

France Hair

Cu 23

Fe 10
Se 0.2 ak.

* Good water

* Pb ↑ wine

Mu ↓



9

Study of the Age and Sex Dependence of Trace Elements in Hair by Correspondence Analysis

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ABSTRACT

The aim of the study was to examine the potential of multidimensional analysis, and in particular of correspondence analysis (CA), in bringing to light the influence of sex and age on trace element (TE) concentrations in hair from an unselected French population. Sixteen elements (S, Hg, Se, Zn, Pb, Cd, Ni, Co, Mn, Fe, Cr, Mg, Al, Ca, Cu, Ag) were assayed by inductively coupled argon plasma (ICAP) emission spectroscopy in the scalp hair of 135 men and 346 women. In spite of the high background noise, CA was able to reveal the differing patterns in males and females. For instance, in this population, higher relative levels of the essential elements, Ca, Mg, Zn, and Cu, but also of Ag, characterized women's hair, whereas higher relative levels of the heavy metals, Fe and Pb, were associated with men's hair. Al and Ag were unexplainedly high in the hair of the youngest members of the population. The Cu and Co of youth seemed to give way to a predominance of Zn in maturity. The hair of individuals in their forties tended to be richest in Ca and Mg, but these elements decreased with advancing age. Heavy metals (Hg, Pb, Fe) accumulated with age, whereas Se, Mn, and Cr seemed independent of age. CA is manifestly a very useful tool for revealing underlying di-

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mensions in complex dynamic systems and unsuspected relationships among variables. Clearly, the significance of the high Al and Ag contents in the hair of certain members of the population, especially of the very young, needs to be investigated from both physiological and toxicological aspects.

Index Entries: Hair; trace elements; minerals; plasma emission spectroscopy; multivariate analysis; correspondence analysis; minimum spanning tree.

INTRODUCTION

Several studies have attempted to relate the changes in trace element (TE) composition of human hair to factors, such as exposure to pollutants in urban and occupational settings (e.g. lead poisoning, anthrasicosis [1]) or to disease (e.g., diabetes, cancer, arterial hypertension, respiratory disease [2-6; for review 7]). Invariably, the validity of the conclusions of such studies depends on the sensitivity of the assay method, the highly systematic nature of the screening, and an appropriate analysis of the results. It is on this last point that we wish to focus in this study.

There are many advantages to the use of hair as a study material:

1. Hair is considered an excretory organ for TE;
2. It is readily available at virtually no cost and is therefore an ideal screening tool;
3. There are several high-performance, yet routine, assay methods to analyze TE which are based on emission, absorption, or mass spectrometry, or on X-ray emission or fluorescence; and
4. The TE composition of hair reflects a response integrated over several months, i.e., it remains constant for a long time.

However, there are also major problems in interpreting the results of TE analyses of hair, such as:

1. The number of factors that might influence TE status in the general population (e.g., age, sex, ethnic origin, geochemical conditions, diet, beauty care, environmental exposure, and so on);
2. The need to obtain reference values from a large number of "normal" subjects matched for confounding factors. Differences in TE levels are, in fact, often more relevant than absolute TE values;
3. The non-Gaussian TE distributions obtained even after log transformation of the data; and
4. The low informational value of a single TE compared to the analysis of correlations among clusters of TEs.

For all these reasons, it is necessary to apply sound, yet simple, statistical methods to analyze the results of TE studies. Among the methods used so far are unsupervised pattern recognition by Q-type factor analysis (1,8), multivariant factor analysis (9), and various types of discriminant analyses (3,5). To the authors' knowledge, there are no TE studies using a factorial mapping method termed correspondence analysis (CA), which has been successfully employed by several teams to highlight correlations among variables in a variety of scientific disciplines (10–14 and references therein; 15): to mention but three—clinical medicine (16–18), structure–activity studies in pharmacology and toxicology (14,19–21), and agronomy (22). CA achieves appropriate data reduction, filters out the noise within the data set, objectifies correlations among the variables (discrete and/or nondiscrete), and depicts the results of the analysis in the form of factorial biplots or “maps.” Maps are easier to grasp than tables, have a holistic quality, and appeal to visual memory.

The present article describes the CA of the age and sex dependence of 16 TEs in the hair of 500 individuals in the wake of published studies using other approaches (23–28).

EXPERIMENTAL METHODS

Hair was collected from individuals, most of whom lived in the western regions of central France, according to strict instructions from the Laboratoires Patrick Poirier (Esvres-sur-Indre). Lengths of 2–3 cm of occipital hair were cut at different sites from the nape of the neck as close to the scalp as possible and sent in a special envelope to the laboratory. Each hair sample (approx 0.5 g) was accompanied by a completed questionnaire giving data on sex, age, occupation, residence (urban or country), hair care, diet, medication, and lifestyle.

The hair sample was placed in a 20-mL plastic tube, carefully washed in turn with ultrapure demineralized water and acetone (6×10 min), and oven-dried for 48 h at 60°C. A 200–400 mg accurately weighed aliquot of dried hair in a Teflon tube (prewashed with EDTA) was acid-digested in $\frac{1}{3}$ dilute 10 N HNO₃ RP (Carlo Erba, Italy) over 4 h at a temperature that was increased stepwise to 65°C. The tube was then cooled to give a yellow liquid that was made up to 10 mL and stored until analysis.

TE analysis was performed by inductively coupled argon plasma (ICAP) emission spectroscopy (JY 50 Poly Scan Jobin-Yvon, Longjumeau, France) according to the manufacturer's instructions. The spectrometer was equipped with a plasma therm generator operated at 40.68 MHz. Calibration of the ICAP system was accomplished with aqueous TE standards from Merck (Darmstadt, Germany). The top end of the five-point calibration curve corresponded to a value double the maximum recorded in our laboratory for each TE, and the low end to the nitric acid solution

that was used to digest the hair. Each run included several blanks and a control sample made up of a mixture of standard element solutions excluding S, which was run separately because of its high concentration in hair. We thus obtained results for 16 elements expressed in $\mu\text{g/g}$ hair. The detection limits for each element are as follows in ppb : S (60), Hg (17), Se (20), Zn (2), Pb (20), Cd (2), Ni (6), Co (5), Mn (0.5), Fe (2), Cr (3.5), Mg (2), Al (12), Ca (7), Cu (2), Ag (4).

STATISTICAL ANALYSIS

The present study is a retrospective analysis of 500 files randomly taken from among 5000 files collated between 1988 and 1990. Of the 500 files, 481 proved to be complete and to refer to distinct individuals. Elemental data were the means of triplicate determinations.

The statistical method, CA, is described in detail elsewhere (11-14). CA is an appropriate exploratory approach because:

1. It extracts factors and eliminates background noise (i.e., the influence of confounding variables);
2. It is based on χ^2 -metrics, not covariance, and thus compares TE profiles rather than absolute TE values;
3. It reveals the multidimensional latent structure within the data;
4. It does not assume response linearity; and
5. It translates numerical data into easy-to-read maps highlighting clustering (i.e., correlations) among TEs and individuals.

The data were centered around the population mean for each TE in order that each TE may have the same statistical weight. The TEs were projected into the multidimensional space made up by the individuals, and the individuals were projected into the space made up by the TEs. The two clouds of points thus obtained were plotted using principal orthogonal projection axes (factorial axes) calculated by an Eigenvalue-Eigenvector routine. The procedure involves construction of a symmetrical semisquare probability matrix of the χ^2 distances between pairs of TEs or individuals, diagonalization of the matrix, and calculation of the coordinates of all the variables for the factorial axes. Commercial CA software is available from several sources, but we ran a more flexible in-house program adapted for BASIC from FORTRAN ANACOR software.

The output of our CA includes:

1. The mean marginal weights of the variables;
2. Their distances from the center of gravity of the multidimensional system;
3. The distribution of the variance (τ) over the number of factorial axes required to describe the data set (these axes are orthogonal and thus independent);

4. The coordinates of all variables to each factorial axis; and
5. Their absolute (AC) and relative (RC) contributions to these axes.

The coordinates were used to plot single factorial axes or to construct biplots. The factorial axes describe ever-decreasing proportions of the total variance (information content) of the data set. The main factorial biplot or "map" ($\phi_1\phi_2$) reveals the strongest correlations among the variables, the $\phi_3\phi_4$ map weaker, but equally meaningful correlations, and so on. The contributions attribute meaning to the axes. The ACs of the variables describe how well a particular axis represents the variance of the system ($\Sigma ACs = 100\%$) and the RCs describe how a variable is dispersed across all the axes ($\Sigma RCS = 1$). A theoretical index (λ) describes the quality of the factorial representation. A maximum value of 1 for λ means that the two study fields are totally distinct.

In a factorial map, clustering of variables indicates how correlated (or alike) they are. If an individual lies close to a TE, this means that this TE is particularly relevant to this individual (or that the TE is rejected by an individual in an opposing quadrant of the factorial map). Variables near the origin of the axes are unrelated to the others; those in diametrically opposed positions with respect to the origin are anticorrelated. The interpretation of proximity is equivocal only when the variable is well above or below the plane of the 2D plot. This information is given by its ACs and RCs to the factorial axes.

The authors' CA software also includes subroutines for ascending hierarchical classifications and minimum spanning trees (MST) (29), which are applied to the same matrix of χ^2 distances as used for the CA. The aggregation coefficients of Lance and Williams (30) were used to construct the hierarchical classification. Prim's algorithm (31) for an MST gave the shortest distance linking all TEs (or all individuals) into a tree-like network.

RESULTS

Description of the Study Population

The study population consisted of 346 women (72%) and 135 men (28%) whose ages ranged from 2–83 yr. The age distribution, regardless of sex, was Gaussian with a maximum between 35 and 45 yr. Only 3.7% of the population were children under 10 yr of age. The sex ratio was fairly constant throughout the age groups (male-to-female ratio 1 to 2), but with a trend toward a higher percentage of males among the under 20 yr old (45%).

The files held information on many factors that could influence TE concentrations:

1. Habitat: Residence was fairly evenly distributed among large towns (23%), small towns (38%), and country (39%);
2. Hair care: 38% of the women used dyes, 38% permanents, and 15% both. The TE most often present in these products is Zn;
3. Smoking: Heavy smoking (≥ 20 cigaret/d) reputedly increases body Cd and Cu; 17% were smokers and 4% heavy smokers;
4. Amalgam dental fillings: During the year before TE analysis, 36% of the population had had new dental fillings;
5. Pool water: 12% regularly attended swimming pools. Cu can be present in the products used to purify the water;
6. Diet: 43% regularly ate fish and seafoods, which can be enriched in Se, but can also concentrate toxic metals, such as Hg and Cd. However, consumption was high in only 4% of the population. A majority of individuals (60%) regularly drank different brands of mineral water;
7. Medication: Several home therapies and ethical drugs might represent potential sources of TEs and had been ingested on occasion by 7.5% of individuals.

Mean Hair TE Levels in the Study Population

Fifteen of the 16 elements under study can be divided into three mutually nonexclusive categories: essential macroelements (S, Ca, Mg), biocatalysts (Fe, Cu, Zn, Se, Co, Cr, Ni, Mn), and toxic heavy metals (Cd, Hg, Pb, Al). Ag is in a class of its own. The means, standard deviations, and ranges we recorded for each element are given in Table 1. Although "normal" values of hair metal levels are difficult to set because of differences in populations and assay technique (washing and analysis), our means in Table 1 were consistent with the reference ranges derived by Iyengar and Woittiez (32) from a large collection of literature reports (*see also* Di Pietro et al. [25]).

S was by far the most abundant element (94.6%), since it is a constituent (disulfide bonds) of the keratin structure of hair. Next in ranking order were five divalent cations (Ca [4.6%], Zn, Mg, Cu, Fe) that play important roles either as "cofactors" in physiological and metabolic processes or as coordination metals in the maintenance of protein conformations. The most abundant heavy metals were Pb (mean 6.6 ppm) and Al (high upper limit of 65 ppm), which are very common pollutants (e.g., car fumes, batteries, cans, deodorants, and so on), followed by Ni (mean 1.2 ppm) and four TEs with similar mean levels (Cd, Co, Mn, and Ag [mean 0.3–0.4 ppm]). Mean Cr, Hg, and Se levels were about 0.2 ppm.

Distribution of TEs in Hair

The distributions (not shown) of only four elements—those with very high (S, Zn) or very low (Hg and Se) mean concentrations—were

Table 1
Element Contents in Hair^a

	Study population				Reference range (32)
	Mean	SD	Mini	Maxi	
S	✓ 32087	6677	4650	57650	NA ^b
Ca	✓ 1568	1450	40.40	7250	NA
Zn	✓ 133 ✓	39.63	21.20	577	124-320
Mg	✓ 70.43	77.8	2.0	510	NA
Cu	✓ 22.83	27.79	2.05	445	6.8 -39
Fe	✓ 9.95 ✓	7.73	2.86	86.4	13 -177
Pb	6.59 ↑	3.83	0.10	28.1	4.2 -52
Al	✓ 1.67	4.63	0.001	65.0	NA
Ni	1.17 ↑	0.84	0.001	7.57	0.002-1.25
Cd	0.41 ↑	0.44	0.001	5.80	0.35-2.43
Co	✓ 0.36	0.29	0.001	3.28	0.004-0.5
Mn	✓ 0.36 ↓	0.60	0.001	8.31	0.2-4.4
Ag	✓ 0.34	0.67	0.001	7.21	NA
Hg	✓ 0.24	0.06	0.04	0.74	0.5-12.2
Cr	✓ 0.24	0.34	0.001	5.64	0.06-4.1
Se	0.20 ↓	0.09	0.02	1.14	0.2-1.4

^aValues are given in µg/g (or ppm).

^bNA = not available in ref. (32).

alcohol

truly Gaussian. The remaining distributions were skewed in a variety of ways: peak shifted toward low concentrations (Mn, Fe, Mg, Ca, Cu, Cd), extra peak at very low concentrations (Pb), a fairly flat distribution (Al, Ag), several peaks (Ni), and a highly irregular pattern (Cr, Co). Because of this skewness often uneliminated by log transformation, the data were centered around the means and converted into normalized profiles using χ^2 metrics.

Correlations Among TEs

Figure 1 shows a hierarchical ascending classification (A) and a minimum spanning tree (MST) (B) of the normalized profiles obtained for each TE (the profile is given by the percent participation of each individual to a TE; total of 16 profiles). In panel A, the TEs below the lowest node heights are correlated, whereas in panel B, those separated by the shortest distance are most similar. The two classification methods yield

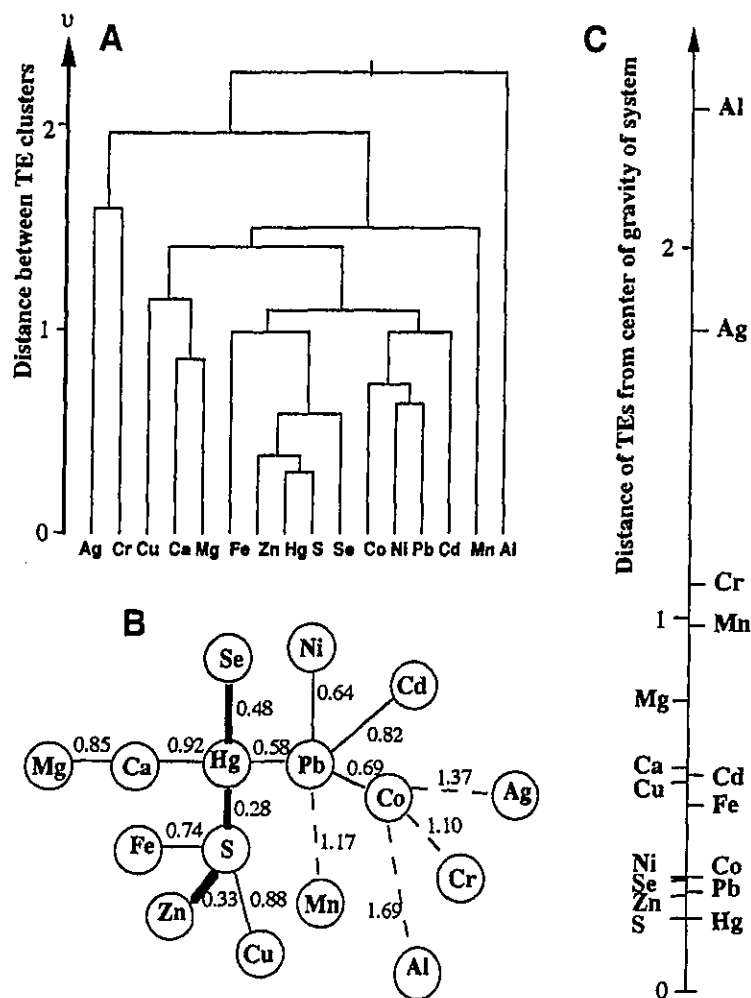


Fig. 1. Correlations among elements. (A) Hierarchical ascending classification. (B) MST giving distances between TEs. (C) Distance of the TEs from the center of gravity of the multidimensional system. (All calculations were performed on the χ^2 -distance matrix.)

coherent results. Hg and S are highly correlated and closely linked to Zn and Se; Ni, Pb, Co, and Cd form a foursome; Ca and Mg are correlated; Al, Mn, Ag, and Cr are outliers. The only major discrepancy is in the position of Cu, which is linked to the alkali group 2A elements, Mg and Ca, in the ascending hierarchical classification and to S in the minimum spanning tree.

The calculation of the χ^2 distance of each TE from the center of gravity of the multidimensional space (Fig. 1C) distinguishes TEs with highly atypical behavior (e.g., Al, Ag) from the TEs that are the most stable within this particular study population (S, Hg).

Associations Among Individuals

The authors also applied the MST algorithm to the normalized TE profiles for each individual (the profile is given by the percent participation of each TE to an individual—a total of 481 profiles) and obtained Fig. 2. Clearly, sex is a discriminating factor with regard to hair TE content, since most men, highlighted by a thick contour, are clustered within the center branches. Women are separated into two main subgroups, one at the top and one at the bottom of the tree. The hair of individuals at branch tips is least typical and, in Fig. 2, individuals whose hair presented exceptionally high contents for a given TE are shaded. The hair of the top female subgroup is characterized chiefly by high Mg, Ca, and also Cu levels, whereas that of the bottom subgroup tends to be richer in Ag, Al, and Mn. Several men are dispersed within this lower subgroup and lie at, or very close to, the branch tips (*see* Mn and Al). Cr and Cd seem to be more closely correlated with men's than women's hair.

No specific patterns emerged when we identified age groups in the MST or when we separated smokers from nonsmokers, although smokers tended to be at branch tips.

Correlating TEs and Individuals by CA

The above classification methods can only be applied to the two study fields, TEs and individuals, independently. To depict TEs and individuals simultaneously, we performed a CA, which reduces the matrix of correlations to a few main factors that retain as much information as possible. CA yields factorial maps of the two study fields that are totally superimposable (*see* panels A and B, and panels C and D of Fig. 3). The $\phi_1\phi_2$ factorial map depicts the most discriminating 43.6% of the information (25.0% for ϕ_1 and 18.6% for ϕ_2) and reveals that the least typical profiles (the most excentric) are those of Al and Ag. A few people (*see* panel B) have either very high (or very low) Al or Ag levels in their hair; others have both untypical Al and Ag levels as shown by their intermediate position between these two TEs. In the presence of Al, there is a tendency for Cr to be present; in the presence of Ag, there is a tendency for Mn to be present. Most of the TEs are very near to the origin of the axes and do not intervene in the discrimination described by this factorial map.

Panels C and D of Fig. 3 depict an extra 22% of independent information (11.9% for ϕ_3 and 9.9% for ϕ_4). They illustrate the correlations among TEs and individuals that underly the highly discriminatory influence of Ag and Al observed in the main factorial map. In panel 3C, Al is part of a central pool of TEs that does not characterize any particular set of individuals, whereas Ag (slightly excentric) displays a facet different from the one it has in panel 3A. The most excentric TEs are those that define particular subgroups of individuals, which we shall identify by working anticlockwise from 3 o'clock. There is a subgroup of individuals with high Mg and Ca

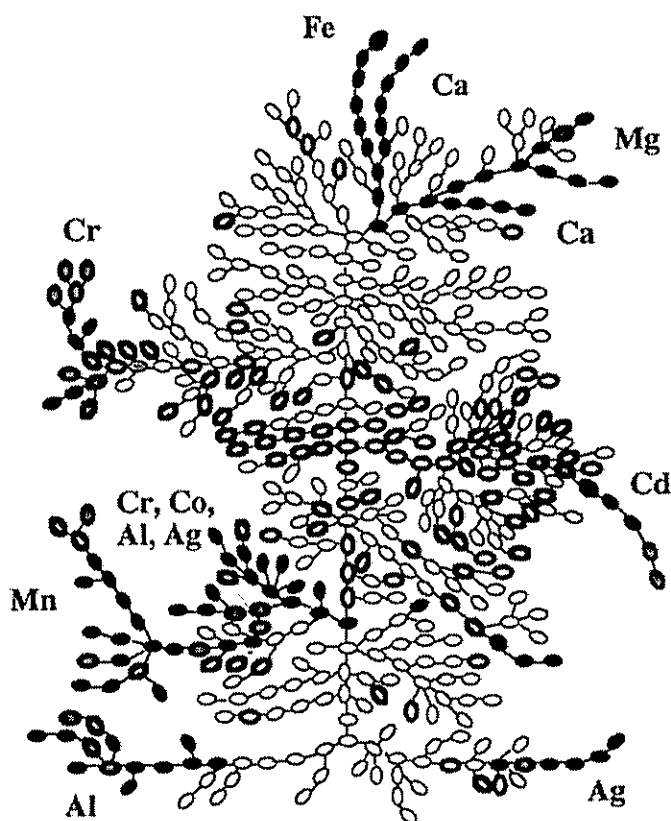


Fig. 2. Minimum spanning tree linking all 481 individuals into the shortest distance network based on the similarities in their TE profiles. The ovals with bold outlines refer to men; the others to women. The shaded ovals refer to individuals with exceptionally high values for certain TE concentrations.

levels in their hair along the ϕ_3 axis (top right-hand quadrant); relatively few, rather isolated, individuals with high Cr levels (top left-hand quadrant); a throng of people in whom the influence of Fe and of the toxic elements, Cd and Pb, outweighs that of the elements in the diametrically opposed position (Ca and Mg); a fairly large subgroup of individuals in whose hair Mn, Ag, and Cu predominate.

The fifth factorial axis holds 8.6% of independent information (not shown) and just over a quarter (26%) resides in the sixth to fifteenth axes. Most of this underlying information no longer represents general trends, but background noise.

Influence of Sex

The factorial plots reveal how each individual lies in relation to all his or her fellow men/women under study and what the governing TE

forces are that determine his/her position. No preliminary hypothesis was formulated. An individual by individual analysis is fastidious and only necessary to place a specific individual in the context of the overall population. Instead, as a follow-up to the MST of Fig. 2, the authors calculated the "barycenters" (centers of gravity) of the male and female populations, and introduced them into Fig. 3D used as a mathematical model (14,21). As suggested by the MST, TEs in the hair of this population of individuals tend to display contrasting patterns according to sex, the barycenters lying on either side of the origin of the third factorial axis.

To probe this difference between the sexes, the authors performed a simplified CA based this time not on the actual TE values recorded for the hair of each person, but on the number of people whose hair TE contents fell within the bounds of five ranges defined as follows: The hair TE content was more than 2 standard deviations (SD) below the mean (group 1); 1-2 SD below the mean (group 2); 1 SD below the mean (group 3); 1 SD above the mean (group 4); more than 1 SD above the mean (group 5). The projections of the TEs on the ϕ_1 axis of this CA are shown in Fig. 4, which reveals that exceptionally high contents of essential elements with a physiological role (Ca, Mg, Cu, Co, Ni, and Zn) as well as of Ag characterize women's hair. The highest levels of these elements (group 5) fall clearly on the female side. On the other hand, Fe and Pb are more specific to men's hair.

Influence of Age

A similar analysis was undertaken with respect to age. Individuals were separated into nine age groups (decades). The data were normalized twice, once by centering around 100 and then by dividing by the mean of each group. This procedure evens out the high dispersion within the population in a way that discriminant analysis cannot do. The plot of the first two axes obtained in the CA is shown in Fig. 5. The most discriminatory influence is that of Ni, which has an overwhelming absolute contribution (not given) to the principal ϕ_1 axis. In fact, the position of the above 80-yr-old group has to be interpreted with care, since this group was composed of only two individuals with hair samples showing exceptionally high Ni and Cd contents.

In Fig. 5, the age groups follow a typical Guttman curve (33) along the ϕ_2 axis, which is clearly accounted for by age. The order of the projections of the decades onto this axis is coherent (from right to left) apart from a minor inversion between two decades (51-60 and 61-70 yr). Figure 5 prompts the following comments:

1. The characteristic feature of the hair of the under 10 age group, which was small, is the presence of high relative Ag, and especially Al, contents;

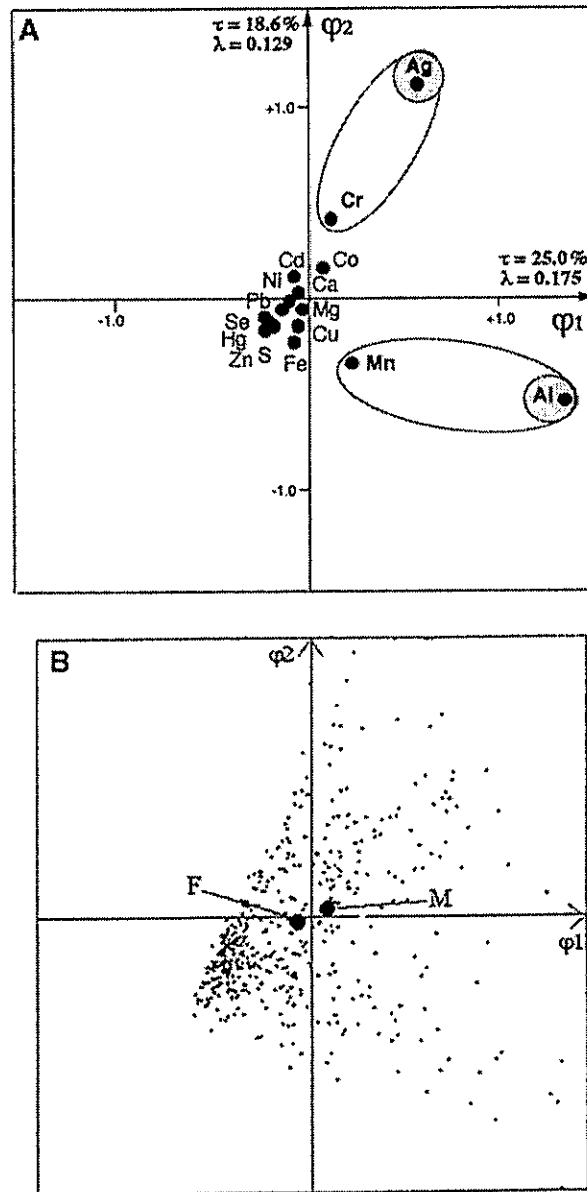


Fig. 3. CA of the matrix (16 TEs \times 481 individuals). (A) $\phi_1\phi_2$ factorial plot of TEs; (B) $\phi_1\phi_2$ plot of individuals superimposable upon panel A; τ , variance of factorial axis; λ , theoretical index describing data dispersion. Panel C gives the barycenters of the male (M) and female (F) populations.

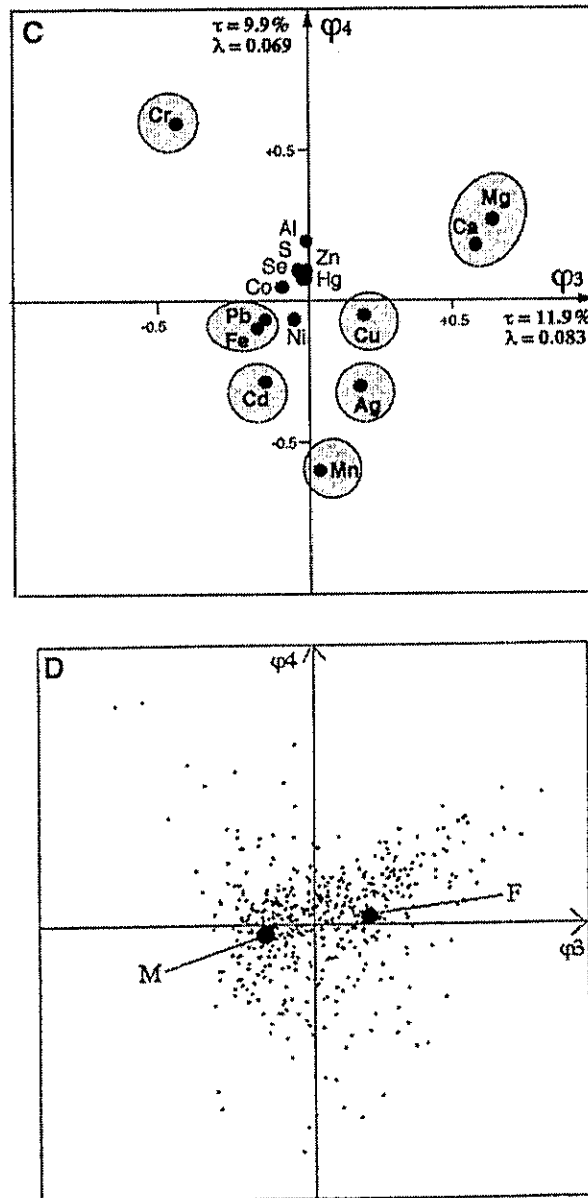


Fig. 3. (continued) (C) $\phi_3\phi_4$ plot of TEs; (D) superimposable $\phi_3\phi_4$ plot of individuals. (τ = variance of factorial axis; λ = theoretical index describing data dispersion). Panel D gives the barycenters of the male (M) and female (F) populations.

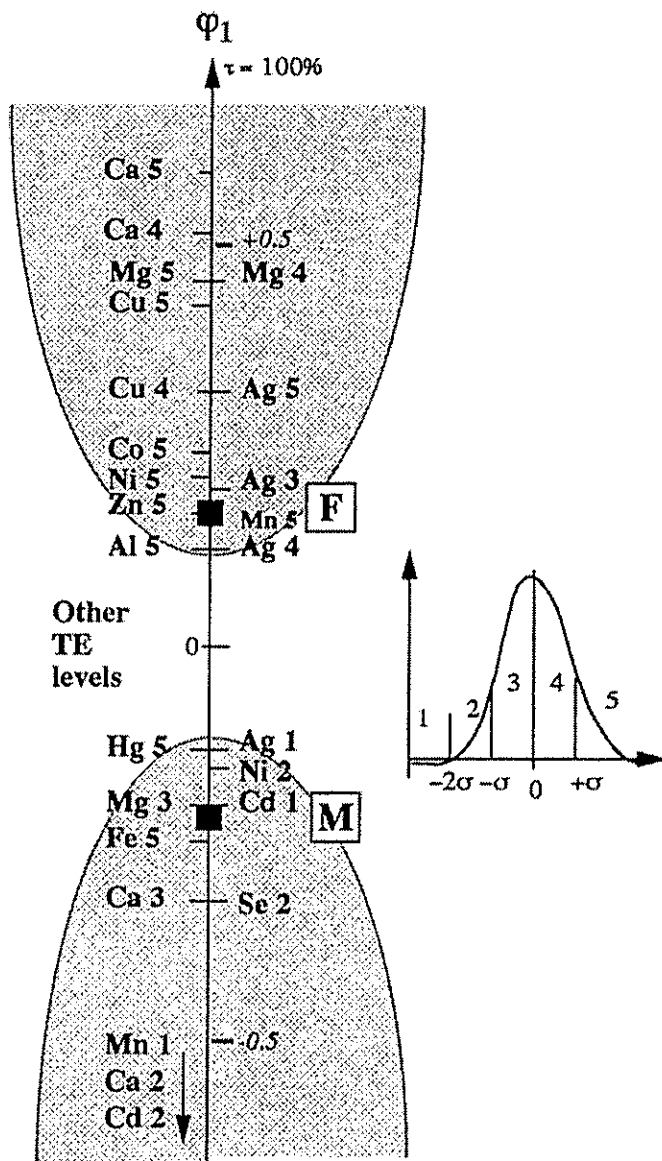


Fig. 4. CA of the matrix (5 ranges of 16 TEs \times sex). Only the projections of the TE ranges (levels 1-5 as shown in insert) and of sex (M, F) onto the main ϕ_1 axis are shown.

2. The hair of individuals in maturity is rich in Ca and Mg, which are correlated and decrease with advancing age (distant from old age);
3. The Cu and Co (vitamin B₁₂) associated with the hair of individuals before middle age seem to give way to a predominance of Zn in later years.

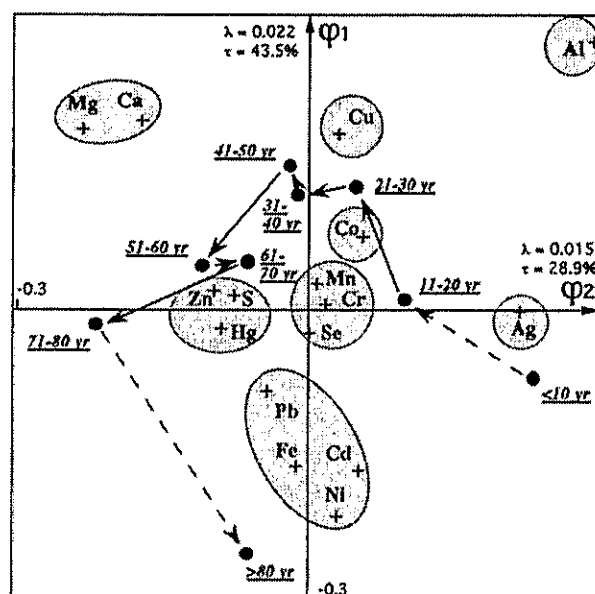


Fig. 5. CA of the matrix (16 TEs \times 9 age categories).

4. Heavy metals (Ni, Cd, Pb, Hg), located in the lower left-hand quadrant, accumulate in hair with age.
5. Se, Cr, and Mn lie near of the origin of the axes and are thus independent of age in this population; and
6. The part played by the structural element, S, increases with age at the expense of other TEs.

CONCLUSIONS

Several teams have studied the influence of sex and age on TE levels in human hair (23–26), especially in young children (27,28), but nearly all have relied on classical probabilist statistics and traditional tables and graphs to arrive at their conclusions. None of these studies offers a visual, objective description of their conclusions that is based on a statistical analysis that does not preselect an independent variable and that concentrates the most discriminatory information, after elimination of background noise, into all-embracing displays.

The conclusions of our correspondence analyses confirm many published observations. The correlation between high Al content and early childhood is consistent with Paschal et al.'s (26) observation that Al in hair decreases with age. Although the authors have no explanation for the highly discriminatory nature of either Al or Ag in the hair of this population, it should be noted that Al is a highly prevalent metal in the atmosphere (34), and that infants are considered to be particularly sus-

ceptible to Al accumulation and toxicity (35). Studies on Al distribution in the brains of growing, mature, and aging rats have detected the highest Al concentrations in young rats (36).

Sex proved to be a highly discriminatory factor. In an MST analysis, two female subpopulations with slightly different TE profiles were identified; their hair TE profiles differed from those of men. According to the CA, the main difference was a higher relative content of certain essential TEs, in particular of Ca, Mg, Zn and Cu, in women's hair. In a small study, Deeming and Weber (23) found higher Zn in women's hair and Shuhmacher et al (27) detected higher Zn and Cu levels in the hair of young girls. A discrimination due to Ca, Mg, and Cu has also been noted by Perrone et al. (28).

Age was also a discriminatory factor. We confirmed the Ca and Mg peak in middle age, the decline in Zn after middle age, and the association of Cu with hair from the young (24,27,28). In animal studies, a deficiency in Cu leads to a depigmentation of hair involving the enzyme tyrosinase and, interestingly, Cu is a cofactor of tyrosinase. Ni was most closely associated with the oldest age group. According to observations relating natural hair color and metals, Ni is preferentially present in non-pigmented white hair, which is characteristic of the aged. Overall, the TE profiles did not seem to evolve linearly with age, but according to specific life stages (youth, maturity, and old age).

The authors' analyses have demonstrated the power of CA in extracting the underlying dimensions from a highly complex system where the variables measured (TE levels) are influenced by many external factors (environment, diet, and so forth). They chose CA because it uses χ^2 metrics and not Euclidian metrics for the reduction of dimensionality, and can therefore manage both discrete and categorical variables (e.g., TE levels and age groups), analyze linear and nonlinear relationships (it makes no assumptions about normal distributions), and depict row and column variables on a single factorial map (e.g., TE levels, individuals, and/or age groups).

Of course, there are caveats to this, as with other studies on hair TE content. Although CA minimizes the influence of confounding variables, the differential use of hair care products (dyes, tints, permanents) by the sexes could account for part of the sex difference and also for the two distinct female populations in the MST. Future CA studies should include this variable in the analysis. Furthermore, TE excretion profiles may be a function not only of TE levels, but also of hair follicle activity, which varies with sex and age. This aspect needs consideration in the interpretation of the significance of TE levels not so much in toxicity studies as in clinical studies.

In conclusion, the demonstration of the feasibility and high performance of CA under the most difficult conditions of very high background noise now incites us to use—and recommend the use of—CA for the analysis of TEs under specific environmental and medical situations.

REFERENCES

1. B. Li, S. Jiang, S. Xu, and J. Shen, Q-type stepwise informational cluster analysis for the elements of silicotics, *Jisuanji Yu Yingyong huaxue* **12**, 285–288 (1995).
2. M. W. Tsang, H. S. Lau, and P. L. Leung, Trace element study by use of X-ray fluorescence spectrometry in Chinese diabetes patients, *Int. Congr. Ser. 1100 (Diabetes 1994)* 1125–1127 (1995).
3. Y. Sha, P. Lin, P. Zhang, Y. Dong, Z. Yang, Y. Wu, J. Li, D. Liu, Y. Wang, and D. Zhang, Diagnostic significance of elements in hair of people with esophageal cancer by computerized pattern recognition and PIXE, *Hejishu* **17**, 164–167 (1994).
4. C. K. Man, Y. H. Zheng, and P. K. Mak, Trace element profiles in the hair of nasopharyngeal carcinoma (NPC) patients, *J. Radioanal. Nucl. Chem.* **212**, 151–160 (1996).
5. I. Shtangeeva and V. Kulikov, Study of chemical element behavior in health and disease by means of neutron activation analysis and multivariate statistics, *Nutrition (Suppl.)* **11**, 592–594 (1995).
6. A. Sonnevile and F. Poirier-Duchêne. Les hypersensibilités non spécifiques par instabilité membranaire des cellules cibles ont-elles pour support un déséquilibre en oligo-éléments? *Allergie Immunol.* **22**, no. 1 (1990).
7. A. Chatt and S. A. Katz, *Hair Analysis: Applications in the Biomedical and Environmental Sciences*, VCH, New York (1988).
8. V. A. Batzevich, Hair trace element analysis in human ecology studies, *Sci. Total Environ.* **164**, 89–98 (1995).
9. R. E. Jervis, B. T. Kua, and G. Hercz, Trace elements in kidney dialysis patients by INAA, *Biol. Trace Element Res.* **43–45**, 335–342 (1994).
10. J. P. Benzécri, *L'Analyse des Données. Tome I: La Taxinomie. Tome II: L'Analyse des Correspondances*, 1st ed., Bordas/Dunod, Paris (1973).
11. M. J. Greenacre, *Theory and Applications of Correspondence Analysis*, Academic, New York (1984).
12. L. G. Underhill and M. Peisach, Correspondence analysis and its application in multi-elemental trace analysis, *J. Trace Microprobe Techn.* **3**, 41–65 (1985).
13. M. Jambu, *Exploratory and Multivariate Data Analysis*, Academic, New York (1991).
14. J. C. Doré and T. Ojasoo, Molecular taxonomy by correspondence factorial analysis (CFA), in *Advanced Computer-Assisted Techniques in Drug Discovery*, vol. 3, *Methods and Principles in Medicinal Chemistry*, H. van de Waterbeemd, ed., VCH, Weinheim, pp. 190–227 (1994).
15. J. Blasius and M. J. Greenacre, eds., *Visualization of Categorical Data*, Academic, New York, in press.
16. G. Sandor, P. Lenoir, and M. Kerbaol, Une étude en ordinateur des corrélations entre les modifications des protéines sériques en pathologie humaine, *C. R. Acad. Sci.: Paris* **272**, 331–334 (1971).
17. J. C. Doré, J. Lacroix, R. Lacroix, and T. Etienne, Multivariate analysis of clinical chemistry standard profiles and biological typing of a psychotic population, *J. Pharm. Clin.* **10**, 257–263 (1991).
18. T. Ojasoo, J. Fiet, J. P. Raynaud, and J. C. Doré, A multivariate approach to the description of patient populations—Example of the analysis of the hormone profiles of patients with advanced prostate cancer, *J. Steroid Biochem. Mol. Biol.* **46**, 183–193 (1993).
19. C. Lugnier, J. C. Doré, and C. Viel, Inhibition of cyclic nucleotide phosphodiesterases from bovine aorta by papaverine analogues: a multivariate approach. *Eur. J. Med. Chem.* **29**, 723–734 (1994).
20. J. Gilbert, J. C. Doré, E. Bignon, M. Pons, and T. Ojasoo, Study of the effects of basic di- and tri-phenyl derivatives on malignant cell proliferation. An example of the application of correspondence factor analysis to structure-activity relationships, *Quant. Struct. Act. Rel.* **13**, 262–274 (1994).
21. T. Ojasoo, J. P. Raynaud, and J. C. Doré, Correspondence factor analysis of steroid libraries, *Steroids* **60**, 458–469 (1995).
22. I. P. de A. Miranda, V. Fortin, J. C. Doré, L. Derouet, F. Poirier, M. C. Carbonnier-Jarreau, M. L. Absy, M. T. Cerceau-Larrival, and C. Viel, Analyse multiparamétrique

- de la composition minérale des pollens d'une population de *Bactris gasipaes* (Arecaceae), *Grana* **34**, 434-440 (1995).
23. S. B. Deeming and C. W. Weber, Hair analysis of trace minerals in human subjects as influenced by age, sex, and contraceptive drugs, *Am. J. Clin. Nutr.* **31**, 1175-1180 (1978).
 24. G. F. Gordon, Sex and age related differences in trace element concentrations in hair, *Sci. Total Environ.* **42**, 133-147 (1985).
 25. E. S. Di Pietro, D. L. Phillips, D. C. Paschal, and J. W. Neese, Determination of trace elements in human hair, *Biol. Trace Element Res.* **22**, 83-100 (1989).
 26. D. C. Paschal, E. S. Di Pietro, D. L. Phillips, and E. W. Gunter, Age dependence of metals in hair in a selected U.S. population, *Environ. Res.* **48**, 17-28 (1989).
 27. M. Schumacher, J. L. Domingo, J. M. Llobet, J. Corbella, and J. B. Marti, Chromium, copper, and zinc concentrations in hair of school children from Southern Catalonia Spain, *Trace Elements Med.* **10**, 21-26 (1993).
 28. L. Perrone, R. Moro, M. Caroli, R. di Toro, and G. Gialanella, Trace elements in hair of healthy children sampled by age and sex, *Biol. Trace Element Res.* **51**, 71-76 (1996).
 29. J. C. Gower and G. J. S. Ross, Minimum spanning trees and single linkage cluster analysis, *Appl. Stat.* **18**, 54-64 (1969).
 30. G. N. Lance and W. T. Williams, Computer programs for hierarchical polythetic classification ("similarity analyses"), *Computer J.* **9**, 60-64 (1966).
 31. R. C. Prim, Shortest connection networks and some generalisations, *Bell Syst. Technol. J.* **36**, 1389-1401 (1957).
 32. G. V. Iyengar and J. Woittiez, Trace elements in human clinical specimens: evaluation of literature data to identify reference values, *Clin. Chem.* **34**, 474-481 (1988).
 33. L. Guttman, The principal components of scale analysis, in *Measurement and Prediction*, S. A. Stouffer et al., eds., University Press, Princeton (1950).
 34. H. Ozkaynak, J. Xue, J. Spengler, L. Wallace, E. Pellizzari, and P. Jenkins, Personal exposure to airborne particles and metals: results from the Particle TEAM study in Riverside, California, *J. Expo. Anal. Environ. Epidemiol.* **6**, 57-78 (1996).
 35. T. P. Flaten, A. C. Alfrey, J. D. Birchall, J. Savory, and R. A. Yokel, Status and future concerns of clinical and environmental aluminum toxicology, *J. Toxicol. Environ. Health*, **48**, 527-541 (1996).
 36. J. L. Domingo, J. Llorens, D. J. Sanchez, M. Gomez, J. M. Llobet, and J. Corbella, Age-related effects of aluminum ingestion on brain aluminum accumulation and behavior in rats, *Life Sci.* **58**, 1387-1395 (1996).

Trace element determination in hair by synchrotron x-ray fluorescence analysis: application to the hair of Napoleon I

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Several hairs belonging to **Napoleon I** were analyzed through synchrotron induced x-ray fluorescence analysis. This study revealed the presence of many toxic elements, often far above the lethal content, and with important concentration variations from one hair to another and even from one place to another in a same sample. This does not seem to favor the simple hypothesis of poisoning through arsenic ingestion. Copyright © 2005 John Wiley & Sons, Ltd.

INTRODUCTION

Hair has attracted interest as an alternative biological medium for forensic toxicology. A question is whether or not intense or chronic intoxication by mineral poisons can be demonstrated from such a matrix. For more than 100 years, it was thought that owing to its regular growth with time and the apparent stability of this medium compared with other biological matrices, the mineral content along a hair could be used as a 'fingerprint' for the identification of a person. Unfortunately, the lack of reliability in the determination of such profiles forced this idea to be abandoned.

In this work, our main objective was to use a multielemental and truly non-destructive analytical method in order to preserve the sample's integrity for further investigations, capable of identifying (and if possible quantifying) trace elements (at the mg kg^{-1} level or lower) in very small samples (of the order of a few micrograms). We proposed to use z-ray fluorescence analysis induced by synchrotron radiation (SXRF). At the same time, we wished to examine the potential reliability of hair as evidence for arsenic intoxication in a forensic or historical context. This was achieved by analyzing a set of hairs attributed to Napoleon Bonaparte, the arsenic intoxication of whom has been the object of investigation for over 40 years, with a number of assertions, more or less argumentative, founded on several technically serious analyses, all of them reporting variable and sometimes important arsenic concentrations.^{1–7}

Ten hairs out of 19 were taken from several locks cut at different periods ranging from 1805 to the day after the death of Napoleon I on 6 May 1821. Three hairs from his sisters were also analyzed. They came from two different collections (P. J. Chalençon and J. Tranié) and, as far as we know, their origin seems historically well established.

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EXPERIMENTAL

Choice of SXRF

Because of its sensitivity, XRF analysis is a widespread analytical method for trace element studies. Furthermore, the fact that it can be non-destructive is of interest as a first step in an investigation and was one of the deciding factors in our choice for the study of such precious samples. However, detection limits are typically in the mg kg^{-1} range for samples of about 1 g, which would rule out the possibility of analyzing a single hair.

For about 30 years, synchrotron radiation (SR) emitted from bending magnets of powerful electron accelerators has been used as an excellent x-ray source. It can be used to excite x-ray fluorescence and this approach leads to interesting features and especially very low detection limits on very small samples. Typically the latter lie in the 0.1 mg kg^{-1} range for samples of no more than a few micrograms. This means that 1 mm of hair is sufficient to investigate trace elements.

Experimental set-up

We used the XRF set-up D15 at LURE (Orsay).⁸ The white beam from the accelerator passes first through a pair of horizontal and vertical slits and then into a monochromator made of two highly oriented mosaic pyrolytic graphite crystals. The first is a plane crystal that chooses energies between 9 and 23 keV. The second is curved and concentrates the monochromatic beam in the horizontal direction to a spot of about 1 mm diameter. It is important to note that the photon distribution in the beam spot is not homogeneous but has a more or less Gaussian aspect. Note also that the beam intensity decreases with time owing to the finite half-life of the electron bunch stored in the ring. Furthermore, from one shift to another the beam intensity can be very different owing to the exact size of the slits and the parameters of the electron orbit.

Before analysis, each hair was decontaminated following IEAE recommendations,⁹ that is, four successive 5 min washings with 2 ml of acetone followed by two washing with water with a final 5 min washing with acetone under ultrasound. Water and all reagents were used for blank determinations and all washing wastes were collected and evaporated for arsenic determination by atomic absorption spectroscopy. We found arsenic concentrations ranging between 140 and 1280 mg kg⁻¹.¹⁰

Each hair is maintained tight with adhesive tape at both ends on a strong cardboard frame. Typically five hairs are mounted on the same frame with about a 5 mm spacing between each. The frame is then fixed on a three-stage translator (X, Y and Z), driven by remote-controlled stepper motors with 1 μm steps. This allows setting of the sample in the beam and a microscope equipped with a video camera is used to view its exact position from the control room.

The fluorescence spectrum is recorded with an Si(Li) detector of 150 eV energy resolution. It is set in the horizontal plane and at 90° from the incident beam to minimize scattering. Signals are processed through a Tennelec 744 amplifier and stored in the memory of a Nucleus PCA3 acquisition card installed in a PC. A special program allows automatic placing of the sample at a given position whose coordinates are stored in a file, start the acquisition for a preset time, store the result and then start this cycle again for every position of the file.

Each spectrum is then processed with a deconvolution program.¹¹ This program gives the identification of each peak, together with its net area after correction for air absorption and detector efficiency.

For protection against irradiation, the monochromator, sample holder, microscope and detector are in a special hutch that cannot be entered when the beam is on. Visualization and displacements of the sample, as well as data acquisition and reduction are done in a separate control-room, next to the experimental hutch.

Experimental conditions

Excitation energy

A first series of measurements was performed under 14.6 keV photon excitation. This immediately revealed the presence of arsenic as well as lead in quite unusual quantities. These two elements present a very serious spectral interference in XRF since the As K_α line at 10.54 keV overlaps with the L_α line of Pb at 10.55 keV. Although our deconvolution program can in principle easily get around this difficulty by taking into account the K_β and L_β line of these elements we preferred to completely eliminate the problem so that no doubt could be left concerning the arsenic identification. This is possible with SR since the monochromator can be continuously tuned to adjust the excitation energy. As the K absorption edge of arsenic is at 11.718 keV whereas the L₃ edge of lead is at 13.035, choosing 12.4 keV photons will excite most favorably arsenic since we are just over its K edge and not excite lead at all.

Quantitative aspects

Theory. In principle XRF can be considered as a quantitative analytical method. In practice things are not always as simple.

For a homogenous slab of thickness l , the number of K α lines of element Z, that is, the peak area recorded on the fluorescence spectrum, $N_{(K\alpha,Z)}$, can be expressed by

$$N_{(K\alpha,Z)} = I_0 C_Z \mu_{(K\alpha,Z,E_0)} \frac{\rho l}{\sin \alpha} G_Z A_{(M,Z,E_0)}$$

where

I_0 = number of incident photons of energy E_0 striking the sample;

$\rho l / \sin \alpha$ = mass per unit area of the irradiated part of the sample;

C_Z = concentration of element Z in g g⁻¹;

G_Z = geometrical factor including absorption through air and detector efficiency for element Z;

$\mu_{(K\alpha,Z,E_0)}$ = mass absorption coefficient for K α fluorescence, defined as

$$\mu_{(K\alpha,Z,E_0)} = \left(\frac{J_K - 1}{J_K} \right)_Z (\omega_K)_Z \left(\frac{I_{K\alpha}}{I_{K\alpha} + I_{K\beta}} \right)_Z \mu_{(\Phi,Z,E_0)}$$

where

J_K = K absorption jump ratio;

ω_K = K-shell fluorescence yield;

K α /(K α + K β) = relative K α emission rate;

$\mu_{(\Phi,Z,E_0)}$ = photoelectric mass absorption coefficient for element Z at energy E_0 ;

$A_{(M,Z,E_0)}$ = auto absorption correction factor for element Z in matrix M at energy E_0 :

$$A_{(M,Z,E_0)} = \frac{1 - e^{-[\mu_{(M,E_0)} + \mu_{(M,EZ)}]} \frac{\rho l}{\sin \alpha}}{[\mu_{(M,E_0)} + \mu_{(M,EZ)}] \frac{\rho l}{\sin \alpha}}$$

where

$\mu_{(M,E_0)}$ = matrix mass absorption coefficient for energy E_0 ;

$\mu_{(M,EZ)}$ = matrix mass absorption coefficient for energy of the K α line of element Z.

For elements Au, Hg, Pb and Bi, the analysis is made through the L α line. We then have a similar expression in which we take into account the various Coster-Kronig coefficients, depending on the relative value of the incident energy with respect to the three L absorption edges.

For these same elements and also for As, whose x-ray energy is >10 keV and owing to the size and composition of the sample, the auto-absorption correction factor is very nearly equal to unity so that the sample can be considered as a 'thin target'.

In our case, the sample can be considered like a cylinder of about 60 μm diameter and 5–10 cm long while the beam spot is a 1 mm diameter disk with a nearly Gaussian distribution. Then, depending on the exact position of the hair in the beam, the number of photons striking the sample and the mass of irradiated hair can vary considerably. Considering the number of analyses (we measured more than 1000 spots), an exact positioning for each measurement is impossible.

We then decided to use an internal standard to monitor the beam and sulfur, which is a constituent of keratin,

seemed to us to be a satisfactory indicator of the exact irradiation conditions (sample mass and beam intensity). Then, after correction for absorption and detector efficiency, we introduced the normalized peak area $\Sigma_{(K\alpha,Z)}$, defined as

$$\Sigma_{(K\alpha,Z)} = \frac{N_{(K\alpha,Z)} G_S}{G_Z N_{(K\alpha,S)}}$$

$$\Sigma_{(K\alpha,Z)} = \frac{C_Z \mu_{(K\alpha,Z,E_0)} A_{(M,Z,E_0)}}{C_S \mu_{(K\alpha,S,E_0)} A_{(M,S,E_0)}}$$

In principle, concentrations could be extracted for all elements from this last equation, but owing to the errors on the parameters (some of them exponential) and the approximate equation used for $A_{(M,Z,E_0)}$, we preferred to use an external standard to enhance the accuracy for arsenide concentration, which was our main objective. We then used a hair that we had left 24 h in an arsenic solution. After uniform arsenic fixation verification, this element was quantified on one of the hair by atomic absorption spectroscopy. Then arsenic concentration in the sample, $C_{(As,Spl)}$, is obtained directly from the ratio of the normalized arsenide peak area of the sample, $\Sigma_{(As,Spl)}$, and the standards, $\Sigma_{(As,Std)}$:

$$C_{(As,Spl)} = C_{(As,Std)} \frac{\Sigma_{(As,Spl)}}{\Sigma_{(As,Std)}} = K_{(As,Std)} \Sigma_{(As,Spl)}$$

where $K_{(As,Std)} = C_{(As,Std)}/\Sigma_{(As,Std)}$ is a constant for a given standard and excitation energy. We have verified on different standards and in different experiments that this assertion was exact.

The concentration of other elements can then be calculated from this estimate of the arsenic content from the following equation:

$$C_Z = K_{(As,Std)} \Sigma_{(K\alpha Z)} \frac{\mu_{(K\alpha,As,E_0)} A_{(M,As,E_0)}}{\mu_{(K\alpha,Z,E_0)} A_{(M,Z,E_0)}} = F_{(M,Z,E_0)} \Sigma_{(K\alpha Z)}$$

where F is a factor which for each element depends upon the matrix and the excitation energy. It is the factor used to

transform the normalized area of the characteristic line of an element into its concentration in mg kg^{-1} . Table 1 gives the measured values of $K_{(As,Std)}$ for the standard containing 6.9 mg kg^{-1} of arsenide. This hair was chosen because we had verified that the As concentration was fairly homogeneous over all its length. From a set of measurements at 12.4 keV and at different locations along the sample, we calculated a standard deviation of 11% for this coefficient, mainly due to concentration fluctuations along the hair. Values for 14 and 20.5 keV photons were calculated from this first value using the proper cross-sections. The standard deviation for these last values should also be near 11% since errors on cross-section ratios from the same reference are usually very small.

Application. Fourteen hairs (eight in a first set and six in a second set) taken from contemporary people of different sex, age and color constituted our control set to test the validity of our quantitative approach. Set one was analyzed under 12.4 and 20.5 keV photon excitation whereas set 2 was only analyzed at 12.4 keV. All results were compared with values found in the literature for various elements. All results are summarized in Fig. 1.

The large empty boxes represent the range of concentration found for each element on our control set 1 at 20.5 keV and the medium slashed boxes represent the same set at 12.4 keV. The medium slashed boxes are for set 2 at 12.4 keV

Table 1. $K_{(As,Std)}$ values used in this experiment (see text for explanation)

Energy (keV)	$K_{(As,Std)}$ for 6.9 mg kg^{-1}
12.4	190
14.6	166
20.5	148

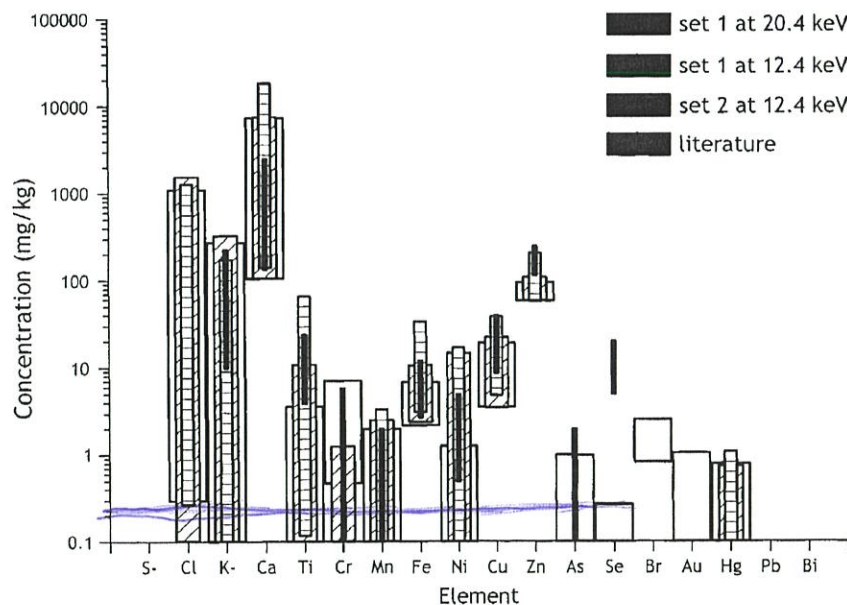


Figure 1. Concentration profile for 18 elements found in each hair of the control set compared with other studies.

and the full bars represents extreme values taken from the literature.¹²

First we note the very large concentration variation for most elements, sometimes of more than an order of magnitude from one sample to another. Nevertheless, results for set 1 at both energies are very consistent with results for set 2 at 12.4 keV, especially if we acknowledge that we certainly have not analyzed exactly the same spots in each experiment. Moreover, our values are also in very good agreement with those in other studies.

The concentration variations in our measurements are usually much larger than values found in the literature. This comes from the fact that we analyzed only 1 mm of hair whereas other workers used up to 1 g of hair and therefore measured a relatively reliable average value. For this reason, we will compare the measurements on Napoleon's hair with our complete control set.

All these remarks give credit to our quantitative estimate, especially if it is remembered that our main interest is in As, Au, Hg and Pb, for which relative corrections for various absorptions are almost negligible in our samples.

RESULTS AND DISCUSSION

General elemental content

Figure 2 present two typical spectra: the solid line is one of the 600 spectra representative of a 1 mm spot on one of Napoleon's hairs and the dotted line is from a control hair. Both have been normalized on the sulfur $K\alpha$ peak. Their comparison leads to some important remarks:

1. Although both spectra were taken during different shifts, after normalization on sulfur the large peak corresponding to scattering processes of the incident photon beam on the sample overlaps perfectly. This is a strong argument in favor of considering sulfur as a reliable internal standard.
2. The relative intensities of the most common trace elements usually found in hair (Ca, Fe, Cu, Zn) are very similar in both samples.
3. The characteristic lines of some elements are considerably enhanced on Napoleon's hair (Mn, Ni and Hg) and,

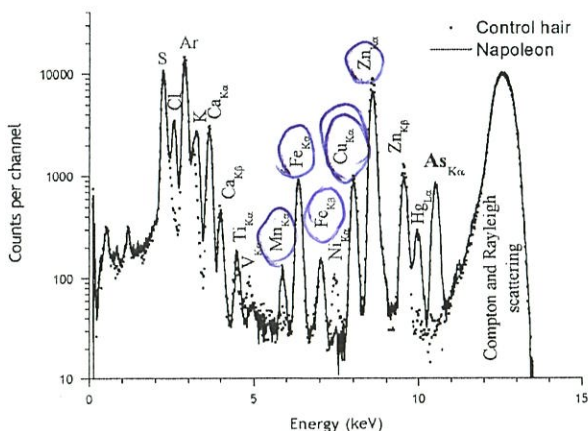


Figure 2. Typical SXRF spectrum of a control hair and one of Napoleon's hairs, normalized on the sulfur $K\alpha$ peak.

moreover, some intense peaks (Cl, which is observed on people living near the sea, K and As) seem completely below the detection limit on the control hair.

Comparison with other spectra of the whole set of Napoleon's hair (more than 600 measurements) suggests two other remarks:

1. Gold was found at a high concentration in some hairs, but not all.
2. As already stated, a first set of experiment performed under 14.6 keV photon excitation had revealed the presence of a noticeable quantity of Br (coherent with the high Cl content), Pb and traces of Bi on all hairs.

To our knowledge, up to now, most investigations concerning Napoleon's hair were undertaken with a view to proving his death through arsenic poisoning, hence As was the single element searched for. A great advantage of XRF is its multielemental character, which shows that As is present together with high (and probably lethal) quantities of other toxic elements such as Hg, Au and Pb.

Longitudinal profile

As a 1 mm length of hair is sufficient for our analysis, we can perform analyses all along each hair and, owing to its growth, find a chronology of a possible toxic element fixation. This was done on most of the samples but not all owing to finite beam time allocation. Figure 3 give some representative results and shows how they can differ from one sample to the other.

For example, 1 mm of hair number 4 was analyzed every 0.5 mm for 45 mm. This means, taking into account an average growth rate of 1 cm per month, that we made a scan every 1.5 days over 4.5 months, each point being representative of the average of a 3 day intake. The arsenic profile is reasonably smooth, in contradiction with what could be expected for occasional ingestion on particular periods, as we have shown that the concentration profile along the hair follows very precisely the arsenic intake.¹³ The average concentration is fairly high, from 20 to 40 mg kg^{-1} , which corresponds to an unquestionable chronic intoxication

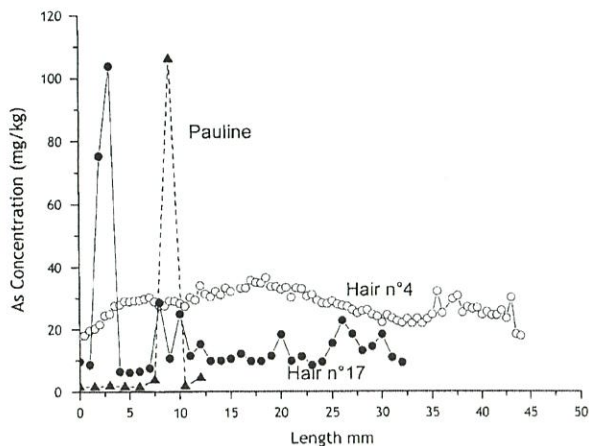


Figure 3. Longitudinal profile of arsenic concentration along different hairs from Napoleon and one of his sister's hairs.

if we can be certain that there was no external contamination. Most samples presented such a longitudinal profile.

The profile of hair number 17 is completely different. Here we performed 33 measurements every 1 mm, representing a 3 month period. We observe a fairly smooth background, around 12 mg kg^{-1} , with sharp peaks superimposed, one of them lasting about 1 week and reaching 100 mg kg^{-1} . This is what could be expected for a hair from a person who had absorbed a high dose of arsenic (possibly lethal) for a short period (about 3–4 days), but is in contradiction with the result for hair number 4, which suggested a chronic intake. Both hairs came from the same lock! This profile also raises a delicate question: what would be the meaning of an average arsenic concentration on this hair? Neutron activation or atomic absorption methods generally need a few millimeters of hair to perform an analysis. Then sampling at the beginning of hair number 17 would lead to an arsenic concentration around 50 mg kg^{-1} , whereas 1 cm further we would only find 12 mg kg^{-1} ! Then, considering the spatial resolution of our method and the fact that we did not analyze the whole length of each hair, we prefer to give the smallest and the highest concentration found in a given hair rather than a meaningless average.

Figure 3 also shows the arsenic profile for a 2 month period on a hair from Pauline (one of Napoleon sisters). The arsenic background is much lower, around 2 mg kg^{-1} . This can be considered as an abnormally high concentration nowadays but maybe not during that period, since Dale *et al.*¹⁴ reported an average concentration of 3.8 mg kg^{-1} for hairs from Napoleon's contemporaries. The interesting fact is that this hair also shows a single, narrow, prominent peak up to 100 mg kg^{-1} .

Elemental profile

Eighteen different elements were found in all the hairs we analyzed (with the exception of scandium, all elements from

phosphorus to arsenic where identified by their K x-ray spectra and Au, Hg, Pb and Bi by their L x-ray spectra).

In Fig. 4 we report the calculated concentrations of 18 elements found in a set of 14 control hairs, eight of Napoleon's hairs analyzed at 12.4 keV and five others analyzed at 14.6 keV (most of these hairs were cut at the time of Napoleon's death, but some were taken as soon as 1805 and 1814). This represents more than 600 different measurements. For each element, the full horizontal line represents the concentration range found in the 14 control hairs. The empty boxes frame the minimum and maximum values found for Napoleon's hair at 12.4 keV and the vertical strip box is the same for 14.6 keV. In this figure, values <0.1 should be taken as zero (the element is below the detection limit in at least one measurement for this set of samples).

Concerning the set of control hairs, Fig. 4 shows the well-known large variation in concentration for most elements in this material. This variation is fairly small for zinc but can extend over several orders of magnitude (Cl, Ni, Au, Hg, etc.). Note that for gold and mercury the rather high maximum value for the control hair is the fact for a small part of a single hair (not the same for both elements and in each case no explanation of the presence of these elements could be found). We could have disregarded these exceptional results, but we preferred not to do so in order to show how great the qualitative and quantitative variability can be in such a material.

Considering the set of Napoleon's hairs, the dispersion is fairly similar to that of the control set for some elements but not for others. This is the case for Ti, Cr, Ni, Cu, Zn and to a lesser extent Ca, Mn and Fe. In contrast, Cl, K, As, Au and Hg lie well above the control set, sometimes more than 100 times higher. In particular, As is always much above the control set. The same holds for Hg apart from a single measurement already mentioned. The case of Au is slightly different; either we find a very high gold concentration, or we do not find gold at all.

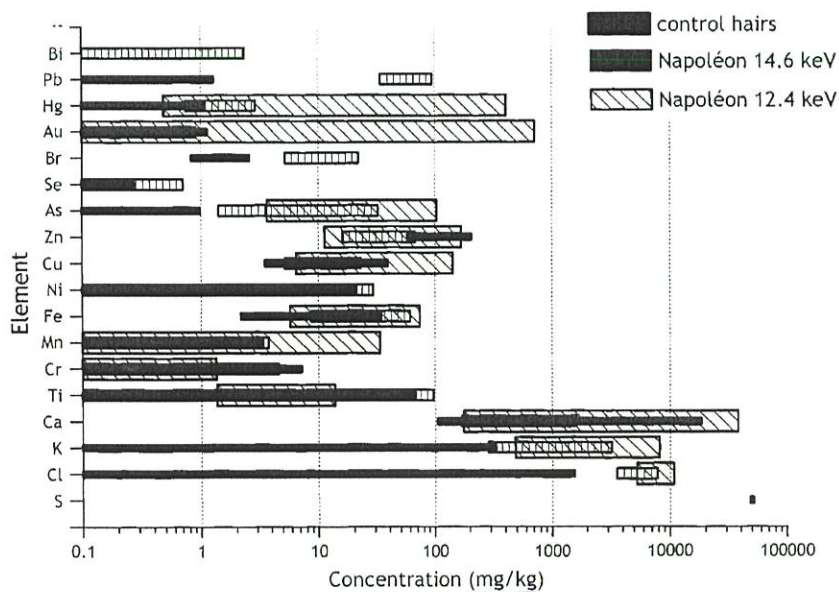


Figure 4. Elemental profile in a set of control hair and of Napoleon's hair (see text for explanation).

mg/kg ÷ 10 = mg/g (ppm)

Finally we can see that the results at 12.4 and 14.6 keV are very consistent, although not exactly the same, which is not surprising since we purposely used a different set of hairs in order to have a statistically more representative collection.

CONCLUSION

The most relevant conclusion of this study is that the set of hairs we were given and stated to belong to Napoleon I all contain significant quantities of arsenic. Is this sufficient to assert, like other experimentalists have done previously, that Napoleon died of arsenic poisoning? We think certainly not, since our study has revealed many confusing facts.

First, we found an important arsenic concentration in the waste water recovered after washing of Napoleon's hair.

Second, the arsenic concentration profile is very different, both in shape and in concentration, from one hair to another (even when they come from the same lock), and usually not consistent with the suggestion of a discrete intake of arsenic. Further, how could we explain that really characteristic clinical signs concerning arsenic poisoning are never mentioned, either in medical reports or in the memoirs published by the Emperor's exiled companions, whereas we found a high content of arsenic in hair cut in 1805 and 1814, suggesting a chronic intoxication for more than 10 years?

Moreover, other toxic elements were always found, also at high concentration, in all hairs analyzed. This is the case for Hg and Pb.

Gold can be found in certain but not all hairs and can reach surprisingly high concentrations at very precise positions.

Finally, the sister's hair also contained arsenic, but it has never been suggested that they had been poisoned.

We must remember that these hairs have been very carefully preserved for over 200 years by passionate collectors for their historical value and in no way as evidence for further forensic investigations. That means they were preserved with considerations of integrity and their nature as relics, but not with regard to their chemical composition. Hence it can be imagined that some external contamination, impossible to prove or disprove, could have led to erroneous interpretations. This could be the case for arsenic found in these hairs, as many products used for conservation would contain this element.

Note added after completion of the manuscript

The question arises of whether the auto absorption correction calculated for a slab can be applied to our samples. This approximation, which leads to a simple analytical equation for the correction and avoids computation, can be justified in our particular study: (i) the elemental concentration profile of the control set of hairs obtained with these corrections is completely coherent with literature values; (ii) above 10 keV, that is, in the energy region in which we are interested with the characteristic lines of As, Hg, Au and Pb, the auto absorption corrections are negligible and would be even

less for a cylindrical geometry; and (iii) we further used a standard sample for As.

If we were interested in light elements, this approximation would be much more questionable and more realistic calculations such that suggested by Török *et al.*¹⁵ would have been used.

As the standard sample was prepared using an As solution in which the hair samples were left for 24 h, one might ask if the As penetrated within the hair or was simply deposited on the surface. This is not very important for our study since we have seen that auto absorption corrections were negligible for this element in hair. Further, x-ray microtomography of hair from a patient who had received arsenic intake for leukemia treatment showed^{16,17} that this element would concentrate in the periphery of the hair, which is as could be expected from the standard sample mode of preparation.

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REFERENCES

- Smith H, Forhuvud S, Wassen A. *Nature* 1962; 194: 725.
- Forshufund S, Smith H, Wassen A. *Arch. Toxicol.* 1964; 20: 210.
- Leslie ACD, Smith H. *Arch. Toxicol.* 1978; 41: 163.
- Ayrault S, Picot D. Cited in Maury R, de Condé-Montholon F. *L'Énigme Napoléon Résolue*, Michel A (ed). Paris, 2000; 221.
- Lewin PK, Hancock GV, Voynovich P. *Nature* 1982; 299: 627.
- Lin X, Henkelmann R. Cited in Maury R, de Condé-Montholon F. *L'Énigme Napoléon Résolue*, Michel A (ed). Paris, 2000; 223.
- Kintz P, Coullé JP, Fornes P, Ludes B. *Ann. Toxicol. Anal.* 2001; 13: 243.
- Brissaud I, Wang JX, Chevallier P. *J. Radioanal. Nucl. Chem.* 1989; 131: 399.
- Valkovic V. *Human Hair*, vol. 1. CRC Press: Boca Raton, FL, 1988; 91.
- Ricordel I, Pirnay S, Marechal A, Chevallier P, Meyer G, Milan N, Plesse J. Presented at the FBI-TIAFT Conference, Washington, DC, August 2004.
- Wang JX, Piccot D, Chevallier P, Legrand F, Abbas K. *VIRESYME: Logiciel d'Exploitation des Spectres de Fluorescence X*. Note CEA-N-2756, CEA, Saclay, France 1993; 231.
- Valkovic V. *Human Hair*, vol. 1. CRC Press: Boca Raton, FL, 1988; 89.
- Nicolis I, Dacher P, Guyon F, Chevallier P, Curis E, Benazeth S. *J. Trace Microprobe Tech.* 2002; 20: 565.
- Dale IM, Lenihan JMA, Smith H. In *Proceedings of the 2nd International Conference on Nuclear Methods in Environmental Research*, University of Missouri, Columbia, July 1974; Organized by: US Energy Research and Development Administration, 187.
- Török Sz, Van Dyck P, Van Grieken R. *X-Ray Spectrom.* 13: 27 1984.
- Bohic S, Susini J, Ortega R, Boruchowska M, Lankosz M, Nicolis I, Benazeth S, Adamek D, Krygowska-Wajs A, Tomik B. *Microsc. Microanal.* 2003; 9(Suppl. 2): 1484.
- Bohic S, Susini J, Ortega R, Boruchowska M, Lankosz M, Nicolis I, Benazeth S, Adamek D, Krygowska-Wajs A, Tomik B. *Phys. Scr.* 115: 870 2005.