THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 66:371–375, 1976
Copyright © 1976 by Williams & Wilkins Co.

Vol. 66, No. 6
Printed in U.S.A.

TRACE-ELEMENT CONCENTRATIONS IN HUMAN HAIR MEASURED BY PROTON-INDUCED X-RAY EMISSION

D. RENDIĆ, PH.D., S. HOLJEVIĆ, B.SC., V. VALKOVIĆ, PH.D., T. H. ZABEL, M.A., AND G. C. PHILLIPS, PH.D.

Ruder Bošković (DR), Zagreb, and FIP, University of Rijeka (SH), Rijeka, Yugoslavia; and T. W. Bonner Nuclear Laboratories (VV, THZ, GCP), Rice University, Houston, Texas, U. S. A.

Proton-induced x-ray spectroscopy has been used to measure trace element concentration ratios for 10 elements relative to Zn on a group of the population in the harbor city of Rijeka, Yugoslavia. The average biologic levels of elemental ratios for the inhabitants of this region have been established. The presence of pollutants has been confirmed by studying the variation of elemental concentration ratios along single samples of hair.

Hair is a unique biologic material which reflects the biomedical and environmental history of the subject. Since it is convenient to handle and sample, and since it has relatively high concentrations of metals, trace-element analysis of human hair has been applied widely [1–7]. It has been shown [8,9] that the diagnosis of chronic plumbism in children can be confirmed by hair lead. Poisoning by other heavy metals and environmental pollution should also be accompanied [10] by elevated levels in the growing hair. Therefore, measurements of trace-element concentrations in hair might give an indication of environmental pollutants.

Hammer et al [11] related hair trace-metal levels to environmental exposure in a study of fourth-grade school boys in 5 cities in the U. S. A. It was shown that mean hair metal levels for arsenic, cadmium, and lead accurately reflected community exposures. On the other hand, copper and zinc concentrations were not influenced by environmental effects, apparently due to the high concentrations of these elements in human diets.

The concentration of trace elements in human hair may vary for most of the elements from individual to individual, but some correlation due to sex, age, and color has been found [12–16] if the sample preparation was adequately performed [17]. Assuming that the growth of hair is approximately constant and continuous during the lifetime of the subject, the concentrations of trace elements should differ with increasing distance from the scalp. The concentrations of most trace elements should increase with increasing distance from the scalp if the exposure of hair to the elements of the environment is constant. Therefore, the influence of the environment on a population group can be indicated by trace-element analysis. Renshaw et al [18] have studied the concentration of lead along a single hair and found a nearly linear increase in concentration with distance from the scalp.

Klevay [19] and Hambidge et al [20] found the amount of zinc in hair to vary with age of the subjects. Zinc content fell during the first decade of life and increased during the second. No difference due to sex was noted. Zinc deficiency may occur in otherwise normal children and severe zinc depletion accompanied by low hair zinc concentrations may occur in acrodermatitis enteropathica [21]. However, zinc concentration in sections of the hair shafts immediately adjacent to the scalp does not differ significantly from concentrations 30 cm distant from the scalp [20]. This suggests that zinc in the external environment does not contribute to the hair zinc content. Obrusnik et al [2] and Eads et al [15] also showed that zinc variations were small along single hairs. Thus, to obtain the distribution of elemental concentrations along the hair, the elemental concentrations relative to zinc may be measured. Zinc is eliminated from the measurement which limits the diagnostic and clinical value of the data, but such measurements can be performed easily on a large group of subjects.

EXPERIMENTAL METHODS

Proton-induced x-ray emission spectroscopy, suitable for such relative-concentration measurements [22], is described elsewhere [23,24] in detail. For these studies a system for trace-element analysis by proton-induced x-ray emission spectroscopy developed at the T. W. Bonner Nuclear Laboratories at Rice University [24] was used. Hair samples of different lengths were obtained by cutting a lock of hair from each of 101 school children from three schools at different locations in the harbor city of Rijeka, Yugoslavia. School M is located in the downtown area, school V in a suburb of the city, while school J is in a small village in the hilly country of Gorski Kotar, approximately 30 km from the downtown areas. Targets were prepared by cutting the samples into pieces approximately 3.5 cm in length. Depending on the length of hair,

Manuscript received September 26, 1975; in revised form February 9, 1976; accepted for publication February 25, 1976.

This work was supported in part by the City Council of Rijeka, Yugoslavia, and the U. S. Energy Research and Development Administration.

Reprint requests to: Dr. V. Valković, T. W. Bonner Nuclear Laboratories, Rice University, Houston, Texas 77001.

1 to 14 targets (labeled A, B, C, etc.) were obtained from each hair sample and each was mounted on an aluminum holder with a central hole 1.5 cm in diameter. The sample was washed carefully with triple-distilled water. A 0.1-cm polystyrene absorber was placed in front of the detector to eliminate x-rays from elements below potassium, as well as secondary electron-induced *bremsstrahlung* below 3 kev. The low-energy background was greatly reduced. The signals obtained from the detector preamplifier were amplified and processed by an IBM-1800 computer.

The number of counts in x-ray peaks was evaluated by programming the computer to "fit" each peak to a Gaussian plus quadratic background function. Concentration ratios relative to zinc were determined using the ratios of the integrated K peaks in the samples normalized to the same ratios obtained from the measurements of standards prepared by the deposition of predetermined solutions of elements onto aluminum-Formvar backings.

RESULTS AND DISCUSSION

Figure 1 shows 2 characteristic spectra obtained with 2 different hair samples. Differences in yields of some peaks are seen. While the K_α and K_β lines from iron show a significant yield in the lower spectrum, the same lines are hardly visible in the upper spectrum.

Fourteen elements were found in almost all samples. Elements Mn, Fe, Ni, Cu, As, Se, Br, Sr, and Pb were extracted in concentration ratios to zinc for all targets. Element Rb was found in a few samples in concentration ratios to zinc of 5×10^{-3} or less. Elements K, Ca, and Ti were not analyzed because their yields were influenced by the attenuation of the polystyrene absorber in front of the detector.

Figure 2 represents the distributions of element/zinc concentration ratios for 9 elements for all subjects in the group under investigation sorted according to the sex of the subjects. The A samples (close to the scalp) have been plotted. It can be noted that all distributions except a few are grouped. While the Cu/Zn distribution is nearly symmetric, and the Cu/Zn ratio changes very little from subject to subject, the Fe/Zn and Pb/Zn ratios show an asymmetry towards higher concentration ratios caused by the high concentration ratios found for some individuals, probably representing the biologic level and/or environmental effects on these particular subjects. It can also be noted that most of the deviations towards higher Pb/Zn concentration ratios are associated with the samples taken in school M and are due to lead in the downtown area, coming probably from the automobile traffic [25].

While the Mn/Zn, As/Zn, and Sr/Zn concentration ratios show a slight asymmetry towards higher ratios, the Se/Zn ratio does not show any tendency towards peaking; this might be due to the low concentration ratios with large errors. It may be concluded that, for most of the elements, a normal concentration ratio can be established, so any significant departure from this concentration ratio

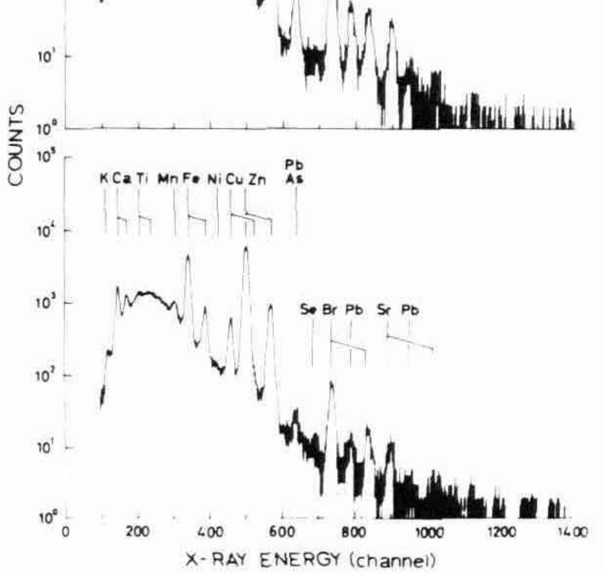

FIG. 1. Two x-ray spectra obtained by proton bombardment ($E_p = 3$ MeV) of hair samples.

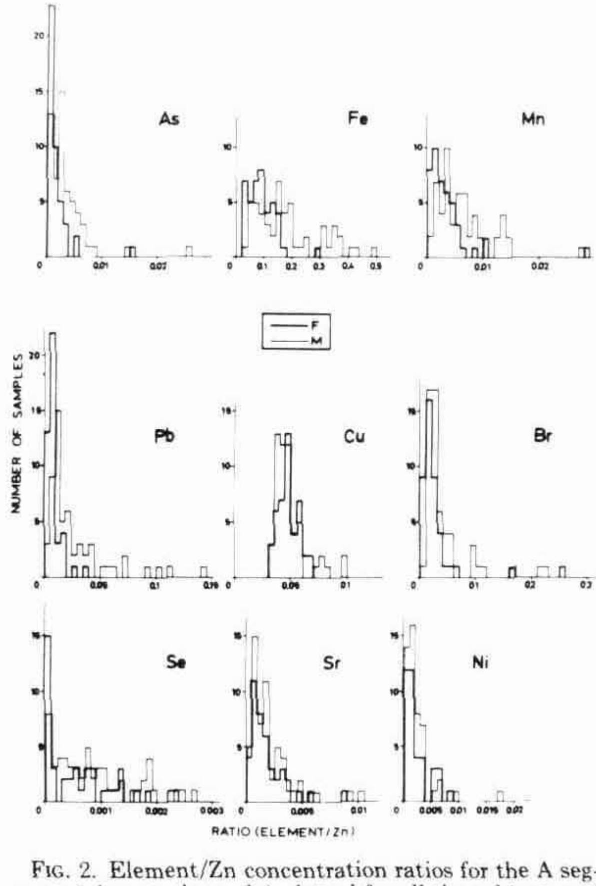

FIG. 2. Element/Zn concentration ratios for the A segments (close to the scalp) plotted for all the subjects and sorted following the sex of the subjects. Note that the abscissa for all the distributions is not the same.

can be spotted easily. The Table shows the arithmetic mean values of element/Zn concentration ratios with their standard deviations for each school separately, as well as the overall mean values. To calculate these average values, the A hair samples were chosen, since it was expected the environmental effects had not significantly influenced the samples. The calculated average values closely reproduce the biologic levels of element/Zn concentration ratios in human hair for the population in this area. It is also worth noting that the variations of the Cu/Zn concentration ratio throughout the whole group of subjects under investigation is rather small. All other ratios exhibit much larger variations.

It can be seen that Cu/Zn, Ni/Zn, Sr/Zn, and Br/Zn concentration ratios do not show any remarkable difference between the distributions for male and female samples. On the other hand, the Pb/Zn, As/Zn, Fe/Zn, and Mn/Zn distributions from female samples are shifted towards lower values.

In the group of children studied, there were 22 female subjects with hair long enough that 5 or more targets could be made. A thorough investigation of the variation of elemental concentrations along hair samples was made in order to investigate possible regularities. In our measurements, element/Zn concentration ratios were measured as functions of distance from the scalp. The functional relationship between element/Zn concentration ratio, R, and distance from the scalp, d, was approximated by a straight line or by a parabola. The most probable values for the coefficients a and b, considering the functional relationship $R = f(d)$ to be linear and of the form

$$R = a + b \cdot d$$

were determined using the standard method of least squares. The quantity χ^2 is defined to be sum

$$\chi^2 = \Sigma\, [(R_i - a - b \cdot d_i)/\Delta R_i]^2$$

where ΔR_i is the uncertainty in the determination of concentration ratio. The method for finding the optimum fit to data consists of minimizing this weighted sum of squares of deviations χ^2 and, hence, finding the fit which produces the smallest sum of squares or the least-square fit. (For more details on statistical interpretation of the data see [26]) The following conclusions were obtained:

The Cu/Zn ratio was nearly constant, showing a slight tendency towards decrease in some cases. The χ^2 fits of concentration ratios to a straight line are good with the χ^2 values (per degree of freedom) between 0.7 and 3.5, and with the b/a ratio in the range of 0.003 to 0.014 cm^{-1} (68% of the samples have a b/a ratio smaller than 0.007 cm^{-1}). It is interesting to note that school M is one with all but 1 sample having a constant Cu/Zn ratio along the hair.

The Pb/Zn ratio showed an increase away from the scalp (in 90% of the samples), and indicated a tendency towards very rapid exponential increase in a few cases. Fits to a straight line show the b/a ratios in the range from 0.14 to 2.26 cm^{-1}. The samples from school M show a much faster average increase ($(b/a_{av.} = 0.76\ cm^{-1}$) than the other two schools (0.33 cm^{-1} and 0.25 cm^{-1} for J and V schools, respectively). All these values were calculated only for those samples which gave a relatively good fit to a straight line.

The Fe/Zn ratio was almost constant. Forty percent of the samples have the b/a ratio smaller or equal to 0.007 cm^{-1}, while most of the remaining samples show a slight tendency towards increasing or decreasing. In only 2 cases was a strongly increasing or decreasing variation found. The parabolic polynomial was found to be the best fit to the concentration ratio data in the other 2 cases, since they exhibited shallow minima in the middle of the hair.

The Br/Zn ratio showed very different variations. In most of the cases studied, χ^2 values are very high, indicating that the data are very scattered. Apparently, excitation of x-rays by protons is not suitable for the analysis of bromine, probably because of its evaporation induced by heat dissipation of the proton beam. However, it might be concluded that most of the samples taken in school M show a tendency towards increasing, while in the other two schools, the concentration ratios are rather constant or decreasing towards the end of the hair.

The Ni/Zn ratio was predominantly constant (in 80% of the samples the b/a ratio was $\leq 0.046\ cm^{-1}$ with only 3 cases showing an increasing tendency; all of them in school M). Because of the low values of the concentration ratios, the variation having the b/a ratio $\leq 0.046\ cm^{-1}$ seems to be fairly constant. The χ^2 values are rather low, partly for the same reason.

The Mn/Zn concentration ratios generally had large statistical errors. The ratios are fairly constant (in 60% of the samples), showing a slight increase in the other cases. Because of the large statistical errors, it is not possible to draw conclusions on the limits for b/a ratios.

TABLE. *Average values for concentration ratios (element/Zn) × 100*

School \ Element	Fe	Ni	Cu	Br	Sr	Pb	Mn	As	Se
V	13.7 ± 9.4	0.17 ± 0.14	4.7 ± 1.1	2.3 ± 2.9	0.2 ± 0.1	1.4 ± 1.5	0.36 ± 0.20	0.24 ± 0.18	0.05 ± 0.06
J	20.7 ± 14.0	0.24 ± 0.15	4.9 ± 1.1	1.7 ± 1.0	0.2 ± 0.3	1.5 ± 1.4	0.93 ± 0.79	0.14 ± 0.17	0.04 ± 0.40
M	13.3 ± 12.5	0.19 ± 0.21	5.0 ± 1.1	2.1 ± 1.6	0.5 ± 0.4	1.7 ± 1.6	0.36 ± 0.31	0.27 ± 0.18	0.19 ± 0.25
Average	15.2 ± 6.6	0.21 ± 0.09	4.9 ± 0.6	1.9 ± 0.8	0.22 ± 0.09	1.5 ± 0.9	0.38 ± 0.16	0.21 ± 0.10	0.06 ± 0.06

The Sr/Zn concentration ratios showed very interesting features. The 50% of the samples, most of them from schools V and J, the ratios show a strong tendency to increase away from the scalp. The rest of the samples, mostly from school M, show very low concentration ratio values at the scalp, a fast increase, and then a leveling off further along the hair, and, in a few cases, a tendency to decrease towards the end of the hair. Apparently the presence of Sr in the environment, most probably from sea water, and the mechanism of its deposition onto the hair causes such a variation of the concentration ratios.

The As/Zn ratios showed a slight increase along the hair, indicating that this element was a possible contaminant from the environment. Samples from school M show a faster increase than those from the other two schools.

The Se/Zn concentration ratios were in general very low, but it may be concluded that they are very constant along the hair. No significant increase was observed in all the samples, although the values of a few of the concentration ratios are rather high.

Five of the concentration ratios (Cu/Zn, Fe/Zn, Ni/Zn, Mn/Zn, and Se/Zn) were constant along the hair. Apparently, the measured concentrations for these elements relative to Zn more or less represent the biologic level of the element in the human body. However, 4 other concentration ratios show a marked increase along the hair. This effect is especially pronounced in the samples from school M, indicating in this way that elements Pb, Br, Sr, and As are contaminants in the downtown environment where the school is located. The differences in the data obtained for subjects from the other two schools clearly indicate that the environment in the regions of those schools is much less polluted with the mentioned elements. The Pb and Br come from burning gasoline in automobile engines; Sr comes from aerosols from sea water; but the source of As has yet to be determined.

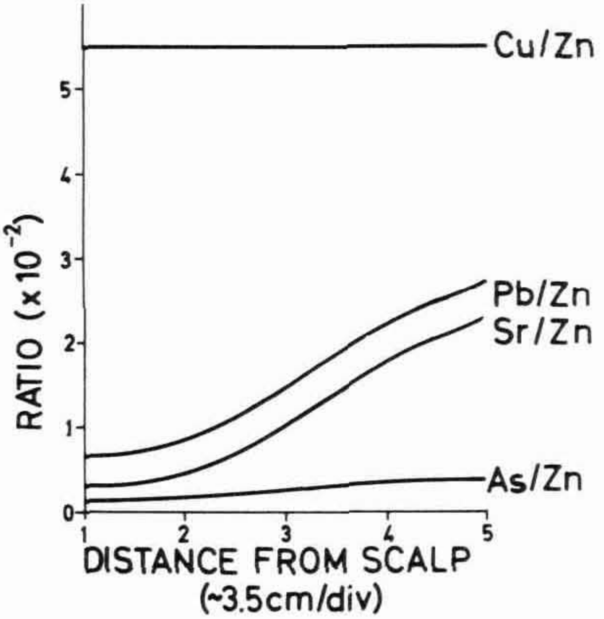

FIG. 3. Median concentration ratio values as a function of distance from the scalp for the distributions of element/Zn concentration ratios for the first 5 segments (A to E) of the hair.

Figure 3 represents the median concentration ratio values for the Cu/Zn, As/Zn, Pb/Zn, and Sr/Zn distributions for the first 5 targets (A to E) of each hair sample, as a function of distance from the scalp. It is seen that the value of the Cu/Zn concentration ratio is remarkably constant. The medians for Pb/Zn, Sr/Zn, and As/Zn increase along the hair. Moreover, the Pb/Zn and As/Zn distributions of the concentration ratios for segments D and E show a faster increase than is observed in other groups; these are samples from the children in school M, in the downtown area. This confirms our previous conclusion [24] that these 3 elements are present in the environment as pollutants.

CONCLUSION

It has been shown that x-ray emission spectroscopy is a very suitable technique for studying elemental concentrations in hair samples. Since only the relative concentrations to Zn have to be measured, the target-preparation procedure is very simple because no internal standard is necessary. This protects the sample from any contamination that can be introduced during the target preparation.

The results in this paper indicate the average concentration values for 9 elements relative to Zn for the area around the harbor city of Rijeka. Five elements show constant concentrations along the hair, and their concentration ratios most probably represent the biologic level of these elements in the hair of the inhabitants in this area. The tendency of the other 4 elements to increase along the hair indicates the presence of these elements as pollutants in the environment. The measurements of concentrations along the hair can undoubtedly be used as a method of identifying pollutants in an area, as well as for extracting information on the exposure of subjects in such an environment.

The authors appreciate the help of a group of students from the Faculty of Industrial Pedagogy, University of Rijeka, during the sample collection period.

REFERENCES

1. Perkons AK, Jervis RE: Trace elements in human head hair. J Forensic Sci 11:50–63, 1966
2. Obrusnik I, Gislason J, McMillan DK, D'Auria J, Pate BD: The variations of trace element concentrations in single human head hairs. J Forensic Sci 17:426–439, 1972
3. Strain WH, Steadman LT, Lankau CA, Berliner WP, Pories WJ: Analysis of zinc level in hair for the diagnosis of zinc deficiency in men. J Lab Clin Med 68:244–249, 1966
4. Prasad AS, Miale A, Faird A, Sanstead HH, Shulert AR: Zinc metabolism in patients with syndrome of

iron deficiency anemia hepatosplenomegaly, dwarfism, and hypogonadism. J Lab Clin Med 61:537–549, 1963
5. Rice WE, Goldstein NP: Copper content of hair and nails in Wilson's disease. Metabolism 10:1085–1087, 1961
6. Mahler DJ, Scott AF, Walsh JR, Haynie G: Study of trace metals in fingernails and hair using neutron activation analysis. J Nucl Med 11:739–742, 1970
7. Ventakavardan VS: Trace elements in hair. Nuclear India 12:4–5, 1974
8. Kopito L, Byers RK, Schwachman H: Lead in hair of children with chronic lead poisoning. N Engl J Med 276:949–953, 1967
9. Kopito L, Briley AM, Schwachman HJ: Chronic plumbism in children. JAMA 209:243–248, 1969
10. Flesch P: Physiology and Biochemistry of the Skin. Edited by S Rothman. Chicago, University of Chicago Press, 1954, p 601
11. Hammer DI, Finklea JF, Hendricks RH, Shy CM, Horton RJM: Hair trace metal levels and environmental exposure. Am J Epidemiol 93:84–92, 1971
12. Schroeder HA, Nason AP: Trace metals in human hair. J Invest Dermatol 53:71–78, 1969
13. Shabelnik DJa: Relationship between the colour of the hair, sex, and the hair microelement content. Sud Med Ekspert 9:7–9, 1966
14. Petering HG, Yeager DW, Wintherup SO: Trace metal content of hair. Arch Environ Health 27: 327–330, 1973
15. Eads EA, Lambdin CE: A survey of trace metals in human hair. Environ Res 6:247–252, 1973
16. Dutcher TF, Rothman S: Iron, copper, and ash content of human hair of different colors. J Invest Dermatol 17:65–68, 1951
17. Bate LC: Absorption and elimination of trace elements in human hair. Int J Appl Radiat Isot 17:417–423, 1966
18. Renshaw GC, Pounds CA, Pearson EF: Variation in lead concentration along single hairs as measured by non-flame atomic absorption spectrometer. Nature (Lond) 238:162–163, 1972
19. Klevay LM: Hair as a biopsy material. I. Assessment of zinc nutritive. Am J Clin Nutr 23:284–289, 1970
20. Hambidge KM, Hambidge C, Jacobs M, Baum JD: Low levels of zinc in hair, anorexia, poor growth, and hypogensia in children. Pediatr Res 6:868–874, 1972
21. Amador M, Pena M, Garcia-Mirande A, Gonzales A, Hermelo M: Low hair zinc concentrations in acrodermatitis enteropathica. Lancet 1:379, 1975
22. Valković V, Miljanić D, Wheeler RM, Liebert RB, Zabel T, Phillips GC: Variation in trace metal concentrations along single human hairs or measured by proton induced x-ray emission photometry. Nature (Lond) 243:543–544, 1973
23. Valković V: X-ray emission spectroscopy. Contemporary Physics 14:415–439, 1973
24. Valković V, Liebert RB, Zabel T, Larson HT, Miljanić D, Wheeler RM, Phillips GC: Trace element analysis using proton-induced x-ray emission spectroscopy. Nuclear Instruments and Records 114:573–579, 1974
25. Valković V, Rendić C, Phillips GC: Elemental ratios along human hair or an indicator of the exposure to environmental pollutants. Environmental Science and Technology 9:1150–1152, 1975
26. Bevington PR: Data Reduction and Error Analysis For the Physical Science. New York, McGraw-Hill, 1969.