Bull. Environ. Contam. Toxicol. (1990) 45:478–485

Bulletin of Environmental Contamination and Toxicology

Lead, Iron, Copper, Zinc and Ash in Deciduous Teeth in Relation to Age and Distance From a Lead Smelter

Maja Blanuša,[1] Nikola Ivičić,[1] and Vladimir Simeon[2]

[1]Institute for Medical Research and Occupational Health, University of Zagreb, M. Pijade 158, 41000 Zagreb, Yugoslavia and [2]Faculty of Science, University of Zagreb, Marulićev trg 19, 41001 Zagreb, Yugoslavia

As lead is very easily deposited in calcified tissues throughout life, tooth lead level is a representative and reliable indicator of cumulative long-term exposure to lead and probably also to other trace elements (Needleman et al. 1972). Since children present a high risk population group in respect to lead exposure an international study on "Biological indicators of lead neurotoxicity in children" was undertaken in 1985, sponsored by WHO/CEC. In this country the study group were children living in a lead smelter area. In the same region various environmental and biological parameters were measured over a period of years (Prpić-Majić et al. 1988). The lead in deciduous teeth of the group of children under study was found to be related to some other biological parameters indicating recent lead exposure like blood lead, erythrocyte delta-aminolaevulinic acid dehydratase, zinc-protoporphyrin and haemoglobin levels (Telišman et al. 1987).

The purpose of the present study was to determine in the same teeth not only the concentrations of toxic (Pb) but also of some essential trace elements (Fe, Zn, Cu) and to relate these data to children's age and residential distance from the lead smelter. An exploratory principal-components statistical analysis was performed.

MATERIALS AND METHODS

Incisors were collected from a total of 40 children, 21 boys and 19 girls living in the polluted area around the smelter and from 9 children, 7 boys and 2 girls from the control area. The children were 5.8-8.4 years old. As the average lead content of the shed teeth may be taken as an integrated measure of the

Send reprint requests to M. Blanuša at the above address.

total lead exposure during early life, the lead content of teeth with partially or completely resorbed roots was corrected for the part resorbed. Namely, the length of the root is normally twice the length of the crown and by carefully measuring the heights of the crown and the rest of the root, the percentage of resorption can be estimated (corrected tooth values).

Whole extracted teeth were dried at 105°C, dry ashed in muffle furnace at 450°C and dissolved as described earlier (Ivičić and Blanuša, 1988). Lead, iron and copper concentrations were determined by electrothermal atomic absorption spectrophotometry (ET-AAS) on Varian-375, a carbon rod atomizer (CRA-90), an automatic sample dispenser (ASD-53) and a recorder (Mod. 9176). Nitrogen gas flow and deuterium background correction were applied. Zinc was determined by flame AAS and deuterium background correction. Results of the analyses for all elements are expressed in µg per gram of dry weight of tooth. Lead was additionally expressed in µg on the whole corrected tooth.

Quality control of tooth lead analysis was performed within the WHO/CEC project "Lead Neurotoxicity in Children". The quality of assessment of other elements was tested within "Intercomparison of Minor and Trace Elements in IAEA Animal Bone, H-5" (IAEA, 1982). All our values were within the range of accepted laboratory averages.

Factor analysis was performed by using SPSS/PC program package. Eight variables from 49 observations were analyzed: Pbd (expressed in µg/dry tooth), Pbc (in µg/whole corrected tooth), Fe, Zn, Cu (in µg/g dry tooth), ash (as percentage of dry tooth), age (months) and distance from the lead smelter (1, 2, 3 or 4). The children living at various distances from the main source of pollution (i.e. 150-180 m, 1200-3400 m, 4200-6500 m and the control group at about 7 km) were assigned four ranks for statistical evaluation and used as coded values.

RESULTS AND DISCUSSION

The concentrations of lead, iron, zinc, and copper, and the amount of ash in tooth samples are tabulated according to the distance from the lead smelter and age (Table 1). All trace elements show the tendency to decrease with the distance from the smelter. Product moment correlations of the contents of various metals, age and distance from the smelter are presented in Table 2. The correlation matrix shows significant and positive correlation of zinc and iron with copper, but

Table 1. Concentrations of lead, iron, zinc, copper and quantity of ash in deciduous teeth of children living at different distances from the lead smelter

	Pb_d (µg/g)	Pb_c (µg/corr. tooth)	Fe (µg/g)	Zn (µg/g)	Cu (µg/g)	Ash (%)	Age (month)
			150-180 m				
Mean	34.6	11.6	36.2	319.7	2.5	81.7	84.0
SE	5.8	1.9	4.2	20.7	0.8	0.9	2.9
max	85.1	29.3	55.7	407.7	10.5	86.6	101
min	16.6	6.7	8.4	233.9	0.4	78.1	79
n	11	11	11	11	11	11	11
			1200-3400 m				
Mean	19.3	8.3	42.9	270.7	1.7	81.3	75.5
SE	2.2	1.1	5.8	10.1	0.4	0.8	0.9
max	30.6	13.0	100.2	342.0	5.0	84.2	80
min	8.7	4.2	40.9	219.5	0.5	73.9	69
n	12	12	12	12	12	12	12
			4200-6500 m				
Mean	19.5	6.3	37.8	290.6	1.7	80.5	75.7
SE	3.4	1.4	6.4	11.5	0.3	0.6	0.7
max	58.0	21.9	105.0	428.5	5.2	85.7	80.0
min	5.0	1.1	8.5	222.6	0.5	76.8	71
n	17	17	17	17	17	17	17
			7 km				
Mean	7.4	1.3	21.1	193.6	0.8	84.4	86.4
SE	0.7	0.2	3.2	7.7	0.1	0.6	1.5
max	12.5	2.9	35.0	240.1	1.3	86.6	91.0
min	3.5	0.9	5.6	180.2	0.3	81.3	78
n	9	9	9	9	9	9	9

Results are expressed in µg per g of dry tooth weight.

Table 2. Product-moment correlations of the contents of various metals, ash, age and distance from the lead smelter in deciduous teeth

Variables	Pb_d	Pb_c	Fe	Zn	Cu	Ash	Age	Distance
Pb_d	1.0000							
Pb_c	0.8851**	1.0000						
Fe	0.3451*	0.3356*	1.0000					
Zn	0.5716**	0.5736**	0.3282	1.0000				
Cu	0.3148	0.3990*	0.5182**	0.5805**	1.0000			
Ash	-0.3241	-0.2969	-0.1499	-0.1680	-0.1088	1.0000		
Age	0.170	-0.0386	-0.3994*	-0.1595	0.1675	0.4062*	1.0000	
Distance	-0.5352**	-0.5755**	-0.2216	-0.5374**	-0.3208	0.1976	0.0316	1.0000

a) Significance: (*) $\leq$ 0.01, (**) $\leq$ 0.001

Table 3. Principal components (Pct) of correlation matrix from Table 2: factor significance (x) was inferred by means of Kaiser criterion; cummunalities of variables were estimated from three factors taken as significant

Factor No.	Eigenvalue	Pct. of variance	Cum. pct variance
1	3.55760	44.5	44.5
2	1.41338	17.7	62.1
3	1.07412	13.4	75.6
4	0.63016	7.9	83.4
5	0.53030	6.6	90.1
6	0.43056	5.4	95.5
7	0.29946	3.4	98.8
8	0.09443	1.2	100.0

Table 4. Factor structure/pattern matrix of Varimax rotated PC solution (cf. Tables 2,3): loadings > 0.5 are marked with an asterix

Variables	Factor 1	Factor 2	Factor 3	Cummunality
Pb_d	0.90217*	0.11885	0.11718	0.84177
Pb_c	0.89442*	0.18135	0.11002	0.84497
Fe	0.14138	0.78795*	0.24986	0.70380
Zn	0.64467*	0.51159*	0.00204	0.67732
Cu	0.30602	0.81099*	0.05273	0.75413
Ash	-0.32226	0.10775	0.84360*	0.82712
Age	0.17187	-0.41776	0.78099*	0.81401
Distance	-0.74401*	-0.16780	0.01638	0.58198
	lead factor	transition metal factor	mineralization factor	

a negative correlation of lead, iron, zinc and copper with the distance.

To gain insight into the interrelations of the variables measured an exploratory principal-components analysis was performed. On the ground of the scree plot and Kaiser criterion three factors were retained explaining as much as 76 per cent of total variation. The results are given in Table 3.

Upon rotating this solution into Varimax position (see Table 4) a logical and clear enough separation of variables was achieved. Closely related variables (Pb)dry and (Pb)corr clustered near Factor 1 ("Lead factor") together with the distance from the lead smelter and - to a somewhat lesser extent - with (Zn). The contents of iron, copper and zinc centered around Factor 2 ("Transition-metal factor"), while ash content and age came close to Factor 3 ("Mineralization factor"). Oblimin rotation yielded the same clustering of variables as Varimax.

It was previously shown (Blanuša et al. 1985) that factor analysis as a statistical approach enables better insight into the mutual dependence and interaction of variables. The group of children residing in the lead polluted area was chosen because of expected high lead deposition in their teeth and higher environmental exposure to lead and other elements. Longitudinal studies in that area of lead, zinc and cadmium levels in samples of aerosols and soil showed them to be higher than in the control area (Hršak, 1987). The zinc content of teeth has been found to vary with dietary zinc levels (Attramadal and Jonsen, 1976) and slightly also with environmental levels (Hambidge, 1986). This was proved in an experimental study on mice (Fosse and Justesen, 1987) where it was concluded that human deciduous teeth can be used as indicators of (hyperoptimal) high zinc absorption but not of high copper absorption. However, high correlations were found between lead and copper in permanent and deciduous human teeth (Kiyoshi, 1983). Iron was found to be taken up by teeth and bones mostly as a trivalent iron compound in an vivo process. In vitro, iron was absorbed on the surface of dental enamel as amorphous or colloidal layer (Bauminger et al. 1985). Correlation was also found between iron content in the enamel and the dentine (Masao et al. 1982). There are no literature data about the relationship between iron intake and tooth iron level. Unfortunately, data on the environmental level of the elements measured and data on their dietary intake in the population examined are lacking.

Our statistical evaluation pointed an association of zinc, copper and iron concentrations in teeth around the "transition-metal factor" as well as of lead and zinc in the "lead factor". This might be the consequence of higher population exposure to these metals or to some biochemical mechanisms which cause higher teeth uptake of essential elements in conditions of higher lead uptake (metal-metal interaction ?).

In "lead factor" tooth lead level was expressed in two different ways, as concentration (in µg/g dry tooth weight) and as quantity (in µg/whole tooth), because the teeth in our study were extracted at different stages of root absorption. As root contains more lead than the rest of the tooth, this may offset the gain in lead which comes with age (Mackie et al. 1977) and which was found in other studies (Stewart, 1974). However, in our "corrected teeth" values no association with age was found. The reason for lack of lead and age association was probably the rather narrow age interval in our study i.e. 5.8-8.4 years. However, in spite of this, age was closely correlated with the ash content in the "mineralization factor". This finding is supported by other findings of positive association of deciduous teeth formation and somatic growth (Karlberg et al. 1976) and the possibility of using primary teeth development for estimating age (McGregor et al. 1968).

Our statistical approach shows once again the advantage of this method, especially when the number of observations is rather low. The results seem to be in accordance with the physiological properties of the metals under study as well as with their chemical properties and positions in the periodical table.

Our results seem to indicate that deciduous teeth might be a good indicator of environmental exposure to several trace elements. However, more data on actual intake of these elements in children are needed before making final conclusions.

Acknowledgments. This work was supported by Research Council of Croatia. We appreciate the help and support of Professor K. Kostial in this work. Our thanks are due to Mrs Đ. Breški and M. Ciganović for their excellent technical assistance.

REFERENCES

Attramadal A, Jonsen J (1976) The content of lead, cadmium, zinc and copper in deciduous and permanent human teeth. Acta Odont Scand 34:127-131

Bauminger E, Ofer S, Gedalia I, Horowitz G, Mayer I (1985) Iron uptake by teeth and bones: a Mossbauer effect study. Calc Tiss Int 37:386-389

Blanuša M, Kralj Z, Bunarević A (1985) Interaction of cadmium, zinc and copper in relation to smoking habit, age and histopathological findings in human kidney cortex. Arch Toxicol 58:115-117

Fosse G, Justesen NPB (1978) Zinc and copper in bone and teeth of mice. Int J. Environ Stud 12:111-120

Hambidge KM, Casey CE, Krebs NF (1986) Zinc,.In: Mertz W (ed) Trace Elements in Human and Animal Nutrition Vol. 2, Fifth edition, Academic Press Inc. Orlando, pp 1-109

Hršak J, Fugaš M (1987) The fate of lead, zinc and cadmium from lead smeltery emissions in the environment. In: Trace Elements in Human Health and Disease, Second Nordic Symposium, Odense, Denmark 1987, Abstract E10

International Atomic Energy Agency (1982) Intercomparison of Minor and Trace Elements in IAEA Animal Bone (H-5). Progress Report No. 1.

Ivičić N, Blanuša M (1988) Comparison of ETA-AAS and DPASV methods for the determination of lead in deciduous teeth. Fresenius Z Anal Chem 330:643-644

Karlberg P, Taranger J, Engstrom I, Lichtenstein H, Svenberg-Redegren I (1976) The somatic development of children in a Swedish urban community. A prospective longitudinal study. III. Dental development from birth to 16 years. Acta Pediatr Scand, Suppl 258:83-97

Kiyoshi O (1983) A study of trace elements in human dental tissues. On concentrations of copper and lead. Shika Igaku 46:65-78

Mackie AC, Stephens R, Townshend A, Waldron HA (1977) Tooth lead levels in Birmingham children. Arch Environ Health 32:178-185

Masao M, Takashi H, Kazuo S (1982) Concentration distribution of trace metals in human teeth. Nippon Eisegaku Zasshi 37:499-504

McGregor IA, Thomson AM, Billewicz WZ (1968) The development of primary teeth in children from a group of Gambian villages, and critical examination of its use for estimating age. Br J Nutr 22:307-314

Needleman HL, Tuncay OC, Shapiro IM (1972) Lead levels in deciduous teeth of urban and suburban American children. Nature 235:111-112

Prpić-Majić D, Hršak J, Skender Lj (1988) Biological and environmental monitoring in a lead smelter community. In: Astruc M, Lester JN (eds) Heavy Metals in the Hydrological Cycle, Proceedings of the International Conference Chemicals (Heavy Metals) in the Environment, Lisbon, Portugal 1988, Publications Division, Selper Ltd., London, pp. 19-26

Stewart DJ (1974) Teeth as indicators of exposure of children to lead. Arch Dis Child 49:895-897

Telišman S, Ivičić N, Pongračić J (1987) Lead in deciduous teeth in relation to other biological indicator of exposure and effect of inorganic lead in children. In: Lindberg SE, Hutchinson TC (eds) International Conference Heavy Metals in the Environment, New Orleans 1987, CEP Consultants Ltd,Edinburgh, pp 300-302

Received January 11, 1990; accepted May 31, 1990.