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REFERENCE VALUES FOR ELEMENTS OF TOXICOLOGICAL, CLINICAL AND ENVIRONMENTAL INTEREST IN HAIR OF URBAN SUBJECTS

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Summary. - *A monitoring campaign has been undertaken to ascertain the reference concentration ranges for a number of minor and trace elements in hair of healthy urban subjects under age 15. An outline of criteria and methods lying at the root of a sound and reliable experimental approach are presented with special regard to such crucial steps as study planning, sampling, storing, pretreatment, analysis and evaluation of results. Determinations were carried out mainly by inductively-coupled plasma atomic emission spectrometry (ICP-AES) given the wide investigative potential and inherently multielemental character of this technique. The results obtained so far regard 100 youngsters allowing reference intervals for Al, As, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, P, Pb, Se, Ti, V and Zn to be established for the population group tested. Finally, further developments of this activity are highlighted, particularly emphasizing the diagnostic capabilities of hair analysis.*

Riassunto (Valori di riferimento per elementi di interesse tossicologico, clinico ed ambientale in capelli di soggetti urbani). - *E' stato intrapreso uno studio a vasto raggio allo scopo di accertare gli intervalli di riferimento per le concentrazioni di numerosi elementi, sia a basso contenuto che in traccia, nei capelli di soggetti urbani sani di età non superiore ai 15 anni. Vengono discussi i criteri ed i metodi che sono alla base di una strategia sperimentale coerente ed attendibile, specialmente per quanto riguarda fasi critiche come la progettazione della ricerca, il campionamento, la conservazione, il pretrattamento, l'analisi e la valutazione dei risultati. Le determinazioni sono state condotte di norma tramite la spettrometria di emissione atomica a plasma induttivo (ICP-AES) in considerazione delle ampie capacità investigative e del carattere intrinsicamente multielementare di questa tecnica. I risultati ottenuti finora riguardano 100 soggetti per i quali sono stati determinati gli intervalli di riferimento relativamente a Al, As, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, P, Pb, Se, Ti, V e Zn, validi per la sottopopolazione urbana esaminata. Vengono infine delineati gli ulteriori sviluppi di questa attività, in particolare per quanto riguarda le possibilità diagnostiche dell' analisi dei capelli.*

Introduction

The determination of trace elements in biological fluids and tissues can be not only a means of evaluating actual exposure events, but it can potentially reveal and reconstruct past health episodes long after their action has ceased. The ability to serve in this detective story is exhibited to a significant extent by hair, a highly stable material that can be painlessly collected and easily stored. This kind of tissue lends itself to the performance of biomedical, epidemiological and environmental studies to monitor and assess exposure to noxious pollutants [1-3]. In fact, there are at least two main advantages to this type of analysis: a) the fact that hair may reflect the total body intake of certain elements better than the more frequently employed blood and urine; b) the accumulation undergone by most inorganic components over extended periods of time leading to analyte concentrations higher as a rule by one order of magnitude or more than in biological fluids. In spite of this, hair analysis has not yet gained the popularity and general acceptance it deserves. Historically, the first discipline to benefit from hair analysis was forensic medicine in that it was deemed possible to reconstruct a given crime by identifying the elements present in

a few strands of hair. Far from being simply a literary artifact to highlight the perspicacity of the protagonist of a mystery novel, hair analysis has often played a major role in unravelling the plot, as it recently occurred in an As poisoning case [4].

Elements can find their way into hair through a multiplicity of sources, both endogenous and exogenous [5, 6]. Insofar as the former are concerned, these are definitely more important than the latter for evaluating an individual's health status as a consequence of physiological anomalies, nutritional unbalances or uptake of environmental toxicants. From this point of view substances transported by the blood stream dramatically contribute to the build up of elemental burden, albeit the hygroscopicity of hair greatly favours the incorporation of contaminants from external agents such as water, shampoos and cosmetic preparations of all sorts. The main parts of a hair shaft are illustrated in Fig. 1. As reported by Hopps [1], trace elements can enter the hair continuously during its growth and thus record along its axis the variations occurring in the element concentrations of circulating body fluids.

Specifically significant are the amounts of elements conveyed by the sebaceous excretion as well as through eccrine sweat, the latter also acting as a leaching factor. Incorporation of elements into the keratin structure of hair takes place through binding to the sulphydryl groups abundantly present in the follicular proteins. Detergents, such as soaps and shampoos, cold-waving lotions, hair bleachers and dyes actually compete with the complexing ability of these reactive sites and thus lead to a significant depletion of elements from the bulk shaft. On the other hand, the presence of a substantial load of inorganic contaminants in the various environmental compartments inevitably poses the conditions for their deposition onto, and eventually absorption through, the hair surface. This phenomenon adds to the exogenous accumulation caused by the already mentioned (and intentional) cosmetic treatments as well as by the contact with desquamating epidermis. Which kind of hair samples should be selected is also a matter of debate, whether pubic, axillary, limb or scalp. Each shows its own analytical merits, but is also plagued by some disavantages. Any sound decision in this sense relies on the elements to be analyzed and the suspected impact of exogenous agents. Although scalp hair is more prone to contamination through environmental conditions and cosmetic treatments, it is still preferable to hair from other parts of the body in that natural excretion affects it to a lesser degree.

Multielemental analyses of hair are needed due to a couple of equally important reasons, the first being that the number of elements with potential toxicity is high, while this approach also permits antagonistic and synergistic aspects to be taken into account [7, 8]. "Normality" is a frustrating concept that lacks a satisfactory definition. What should be considered normal concentrations for certain elements in hair strongly depends on the assumed absence of perturbing factors such as local emissions and toxicants, diet unbalances, pathological conditions and the

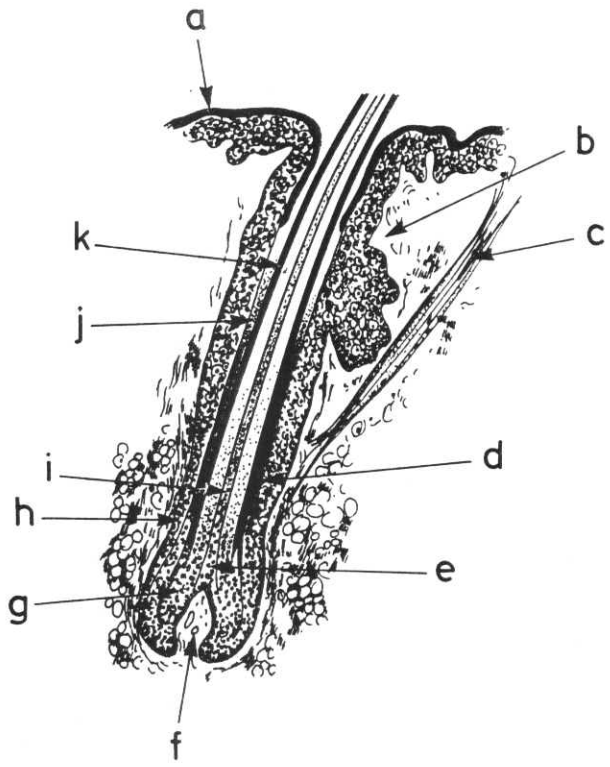

Fig. 1. - Cross-sectional view of a hair shaft: a) epidermis; b) sebaceous gland; c) muscle *arrector pili*; d) internal root sheath; e) matrix; f) connective tissue papilla; g) hair bulb; h) connective tissue sheath; i) medulla; j) hair cuticle; k) cortex.

like. Thus, it makes much more sense to speak generally of reference values for the element concentrations, i.e. those which can be attached to individuals whose environmental exposure and health risk on the whole do not affect the standards considered to date still acceptable in terms of welfare and life expectancy.

As for all other tissues and organs, a reliable use of element analysis in hair is unavoidably bound to the knowledge of such reference intervals for the analytes of interest for well-identified and characterized population groups. Several national and international organizations have undertaken programmes to collect such data. Among these mention should be made of the activities of the International Atomic Energy Agency (IAEA), which started in 1977 a special subprogramme named "Health-related environmental research". This has also led to the set-up of the "nuclear-based methods for the analysis of pollutants in human hair" project [9]. Another example of striking importance is the initiative taken a few years ago in the UK to assess the suitability of this kind of investigations to gain information on the health status of the fetus in the early pregnancy phase relating it to period before conception [10].

More recently a campaign was promoted by our research group to establish the reference values for a number of minor and trace elements in healthy subjects up to 15 years of age in the urban area of Rome. Given the young age of the donors it is thought that the results will better reflect the intrinsic concentration ranges as these are less likely to be influenced by cosmetic treatments. At the same

time, differences with adult and elderly groups will be reliably detected when the study is extended to other age categories, as is planned. The strategy and the results obtained so far in the course of this investigation are illustrated in detail in the following sections.

Experimental methods

Sampling. - The collection campaign was carefully planned in all its stages, and can be summarized by the main issues:

a) a number of nursery, primary and secondary schools were selected in central and suburban parts of Rome in order to set up a network of sampling sources which might reasonably reflect the average urban exposure not affected by any specific emission of pollutants;

b) the subjects selected for sampling were in no case older than 15 years and were with reasonable certainty not bearers of major or minor pathologies. In this respect, an interview was carried out to collect general information on each youngster by filling in the form shown in Table 1;

c) the hair bundle was always cut from the same zone of the scalp for all subjects, namely the occipital region, at a distance of approximately 1 cm from the scalp itself. Titanium nitride-coated scissors were employed throughout the campaign to minimize possible releases of contaminating elements during this crucial phase of the overall procedure;

d) 1 to 2 g of hair thus cut were immediately placed in polyethylene bags, accurately sealed and labelled with a progressive number, subject's name and date;

e) all specimens were stored in a dry, cool and ventilated environment until they were delivered to the laboratory and kept then in desiccators up to the moment of analysis.

The above steps are rigorous enough to fully guarantee the reliability and validity of the operational approach adopted while at the same time not posing an excessive load in terms of practicability.

Sample treatment. - One of the most delicate phases of sample pretreatment consists in the removal of exogenous substances from hair surface. Sample preparation is critical, as washing procedures may well leach a number of elements at different rates. Dirt and dust, on the other hand, are relatively easy to remove. In general no standard procedure for hair washing and pretreatment can be prescribed, it can be only suggested that it should be kept as simple as possible and that the ultimate goal should not be the complete removal of environmental contaminants for the reasons mentioned above. Furthermore, the selection of one or the other strategy also depends on the analytical technique to be employed as this determines the final form in which the samples must be presented. A variety of chemical and physical treatments have been devised so far to circumvent the difficulties inherent in hair washing, as abundantly described in the relevant literature (Ward, personal communication; Mineral Laboratory, USA). Among these, mention should certainly be made of the rather popular use of EDTA as a complexing agent to detach loosely adhering metals. This treatment should however be applied with the greatest care, as otherwise it could bring about a dramatic waste of the information possessed by the inner layers of the hair shaft. Under the constraints of two contrasting needs - cleaning of the samples without sensibly altering their real element content - a mild, yet efficient procedure has been deviced. This involves a thorough washing of about 0.5 g of hair, cut in pieces not longer than 1 cm, with a mixture of ethyl ether and acetone (3+1, v/v) under continuous stirring for 10 min; drying at 85 °C for 1 h; treatment with a diluted (5%) aqueous solution of EDTA for 1 h; repeated rinsing with doubly-distilled water; and finally drying at 105 °C for 24 h in an oven to determine the sample dry weight just before the subsequent attack be started. Hair digestion has also been carried out resorting to a multiplicity of wet and dry ashing type methods. Given the high number of samples to be treated (several hundreds) preference has been addressed in this study to a relatively new digestion approach based on the irradiation with a microwave (MW) field at 2.45 GHz which considerably cuts down ashing time, by one order of magnitude as a rule. The system, supplied by the CEM Corporation (USA), allows programmable cycles of MW irradiation to be done minimizing both duration and risk of contamination. The main results on this procedure are summarized in Table 2.

Analytical determinations. - The method chosen for the quantitation of elements in hair adopted in this study has been in most cases inductively-coupled plasma atomic emission spectrometry (ICP-AES) given its suitability for this kind of investigation (multielemental capability, wide dynamic range, adequate detection power and relative freedom from matrix interferences, among others). From time to time also atomic absorption spectrometry (AAS) measurements have been performed to check the reliability of data for certain elements particularly prone to spectral

Table 1. - *Information form employed in the sampling campaign*

Subject no. _____
Surname: _____ Name: _____
Address: _____
City: _____ Province: _____
Sex: (male) ___ (female) ___ Age: ___ (years) ___ (months)
Weight: ___ (kg) ___ (hg) _____ Height: ___ (m); ___ (cm)

Health condition (brief description): _____

Comments on food habits and life customs in general (brief description): _____

Specific remarks (e.g., type of shampoo normally used, frequency of application, and the like): _____

Table 2. - *MW digestion sequence*

Sample weight:	approximately 0.5 g
Digestion containers:	white teflon, pressure-tight vessels capable of sustaining about 6-10 atm
Treatment steps:	overnight predigestion with 5 ml of high-purity, concentrated HNO_3, 1 h stage at an MW power of *ca.* 180 W; 1 h cooling; addition of 1 ml H_2O_2; further 1 h stage at *ca.* 300 W; quantitative transfer into polypropylene tubes and dilution up to 20 ml
Capacity:	12 vessels placed on a continuously rotating carousel

Table 3. - *Instrumental (A) and analytical conditions (B) for ICP-AES*

A
ISA instruments Jobin-Yvon 32+38 VHR spectrometer, consisting of a polychromator (HR 1000 M, focal length 0.5 m, Paschen-Runge mounting, holographic concave grating of 3600 grooves/mm linear dispersion in the first order 0.55 nm/mm, spectral range 170-410 nm, with 31 channels) and a monochromator (HR 1000 M, focal length 1 m, Czerny-Turner mounting, holographic plane grating of 3600 grooves/mm, linear dispersion in the first order 0.27 nm/mm, theoretical resolution 504,000, spectral range 170-470 nm)
HF Generator DURR-JY 3848, with a frequency of 56 MHz and a nominal output of 2.2 kW
INSA demountable torch
Hydride generator system PS analytical with continuous flow
Computer Apple II, Version Pascal 2-5
B
Torch argon flows, 16 l/min (outer) and 0.6 l/min (intermediate)
Nebulizer flow, 0.6 l/min
Entrance slits, 50 μm (polychromator) and 40 μm (monochromator)
Exit slits, 50 μm (polychromator) and 40 μm (monochromator)
Spectral lines (in nm), Al (I) 396.2, As (I) 193.7, Ca (II) 317.9, Cd (II) 226.5, Co (II) 238.9, Cr (II) 267.7, Cu (I) 324.8, Fe (II) 259.9, Mg (II) 279.6, Mn (II) 257.6, Mo 292.3, Ni (II) 231.6, P (I) 214.9, Pb (II) 220.4, Se (I) 196.0, Ti (II) 336.1, V (II) 292,4, Zn (I) 213.9

interferences with ICP-AES, as Cr and Ni. The working parameters for this technique are summarized in Table 3.

The entire analytical procedure was tested for both precision and measurement accuracy in order to assess the degree of reliability which can be bestowed on the data generated by this investigation. The level of accuracy has been continuously monitored by adding to each series of unknowns one of the two reference materials available, namely the NIES (National Institute of Environmental Sciences, Tokyo, Japan) no. 5, certified for As, Cd, Co, Cu, Fe, Mg, Mn, Pb, Sb, Se and Zn, and the BCR (Community Bureau of Reference, Bruxelles, Belgium) no. T7. The other basic qualifying requirement, i.e. precision, was ascertained by replicating the determinations an adequate number of times (not less than 10). Although both parameters are of fundamental importance, it should be stressed here that the very nature of the study undertaken makes accuracy all the more crucial in that it affects the confidence given to the obtained reference values. Since the latter are the starting point for any further progress in the exploitation of hair analysis for diagnostic purposes, it is immediately understandable why accuracy is, from this standpoint, of paramount importance. On the other hand, given the high number of subjects considered, individual variability makes the achievement of the highest possible precision not essential as this will be probably obscured by the much wider range of values expected for each element in the population examined.

Results and discussion

The data obtained so far refer to approximately 100 subjects and can therefore provide a preliminary and sufficiently reliable indication of the concentration ranges to be established for the elements under test. An overall view of the main outcomes of this pilot study is set forth in Table 4, wherein the data reported refer to the entire population tested. The uncertainty affecting each average value is in some cases even larger than the mean itself thus implying negative figures for the lower extreme of the resulting interval. This is obviously a purely mathematical consequence of the statistical treatment applied to the experimental measurements which has no physical meaning as concentration cannot be negative. Therefore, it should be kept clearly in mind that the calculated standard deviations simply reflect the distribution of the element contents for different individuals, i.e. the biological variability, rather than the precision of a set of measurements repeatedly carried out on one and the same quantity. The real range of concentrations encountered for each element is shown in the last column of Table 4. On the other hand, the data listed in Table 5 seem to support the view that there are no striking differences for most elements between males and females. In a few cases, however, a borderline situation is apparent, as for Cd, Mg, Mo and Pb, for which variations according to sex are appreciable. For Ca this difference is even more striking. Definitely more interesting information can be deduced by comparing subgroups of subjects following the criterion of age in combination

Table 4. - *Element content of hair for all subjects*

Element	Mean (μg/g) *(a, b)*	Median (μg/g) *(a)*	Percentiles (μg/g) *(a)* 10%	90%	Range spanned (μg/g) *(a)*
Al	13.17 ± 6.83	13.85	4.63	21.69	0.5 + 31
As	0.164 ± 0.16	0.13	0.051	0.271	0.014 + 0.853
Ca	360 ± 166	327.5	185.6	591.5	163.2 + 1173
Cd	0.14 ± 0.13	0.09	0.043	0.213	0.04 + 0.28
Co	0.410 ± 0.62	0.19	0.041	0.72	0.024 + 3.09
Cr	0.67 ± 1.63	0.32	0.090	0.70	0.03 + 11.3
Cu	26.60 ± 50.80	9.5	7.71	89.40	4.25 + 279.6
Fe	14.14 ± 5.54	13.85	7.26	21.28	3.54 + 32.07
Mg	22.25 ± 17.55	17.5	5.95	42.98	0.32 + 116.5
Mn	0.34 ± 0.45	0.27	0.083	0.52	0.039 + 4.04
Mo	0.482 ± 0.785	0.25	0.062	1.32	0.040 + 5.11
Ni	0.43 ± 0.38	0.32	0.090	0.996	0.027 + 2.03
P	122 ± 22	123.35	91.15	147.9	68.10 + 180.0
Pb	8.20 ± 4.73	7.94	2.25	14.0	0.977 + 22.4
Se	1.05 ± 1.53	0.57	0.096	0.996	0.049 + 9.80
Ti	1.17 ± 0.86	1.01	0.198	2.31	0.05 + 4.56
V	0.13 ± 0.19	0.06	0.035	0.205	0.03 + 1.16
Zn	110 ± 59	99.60	53.5	159.0	23.80 + 476.8

(a) dry weight

(b) each mean value is accompanied by its standard deviation to account for measurement distribution

Table 5. - *Element content of hair according to sex*

Element	Mean concentration (μg/g) *(a, b)* Males	Females	Median concentration (μg/g) *(a)* Males	Females	p *(c)*
Al	12.53 ± 6.68	13.88 ± 6.92	13.77	13.10	> 0.1
As	0.172 ± 0.198	0.155 ± 0.095	0.13	0.13	> 0.1
Ca	315.7 ± 163.8	409.4 ± 154.1	269.6	357.3	< 0.01
Cd	0.106 ± 0.070	0.186 ± 0.171	0.08	0.14	< 0.1
Co	0.323 ± 0.421	0.528 ± 0.796	0.138	0.200	> 0.1
Cr	0.899 ± 2.22	0.445 ± 0.599	0.236	0.385	> 0.1
Cu	30.96 ± 61.94	21.77 ± 33.95	9.15	10.23	> 0.1
Fe	13.40 ± 6.00	14.95 ± 4.86	12.36	13.93	> 0.1
Mg	18.93 ± 18.38	25.82 ± 15.85	14.60	20.90	< 0.1
Mn	0.295 ± 0.199	0.378 ± 0.610	0.27	0.27	> 0.1
Mo	0.340 ± 3.48	0.716 ± 1.16	0.18	0.31	< 0.1
Ni	0.359 ± 0.292	0.499 ± 0.443	0.280	0.346	> 0.1
P	123.4 ± 25.0	121.2 ± 17.9	127.4	120.4	> 0.1
Pb	7.24 ± 4.76	9.25 ± 4.48	6.37	8.78	< 0.1
Se	0.834 ± 0.899	1.30 ± 2.00	0.516	0.771	> 0.1
Ti	1.21 ± 0.74	1.23 ± 0.98	0.98	1.10	> 0.1
V	0.095 ± 0.124	0.177 ± 0.279	0.062	0.089	> 0.1
Zn	105.1 ± 37.2	114.7 ± 75.2	95.32	100.3	> 0.1

(a) dry weight

(b) each mean value is accompanied by its standard deviation to account for measurement distribution

(c) p values deduced by the Student's t-test

with sex. Furthermore, if the concentration values are split on the basis of age, as reported in Table 6, additional interesting remarks are possible. In fact, it is clear that significant differences exist between the two groups for Al, Co, Cr, V and Zn, none of these elements coinciding with any of those which have a different behavior within the two series. This should lend support to the hypothesis that the accumulation mechanisms do vary in still unknown fashion depending on at least three parameters, namely sex, age, and nature of the element. Albeit this is certainly acceptable in principle, the information obtained so far is too scanty to corroborate it and much more than

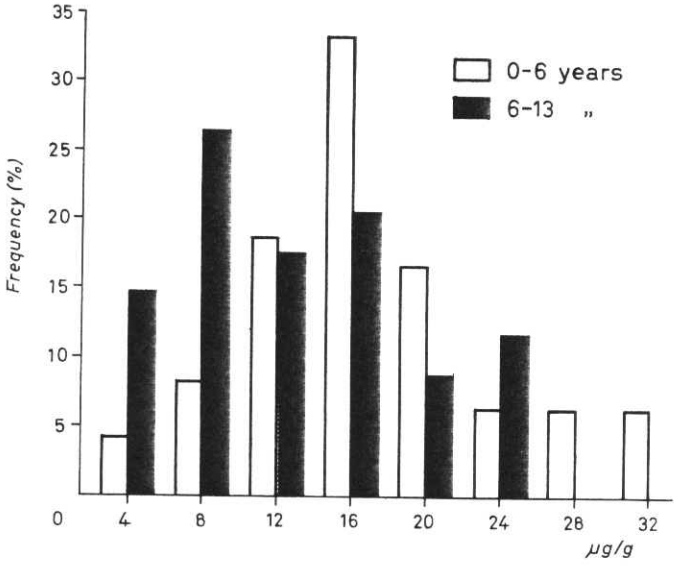

Fig. 2. - Distribution patterns of Al in hair as a function of age.

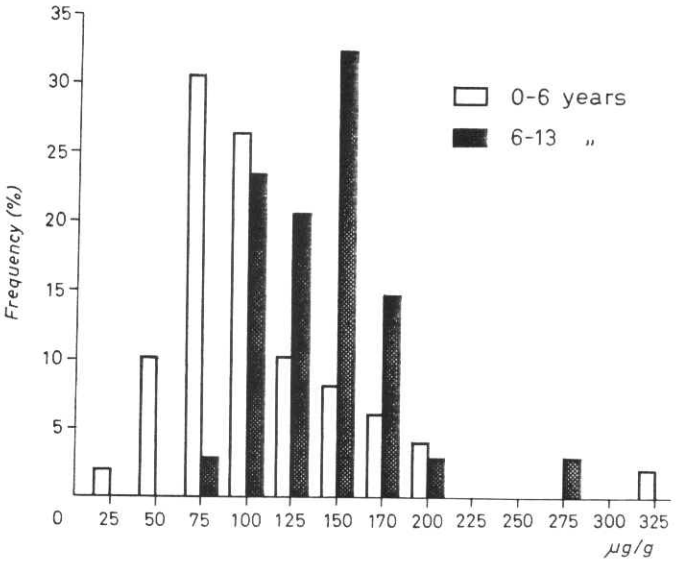

Fig. 3. - Distribution patterns of Zn in hair as a function of age.

Table 6. - *Element content of hair according to age*

Element	Mean concentration (µg/g) *(a)*		Median concentration (µg/g) *(a)*		p *(b)*
	0-6 years	9-13 years	0-6 years	9-13 years	
Al	15.11 ± 6.61	10.44 ± 6.16	13.80	11.26	< 0.001
As	0.131 ± 0.16	0.19 ± 0.16	0.099	0.154	> 0.1
Ca	333.9 ± 135.6	395.2 ± 195.1	293.4	357.3	> 0.1
Cd	0.129 ± 0.104	0.182 ± 0.188	0.090	0.115	> 0.1
Co	0.564 ± 0.716	0.140 ± 0.190	0.393	0.063	< 0.001
Cr	1.034 ± 2.196	0.027 ± 0.176	0.400	0.200	< 0.05
Cu	29.42 ± 44.30	31.06 ± 58.48	9.50	9.60	> 0.1
Fe	13.47 ± 5.36	15.08 ± 5.66	13.4	13.92	> 0.1
Mg	20.85 ± 14.38	24.27 ± 21.25	16.0	20.90	> 0.1
Mn	0.370 ± 0.57	0.280 ± 0.160	0.28	0.27	> 0.1
Mo	0.801 ± 1.050	0.196 ± 0.100	0.56	0.18	> 0.1
Ni	0.398 ± 0.36	0.470 ± 0.405	0.294	0.406	> 0.1
P	124.70 ± 21.01	119.1 ± 22.8	134.8	112.0	> 0.1
Pb	8.88 ± 4.31	7.22 ± 5.12	8.14	7.48	> 0.1
Se	1.06 ± 0.58	1.03 ± 2.10	1.06	0.408	> 0.1
Ti	1.10 ± 0.9	1.04 ± 2.10	0.90	1.10	> 0.1
V	0.20 ± 0.27	0.06 ± 0.04	0.13	0.48	< 0.05
Zn	98.12 ± 67.64	126.03 ± 37.24	82.7	127.2	< 0.05

(a) dry weight
(b) p value as deduced by the Student's t-test

the present 100 subjects would be necessary to that purpose. Figs 2-5 illustrate some instances where the above differences are more impressive.

Finally, the values obtained in this study are compared with those available in the international literature, as is cumulatively shown in Table 7. Since there is definitely no abundance of the latter the data reported therein are not homogeneous in terms of age, sampling procedure and life conditions. Nonetheless, the comparison seems to point to concentration ranges which overlap fairly well for the majority of elements, at least insofar as the order of magnitude of concentration is concerned. This holds true especially for essential elements like Ca, Cr, Cu, Fe, P, Se and Zn. On the other hand, the rather spread values obtained for Ca from country to country is to a certain extent surprising, although the Italian range falls in the

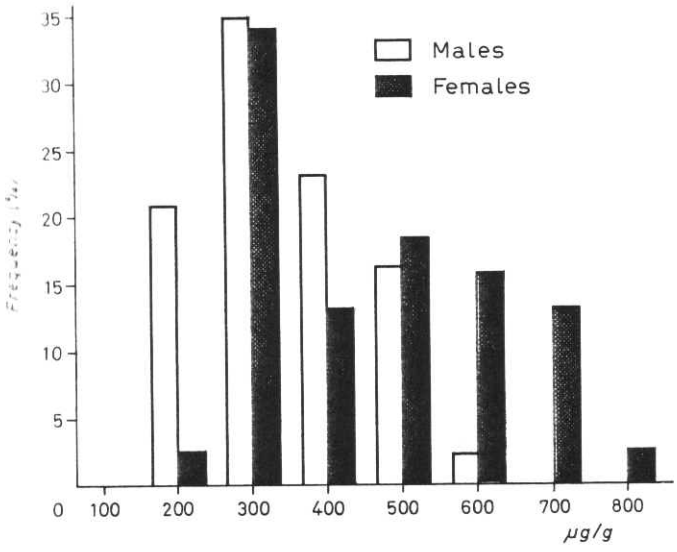

Fig. 4. - Distribution patterns of Ca in hair as a function of sex.

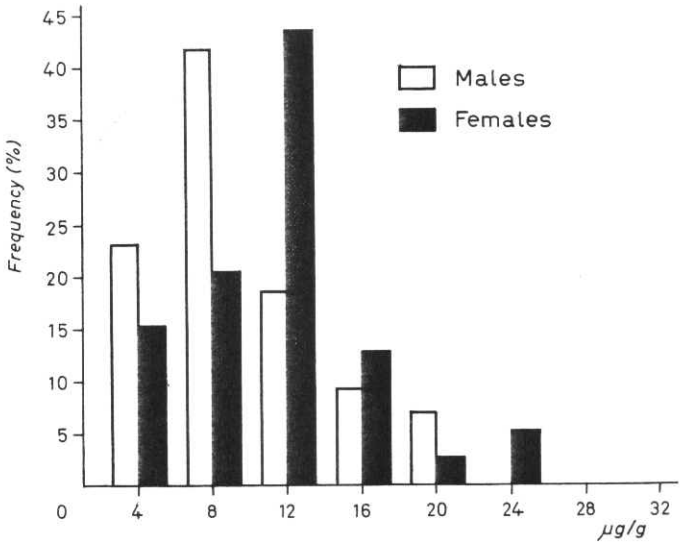

Fig. 5. - Distribution patterns of Pb in hair as a function of sex.

Table 7. - *Concentration ranges for elements in the hair of health individuals from different countries*

Element	Concentration (µg/g)						
	Italy (this study)	England *(a)*	USA *(b)*	Canada [11]	Japan [12]	New Zealand [13]	Bulgaria [14]
Al	4.63 - 21.69	1.79 - 9.43	0.60 - 14	1 - 17	0.6 - 36	6.16 - 10.8	2.69 - 21.3
As	0.051 - 0.271	0.10 - 2.41	1.0	-	1 - 2.7	0.279 - 1.05	0.037 - 0.625
Ca	185.6 - 591.5	150 - 1620	1 - 2220	0.7 - 93.1	190 - 3700	250 - 1380	170 - 1900
Cd	0.043 - 0.213	0.112 - 0.994	0.10 - 0.43	-	0.05 - 0.57	0.361 - 1.51	0.561 - 2.71
Co	0.041 - 0.72	0.012 - 0.198	0.20 - 0.23	-	0.13 - 0.49	0.041 - 0.098	0.032 - 0.166
Cr	0.090 - 0.70	0.026 - 1.88	0.20 - 0.41	-	0.20 - 0.77	0.560 - 1.92	0.205 - 1.02
Cu	7.71 - 89.40	4.57 - 19.41	6.5 - 18	4 - 245	6.0 - 69.1	3.42 - 8.12	7.21 - 19.4
Fe	7.26 - 21.28	5.17 - 38.68	4.0 - 15	-	5.5 - 87.4	18.45 - 52.80	12.94 - 96.4
Mg	5.95 - 42.98	30.37 - 81.65	0.06 - 160	-	14 - 567	73.45 - 149.3	25.32 - 128.9
Mn	0.083 - 0.52	0.208 - 3.98	0.06 - 0.36	0.03 - 3.72	0.06 - 4.51	0.573 - 1.68	0.201 - 4.30
Mo	0.062 - 1.32	0.027 - 0.169	-	-	0.20 - 0.59	0.102 - 0.212	0.010 - 0.066
Ni	0.090 - 0.996	0.44 - 7.10	0.40 - 1.3	-	0.17 - 3.0	1.62 - 4.52	0.55 - 3.59
P	91.15 - 147.9	-	110 - 190	-	110 - 250	-	-
Pb	2.247 - 14.00	-	2.0 - 4.0	-	1.4 - 18.0	-	-
Se	0.096 - 0.996	0.340 - 2.83	1.0 - 1.4	0.24 - 87.54	1.0 - 4.9	0.233 - 1.05	0.418 - 2.45
Ti	0.198 - 2.31	1.11 - 8.93	-	-	-	142 - 8.39	2.15 - 5.84
V	0.035 - 0.205	0.011 - 0.080	-	0.005 - 0.564	0.16 - 0.88	0.019 - 0.080	0.012 - 0.360
Zn	53.5 - 159.0	141.9 - 259.6	120 - 220	108 - 357	72 - 327	157.6 - 293.2	143.8 - 284.4

(a) N.I. Ward, personal communication
(b) Data supplied by Mineral Lab., USA

middle of the concentration interval spanned by the others. As regards specific differences, one cannot ignore that the range ascertained for As is one of the lowest, only comparable to that of Bulgaria. An analogous trend can be recognized also for Cd, Mn and Ni, with values close to those of Japan in the first case and to those of USA for the last two elements. What is the actual meaning of the above similarities and discrepancies certainly cannot be elucidated at the moment, even though one is tempted to identify in two different urban environments the causes which lead to them. Whether this assumption is valid or more complex and less understandable aspects should be taken into account is the objective at which the future phases of this investigation will aim.

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