ray fluorescence excited by synchrotron radiation (SRXRF) was used. Additionally, a comparison is given for results obtained for the same samples using SRXRF and using total reflection X-ray fluorescence (TXRF).

2. Materials and methods

2.1. Sampling

For the SRXRF measurements, sampling of clean hair (just after washing the head) was performed without any treatment. The hair was cut near to the skin of the head, *i.e.* so that the bulb of the hair was not included. The samples were taken at the same points on the head of each of the six donors. The sampling points were the top (crown of the head), behind the left and right ear, and the left and right temple. Each sample was washed in acetone. The hair was cut 1.5–2 cm from the root, ground in an agate mortar, and pounded (20–25 mg). The sample for the measurement, a pressed pellet of diameter 8 mm, could be measured repeatedly.

For the TXRF measurements, the hair samples included the bulb of the root. Eight to 10 strands of about 1 mg were pulled out with the roots and dissolved in 100 µL concentrated HNO_3 with an internal vanadium standard added.

Although the samples were taken for both methods at the same time and at the same point, the samples are not identical.

2.2. Methods

SRXRF is an instrumental, multielemental, non-destructive analytical method which uses synchrotron radiation as the primary excitation source. This method has some advantages. The pellet prepared for SRXRF can be measured repeatedly and can be stored for a sample collection. This method gives a total spectral picture of the sample, *e.g.* the presence of all trace components at a given energy of irradiation. A stepwise changing of the excitation energy makes it possible to improve the lower limit of detection (LLD). To our knowledge this is only possible using SRXRF.

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Table 1
Metrological characterizations of SRXRF and TXRF.

n = 7. N/D = no detection.

	S	Cl	K	Ca	Sc	Ti	Cr	Mn	Fe	Co
NiesN5 (mg kg^{-1})	38000	250	34	728	0.05	22	1.4	5.2	225	0.1
LLD (mg kg^{-1}) -SRXRF	500	70	6	6	0.02	0.4	0.32	0.36	0.32	0.01
SRXRF Sr (%)	3	18	28	4	14	4	10	9	3	7
TXRF Sr (%)	2	9	10	2	N/D	N/D	20	8	2	N/D

	Ni	Cu	Zn	Ga	Se	Br	Rb	Sr	Hg	Pb
NiesN5 (mg kg^{-1})	1.8	16.3	169	0.25	1.4	90	0.19	2.3	4.4	6
LLD (mg kg^{-1}) -SRXRF	0.14	0.12	0.12	0.09	0.05	0.05	0.04	0.1	0.1	0.3
SRXRF Sr (%)	10	1.5	2	13	6	1	25	6	5	20
TXRF Sr (%)	5	2.0	2.5	N/D	8	1	N/D	8	12	8

Table 2
Numbers for the concentrations in the individual samples (mg kg^{-1}).

	S	Cl	K	Ca	Sc	Ti	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Se	Br	Rb	Sr	Pb
1top	43100	770	48	280	<0.02	18	1.3	1.9	37	0.08	0.9	9.9	160	0.25	0.43	5.5	0.05	1.0	<0.3
1re	45300	810	150	330	<0.02	21	2.9	5.3	210	0.11	1.0	10.8	145	0.36	0.44	6.9	0.32	1.6	2.7
1rt	41300	730	100	310	0.02	18	3.6	3.5	100	0.09	0.7	9.0	145	0.27	0.45	5.8	0.13	1.3	3.0
1le	50500	990	120	320	0.03	20	1.9	2.2	59	0.09	1.2	9.2	145	0.30	0.47	6.5	0.09	1.1	0.9
1lt	43800	550	25	310	<0.02	20	1.1	1.7	29	0.09	0.6	7.6	130	0.24	0.46	4.2	0.04	1.0	1.1
2top	42800	450	24	670	0.06	18	1.5	3.8	60	0.07	1.2	10.6	190	0.54	0.52	2.4	0.05	3.4	1.0
2re	36100	780	25	570	0.07	17	7.3	5.0	250	0.09	1.2	12.3	180	0.35	0.46	1.7	0.04	4.5	1.8
2rt	42400	710	70	510	0.03	17	1.8	3.1	80	0.09	1.0	10.6	190	0.55	0.56	2.8	0.08	2.6	1.8
2le	41100	90	55	900	0.08	19	1.6	9.0	151	0.10	1.0	9.6	140	0.47	0.60	0.8	0.25	6.1	1.3
2lt	37700	420	32	480	0.02	14	1.0	3.0	60	0.07	0.7	9.4	180	0.60	0.49	2.4	0.07	2.7	1.0
3top	33400	330	330	1950	0.06	24	1.1	8.2	57	0.11	1.8	35	230	0.28	0.32	2.6	0.39	7.8	2.4
3re	48500	640	360	1590	0.18	17	4.2	7.4	38	0.14	3.4	22	200	0.35	0.20	2.4	0.42	8.3	3.4
3rt	45000	520	460	1560	0.35	20	3.3	10.1	42	0.19	4.0	24	225	0.42	0.36	8.5	0.49	9.6	4.1
3le	33400	430	180	1880	0.08	16	2.7	3.4	40	0.10	2.3	26	235	0.23	0.38	2.2	0.32	6.4	4.2
3lt	41300	330	500	1780	0.18	19	6.5	9.2	46	0.15	3.1	26	210	0.21	0.20	7.9	0.34	6.9	7.0
4top	53400	480	75	1430	0.09	32	2.9	6.4	130	0.10	2.1	12.4	200	0.44	0.39	3.1	0.11	3.6	6.8
4re	39800	450	36	591	0.05	22	4.1	3.6	100	0.08	2.2	10.4	190	0.36	0.48	2.7	0.08	2.1	2.0
4rt	49800	950	48	1040	0.08	20	5.8	3.9	110	0.10	1.1	9.4	165	0.27	0.43	1.7	0.02	2.8	6.6
4le	38400	230	43	970	0.08	19	8.4	3.8	90	0.08	4.7	10.7	195	0.53	0.47	2.1	0.08	3.2	1.0
4lt	45400	550	15	1060	0.12	17	10.3	3.9	100	0.08	2.8	9.9	175	0.39	0.48	1.5	<0.04	3.0	<0.3
5top	39000	370	100	610	0.07	19	1.9	6.0	250	0.11	0.6	9.0	190	0.50	0.51	2.3	0.27	2.4	0.6
5re	37000	480	30	420	0.05	21	2.2	1.9	70	0.08	1.2	9.0	195	0.43	0.42	3.4	0.06	1.3	0.9
5rt	40400	580	60	460	0.07	23	2.4	5.9	250	0.12	0.8	8.8	200	0.50	0.51	3.2	0.31	1.9	2.2
5le	40500	760	26	430	0.03	21	1.9	2.1	78	0.09	0.6	8.0	195	0.34	0.48	3.3	0.06	1.1	2.9
5lt	40300	540	20	520	0.06	36	0.8	1.6	60	0.08	0.6	7.7	195	0.46	0.48	3.0	0.04	1.4	0.8
6top	38700	300	30	670	0.03	18	0.6	5.2	65	0.08	0.6	11.2	192	0.37	0.54	2.4	0.14	3.2	0.7
6re	40300	730	150	360	0.03	20	1.4	6.8	200	0.11	0.6	10.5	152	0.34	0.57	5.3	0.75	1.8	1.2
6rt	39100	300	60	630	0.05	20	2.7	6.1	80	0.08	2.2	12.8	195	0.42	0.54	3.0	0.15	3.4	0.6
6le	39000	890	200	380	0.02	17	2.6	5.2	120	0.09	0.5	10.4	141	0.27	0.53	6.5	0.58	1.7	0.7
6lt	40400	340	23	550	0.06	17	0.7	4.2	40	0.08	0.5	12.5	188	0.36	0.52	3.2	0.11	2.6	0.4

The fluorescence radiation was measured on the XRF beamline of VEPP-3 (E = 2 GeV, I = 100 mA), Institute of Nuclear Physics, Novosibirsk, Russia (Trounova *et al.*, 1998). For quality control an international hair reference standard from Japan was used (NIES No. 5).

The parameters of the SRXRF station are as follows: excitation energy, 3–46 keV; detector, Si(Li); detector energy resolution, 150 eV; exposure time, 100–1500 s; detected elements, S, Cl, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Br, Rb, Y, Sr, Mo, Nb, Zr, I, Sn, Sb, Ba, La, Ce, Nd, Pr and Pb. In this work an excitation energy of 17 keV has been used.

For TXRF analysis [a multielemental method which is useful for samples of very low mass (<1 mg)] a sample pre-treatment is necessary (Klockenkämper, 1997). The measurements were performed using the TXRF 8030C spectrometer supplied by ATOMIKA Instruments (Oberschleisheim, Germany). Quantification was performed by internal standardization. In our case V was used as an internal standard. These measurements were carried out at GKSS, Geesthacht, Germany.

Table 1 shows the metrological characterizations of SRXRF and TXRF. The standard deviation, relative standard deviation and lower limits of detection (LLD) were calculated using the following equations,

$$S = \pm\left\{\left[\sum(x_i - \overline{x})^2\right]/(n-1)\right\}^{1/2},$$

$$S_r = S/\overline{x},$$

$$C_{LLD} = C\,3.29\left(N_{bg}/N_p - N_{bg}\right)^{1/2},$$

where S is the standard deviation, S_r is the relative standard deviation, C_{LLD} is the minimal detectable concentration, C is the

concentration of the element in the sample, N_{bg} is the integral area of the background and N_p is the integral area of the signal (net or gross).

The accuracy of the SRXRF method was calculated by simultaneous measurements of standards with similar matrices.

For determination of accurate data in the TXRF measurements the conditions were selected such that the concentrations of trace elements in the samples were in the same range as in the standard.

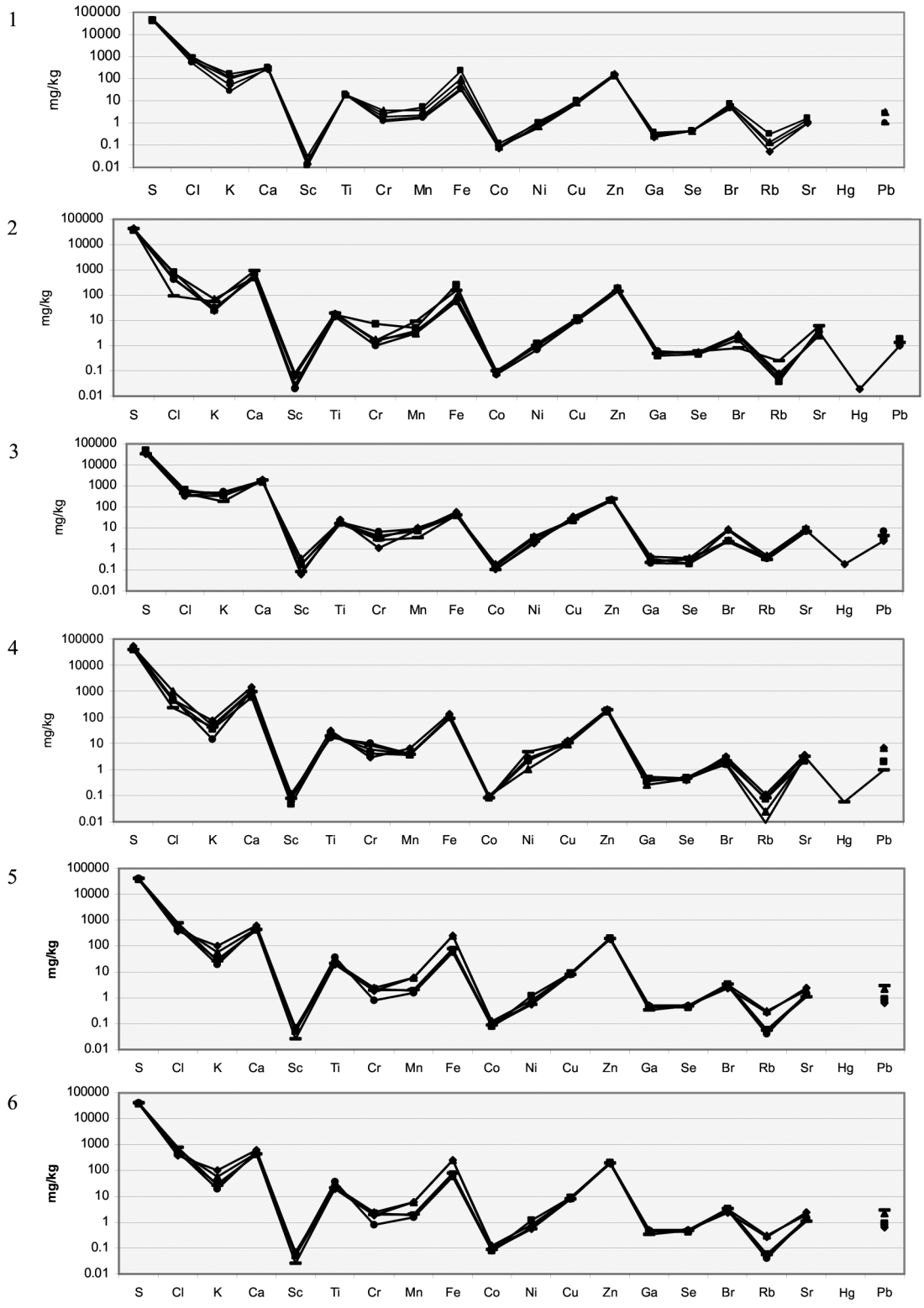

Figure 1
The distribution of 20 trace elements at five points from the heads of six people (1, 2, 3 female; 4, 5, 6 male).

3. Results and discussions

Fig. 1 shows six diagrams which characterise the distribution of 20 trace elements from samples taken at five points from the heads of six people (the ordinate is given on a logarithmic scale). Persons 1, 2 and 3 are female, and 4, 5 and 6 are male. The numbers for the concentrations in the individual samples are given in Table 2. The abbreviations are as follows: 1le = sample number 1, left ear; 1re = right ear; 1lt = left temple; 1rt = right temple; 1top = crown of the head. The content of Hg was at the level of LLD and so is not given in Table 2.

At first sight all six diagrams show the same character for the distributions of 20 trace elements. Looking at each diagram more closely, however, reveals a non-identical behaviour. The elements detected in this experiment can be separated into three groups.

The first group of elements can be characterized by a concentration deviation of a factor of five at the different points on the head of a person, *e.g.* Cl, K, Cr, Mn, Fe, Rb and Pb. For the elements Cl, K, Cr and Pb the relative standard deviation (S_r) is relatively high; nevertheless, the distributions of the results at the various sampling points exceed S_r. The uneven distribution of Fe is explained by Bos *et al.* (1985) with individual physiological characteristics of the person (injury cuticle of hair and sebaceous glands). It is outlined in the same paper that the main contribution of Pb is of exogenic character, which is precipitated on the surface of the hair and diffuse inside afterwards.

The second group is characterized by elements which differ in concentration at the different points on the head of a person by a factor of less than two, *e.g.* Ca, Sc, Ni, Br and Sr (see Tables 1 and 2). The variation of the concentrations of Ca and Sr correlates for all samples. This special behaviour of Ca and Sr was shown in the literature for a large number of people (Trounova *et al.*, 2002).

The third group can be characterized by elements whose concentrations differ in the range of the relative standard deviation, *e.g.* S, Ti, Co, Cu, Zn, Ga and Se. For nearly all sampling points the concentrations of these elements tend to a constant value.

Fig. 2 shows SRXRF and TXRF results of hair samples from different places on the head from one person (mg kg^{-1}). As the

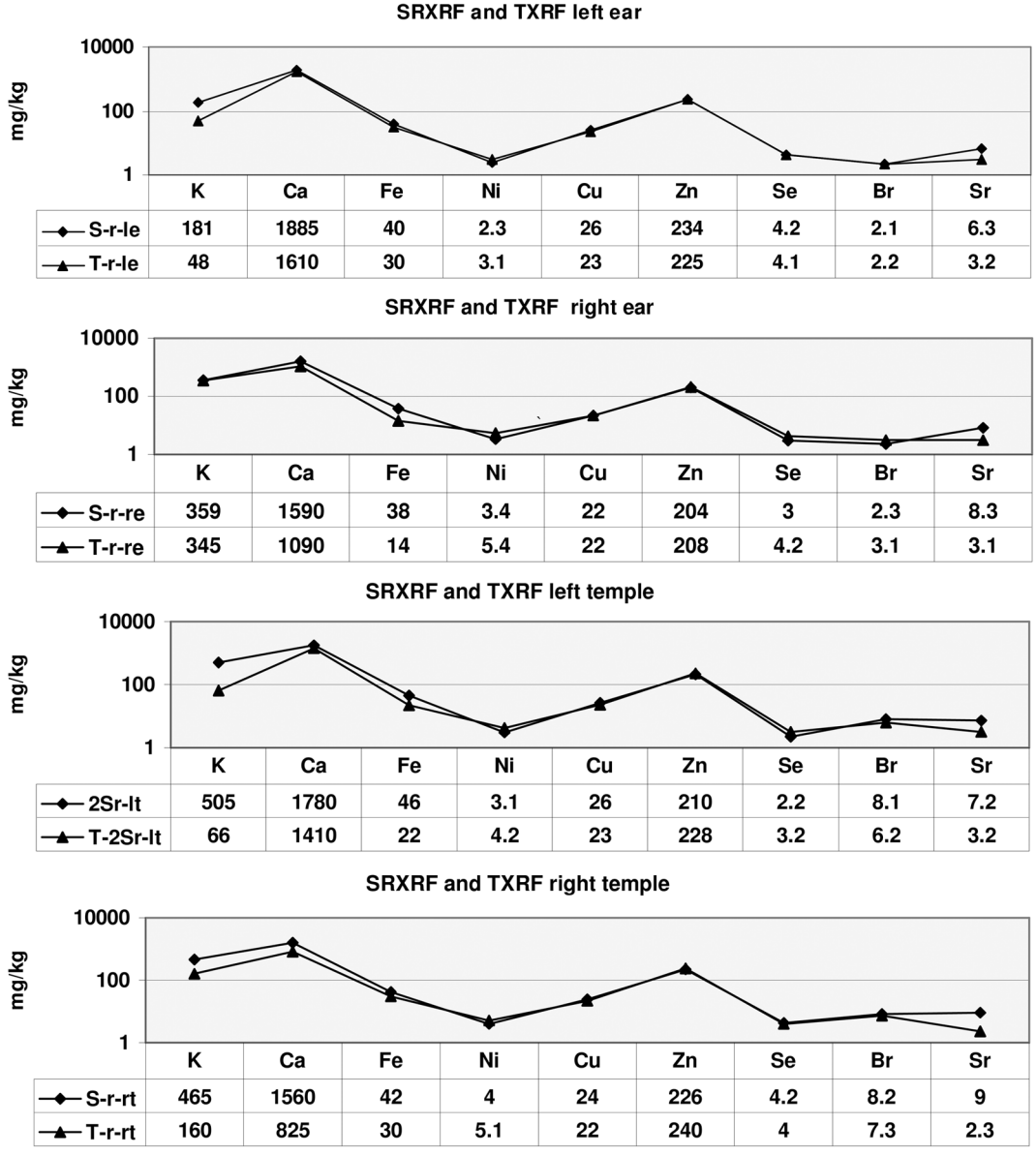

Figure 2
SRXRF and TXRF results of hair samples at four points from the heads of one person (Se/10).

ordinate is given on a logarithmic scale the concentration of Se was multiplied by ten. Two samples were taken from the right-hand and left-hand side of each head. The samples were collected from the temple and from behind the ears.

For all four examples it can be stated that some elements (Ni, Cu, Zn, Se and Br) behave more constantly than others (K, Fe and Sr). It is important to note that the samples for both methods were taken at one point and at the same time but they are not identical. However, the results for the elements Ni, Cu, Zn, Se and Br are identical for both methods within the limits of error. For the other elements, K, Fe and Sr, there are more serious deviations.

All these diagrams show identical results, within the experimental uncertainties, obtained using both methods. The behaviour of the trace elements for the different sampling points described above is confirmed. Namely, the content of Ni, Cu, Zn, Se and Br is practically constant although the samples are not identical. In contrast, the concentrations of the elements K, Fe and Sr vary, as shown in Fig. 1 and Table 2.

4. Conclusions

As was outlined above, the concentrations of microelements in hair given in the current literature (Spyrou *et al.*, 2002) vary by several orders of magnitude for an individual element. There is almost no information on the distribution of traces elements on the area of a head.

We have shown that the behaviour of the trace elements in these areas is not identical. Groups of elements were identified with low variation in concentration with a tendency to a constant value for each individual donor, *i.e.* variation in concentration does not exceed the maximum metering error. In particular, this group of elements can be taken for characterising the state of health of an individual. The other measured elements cannot provide objective information usable for diagnosis.

Comparison of the results using the two methods confirms the existence of a group of elements which characterise a person by their practically constant value.

It is well known that Cu, Zn and Se are analyzed in tissue for diagnosis. These elements are essential for the human organism; they are closely bound to the human immune system. Sulfur reacts on the human metabolism.

In human hair samples, 20 trace elements were determined, *i.e.* S, Cl, K, Ca, Sc, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Se, Br, Rb, Sr, Hg and Pb. However, reliable information on the content of trace elements for a given person can only be obtained from the elements S, Ti, Co, Cu, Zn, Ga and Se at any points.

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