Journal of Radioanalytical Chemistry, Vol. 31 (1976) 267–289

FORENSIC AND ENVIRONMENTAL ASPECTS OF NEUTRON ACTIVATION ANALYSIS OF SINGLE HUMAN HAIRS

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(Received October 6, 1975)

A new analytical procedure consisting of special washing step, irradiation in a thermal neutron flux of $\sim 10^{14}$ n · cm^{-2} · sec^{-1}, and Ge(Li) spectrometry enabled to determine as many as 14 elements in a 3 cm segment of a single human hair by neutron activation analysis (NAA). The criminalistic aspects of hair analysis were studied using a new statistical criterion for elimination/identification and an appropriate computer program was constructed. Hair dimensions as measured microscopically were used as additional individualizing attributes. It was shown that despite the difficulties originating from a relatively large intrinsic variation of the trace element concentration over one head, elimination of most or nearly all of the "suspects" could be achieved in simulated cases. Distincly elevated levels of Au as well as Cu and Ag were found in hair of some groups of persons working under specific conditions thus confirming the importance of the environmental factor related to some kinds of occupation.

Introduction

Long before now, it has been recognized that hair found at the scene of crime can be matched with those taken from the suspect on the basis of its trace element content.[1-5] After the period of initial over-optimism when it was brought to the courts in several countries this method was strongly criticized by other authors[6,7] on the grounds that it lacked credibility because of a considerable variation in the trace element concentration over one head and also as a function of time.

It should be mentioned here, that while a lot of works on NAA of hair may be found in the literature, only a small part of them dealt with single hairs, which are encountered most probably as evidence specimens.

No analytical procedure published so far has gained general acceptance and much controversy arose around the problem of washing of hair before or after irradiation.[2-6, 8, 9]

At the same time the analysis of hair can be of interest in such areas as toxicology,[1, 10-13] medicine,[14, 15] biology,[16-18] study on enviromental pollution[18-23] etc.

Hair is an excretory tissue with unique properties. It can be easily collected, preserved and handled. Trace elements may be incorporated into hair from the bloodstream as well as through sorption or ion exchange from dust, sweat etc., thus reflecting both metabolic processes and external contamination.

An analytical procedure described in this work enables to determine more than 10 trace elements in short segments of single human hairs. The population of over 50 people was examined and the results evaluated with the use of a new statistical criterion to see what are the real prospects for successful individualization of hair for forensic purposes. Simultaneously, the effect of work in some specific environment on the composition of hair was also investigated.

Experimental

Materials and methods

30 mm long hair segments were measured and cut in a single operation using simple plastic device and a quartz knife.[24]

It was assumed that the shape of the hair segment is well approximated by an ellyptical cylinder and the greatest (2a) and the smalest (2b) diameter at each end was measured microscopically.[24] Hair volume could then be calculated from the formula:

$$V = \pi \cdot l \frac{2a_1 + 2a_2}{4} \cdot \frac{2b_1 + 2b_2}{4} \qquad (1)$$

where l - hair length (here 30 mm);

a_1, a_2 and b_1, b_2 - greatest and smallest ellypse semi-axes at each end, respectively.

Each hair segment was washed 2 times for 2 min in doubly distilled benzene and dried between filter paper discs. Hair segments were then placed in individual quartz tubes (3 mm i.d.) open from both ends, 5-6 tubes placed in an all-quartz apparatus[24] and 300 ml of the distilled, doubly deionized water of the temperature of 50 ^{o}C was passed at a flow rate of $\sim$10 ml/min.

As was shown in the previous paper[24] this washing procedure diminished sodium content by approximately two orders of magnitude, while leaving essentially intact other trace elements determined in this work.

5-6 pieces of hairs were irradiated in quartz ampolues in the EWA reactor, together with a set of mixed standards at a flux of $\sim 10^{14}$ $n \cdot cm^{-2} \cdot sec^{-1}$ for 20-24 hrs.

Each hair was then transferred into a standard, flat, plexiglass holder with 2 mm x 30 mm groove and a matching lid. Standards were eluted from the ampoules, diluted to a known volume and appropriate aliquots prepared for counting.

At the first stage of this investigation a 19 cm^3 Ge(Li) detector and a 512 channel pulse-height analyser (system resolution 5.5 keV for the 1332 keV ^{60}Co peak) was used. Later on 70 cm^3 Ge(Li) detector with suitable electronics (Schlumberger) and Didac 4000 PHA (Intertechnique) or 55 cm^3 Ge(Li) detector (Ortec) with Plurimat 20 PHA (based on Multi-8 minicomputer, Intertechnique) were employed. Resolutions of the two systems for the 1332 keV ^{60}Co line were 3.1 and 2.4 keV, respectively,

Table 1

Nuclides identified in the irradiated single human hairs

Element	Radioactive nuclide formed	Half-life	Main gamma-rays,* keV			
Ag	^{110m}Ag	253 d	657.7;	884.7;	937.5;	1384.2
As	^{76}As	26.3 h	559.2;	657.0;	1215.8	
Ar	^{41}Ar	1.83 h	1293.6			
Au	^{198}Au	64.8 h	411.8;	675.9		
Br	^{82}Br	35.6 h	554.3;	619.1;	776.6;	1044.0
Cu	^{64}Cu	12.8 h	511.0;	1345.5		
Hg	^{197}Hg	65.0 h	68.8;	77.6		
	^{203}Hg	46.9 d	279.2			
Ir	^{192}Ir	74.2 d	295.9;	308.4;	316.5;	468.1
	^{194}Ir	19.7 h	328.0			
La	^{140}La	40.2 h	328.6;	486.8;	815.5;	1595.4
Mn	^{56}Mn	2.58 h	846.9;	1810.7		
Na	^{24}Na	15.0 h	511.0;	1368.4;	2753.6	
Sb	^{122}Sb	2.74 d	564.0;	692.5		
	^{124}Sb	60.0 d	602.6;	1690.7		
Se	^{75}Se	120.4 d	121.1;	136.0;	264.6;	279.6
Sr	^{87m}Sr	2.84 h	388.5			
Zn	^{69m}Zn	13.8 h	438.7			
	^{65}Zn	245 h	511.0;	1115.4		

*Photopeaks used for quantitative determinations are underlined.

peak to Compton ratio 25:1 and 31:1, and relative efficiencies with respect to a standard NaI(Tl) detector amounted to 14.1 and 9.4, respectivelly.

Selected peaks of the spectra were printed and peak areas calculated according to Covell,[25] Sterliński[26] and total peak area method cf. Ref.[24] As it was found that the three methods gave very similar results, only the total peak area method was used later on. When computer-based PHA (Plurimat 20) was employed, the net peak areas were calculated using a standard PRM 01A program. Hair samples were first measured 3-6 hrs after the end of irradiation and then usually after 8 and 21 days. Occassionally measurements were made also after other cooling times, e.g. when the identity of a nuclide had to be confirmed by half-time measurements. Counting times varied from 500 sec, to 5000 sec but 2000 sec was usually a good compromise for the first measurement. Standards were counted under similar geometrical conditions as samples and the peak areas were corrected for decay and for interferences from other radionuclides when necessary.

Elements identifified in single human hairs and the photopeaks used for quantitative determinations are shown in Table 1.

Details concerning washing studies, preparation of standards, energy calibration etc, as well as the destructive version of analysis can be found in an earlier work.[24]

Results and discussion

Survey of trace element concentrations in single human hairs

As seen from Table 1, 15 elements were indentified altogether in the spectra of single human hairs. The results of quantitative determinations for the population of 56 people are summarized in Table 2. All concentra-

Table

Concenttration ranges observed in 3 cm segments of single head

Element	Population					
	C_{max}	C_{min}	Medium	C_{max}/C_{min}	$\lg C_{max}/C_{min}$	C_{max}
Zn	1521	71.3 , ND	319.7	>21.3	1.3284	1521
Cu	3237	3,2 ND	22.19	1011.6	3.0047	3237
Au	105.22	0.0078,ND	0.0645	>13489.7	4.1303	105.2
Mn	43.14	0.062, ND	0.829	>695.8	2.8425	43.14
Sb	80.69	0.05 , ND	0.57	>1613.8	3.2076	80.69 66.20
Br	7991	0.47 , ND	4.25	>17002.1	4.2304	7991 8.96
Ag^{v}	2643	46.5 , ND	99.45	>56.8	1.7543	2643
Sr^{+}	113.3	2.4 , ND	16.13	>47.2	1.6739	
Hg^{++}	10.16	5.06 , ND	-	>2.01	0.3032	
As^{+++}	12.97	0.03 , ND	0.38	>432.33	2.6358	
La^{vv}	0.27	0.14 , ND	-	>1.86	0.2695	
Ir^{vvv}	17.0	3.0 , ND	-	>5.67	0.7536	
Se^{+v}	981.7	49.2 , ND	237.34	>19.95	1.3000	

* For at least 5 hairs.

+ Determined only in hairs of 5 persons.

++ Determined only in hairs of 2 persons.

+++ Determined only in hairs of 6 persons.

2

hairs of a population consisting of 56 people, in x 10^6 g/cm^3

Single head hair						
Maximum dispersion			Minimum dispersion*			
C_{min}	C_{max}/C_{min}	$\lg C_{max}/C_{min}$	C_{max}	C_{min}	C_{max}/C_{min}	$\lg C_{max}/C_{min}$
309	4.92	0.6920	239.6	199	1.20	0.0792
242	13.4	1.1271	20.0	14.7	1.36	0.1335
			23.1	17.5	1.32	0.1206
2.21	47.60	1.6776	0.052	0.029	1.79	0.2529
0.466	92.57	1.9664	0.94	0.26	3.62	0.5587
			11.32	4.76	2.38	0.3766
1.67	48.32	1.6841	0.20	0.08	2.50	0.3979
0.63	105.08	2.0216				
81.6	97.93	1.9909	2.52	0.47	5.36	0.7292
0.66	13.58	1.1329	9.57	4.85	1.97	0.2945
401	6.59	0.8189				

[v]Observed only in hairs of 4 persons.

[vv]Determined only in hairs of 4 persons.

[vvv]Determined only in haris of 1 persons.

[+v]Determined only in hairs of 1 persons.

tions are expressed as: $\times 10^{6}$ (g of element per cm^3 of the hair). Normalization of results with respect to hair volume on the basis of microscopic measurements has the additional advantage that the greatest and the smallest hair diameter as well as their ratio can also be utilized as individualizing attributes in forensic considerations.

To enable comparison with literature data where the concentrations are as a rule expressed in terms of ppm (by weight), apparent hair densities were measured in the previous work,[24] and shown to vary from 0.99 to 1.61 g/cm^3. Taking the medium value of 1.30 g/cm^3, concentrations in ppm can be, with satisfactorily good approximation, obtained from the correlation:

$$C_{ppm} = \frac{C_{g/cm^3} \cdot 10^{6}}{1.30\ g/cm^3}\ ppm \qquad (2)$$

Apart from the maximum and minimum concentrations and medium values for the population studied in this work, as a whole, maximum and minimum concentration ranges found for hairs of a single person are also quoted in Table 2, both in linear and logarithmic scale.

In general concentration ranges and medium concentrations for most of the elements studied, agree reasonably well with the results published by other workers.[2-6, 9, 18, 23, 25]

Distinctly elevated levels of Au, Ag and Cu were found in some persons and related to environmental contamination as will be discussed below. Also, rather high concentrations of Sr and Se were detected in some cases.

Iridium was a new element identified in hair, and, surprisingly enough, argon appeared in several of the hair samples analyzed (cf. Fig. 1).

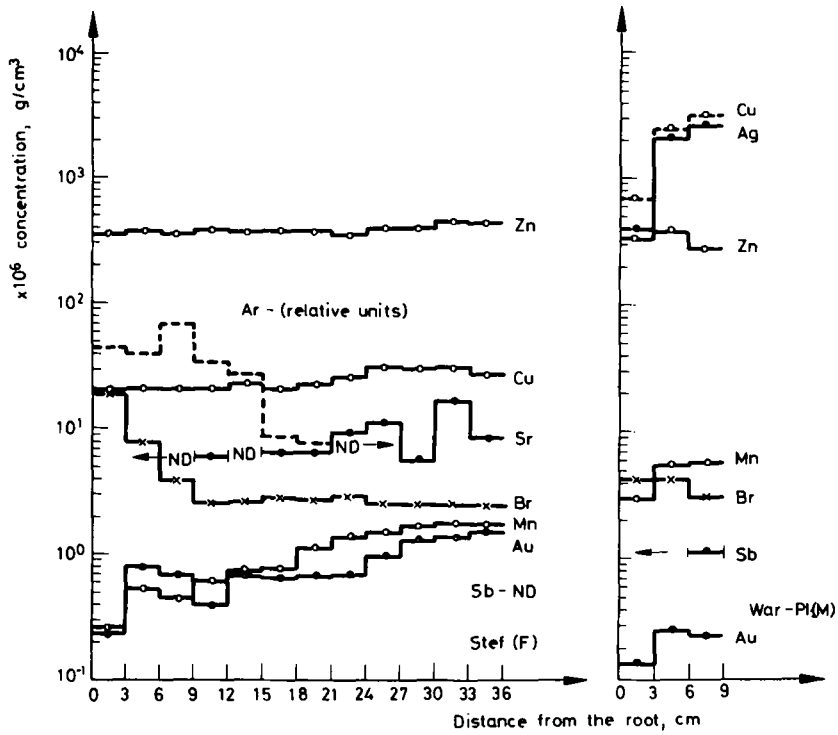

Fig.1. Trace element content in the hair of two different persons as a function of the distance from the root, showing also the presence of argon in hair of Stef(F)

It might be expected that the variation in trace element composition over one head and also within the population found in this work when analysing short segments of single hairs should be considerably larger than in cases when hair samples weighing tens or hundreds of milligrams were used.

Possibilities of using NAA for individualization of single hairs

As it can be concluded from both Table 2 and Fig. 1, although the dispersion of results obtained for a single head is sometimes considerable, it is always significantly smaller than the analogous dispersion for the

population. Hence it can be inferred that there are still not so bad prospects for the successful individualization of hair for forensic purposes.

An attempt was made to examine the chances of hair individualization on the basis of our experimental results. For this aim a yet unpublished criterion for the elimination and identification developed by Golian[26] in this Laboratory was used.

Instead of using directly the results of measurements, Golian uses the ratios d_r of all possible pairs of results for a given attribute (concentration of an element, hair diameter etc.) which fulfil the relation:

$$d_r = \frac{R_{1(S_i, A_j)}}{R'_{1(S_i, A_j)}} \geqslant 1 \qquad (3)$$

where $R_{1(S_i, A_j)}$ = 1 is the result of the measurement of an attribute;

A_j $(j = 1, 2, \ldots, t)$ - object (sample) S_i $(i = 1, 2, \ldots, k)$;

$1, 1' = 1, 2, \ldots, q$ - number of determination of the attribute A_j in the sample S_i.

It is assumed that there exists a criminal sample (object e.g. hair found at the scene of crime) and several control samplex i.e. hairs taken from the suspects. Measurements of each attribute in each sample are repeated several times. It is necessary for correct performance of the criterion that certain minimum amount of data exist for each attribute. 4-5 results for each attribute of a population of 20 - 30 persons is usually sufficient for that purpose.

Once these results become available they constitute a "bank of data" and can be also used later on in next cases, even when the number of real

samples is then considerably smaller. A histogram representing the frequency of occurrence of all the ratios d_r for a given attribute (element) obtained from all the samples in then constructed. The frequencies are next normalized in a way that the value $p_k = 1$ is assigned to the most

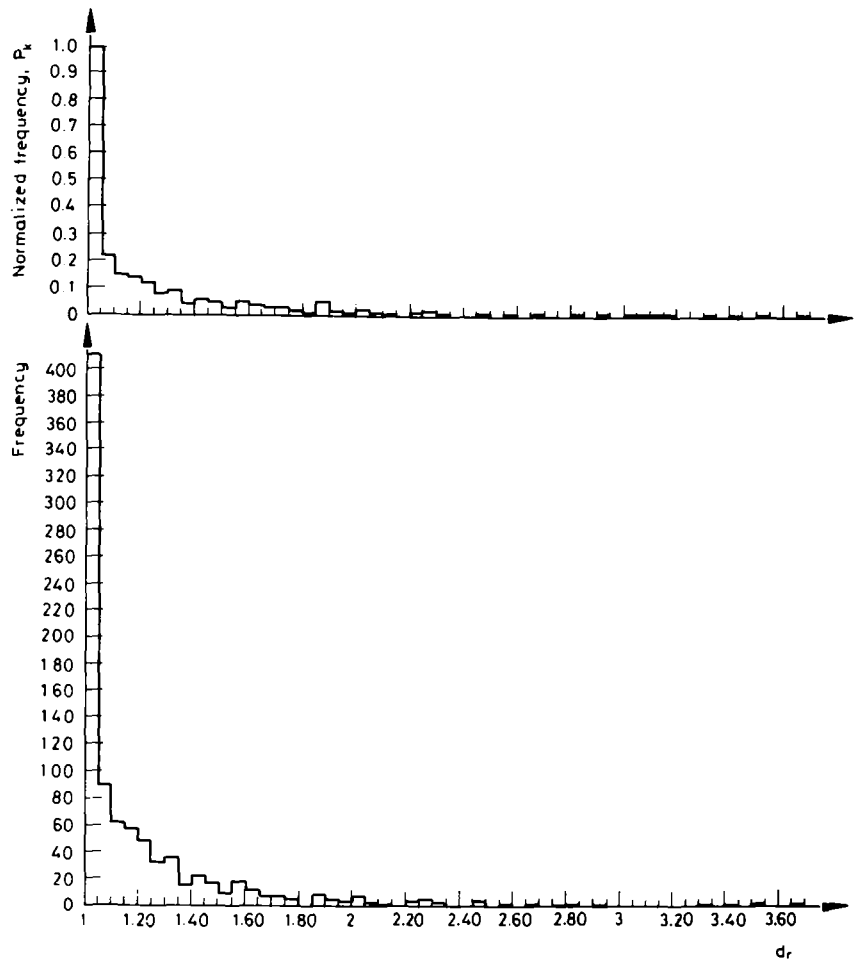

Fig.2. Histograms showing the frequency of occurrence of the ratios of results when determining zinc in single hairs from the same head

probable frequency. As an illustration, the above mentioned histograms obtained in this work for zinc in hair are shown in Fig. 2.

Having the normalized histogram, the so called "index of attachement", G_{S_i, A_j} is calculated for every sample S_i and attribute A_j:

$$G_{S_i, A_j} = \sum_{k=1}^{n} \frac{n}{n^2 + m^2} p_k + \sum_{k=1}^{m} \frac{m}{n^2 + m^2} p'_k \qquad (4)$$

where the first term of the right hand side of the formula represents the ratios d_r for which the histogram assignes $p_k \geqslant 0.1$; n is the number of these ratios and m is the number of the rest of ratios.

In an analogous way as defined by Eq. (3) the new set of the ratios, d_r is then formed for each sample and each attribute by dividing the results for a given control sample (i.e. suspect) by those obtained for criminal sample in all possible combinations and preserving those greater or equal to unity.

Taking this new set of ratios and making use of the normalized histogram, the "comparative index of attachement", GG_{S_i, A_j} is calculated in the same way as the G_{S_i, A_j} [Eq. (4)].

The "index of similarity" W_{S_i, A_j} is defined as the ratio of the two above mentioned values:

$$W_{S_i, A_j} = \frac{GG_{S_i, A_j}}{G_{S_i, A_j}} \qquad (5)$$

If the index of similarity exceeds certain, properly chosen, limiting value it is assumed that the given control sample coming from the suspect is indistinguishable from criminal sample i.e. both may have common

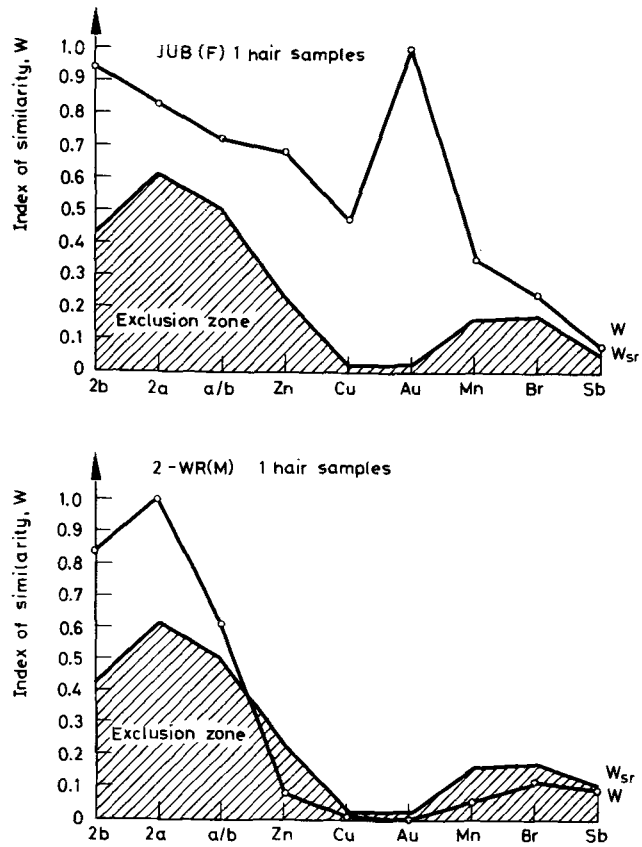

Fig.3. Graphical presentation of the cases of elimination and non-elimination

origin. If W is smaller than the limiting value the control and criminal samples differ significantly.

The illustration of the two cases is shown in Fig. 3.

In Golian's original approach W^L is defined as follows:[26]

$$W^L_{A_j} = \frac{\bar{G}_{A_j} - 2\sigma_{A_j}}{G_{A_j}} \qquad (6)$$

where $\bar{G}_{A_j}$ and σ_{A_j} are the mean and standard deviation, respectively, of the G_{S_i, A_j} values calculated in an usual way. This was used as a limiting value.

In the course of the present study it was found, however, that the use of the mean value of W_{S_i, A_j}:

$$W_{sr} = \frac{\sum_{i=1}^{k} W_{S_i, A_j}}{k} \qquad (7)$$

where k is the number "suspects", results in a much better discriminating power, and this parameter was used as the limiting value later on.

The performance of the criterion was checked by choosing sets of results for 3 samples (persons) which were sufficiently numerous (8 - 12 results for each attribute) and splitting each of them into two halves. These new subsets were then treated as if coming from two different persons i.e. suspect and the criminal. Although the division was sometimes made not at random but rather in a most unfavourable manner for the purpose of identification (results for hair coming from an earlier stage of growth were included into one group and those from later stages of growth to the other), in all investigated cases the calculations classified the samples as indistinguishable with respect to all attributes taken into account [concentrations of Au, Zn, Mn, Cu, Sb and Br as well as the greatest (2a) and the smallest (2b) hair diameter and their ratio a/b]. Only in one case the criterion classified the results for bromine concentration for the two subsets of DOW 8 sample as significantly different. This might be due to analytical error (some troubles were originally encountered with the bromine standard[26]) or to rather high concentration gradient of Br along the hair length. At any rate, however, the similarity between the two

Table 3

Fractions (and percentages) of the total number of eliminated suspects due to individual attributes and all attributes together

Attribute / "Criminal sample"	Au	Zn	Cu	Mn	Sb	Br	2a	2b	a/b	All attributes together
DOW 6	18	12	7	14	17	20	21	12	21	29
	30	30	30	30	30	30	30	30	30	30
	60%	40%	23.3%	46.7%	56.7%	66.7%	70%	40%	70%	96.7%
DOW 7	27	14	28	15	19	15	15	10	20	29
	30	30	30	30	30	30	30	30	30	30
	90%	46.7%	93.3%	50%	63.3%	50%	33.3%	66.7%	96.7%	96.7%
DOW 8	25	14	9	15	20	17	14	10	14	29 *
	30	30	30	30	30	30	30	30	30	30
	83.3%	46.7%	30%	50%	66.7%	56.7%	46.7%	33.3%	46.7%	96.7%

*Bromine concentration was not taken into account as an individualizing attribute for DOW 8 sample.

halves of DOW 8 sample was much higher than the similarity towards any other sample from the population of 30 "suspects".

The numbers (fractions) and percentages of other samples (i.e. suspects) eliminated from the suspicion by the criterion are shown in Table 3.

In each of the three "simulated cases" out of 30 "suspects", 29 actually "innocent" would be exculpated and only one classified as indistinguishable from the "criminal" (which was actually the case).

So the method seems to be quite promising and deserves further investigation. Of course, the more attributes will be taken into consideration, the more certain will be the results of elimination/identification procedure (cf. also Ref.[24]).

As was described above, in Golian's method each attribute is at first treated separately and finally all the results can be examined together e.g. in the form of a graph (cf. Fig.3.)

At this stage some a posteriori considerations and corrections can still be made e.g. when the results for any attribute seem to be doubtful for some particular reason.

In Parker's treatment of the identification problem[29,30] it must be a priori decided which attributes are included for calculation of his "discrepancy index", that has a cumulative character. Any excessive measurement errors etc., for one particular attribute could then spoil the whole statistical treatment and lead to erroneous conclusions.

Hair as indicator of environmental pollution

To investigate how far the composition of hair reflect occupational exposure, hair of several people who were in constant contact at work with some heavy metals was analyzed.

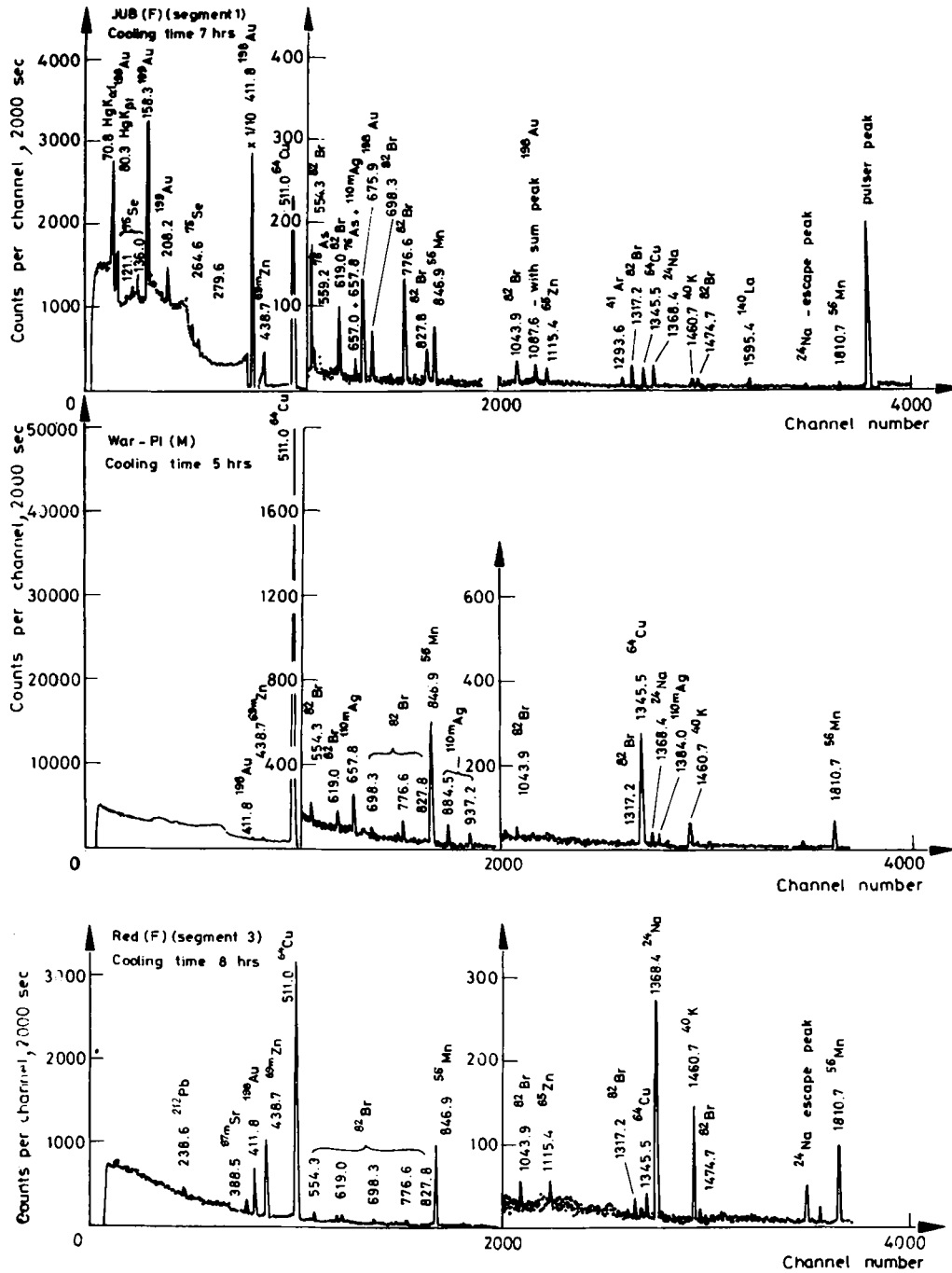

Fig.4. Gamma-ray spectra of the single hairs of three persons exposed to environmental pollution because of occupation

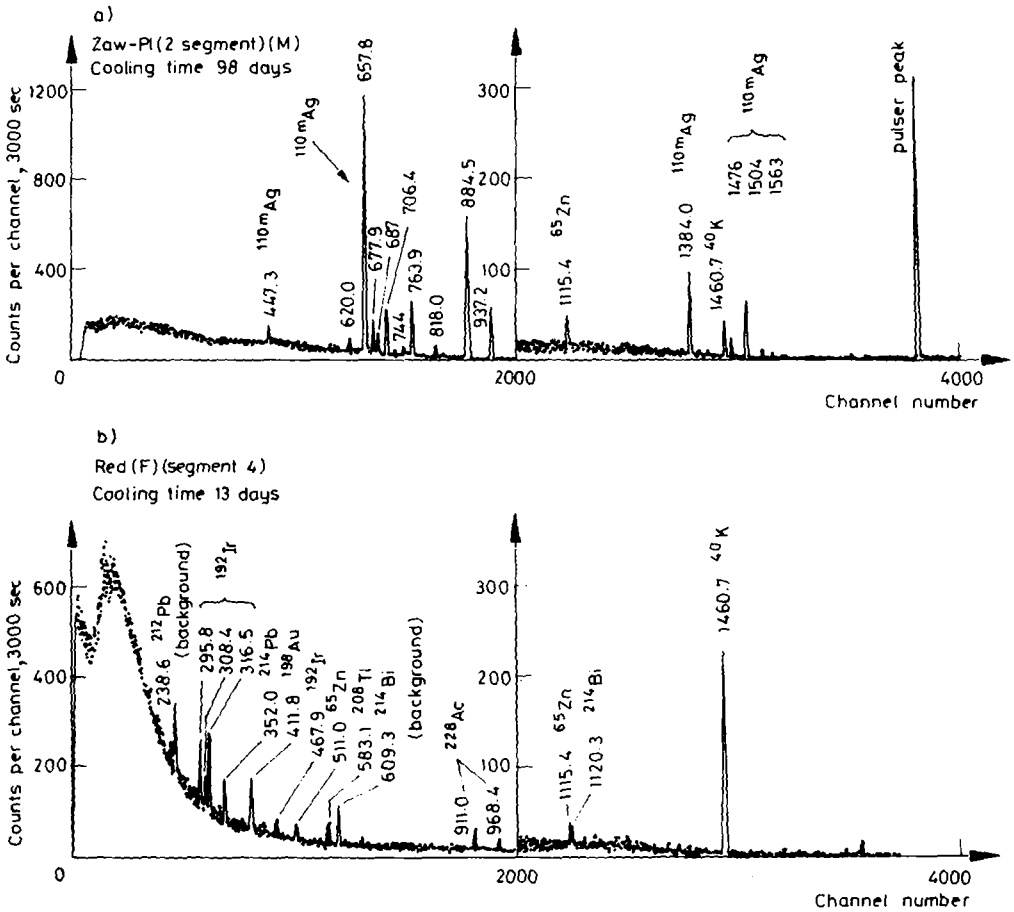

Fig.5. Gamma-ray spectra of the single hairs of two persons (after long cooling) showing the presence of silver (a) and iridium (b)

The following groups of people were included in this study:

(1) Five women (research workers and technicians) working in a chemical laboratory, doing routinely noble metal analyses, with the aid of such techniques as cupellation, wet chemistry, emission spectrography and others. These persons were marked with the following code letters: Red., Kol., GR., Stef. and TNM.

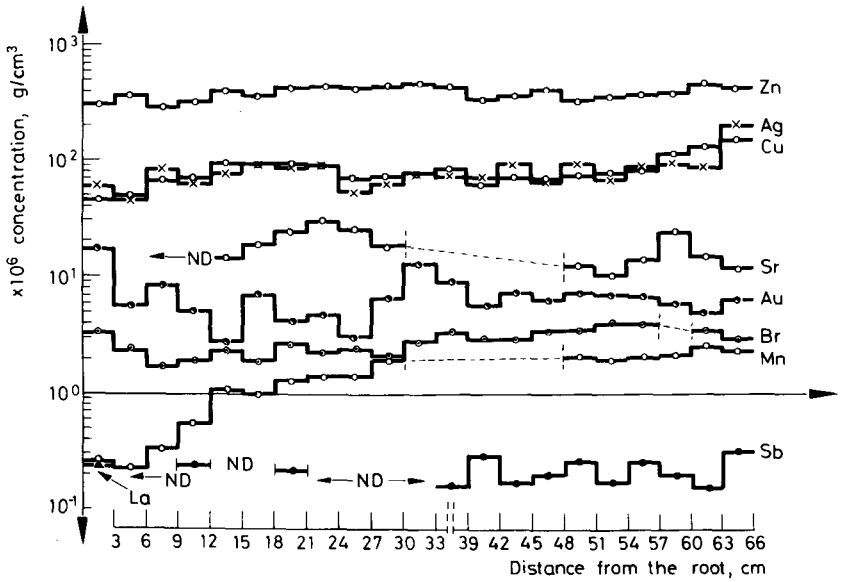

Fig.6. Trace element content of hair of JUB(F); and example of sectional analysis of very long hair

(2) Three men (War-Pl., Sm-Pl., and Zaw-Pl.), workers in a factory producing silver-plated knife, fork and spoons.

(3) One women working in a jeweller's workshop (JUB.).

Gamma-ray spectra of single hairs of some of the above mentioned persons are shown in Figs 4-5, and results of sectional analyses in Figs 1 and 6, respectively.

In Fig. 7 concentration ranges for several elements found in single hairs of the people exposed to heavy metal pollution are shown together with concentration ranges for the rest of the population.

As easily seen, distincly elevated levels of gold were found in hair of all persons doing noble metal analyses and also the one working in jeweller's workshop."Normal" gold concentration were found in hair of factory workers except of slightly elevated level in the hair of War-Pl.,

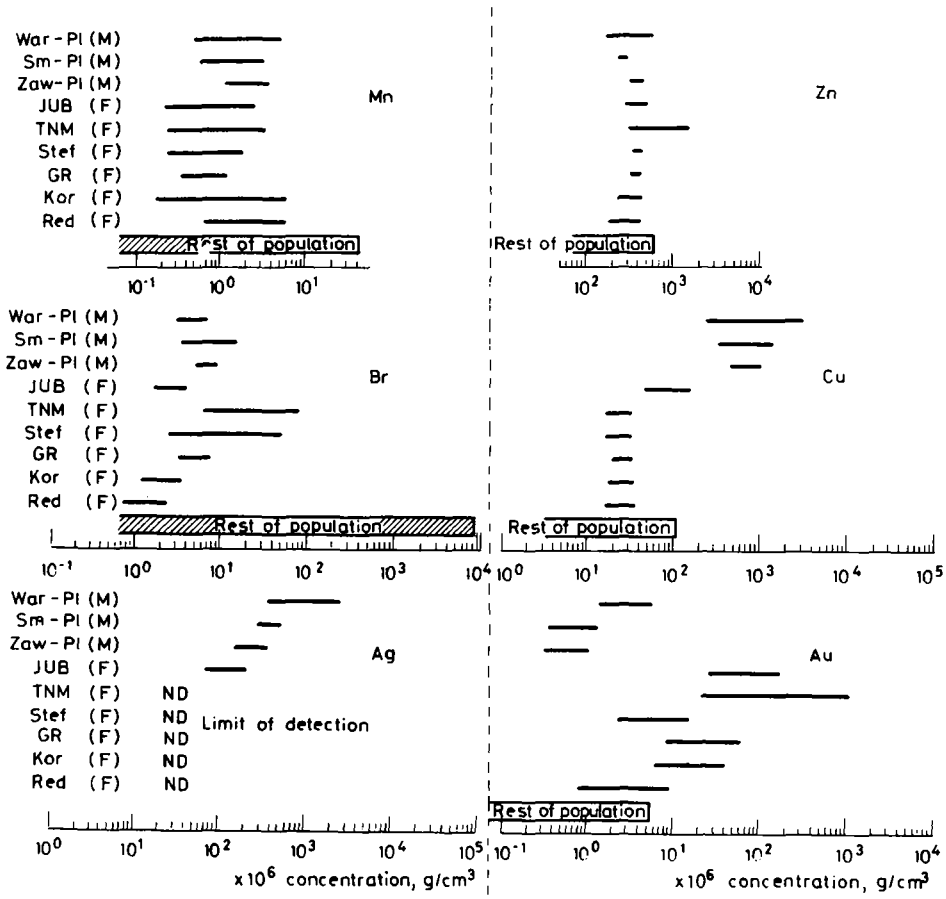

Fig.7. Concentration ranges of several elements in the hair of people exposed to environmental pollution in comparison with analogous data for the rest of the population

who had also the highest silver content. Gold is a common contaminant of silver and heavy and long accumulation of that metal in hair could also result in an increase of Au level in hair. From among the ladies working in chemical laboratory the Head of this Laboratory (Red.,) had relatively the smallest increase in gold content, but was the only person in whose hair iridium was identified (cf. Fig. 5).

In one case enormously high gold content ($105 \cdot 10^{-6}$ g/cm^3), which is nearly three orders of magnitude higher than the "normal" Au level, was found in the hair of TNM.

Silver was found only in the hair of the three men producing table requisites and also in the hair of the woman from the jeweller's workshop, thus obviously indicating contamination from dust, fingers etc.

For the hair of all the other people silver was below the detection limit. The presence of silver in hair was parallelled by the increased concentration of copper originating apparently from the alloys from which knife, fork and spoon and jewellery have been made. As high concentrations as $3237 \cdot 10^{-6}$ g/cm^3 were found in extreme cases.

As the environmental contamination may have direct and often profound effect on the trace element concentration in hair, attempts to correlate the concentration of particular elements with such individual features as mental ability etc. mentioned in literature[27] seem to be rather risky.

Concentrations of other elements like Mn, Br and Zn (cf. Fig. 7) were well within the limits for the rest of population, except in single case of the hair of TNM where parallel to very high gold content also elevated level of zinc concentration was observed.

The results of this study, similary as those published by other workers[19-23, 28] clearly show that activation analysis of human hair can be applied for monitoring of some occupational hazards.

Conclusions

The results of the present study showed that neutron activation analysis enables the determination of 14 elements in short segments of a single human hair. The newly developed washing procedure which selectively removes sodium before irradiation is an essential feature of the method.

With the use of a new statistical criterion for elimination/identification, most or all of the "suspects" could be eliminated and the "criminal" positively identified in simulated cases, thus showing that the method could be valuable to the police at least in the stage of inquiry.

At the same time this method of analysis was shown to be very useful for monitoring occupational exposure.

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The authors wish to thank Mrs. A. KOŁYGA and Mr. J. Kowalski for skilful technical assistance.

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