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Determination of Lead and Copper in Hair by Non-Flame Atomic Absorption Spectrophotometry

The possibility of differentiating between hairs from different people by comparing trace element concentrations (<1000 ppm) in the hair has stimulated a good deal of discussion by forensic scientists in the last decade. Because of its relative ease of removal from the scalp, especially during crimes of aggression, hair is often found at the scene of a crime. Sometimes only a single hair is recovered; and, consequently, sophisticated analytical techniques are required to determine satisfactorily the concentration of trace elements.

There is a characteristic pattern of trace element concentrations in different individuals, which reflects dietary, metabolic, and environmental effects [*1,7,11*]. The probability of establishing either identity or nonidentity in the comparison of two hair samples depends on several factors of utmost importance; (1) the precision of the analysis, (2) trace element concentration variation among specimens from different individuals, and (3) trace element concentration variation among specimens from the same individual. For any given period of time, if the variations in concentration in given individuals are small compared with the range encountered in different individuals then discrimination should be possible. Discrimination will be further enhanced by increasing the number of elements analyzed, provided that they are independent variables.

Emission spectrography [*1*], atomic absorption spectrophotometry [*2*] employing conventional flame techniques requiring prior dissolution of the hair, spark source mass spectrometry [*3*], and neutron activation analysis [*4*] have all been used to measure trace element concentrations in hair. Perkons [*4*] employed neutron activation analysis to study trace element concentrations in the hair of a population of 1200 people. Hair samples were taken from a variety of people, representing different occupations and environments. He concluded that the variations of trace element concentration in hair over the head of one person are larger than the experimental error and, most important of all, that the variations between individuals are at least an order of magnitude larger than those in one person's head hair. A similar study has been carried out by Coleman and co-workers [*5*]. However, they went one stage further than Perkons in that they applied the statistical treatment of Parker [*6*] to their data. From a knowledge of the frequency distribution of mean values of trace element concentrations for the general population and also the variation in trace element concentrations among hairs from a single head, it is possible to decide whether or not hair from a person suspected of committing a crime has a composition comparable with hair recovered from the scene of the crime. In 9 out of 10 simulated

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cases the correct hair was picked out of 10 unknowns as indistinguishable from that from the suspect's head when a limiting value was set so that the combined deviations for 99 percent of the hair on the suspect's head were expected to be less than the limiting value.

One of the biggest problems encountered by the analyst is that of hair sample preparation. Some workers [5,7] maintain that surface contamination should be completely removed from the hair prior to analysis to ensure that the trace element concentrations obtained reflect only those elements within the hair structure and tightly bound to the surface. Too vigorous a washing procedure can remove trace elements from hair. A cleaning method should be just sufficient to remove the oily surface material and extraneous foreign matter. But long exposure to water and ionic detergents should be avoided. A 2 h ether extraction of hair meets these requirements. Bate and Dyer [7] have examined in detail the effect of various washing and soaking procedures on the trace element content of hair. They have suggested that appreciable and highly variable fractions of the trace elements can be removed by extensive washing. Other workers prefer not to wash hair prior to trace element determination [8].

The present study was initiated with the broad objective of measuring trace elements in hair by atomic absorption spectrophotometry, but without prior dissolution of the hair. This entailed the utilization of a non-flame atomizing technique. A carbon rod atomizer [9] has been evaluated and compared with the Perkin Elmer HGA-70 carbon tube furnace. A feasibility study for two elements of interest, namely, copper and lead, has been carried out. The sensitivity of the method is such that it is possible to determine trace element concentrations in sections of hair as small as 1 mm in length (*ca.* 5 μg).

Trace element concentration determinations in hair by other workers are discussed in the light of our present findings concerning the variation of trace element concentrations along the length of the hair. Also, the mechanisms whereby trace elements enter the hair structure are considered.

Experimental

A Perkin Elmer 303 Atomic Absorption Spectrophotometer was used in this study with the carbon rod atomizer (CRA) taking the place of the burner assembly. The absorption signals were measured on a 10 mV strip chart recorder. For a limited number of experiments, as indicated below, a Perkin Elmer 403 Atomic Absorption Spectrophotometer incorporating a HGA-70 carbon tube furnace was made available to us. With these systems detection limits of 5×10^{-11} g and 1×10^{-11} g were attainable for lead and copper, respectively.

The CRA consisted of a 2 mm diameter carbon rod (Morganite Ltd) clamped across two water cooled stainless steel electrodes and could be heated to a temperature in the region of 2500°C within a few seconds by passing a current of about 100 A at 5 V through it. A flow of argon from below the rod inhibited oxidation of the carbon. The cell was enclosed in a glass container with two holes to allow passage of the hollow cathode lamp beam and also to provide an exit for the argon. The sample is placed in a small niche at the centre of the rod. Argon flow rate and the height of the lamp beam above the rod were adjusted to give the maximum absorption signal.

The HGA-70 carbon tube furnace could be heated to a maximum of 3000°C. The furnace control unit could be pre-programmed to give intermediate temperatures and evaporation and pretreatment times prior to final vaporization at the high temperature. Liquid samples were introduced into the tube from a pipette via a hole in its centre and solid samples were placed in the tube through the open end. The whole tube was purged with argon gas.

Hairs were cleaned, prior to analysis, by extracting with ether for 2 h in a Soxhlet apparatus. Sections of hair were cut with a scalpel blade and weighed on a microbalance (Beckman LM 500). At a temperature of about 300°C hair begins to decompose and forms a black carbonaceous deposit. This property enabled the introduction of hair samples of considerable length into the small niche in the carbon rod. The temperature was first raised to about 300°C and the end of the hair was placed in contact with the rod, causing the hair to char. The whole hair sample was then "fed" into the rod in this manner. When all the organic matter had been removed the absorption signal of the element under investigation was recorded at the optimum temperature. A similar procedure was adopted for the HGA-70. For both atomizers standard solutions were introduced via 5–50 μl (5 μl maximum for the CRA) pipettes, and after initial evaporation of solvent the absorption signal determined.

Preliminary experiments with the CRA demonstrated the possibility of preparing calibration curves for lead and copper using 5 μl volumes of standard solutions. However, when sections of hair of varying length were analyzed and the corresponding absorption signals plotted against length of hair, that is, against the amount of trace element contained in that section (millimeter lengths of hair were taken, it being assumed that the concentration of trace element did not vary greatly over up to 1 cm distances), the resulting curve indicated that the absorption sensitivity for hair samples was about one third of that found for solutions. This loss of sensitivity is probably a direct result of the hair decomposition product not covering the whole area of the niche. This problem does not arise with solutions.

On the other hand the same problem did not arise with the HGA-70; the hair sections in this case gave absorption signals which were directly related to the standard solution curves. The atom cloud produced above the hot carbon rod of the CRA is rapidly condensed by the cold argon; whereas, in the HGA-70 tube furnace the atoms are retained for a much longer period; and, consequently, the sampling problems encountered with the CRA are eliminated.

For CRA experiments the trace element concentrations are arbitrarily expressed as percent Absorption per μg^{-1}. For the HGA-70 absolute values of trace element concentration are expressed in ppm.

Results

Variation of Lead and Copper Concentration Along the Length

A hair from the head of a female participant was cleaned, cut into 0.5 cm sections and the sections weighed. The total copper content of each section was determined by atomic absorption spectrophotometry with the HGA-70 accessory. The copper concentrations for the particular sections analyzed are shown plotted in Fig. 1 and demonstrate the variation in copper concentration along the length of the hair. Copper concentration gradients along the hair of other subjects were somewhat lower than the example shown here; but, nevertheless, the gradients did vary from individual to individual.

Lead in hair was determined with the aid of the CRA. Seven subjects (four male and three female) were studied and five or six hairs from each were cleaned and cut into 1 cm sections. Every other centimeter was analyzed for lead. For every centimeter section analyzed, at particular points along the hair, a mean lead concentration was calculated from the values for individual hairs for each subject. These values are shown plotted in Fig. 2 for the seven subjects. In Fig. 3 the results for six hairs from subject 7 are shown

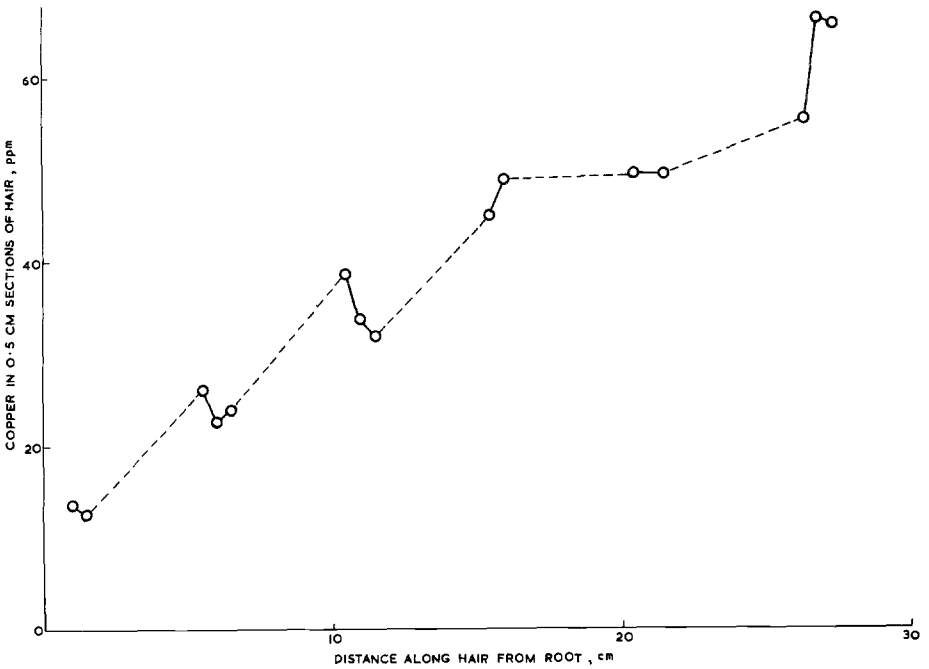

FIG. 1—*Variation in copper concentration along the length of a single hair. The subject was female.*

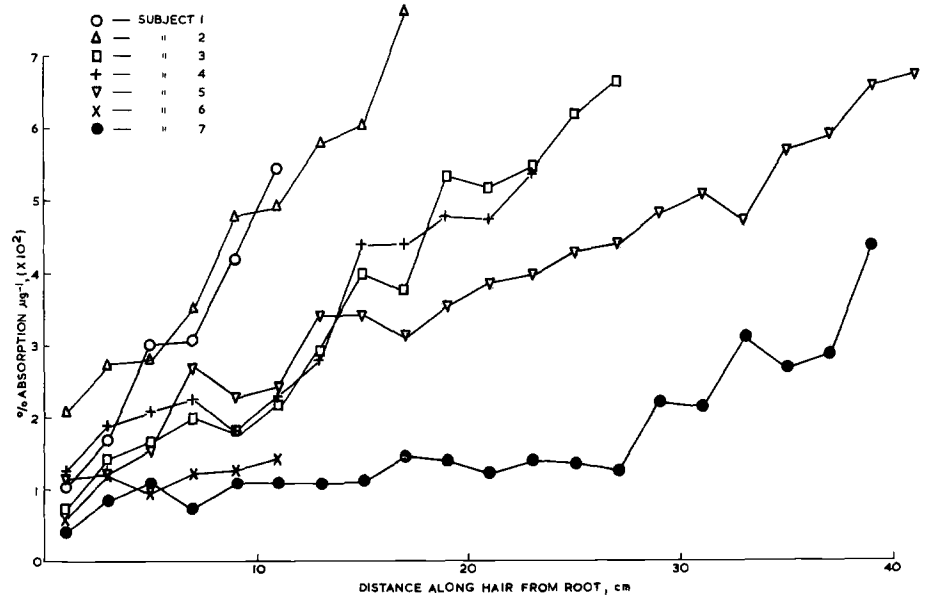

FIG. 2—*Variation in lead concentration along the hair of seven subjects. Each graphical point represents the mean concentration value for the determinations on five or six hairs. Subjects 1, 2, 4 and 6 were male and subjects 3, 5 and 7 were female.*

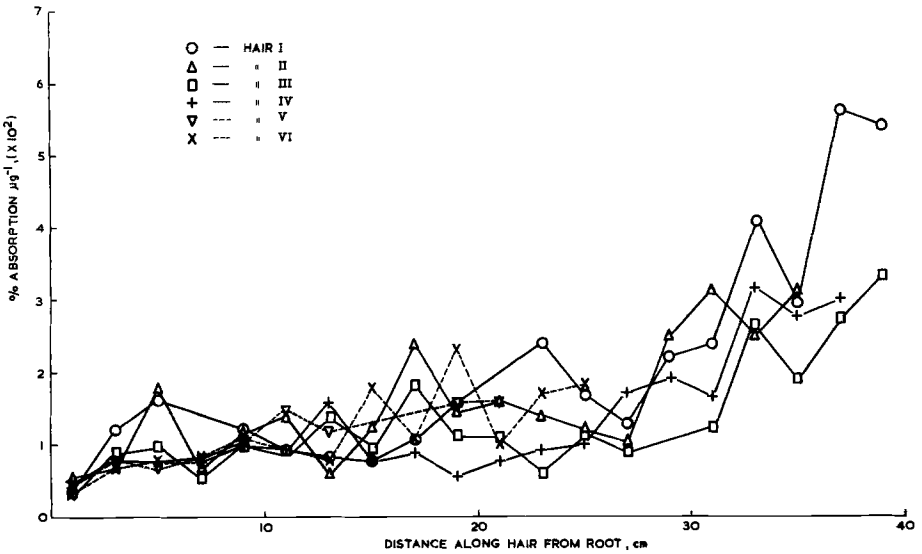

FIG. 3—*Lead concentration variation along the length of six different hairs from the head of subject 7. This figure illustrates the within person variation in lead concentration at various distances from the root. The curves for hairs V and VI are shown in broken lines for clarity.*

plotted and indicate the lead concentration variation over a single head at increasing distances from the root.

Population and Personal Variability of Lead and Copper Concentrations

Hairs from 17 females and 47 males were obtained for analysis to demonstrate the ability or inability of the method to differentiate between hairs from different heads. The variations in copper and lead concentrations in different sections of hair from this group were compared with the variations of these two elements in 20 hairs from 4 of the heads in this same population.

Hairs with roots attached were chosen for analysis and were removed from the head during combing. They were cleaned and weighed and sectioned as follows. The first centimeter from the root end was discarded, the next three 3 mm sections taken for copper analysis, and the next two 1 cm sections for lead analysis. All analyses were performed with the CRA system. The results are shown plotted in the form of histograms in Figs. 4 and 5 for lead and copper, respectively. The data for the variation of copper and lead over individual heads and in the population is summarized in Table 1.

Discussion

Non-flame atomic absorption spectrophotometry has been shown to be a feasible method for the determination of trace elements in human hair. From the experiments so far conducted two conclusions have been drawn: 1) it is possible by this new technique to demonstrate that hairs from different individuals contain different amounts of lead and copper, and 2) the concentrations of both lead and copper increase from root to tip.

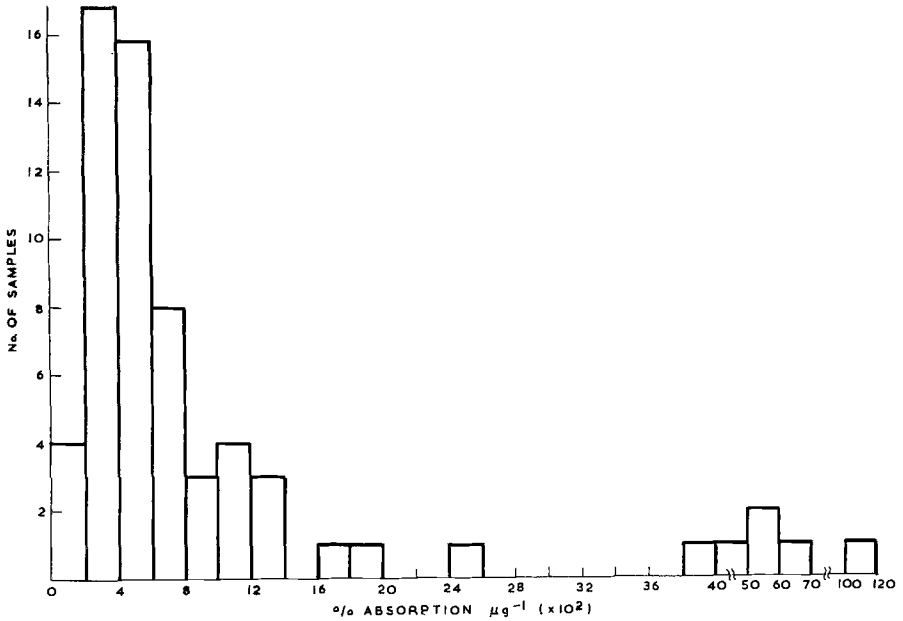

FIG. 4—*Histogram demonstrating the variation in lead concentration in hair from a population of 17 females and 47 males.*

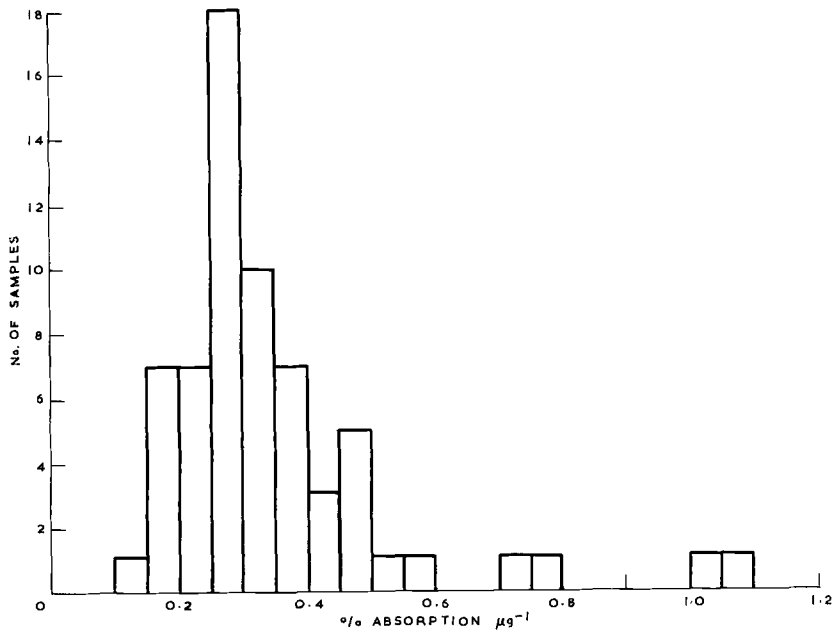

FIG. 5—*Histogram demonstrating the variation in copper concentration in hair from a population of 17 females and 47 males.*

TABLE 1—*Summary of copper and lead data.*

Sample	Element Measured	Number of Samples	Mean, Natural Logarithm of Concentration % of Absorption μg^{-1}	Logaritom of Standard Deviation
F11	Cu	20	−0.333	0.235
	Pb	20	−1.953	0.709
M52	Cu	20	−1.083	0.195
	Pb	20	−3.829	0.423
M51	Cu	20	−1.038	0.180
	Pb	20	−3.178	0.457
M13	Cu	20	−0.512	0.169
	Pb	20	−3.539	0.383
Population	Cu	64	−1.160	0.420
Population	Pb	64	−2.724	0.937

Several interesting observations concerning the analyses are noteworthy.

1. In all cases the lead and copper concentration increased from root to tip. Subject 7 was particularly interesting since the lead concentration here remained more or less constant between 3 and 27 cm and then increased considerably.

2. In the subjects studied there tends to be a greater variation in lead concentration in the distal half of the hair for hairs from different individuals (see Fig. 3) than in the proximal half.

3. Hairs of varying length from the same head show the same lead concentration variation pattern. For example in Fig. 3, hair V, of length 21 cm, shows the same pattern as hair I of 39 cm length.

4. Both the hair-copper and hair-lead distribution curves (Figs. 4 and 5) were positively skewed; and when subjected to a Chi square distribution test, a log-normal behavior was found. The standard deviation of copper and lead concentrations for the population are roughly twice those over a single head (Table 1). It follows therefore, that lead and copper data should prove useful, together with data on other elements, in discriminating between hair samples from different sources. However, this premise will only be true if the hair sample sections for analysis are taken at equal distances from the root, because the variation in concentration along the length of a hair is considerably greater than the concentration variation at a particular distal point along the hair for hairs from the same head (see Fig. 3).

Previous investigators have, in the main, disregarded the possibility of trace element concentration variation along the length of the hair. Schroeder and Nason [*10*] attempted to correlate trace elements with age, color, and sex. They obtained samples of hair from the sides and back of the head of male subjects, that is, relatively short hair. For females they cut samples from the ends of the hair. It is clear from the results presented here that the conclusions they reached, particularly regarding the copper and lead variations, are likely to be very misleading. In a recent paper by Petering et al [*11*] the copper concentrations in the hair of males and females from all age groups were compared and some conclusions drawn. But again the male samples were taken from the sides and back of the head and the female samples from the ends of the hair. In another study Harrison et al [*2*] found a hair-copper value of 13.5 $\mu g/g$. They considered this value to be low compared with the neutron activation value of about 30 $\mu g/g$. However, when one takes into account the fact that their samples were taken from the nape of the neck (short hairs), the low result is not so surprising. Consider the copper results for subject 1 (see Fig. 1). For this

subject a total hair analysis, if carried out by Harrison and co-workers, probably would have shown a copper value of 40 μg/g for a 28 cm hair; but, on the other hand, a 10 cm hair probably would have given a low value of 20 μg/g. Schlesinger et al [*12*] found that when the concentrations of I, Cu, Mn, Zn, Na, Cl and Br were compared with hair length, no significant correlation between length and composition was found, whereas, this work and the results of other investigators [*7,13*] indicate that such a statement is not applicable to lead and copper. A similar incorrect general assumption was made by Coleman et al [*14*] who stated that although it would be advisable to select a portion of hair for the control similar to the crime sample, if this was not possible, the use of whole hairs from the control was not likely to introduce large errors.

In the opinion of the authors, it is imperative that in future investigations the possibility of variation in trace element concentration along the length of the hair should be taken into account. It has been suggested [*17*] that such information would considerably reduce the Q values in Parker's analysis. (The Q value represents that percentage of the population with attributes lying within certain defined limits.) However, there may not be a variation for all elements because this will depend to a large extent on the mechanisms by which the element entered the hair.

Why is there a variation in concentration of lead and copper, and conceivably other elements, along the length of human head hair? This question may be partly answered by a consideration of the mechanisms by which trace elements may enter the hair. There are at least three main pathways:

1. Metabolically essential elements, such as Zn and Cu, enter the hair during the growth stage by way of the blood. The actual elemental concentrations in the body at any one time depend on the physiological and mental [*15*] state of the person. Also, it is known that copper acts as a catalyst in the formation of disulphide cross-linkages in the keratinization process. Trace elements entering the hair by this mechanism are not likely to result in the appearance of a concentration gradient for a particular element along the hair length. Of course, if the body intake of a particular element, for example during poisoning, is elevated at some period then the element will eventually appear in the hair.

2. The apocrine glands, which are associated with the hair follicle, produce perspiration containing trace elements. The perspiration may travel along the surface of the hair by capillary action and on evaporation deposit its trace elements at distal points from the scalp. There is some evidence to support such a hypothesis. Radioiodine given to thyroid patients is found within a week to be strongly adsorbed in the outer ends of the hair [*16*]. Since the iodine at the tip of the hair could not have been incorporated into the growing hair, the iodine must have been adsorbed from perspiration. There will be a variation, depending on the solubility, in the extent to which certain elements are deposited from perspiration.

3. Environmental contamination of the hair results in the elevation of certain trace elements in hair. Hair preparations, dust and air are the main sources of external contamination. Washing techniques might add or remove hair trace elements. It is not presently possible to distinguish in hair between trace elements originating from internal as opposed to external sources.

The variations of copper and lead along the length of hair may be a result of mechanisms 2 and 3 discussed above. External contaminants are dissolved in (or suspended in) the perspiration which then evaporates and leaves trace elements adhering to the hair surface, some of which may become associated with the hair structure. In some cases environmental contamination may be the sole cause. The longer a particular section of the hair is in contact with perspiration and the environment, the greater will be the concentration of trace elements.

Summary

A method has been described for the determination of trace elements in small sections of hair by the application of non-flame atomic absorption spectrophotometry. Lead and copper concentrations have been measured in a population of 64 people and also over a single head. The variations in copper and lead concentrations over the population and a single head were found to be significantly different and useful in discriminating between hairs from different sources. By analyzing sections at varying distance from the scalp it has been demonstrated that the concentration of copper and lead increases from root to tip. Furthermore, the concentration gradient for copper and lead varies from person to person. The consequences of such concentration gradients along the length of human hair have been discussed in relation to the problem of hair individualization.

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