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HAIR AS A BIO-INDICATOR: LIMITATIONS AND COMPLICATIONS IN THE INTERPRETATION OF RESULTS

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As part of a larger occupational exposure study in which the concentrations of 18 elements were measured in head hair and toenail collected from steel plant workers, a number of factors associated with interpreting the data obtained were examined. In this paper, some of the limitations and complications associated with hair and nail analysis that were thereby recognised are discussed. Data obtained from the occupational study demonstrated the potential for misinterpreting hair or nail analysis data either through describing results averaged over a group by arithmetic instead of geometric means or through not accounting for the age range of subjects in groups to be compared. Examples that arose from the study indicated that differences between hair from the same subjects grown at different times can both complicate and assist in interpreting hair analysis results. In an investigation into the addition and removal of metallic powders, it was found that both hair and nail can directly incorporate elements through contact with dust.

Introduction

Hair has been found to be quite attractive as a biological indicator due to both the simplicity of sampling and storage, and the ease of obtaining simultaneous multi-element results through sensitive analytical techniques such as Instrumental Neutron Activation Analysis (I.N.A.A.)[1,2]. The analysis of trace and minor element concentrations in hair has been used in a diversity of applications including nutritional, environmental, medical diagnostic and occupational studies[3,4,5,6]. The use of hair as a bio-indicator has been shown to be quite practical for some of these applications[7,8] while for others, some of the conclusions reached have been controversial. One application that has created a great deal of public controversy[9,10] is the claimed use of hair to assess the nutritional status of individual subjects. Although there is a dearth of publications available focussing on this area, the use of hair as a nutritional diagnostic tool has nevertheless become a very profitable business. The number of unresolved issues associated with interpreting the results of hair analysis would suggest that other than in extreme cases, such use of hair analysis be applied only with utmost caution or avoided completely. For example Golden et al.[11] have reported that rats on a high Zn diet show increased hair Zn concentrations whereas rats on a chronic Zn deficient diet do not show depressed levels of this element in their hair.

The more accepted applications of hair as a bio-indicator of elemental body-status, particularly for heavy metals, have involved comparisons between groups of subjects[12,13]. This approach to hair analysis studies based on groups is less complicated and requires less detailed information than

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assessing the results from individual subjects for a number of reasons including:

i) Questions relating to how well a hair sample represents an individual can be avoided. The concentrations of many elements in hair have been found to vary with the particular time the hair was sampled and the location on the scalp, or along the hair strands, from which the sample was taken[14,15]. A full understanding of to what extent any observed unusual results can be attributed to these factors is required to assess results obtained from the analysis of one subject's hair. In group studies, any differences observed between two groups must be evaluated in relation to the observed variation within the groups. This variation within the groups encompasses the variations both within the hair of the individual subjects and between the subjects. Since in group studies a measure of the overall variation is available, a detailed knowledge of the variation within the hair of the individual subjects is not required.

ii) The dominant reason for consistently abnormal results throughout a group is frequently more obvious than the dominant reason for an abnormal result in the hair of an individual. Consistent abnormal results throughout a group of subjects are usually due to a factor that is common to the group; personal factors which can cause very unusual results for individual hair samples, such as cosmetic hair treatments, are averaged out or can be recognized and eliminated. Consequently, the potential for erroneously inferring the cause for any abnormal results is much lower in group studies.

It is apparent that interpreting the results from hair analysis studies, even studies investigating groups of subjects, can be quite complicated. This is in sharp contrast to the relative ease of obtaining analytical results using hair. In this paper, a number of factors associated with interpreting data obtained from the elemental analysis of head hair and toenail, are considered. Both the limitations and complications that result from these factors are discussed by reference to examples that arose from a study of occupational exposure of steel plant workers in which the concentrations of Al, Au, Br, Ca, Cl, Co, Cr, Cu, I, K, La, Mg, Mn, Na, S, Ti, V, W and Zn were measured in head hair samples and toenail clippings.

The factors considered both for controls and workers included:

i) temporal variations in the hair of individuals, that is variations between hair grown at different times;
ii) the statistical distribution of results from the elemental analysis of hair and nail samples from a population of subjects;
iii) variations associated with the age of subjects; and
iv) the addition and removal of exogenous material from hair and nail as a result of deliberate contamination and subsequent cleaning.

Experimental

The control hair and toenail samples examined in this research were collected from volunteers among the students and staff at the University of Toronto and from friends and relatives of the authors while the occupational group comprised employees at a major Ontario steel plant. The hair samples

were cut from the back of the scalp as close to the root as possible using a clean pair of scissors. The clipped hair was washed prior to analysis using a mild ether-based procedure; the hair was soaked for twenty minutes and then rinsed twice using diethyl ether.

Toenail clippings were also collected from some donors using a clean pair of nail clippers. These nail samples were washed for thirty minutes in deionized water using a ultrasonic bath; the water was changed up to four times during the cleaning. The nails were soaked and then rinsed in methanol and acetone. This washing procedure removed a large part of the outer surface of the nail clippings reducing the sample weights by about 10%.

The samples were all analyzed by I.N.A.A. by irradiating the samples under two sets of conditions in the University of Toronto SLOWPOKE reactor. Short-lived radionuclides were produced by irradiating the samples for ten minutes in a thermal neutron flux of 10^{12} n/cm^2•s. Intermediate and long-lived nuclides were produced through a 16 hr irradiation at a thermal neutron flux of 2.5×10^{11} n/cm^2•s. The induced activity was counted for various lengths of time using one of two Ge(Li) detectors, one having a 12.9% efficiency and the other having a 6.7% efficiency. Two other Ge detectors were used to count the intermediate and long lived radionuclides. These had efficiencies of 10.2% and 21.6%.

The samples were weighed following the counting in inactive polyethylene vials into which the samples had been transferred between irradiation and counting. The hair samples weighed between 50 and 200 mg while the nail samples ranged in weight from 15 to 80 mg.

Elemental concentrations were calculated from the observed sample activities using the comparator method. The required standards of known concentration were prepared from 1000 ppm A.A. solutions. One means used to verify the accuracy of the analysis was to analyze two samples of a standard reference hair from the IAEA: Material HHI[16]. Some representative results of our measurements are presented along with literature values[16] in Table I.

Table 1
Experimental accuracy: The analysis of a Standard Reference Hair

Element	Hair Sample 1	Hair Sample 2	Literature Values[16]
Al	<10	9.0	
Br	3.6	3.7	4.2
Ca	524	566	522
Cl	1990	1993	2270
Co	5.5	5.3	5.9
Cu	10.2	8.9	10.2
I	23	25	
Mg	73	<90	62
Mn	0.9	0.6	0.85
Na	13.7	13.6	12.6
S(%)	4.6	4.4	4.9
Zn	170	176	174

Values are in ppm except for S values

The elemental concentrations determined in two hair samples generally showed good agreement with the literature values. Similar confirmation of acceptable accuracy was obtained through the analysis of NBS standard reference coal 1632a. The analysis of individually washed replicate hair samples indicated an experimental reproducibility well within 20% for most elements. The reproducibility observed for elements with concentrations close to their detection limit such as Mn, I and Mg was generally within 30%.

Results and discussion

1) Temporal variations in hair

Temporal variations in hair can be observed by two approaches: i) by analyzing hair samples taken at the same location on a donors' scalp at different times and ii) by analyzing hair samples taken at the same time at different locations along the length of the hair strands. These variation with time of growth can cause complications when applied to the individual but can also be quite useful in both individual and group studies.

An example of the variation between hair samples taken from the same donor at different times is presented in Table 2. These elemental concentrations were obtained through the analysis of hair from a 21 year old male subject. The hair was collected on four different occasions over a two year period. Each batch of hair was collected through a hair cut and homogenized prior to washing and analysis; at least four samples were analyzed individually for each batch. The mean values presented are arithmetic means while the standard deviations indicate the observed variation between the replicate samples analyzed for each batch of hair.

For a number of the hair constituents, the variation between the batches of hair greatly exceeded the variation within the hair of any one batch. For example, the concentration of Cu varied among the batches by more than a factor

Table 2
Replicate hair samples collected from a subject at four different times

Element	Batch A	Batch B	Batch C	Batch D
Al	26±7	19±3	15±2	6±0.8
Br	4.5±0.5	29±9	2.5±0.2	4.0±0.3
Ca	2160±250	2000±75	1750±150	1535±180
Cl	615±90	395±7	400±46	455±65
Cu	115±4	150±10	35±2	62±15
I	1.7±0.3	0.7±0.5	1.2±0.4	0.4±0.1
Mn	1.1±0.5	1.1±0.3	1.3±0.7	0.6±0.1
Na	220±30	210±22	210±17	136±24
S(%)	4.8±1.0	4.6±0.2	4.3±0.3	4.4±0.2
No.of samples	5	5	7	4
Date of Sampling	June 1981	Sept. 1981	Oct. 1982	June 1983

Values are in ppm except for S values

of four while the concentrations of Br varied by a factor of ten; further, the concentrations of both these elements were considered elevated compared to control values.

The variation observed between the batches indicated that the elemental concentrations in the hair of an individual can change dramatically over as short a period as three months with no apparent change of lifestyle or diet. The reason for these elemental variations is not understood; although the variations could be product of changes in the body status, they could also have been due to exogenous contamination, a factor that will be examined later in more detail.

Human scalp hair grows approximately at a constant rate, and it is believed that only the part of the hair closest to the scalp is affected by changes in the body status[17]. As a consequence, differences in elemental concentrations among different segments along hair strands can also give an indication of temporal variation. An interesting example of such variation was provided by a female subject in her early thirties by age whose hair contained abnormally high Cu concentrations. A bundle of hair strands collected from this subject was divided into five sections each 4 centimeters in length. The concentrations of five of the elements detected, Au, Br, Ca, Cu, and Zn, were found to be significantly elevated and to increase exponentially along the length of the hair as can be seen in Figure I. This variation in the hair, if assumed to have been produced predominantly by changes in the body status of the subject, would suggest that the subject had an elevated body status of these elements and that this status decreased exponentially over time. Two observations lend support to the hypothesis that the elevated concentrations in the hair were due to an elevated body status. Firstly, toenail clippings from the same subject were found also to contain elevated levels of Cu, Br and Au and, secondly, the hair shampoo and cream rinse used by the subject were found not to contain significant amounts of any of these elements. Further, if the exponential increases along the hair in the concentrations of Au, Br, Ca, Cu and Zn had been due to a constant source of contamination, a linear increase in the levels of these elements might have been expected. It should be noted that the subject did not suffer any unusual medical symptoms during the period reflected by the hair sections.

The last two examples give an indication of the difficulty in interpreting the results of hair analysis as applied to individuals. In neither case could it be stated with confidence that the observed temporal variations were due to changes in the body status. Without an understanding of to what extent the elemental concentrations in hair can vary without a a change in the elemental body status, the significance of temporal variations cannot be assessed. These limitations, however, should not be used as a justification for discontinuing further work on using temporal concentration variations in hair as a historical indicator of exposure. In a case of known ingestion[18], it was found that the temporal variations observed in the hair of the subjects corresponded quite well with the time of the increased body status of the element involved.

Temporal variations can be applied to group studies and provide useful information. An example involving the concentrations of Mn in hair from 10 metal welders illustrated this. The welders' hair was sampled on two occasions: the first sampling was shortly after a lengthy plant shutdown and the second five months later. At the time of collection, each hair sample was divided at an appropriate point along the hair strands so as to approximately separate the part of the hair grown while the plant was operating from that

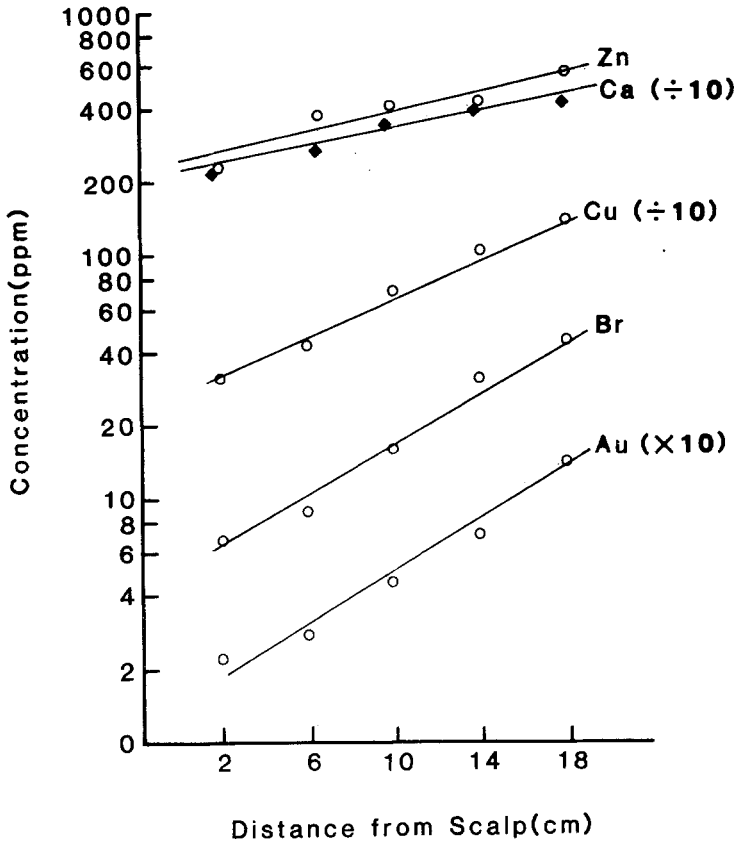

Fig. 1. Concentration variations in a sectioned hair sample as a function of longitudinal distance from the scalp

which had grown while the plant was shut down. The results obtained for several elements in these hair sections are presented in Table 3.

In the initial samples, the hair grown while the plant was operating contained much higher concentrations of Mn than the hair grown while the plant was shut down. This observation, however, could have been attributed either to ingestion of Mn by the welders or to deposition of exogenous material onto the hair in the workplace. However, a comparison between the results obtained for the initial and the second set of hair samples, particularly of the levels of Mn in the hair grown while the plant was shut down, led to the conclusion that no large amount of exogenous Mn was deposited on the hair. The inner part of the initial hair samples was only exposed to the workplace environment for a few working weeks while the outer part of the second set of samples was exposed for about five months. If significant exogenous deposition had been occurring in the workplace a greater concentration of Mn would have been expected in the outer part of the second set of samples than in the inner part of the initial samples.

Table 3
Temporal variations of the concentrations of Mn in the hair of 10 welders

	the initial set of hair samples	the second set of hair samples
inner hair (closer to scalp)	$2.0^{c} \overset{x}{\div} 2.2^{d}$ a	$1.4 \overset{x}{\div} 1.9^{b}$
outer hair (further from scalp)	$5.9 \overset{x}{\div} 2.1^{b}$	$1.4 \overset{x}{\div} 1.9^{a}$

control value = $0.7 \overset{x}{\div} 1.75$

all values are in ppm

[a] = hair grown while plant was shut down
[b] = hair grown while plant was operating
[c] the geometric mean averaged over the 10 welders
[d] the standard deviation of the geometric mean

The lack of any difference between the inner and outer parts of the second set of samples could be a product of the greater degree of exposure experienced by the welders prior to the shutdown as compared to what they experienced after the end of the shut down: after the shutdown ended, the plant was operating at half capacity.

2) The statistical distribution of hair analysis results from a population of subjects

The results in the previous table were presented in the form of concentration geometric means among the 10 workers sampled. This selection of average value is consistent with a recommendation by an IAEA committee[19] and is based on the assumption that the concentrations of most elements in hair are distributed log-normally, to a fair degree of approximation, within a homogeneous population of subjects. This assumption has been empirically supported by the results of authors[20,21] who have presented their experimental data in the form of concentration frequency histograms.

In this study, cumulative frequency diagrams were used as an alternative to examine the statistical distribution of the hair concentration results. Such diagrams are presented in Figure 2 for the levels of Al and Br measured in the hair and nails of 49 control donors. Two plots are presented in each diagram indicating the proximity of the data as measured, and of the logarithms of this data, to normality. It is apparent from these four diagrams that the logarithms of the concentration data produced more linear plots, thus indicating that the data followed log-normal frequency distributions more closely than normal distributions. This same behaviour was found also for Ca, Cu, and Na in control hair.

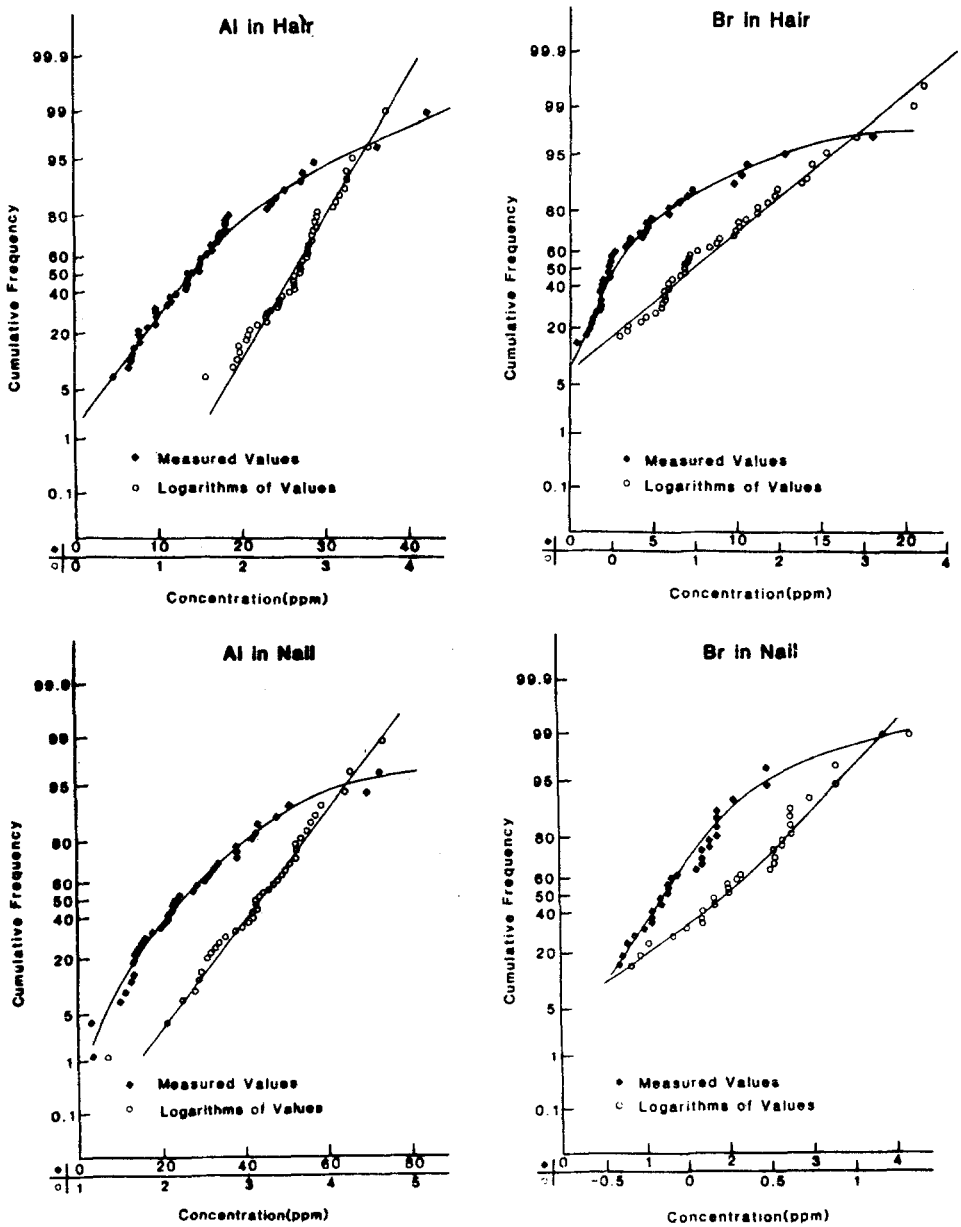

Fig. 2. Cumulative frequency distributions of elemental concentrations, and their logarithms, in hair and nail.

Since log-normal distributions are best represented by geometric means, it is important that geometric means be the mean values used to describe the average elemental content of hair or nail from a group of subjects. In addition all hypothesis testing used to assess the significance of differences between the elemental content of hair or nail from two groups should be done based on geometric means.

3) Variations in hair and nails associated with donor age

Variations associated with age of donors can also complicate the interpretation of hair analysis results. In Figure 3 the concentration of Ca in the hair of 49 male steel plant workers and controls are plotted as a function of the age of the donors. The Ca concentrations determined both in the hair of workers and the hair of controls decreased similarly with subject age over the age range examined. It can be seen that a comparison of the concentration of Ca in hair, between workers of age 30 and controls of age 20 or of age 40 could produce opposite conclusions. The results give an indication of the need to take into account the age range of the subjects in groups to be compared so as to insure that variations with age do not interfere or vitiate such comparisons.

As a further point of interest, the results from the analysis of toenail clippings collected from the same subjects are also presented in Figure 3. The apparent trend for lower levels of Ca in the nails of older subjects is in agreement with the hair analysis results for the same individuals. This would suggest that the variation of the concentration of Ca in hair associated with the age of subjects is due to changes within in the body as opposed to changes in the structure of hair.

4) The addition and removal of exogenous material

In a number of the previous examples, questions relating to the origins of the observed elevated elemental concentrations arose. It has been established in other studies that hair can incorporate exogenous material when placed in contact with aqueous solutions[22,23], although the claims that the solutions used in some of these studies[24] were representative of sweat, have been criticized[25].

In this research, the direct incorporation of metallic dust into hair and nails was examined in conjunction with an investigation of the effectiveness of various techniques for cleaning these tissues. In Table 4 the results of exposing hair to an arbitrary mixture of metallic sulphate powders containing Al, Cu, Mg, Mn and Ti, are presented. This deliberate contamination of hair was achieved through shaking dry hair and the powdered metallic sulphate mixture in a plastic container for about 15 minutes; a static charge was observed following the shaking. The hair was analyzed after washing by the ether-based procedure described in the experimental section of this paper.

It can be seen that a single ether washing of the hair did not remove all the exogenous material; although the exogenous Al, Mg and S appeared to have been completely removed, significant amounts of exogenous Cu and Mn still remained. Repeated washing with ether had no further effects except on the levels of Cu which were reduced to a concentration of 60 ppm as compared to an initial concentration of about 35 ppm. No greater degree of removal was achieved by washing a batch of the same hair using the IAEA recommended acetone based procedure[19].

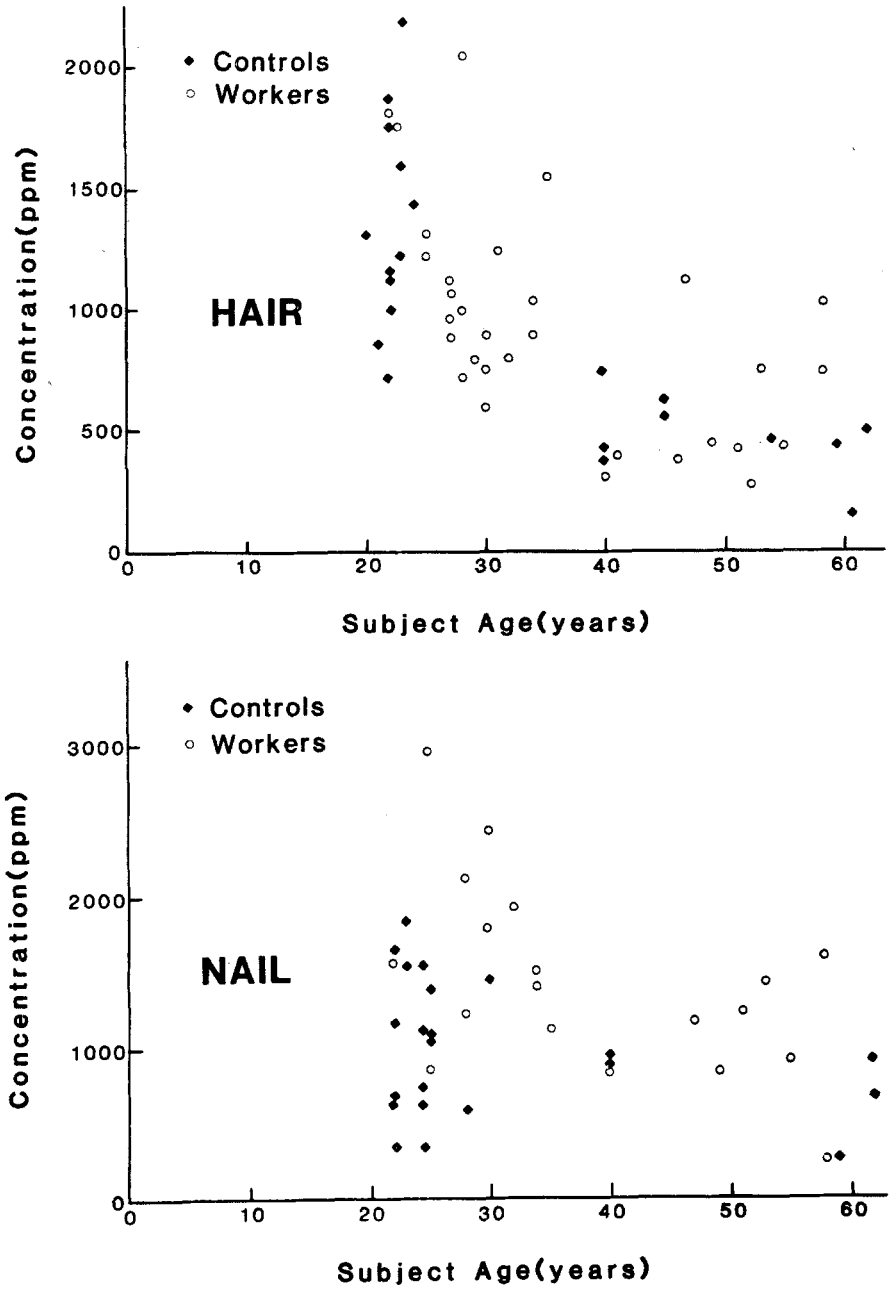

Fig. 3. Ca concentrations in hair and nail as a function of subject age

Table 4
The addition of exogenous material to hair through deliberate contamination

Element	(7)[b] Hair Before Contamination	(1)[b] Hair Following Contamination and Washing
[a]Al	15 ± 2.2[c]	14.3
Ca	1750 ± 150	1670
Cl	400 ± 46	395
[a]Cu	35 ± 2	73
[a]Mg	140 ± 75	≤100
[a]Mn	1.3 ± 0.7	4.6
Na	210 ± 17	240
[a]S(%)[d]	4.3 ± 0.3	3.9
[a]Ti	≤7	≤20

[a] elements present in the metallic mixture used for contamination
[b] (n) where n: The number of replicate samples
[c] arithmetic mean and standard deviation of replicate samples
[d] all results are in ppm except for S for which the results are in percent

In other experiments, hair was contaminated using a mixture of metallic oxides. This resulted in a far greater degree of contamination than in the cases involving the sulphate mixture; following an ether washing, significant amounts of almost all the elements in the oxide mixture (Cu, Mg, Mn, Ti and V) remained in the hair sample. The only exception was Al.

Contacting the hair with either the oxide or sulphate mixtures in glass instead of plastic containers generally reduced the degree of contamination achieved. However, subsequent washing with ether was found to remove all the contaminant only in the case of hair contaminated with the metallic sulphate mixture in a glass container. Attempts were made to clean the hair contaminated in the glass container using a concentrated solution of a commercial shampoo ("Halo", a Colgate-Palmolive product). This cleaning procedure was successful in removing all the contaminant however, large reductions in the hair levels of Al, Ca, and Cl and increases in the levels of Na also occurred independently of which contaminant mixture was used.

It is difficult to assess whether hair would actually ever be exposed, even in an unusual workplace, to as severe contamination as that produced in these experiments. In some occupational situations, such as mining or metal grinding operations, it is possible for hair to get covered with copious dust however, in situations of environmental concern (as distinct from occupational) such exposure would be unlikely. The experiments previously described only served to demonstrate that hair can incorporate exogenous material when contacted by dust, that the degree of contamination varies with both the nature of the contaminant and the contact environment and that mild washing does not insure the removal of all exogenous material.

In order to investigate whether hair actually does incorporate exogenous material when exposed under more realistic conditions, hair was exposed for thirty minutes to welding fumes of concentration 50 mg/m^3 in an environmental

Table 5
The additional of exogenous material to nail through deliberate contamination

Element	Toenail Before Contamination	Toenail Following Contamination and Washing
[a] Al	12[b]	12[b]
Br	2.1	1.4
Ca	1540	1430
Cl	860	570
[a] Cu	13	44
[a] Mg	400	430
[a] Mn	1.2	2.7
Na	930	725
[c] S(%)	2.8	2.4
[a] V	<0.1	0.4

[a] elements present in the mixture used for contamination
[b] all results are from a single analysis
[c] all values are in ppm except for S for which the values are in present

chamber. Following an ether washing, the cleaned hair did not contain increased amounts of any of the elements studied which made up over 80% of the mass of the fumes.

In order to investigate the addition of exogenous material to toenail, a number of clippings were deliberately contaminated in glass containers using the metallic oxide mixture. Following the exposure, the nail sample was washed as described in the experimental section. The results are presented in Table 5.

The contacting of nails with the oxides did result in the addition of Cu, Mn and V to the nails. The elevated levels of these elements produced demonstrate that exogenous material can also be added to toenail such that even a relatively rigorous cleaning procedure cannot entirely remove it.

This observation would suggest that toenails be used preferentially over fingernails in occupational studies due to the relatively lower exposure of toenail to workplace dust.

It was concluded from this study of tissue contamination and subsequent cleaning that exogenous material can be added to hair and nails and that no known cleaning procedure can effectively remove it. However, as described in the section of this paper on temporal variations, in some cases comparisons between samples collected or grown at different times can provide qualitative insight into the degree to which exogenous material is contributing to elevated levels in hair.

Conclusions

1) Interpretation of temporal variations in the hair of individual subjects can be very difficult; however, such variations can provide useful information in group studies.
2) The results of hair or nail analysis using samples from a group of subjects are better represented by geometric means than arithmetic means.
3) Attention should be paid to the age range of the subjects in any groups that are to be compared. Improper age matching between groups can lead to invalid conclusions.
4) Exogenous material can be added to hair and nail and no known cleaning procedure can effectively remove it. It has yet to be established to what degree hair and nail do incorporate material under actual exposure conditions.

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