



Contributions of risk factors to high lead and cadmium levels in deciduous teeth

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Abstract

The aim of this paper is to show the results of lead and cadmium values measured on deciduous teeth of children from the city of Cartagena (Spain), and their relationships with different variables. A total of 834 samples were processed. Lead data shown a positively skewed distribution and fitted to a log-normal distribution, meanwhile cadmium data did not. The true arithmetic mean and standard deviation of cadmium values was estimated censoring data by 10 ng/g, where the cadmium distribution looks log-normal. No statistically significant differences could be observed for lead and cadmium values according to the sex of donor. However, the content of both heavy metals decreased from incisors to molars, and also with the age of shedding, although this last factor was not important when considering a multifactor ANOVA. The use of fluoride and the zone of residence proved to be the most important environmental variables affecting the content of lead and cadmium, meanwhile other parameters, i.e., home antiquity or family socio-economic status were significant for lead values.

1 Introduction

Environmental pollution from lead and cadmium has been recognized to cause adverse effects in children, playing an important role in the development of

intelligence, this correlation specially established for lead [1,2]. Continuous exposure to low lead levels may result in their accumulation in different tissues [3].

Several studies have shown that human tooth is a valuable indicator of the body burden of environmental trace elements, as well as a readily accessible biological tissue for these analyses [4,5,6]. Heavy metals are known to substitute for calcium in the hydroxyapatite crystals of dentine, a calcifying tissue that, unlike bone, once formed remodels very slowly [7].

Different researchers have described associations between children's tooth heavy metal levels and different factors, both environmental and physiological. These are lead pollution from gasoline, the age of the tooth, the zone of residence, the lead content in water, or the ingestion of paint chips in dilapidated houses [8,9].

The aim of this study was to study the behaviour of lead and cadmium levels in deciduous teeth of children living in the city of Cartagena (Spain).

2 Materials and methods

For this purpose, 834 shed deciduous teeth of children from Cartagena were collected during 1995, together with an epidemiological interview obtained through the EPLODIN Project. To encourage participation in the research, children received a toy, i.e. a ball, a doll, or a puzzle. The analytical determination of both heavy metals has been previously described [10]. Statistical analyses were developed using SPSS 9.0 for windows and SYSTAT.

3 Results

3.1 Distribution fitting and main statistical parameters

Deciduous teeth lead concentrations fitted to a log-normal distribution, with a *Z Kolmogorov-Smirnov* test value of 0.825 ($p = 0.503$). For 50 cases with non-detected cadmium level, we assumed a value of 1 ng/g, taking into account that the minimum value was 1.60 ng/g. In this case, the number of non-detects is far to great to follow a normal nor a log-normal distribution, which means that the geometric mean (GM) is not better than arithmetic mean (AM) for displaying the cadmium data. For that reason, lead data were normalised by logarithmic transformation meanwhile cadmium data were not transformed for ANOVA analysis. Table 1 shows the main statistic parameters for lead and cadmium values. When the detection limit for cadmium was raised at 10 ng/g, 116 data were censored, and the other 718 fitted to a log-normal distribution, with value of *Z Kolmogorov-Smirnov* test value of 0.606 ($p = 0.856$). Figure 1 plots the cadmium values in a log scale *versus* the cadmium scores obtained through the ZIF function (*Z inverse function*). The parameter is obtained by applying the ZIF function to the rank of each observation divided by the total number of cases plus 1. Cadmium data are on a perfect straight line indicating that, at this level of censoring, they are log normally distributed. The least squares fit of the line on Figure 1 has an intercept (b_0) value of 3.723 when z is zero. Raising this value to

the power e gives an estimate of the underlying geometric mean of 41.39 ng/g. The slope of the line (b_1) is 0.901. Raising this to the power e gives an estimate of the geometric standard deviation of 2.46. From these values, we can obtain an estimate of the arithmetic mean (\bar{X}) and standard deviation (s) with the following equations [11]:

$$\bar{X} = \exp\left(b_0 + \frac{b_1^2}{2}\right) \quad s = \bar{X}(\exp b_1^2 - 1)^{1/2}$$

The results obtained with both equations were: 62.11 ng/g for the arithmetic mean and 69.50 for the standard deviation cadmium concentrations.

Table 1. Statistic description of tooth lead and cadmium values in the studied population (units: $\mu\text{g/g}$ for lead, and ng/g for cadmium).

	Pb	Cd ^a
Number of samples	834	834
Average	4.17	59.77
Standard deviation	2.64	63.68
Geometric mean	3.57	33.77
Geometric standard deviation	1.74	3.73
Median (P_{50})	3.59	45.00
Range	0.53-22.72	1.00-643.00
P_{25}	2.51	22.00
P_{75}	4.98	77.00

^a Fifty teeth under detection limit were included, using a concentration of 1 ng/g.

3.2 ANOVA analyses

3.2.1 The effect of sex on tooth heavy metals

There appears to be no effect on tooth lead and cadmium levels that can be related to the sex of donor, since no statistically significant differences could be observed for both heavy metals ($F = 0.13$; $p > 0.5$ for lead; and $F = 0.02$; $p > 0.5$ for cadmium). The GM values were 3.60 $\mu\text{g/g}$ and 3.54 $\mu\text{g/g}$ for lead, and AM of 60.08 ng/g and 59.46 ng/g for cadmium values, for boys and girls respectively.

3.2.2 Home antiquity

Children living in younger homes (≤ 25 years-old) shown less lead values than children living in the older ones (> 25 years-old), with GM of 3.39 $\mu\text{g/g}$ and 4.28 $\mu\text{g/g}$, respectively ($F = 22.86$; $p < 0.001$). However, no significant differences could be found with cadmium values according to home antiquity ($p > 0.05$).

3.2.3 The age of shedding

Differences were also examined according to the age of shedding. Both heavy metals shown a similar decreasing tendency, as shown in figure 2, with



statistically significant differences ($F = 14.29$; $p < 0.001$ for lead and $F = 7.46$; $p < 0.001$ for cadmium).

3.2.4 Family socio-economic status

Teeth of children belonging to socially advantaged families have shown less lead values (GM = 3.30 $\mu\text{g/g}$) than teeth from children belonging to socially disadvantaged families (GM = 3.75 $\mu\text{g/g}$) ($F = 10.58$; $p < 0.01$). No statistically significant differences could be noted for cadmium values, although AM for children belonging to socially disadvantaged families was higher (62.03 ng/g) than that for the other group (56.12 ng/g).

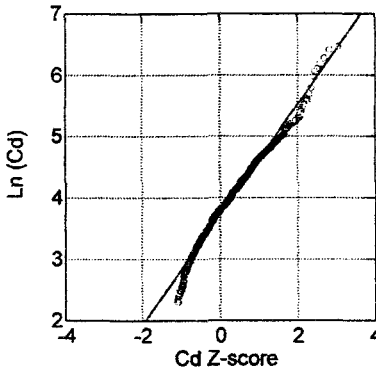


Figure 1: Cadmium values censored at 10 ng/g versus the cadmium scores obtained through the ZIF parameter.

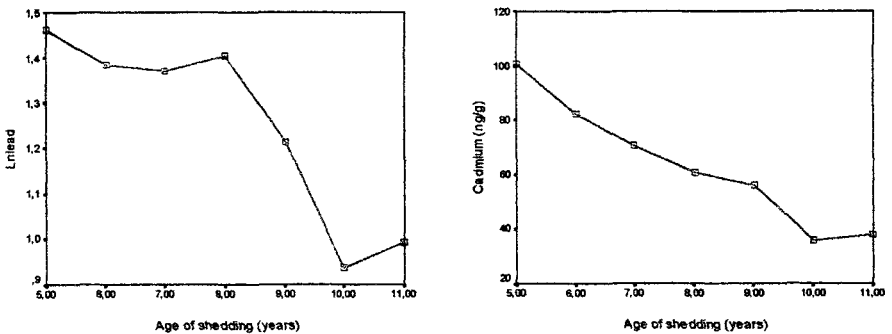


Figure 2: Levels of lead and cadmium in teeth according to the age of shedding.

3.2.5 Parents' smoking habit

According to this variable, children with a non-smoker father displayed lower lead teeth concentrations than children with a smoker one, with GM of 3.38 $\mu\text{g/g}$ and 3.75 $\mu\text{g/g}$, respectively ($F = 6.46$; $p < 0.05$). Mother's smoking habit did not show different groups for lead levels, although the tendency is higher values of

this heavy metal in teeth of children with a smoker mother (GM = 3.62 $\mu\text{g/g}$) than in teeth of children with a non-smoker one (GM = 3.53 $\mu\text{g/g}$). No statistically significant differences appeared with cadmium values, although AM from children with a smoker mother was higher than the other group (64.45 ng/g *versus* 57.59 ng/g).

3.2.6 Thumb sucking habit

Deciduous teeth from children with thumb sucking habit displayed higher lead values (GM = 3.73 $\mu\text{g/g}$) than those without this tendency (GM = 3.41 $\mu\text{g/g}$) ($F = 5.12$; $p < 0.05$). No differences were noted for cadmium groups.

3.2.7 Use of fluoride

The use of fluoride have proved to be important both for lead and cadmium low values. Deciduous teeth from children using fluoride shown a lower lead concentration (GM = 3.47 $\mu\text{g/g}$) than teeth from children who did not use it (GM = 3.83 $\mu\text{g/g}$) ($F = 5.71$; $p < 0.05$). In addition, teeth from children using fluoride also displayed a statistically significant different level of cadmium (AM = 54.36 ng/g) than teeth from the other group (AM = 68.82 ng/g) ($F = 9.30$; $p < 0.01$).

3.2.8 Zone of residence

Teeth of children living in the polluted zone displayed statistically higher lead and cadmium values than children living in the non-polluted or intermediate zone, as shown in table 2.

3.2.9 Differences between jaws

The average lead concentration of teeth differed between the upper and lower jaw. The geometric mean were 3.81 $\mu\text{g/g}$ (upper) and 3.35 $\mu\text{g/g}$ (lower), with statistically significant differences ($F = 11.18$; $p < 0.01$). In addition, cadmium mean value for the upper jaw was higher than for the lower jaw (60.00 ng/g and 59.31 ng/g, respectively), although without statistically significant differences.

3.2.10 Type of tooth

Teeth lead and cadmium values decreased form incisors to molars, with statistically significant differences for both lead and cadmium levels ($F = 28.93$; $p < 0.001$ for lead and $F = 7.82$; $p < 0.001$ for cadmium), as shown in figure 3.

3.3 Multifactor ANOVA

Tables 3 and 4 show the multifactor ANOVA carried out for tooth lead and cadmium levels into contributions due to different variables presenting statistically significant differences in one-way ANOVA tests.

4 Discussion

The variability of lead and cadmium levels in deciduous teeth is without doubt due to a combination of several and complex influences, including both environmental and physiological factors. In contrast to the occurrence of lead in

human teeth, and in studying the factors influencing its presence [5,12]. The comparison of our tooth lead and cadmium average values with those from other studies have been already reported [10,13], being lower than most of reported by other authors.

Table 2. ANOVA for lead and cadmium values by zone of residence.

Zone of residence	Lead		Cadmium	
	N	GM±GSD ($\mu\text{g/g}$) $F=10.68; p<0.001$	N	AM±SD (ng/g) $F=3.90; p<0.05$
Z1 (non-polluted)	383	3.43±1.67	383	55.97±60.27
Z2 (polluted)	211	4.14±1.84	211	70.31±72.47
Z3 (intermediate)	240	3.34±1.72	240	56.58±59.84

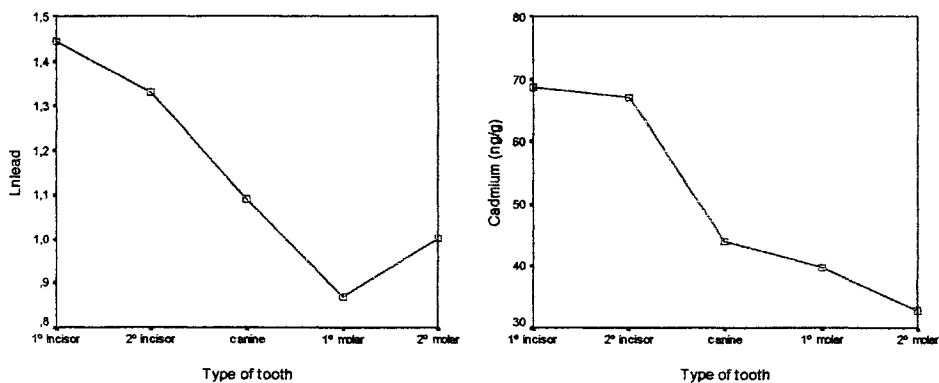


Figure 3: ANOVA results for tooth lead and cadmium values by type of tooth considered.

A t-test was carried out between the arithmetic mean of all cadmium values (59.77 ng/g) and the one estimated censoring data by 10 ng/g (62.11 ng/g), getting a *p-value* greater than 0.05, which means that the null hypothesis of equality of means could not be rejected. This suggests that the assumed value of 1 ng/g for the 50 teeth below the detection limit in Table 1 is reasonable as it results in only a slight underestimation of the true mean.

As in previous studies [8,14], no difference existed between the lead and cadmium levels in the teeth of males and females. However, both heavy metals appeared to be age-dependent, decreasing their values as increasing the age of shedding (Figure 2), as previously reported [8]. However, the opposite effect has been demonstrated for permanent teeth [8,15], increasing the lead value with age. Another important factor for low lead and cadmium levels in teeth has been the use of fluoride. Although the mode of action of fluoride is not entirely clear (fluoroapatite formation or a bactericidal effect) [16], the fluoride content would

reinforce the chemical structure of tooth, avoiding substitution of calcium by heavy metals.

Our results agree with other studies [17] which have found a higher lead and cadmium concentration in the upper than in the lower jaw, although only with statistically significant differences for lead values.

Table 3. Multifactor ANOVA for lead concentrations in deciduous teeth on considered variables.

Factor	N	GM ($\mu\text{g/g}$)	C.I. (95%)	F	p
Home antiquity					
≤ 25 years-old	473	3.27	2.98-3.58	5.53	< 0.05
> 25 years-old	103	3.73	3.27-4.25		
Age of shedding				1.98	N.S.
≤ 5 years-old	5	4.33	2.77-6.78		
6 years-old	71	3.41	2.92-4.00		
7 years-old	149	3.62	3.20-4.08		
8 years-old	142	3.75	3.33-4.23		
9 years-old	82	3.48	3.05-3.98		
10 years-old	56	2.84	2.43-3.31		
≥ 11 years-old	71	3.19	2.80-3.64		
Socio-economic status				2.45	N.S.
≤ 15 (disadvantaged)	351	3.61	3.26-4.00		
> 15 (advantaged)	225	3.37	3.01-3.78		
Father's smoking habit				0.71	N.S.
non-smokers	264	3.43	3.26-4.00		
smokers	312	3.55	3.01-3.78		
Thumb sucking				1.38	N.S.
yes	264	3.58	3.21-3.99		
no	312	3.41	3.07-3.78		
Use of fluoride				0.01	N.S.
yes	372	3.48	3.12-3.89		
no	204	3.50	3.11-3.93		
Zone of residence				6.73	< 0.01
Z1 (non-polluted)	277	3.22	2.89-3.59		
Z2 (polluted)	129	3.92	3.48-4.42		
Z3 (intermediate)	170	3.37	2.99-3.80		
Jaw				5.41	< 0.05
upper	267	3.67	3.28-4.10		
lower	309	3.32	2.99-3.68		
Type of tooth				7.32	< 0.001
1° incisor	210	4.41	3.96-4.90		
2° incisor	179	3.90	3.49-4.34		
canine	103	3.37	2.97-3.83		
1° molar	69	2.71	2.32-3.17		
2° molar	15	3.30	2.52-4.33		

C.I.: confidence interval; N.S.: no-significative



The regular decrease in heavy metal levels from first incisors to second molars is shown in Figure 3. This fact has been explained by different ways, i.e., higher peritubular dentine for incisors or the time of appearance in the oral cavity [14]. Tooth lead and cadmium concentrations shown a similar decreasing tendency for two clearly correlated factors: age of shedding and type of tooth ($r=0.70$; $p<0.001$). The multifactor ANOVA allowed us to distinguish that the age of shedding does not make a significant contribution to tooth lead levels when considered together with all other factors. Similar results were reported by Paterson *et al.* [17], without a residual effect of age when tooth type and jaw were allowed for.

Table 4. Multifactor ANOVA for cadmium concentrations in deciduous teeth on considered variables.

Factor	N	AM (ng/g)	C.I. (95%)	F	p
Age of shedding					
≤ 5 years-old	6	93.38	40.82-145.94	1.69	N.S.
6 years-old	82	72.30	54.69-89.91		
7 years-old	184	67.82	54.42-81.21		
8 years-old	173	56.40	42.87-69.93		
9 years-old	98	55.78	40.95-70.62		
10 years-old	69	38.57	21.23-55.92		
≥ 11 years-old	95	43.78	29.72-57.85		
Use of fluoride				0.30	N.S.
yes	456	59.41	47.66-71.15		
no	251	62.89	50.18-75.60		
Zone of residence				3.84	< 0.05
Z1 (non-polluted)	324	56.15	44.37-67.93		
Z2 (polluted)	171	71.57	58.24-84.89		
Z3 (intermediate)	212	55.73	43.07-68.38		
Type of tooth				1.14	N.S.
1° incisor	260	65.63	54.32-76.93		
2° incisor	219	72.12	60.34-83.91		
canine	122	57.27	43.02-71.53		
1° molar	87	58.66	41.00-76.32		
2° molar	19	52.06	21.16-82.96		

C.I.: confidence interval; N.S.: no-significative

In agreement with previous studies, the home antiquity, the family socio-economic status, and the father's smoking habit were three risk factors clearly associated with larger lead values in teeth, although not statistically related to cadmium values. All these factors disappeared in the multiple ANOVA, except the home antiquity. A crosstabulation allowed us to observe that 46.3% of homes older than 25 years were situated in the polluted zone, meanwhile 84.1% of houses from the non-polluted area had an antiquity of less or equal to 25 years ($\chi^2=48.53$; $p<0.001$). In addition, 74.5% of children living in the polluted zone belong to an economically disadvantaged family ($\chi^2=26.60$; $p<0.001$), and

61.7% of fathers living in the polluted zone had smoking habit ($\chi^2=7.47$; $p<0.05$), belonging 66,3% of them to a socially disadvantaged family ($\chi^2=4.51$; $p<0.05$). Tooth heavy metal variations according to the zone of residence showed a statistical significant higher GM and AM values for the polluted zone. Similar results have been reported by Needleman *et al.* [18], with a different distribution of lead in children from the "lead belt" and from suburbs of Philadelphia. In addition, Shapiro *et al.* [19], observed that teeth from Mexican Indians, living in a zone minimally exposed to lead levels, revealed a mean lead concentration of 4.3 $\mu\text{g/g}$, and Steenhout and Pourtois [20] found increasing tooth lead valued from rural zone, to urban and industrial zone. In our study, the zone of residence has proved to contribute in an important way to large lead and cadmium values, even persisting in the multifactor ANOVA, where the contribution of each factor was measured having removed the effects of all other factor. In the case of cadmium, was the only important factor after the Type III sums of squares was used for the multiple ANOVA.

5 Conclusions

Although a no effect tooth level of lead or cadmium has been reported in the literature, we can conclude that the population of Cartagena presents low values for both heavy metals when compared with other surveys. A larger number of risk factors probed to be related with the variability of tooth lead than for cadmium levels, when analysis of variance was carried out for each factor. However, when all factors presenting significant differences in the single ANOVA were allowed for in the multiple ANOVA, four factors remained with statistically significant differences for lead values, two physiological factors (type of tooth and jaw) and two environmental factors (the home antiquity and the zone of residence). Only the zone of residence appeared to be an important factor for cadmium levels. The high correlations showed between different variables and the zone of residence suggest that this last factor could mask all other contributions.

References

- [1] Needleman, H. L., Gunnoe, C., Levinton, A., Reed, R., Peresie, H., Maher, C. & Barrett, P. Deficits in psychologic and classroom performance of children with elevated dentine lead levels. *The New England Journal of Medicine*, **300**, pp. 689-695, 1979.
- [2] Rabinowitz, M.B., Wang, J.D. & Soong, W.T. Dentine lead and child intelligence in Taiwan. *Archives of Environmental Health*, **46(6)**, pp. 351-360, 1991.
- [3] Khandekar, R.N. & Mishra, U.C. Determination of lead, cadmium, copper and zinc in human tissues by differential pulse anodic stripping voltammetry. *Fresenius Zeitung Analytical Chemistry*, **319**, pp. 577-580, 1984.
- [4] Fergusson, J.E. & Purchase, N.G. The analysis and levels of lead in human teeth: A review. *Environmental Pollution*, **46**, pp. 11-44, 1987.
- [5] Cleymaet, R., Bottenberg, P., Slop, D., Clara, R. & Coomans, D. Study of



- lead and cadmium content of surface enamel of schoolchildren from an industrial area in Belgium. *Community Dental Oral Epidemiology*, **19**(2), pp. 107-111, 1991.
- [6] Nowak, B. Occurrence of heavy metals and sodium, potassium and calcium in human teeth. *The Analyst*, **120**, pp. 747-750, 1995.
- [7] Haller, L.A., Olmez, I., Baratz, R., Rabinowitz, M. & Douglas, C.W. Dentin as a possible bio-epidemiological measure of exposure to mercury. *Archives of Environmental Contamination and Toxicology*, **25**(1), pp. 124-128, 1993.
- [8] Bercovitz, K., Helman, J., Peled, M. & Laufer, D. Low lead level in teeth in Israel. *The Science of Total Environment*, **136**(1-2), pp. 135-141, 1993.
- [9] Lyngbye, T., Hansen, O.N. & Grandjean, P. Lead concentration in deciduous teeth from Danish school children. *Danish Medical Bulletin*, **38**(1), pp. 89-93, 1991.
- [10] Bayo, J., Moreno-Grau, S., Martínez, M.J., Moreno, J., Angosto, J.M., Moreno-Clavel, J., Guillén Pérez, J.J. & García Marcos, L. Electroanalytical determination of cadmium and lead in deciduous teeth after microwave oven digestion. *The Journal of the Association of Official Analytical Chemistry International*, **84**(1), pp. 111-116, 2001.
- [11] Gilbert, R.O. *Statistical Methods for Environmental Pollution Monitoring*, Van Nostrand Reinhold: New York, 1987.
- [12] Oehme, M., Lund, W. & Jonsen, J. The determination of copper, lead, cadmium and zinc in human teeth by anodic stripping voltammetry. *Analytical Chimica Acta*, **100**, pp. 389-398, 1978.
- [13] Bayo, J., Moreno-Grau, S., Martínez, M.J., Moreno, J., Angosto, J.M., Guillén Pérez, J.J., García Marcos, L. & Moreno Clavel, J. Environmental and physiological factors affecting lead and cadmium levels in deciduous teeth. *Archives of Environmental Contamination and Toxicology*, 2001, in press.
- [14] Mackie, A.C., Stephens, R., Townshend, A. & Waldron, H.A. Tooth lead levels in Birmingham children. *Archives of Environmental Health*, **32**, pp. 178-185, 1977.
- [15] Gil, F., Pérez, M.L., Facio, A., Villanueva, E., Tojo, R. & Gil, A. Dental lead levels in the Galician population, Spain. *The Science of Total Environment*, **156**, pp. 145-150, 1994.
- [16] Davies, B.E. & Anderson, R.J. The epidemiology of dental caries in relation to environmental trace elements. *Experientia*, **43**, pp. 87-92, 1987.
- [17] Paterson, L.J., Raab, G.M., Hunter, R., Laxen, D.P.H., Fulton, M., Fell, G.S., Halls, D.J. & Sutcliffe, P. Factors influencing lead concentrations in shed deciduous teeth. *The Science of Total Environment*, **74**, pp. 219-233, 1988.
- [18] Needleman, H.L., Tuncay, O.C. & Shapiro, I.M. Lead levels in deciduous teeth of urban and suburban American children. *Nature*, **235**, pp. 111-112, 1972.
- [19] Shapiro, I.M., Mitchell, G., Davidson, I. & Katz, S.H. The lead content of teeth. *Archives of Environmental Health*, **30**, pp. 483-486, 1975.
- [20] Steenhout, A. & Pourtois, M. Lead accumulation in teeth as a function of age with different exposures. *British Journal of Industrial Medicine*, **38**, pp. 297-303, 1981.