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Study of environmental burden of lead in children using teeth as bioindicator

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ABSTRACT

The south region of Sao Paulo city hosts the Guarapiranga dam, responsible for water supply to 25% of the city population. Their surroundings have been subject to intense and irregular occupation by people from very low socioeconomics classes. Measurements undertaken on sediment and particulate materials in the dam revealed concentrations of lead, copper, zinc and cadmium above internationally accepted limits. Epidemiological and toxicological studies undertaken by the World Health Organization in individuals exhibiting lead concentrations in blood, near or below the maximum recommended (10 µg dl^{-1}), surprisingly revealed that toxic effects are more intense in individuals belonging to low socioeconomics classes. Motivated by these facts, we aimed at the investigation of chronic incorporation of lead, as well as the use of our BIOKINETICS code, which is based on an accepted ICRP biokinetics model for lead, in order to extrapolate the results from teeth to other organs. The focus of our data taking was children from poor families, living in a small, restrict and allegedly contaminated area in São Paulo city. Thus, a total of 74 human teeth were collected. The average concentration of lead in teeth of children 5 to 10 years old was determined by means of a high-resolution inductively coupled plasma mass spectrometer (ICP-MS). For standardization of the measurements, an animal bone certified material (H-Animal Bone), from the International Atomic Energy Agency, was analyzed. The amount of lead in children living in the surroundings of the dam, was approximately 40% higher than those from the control region, and the average lead concentration was equal to 1.3 µg g^{-1} approximately. Grouping the results in terms of gender, tooth type and condition, it was concluded that a carious molar of boys is a much more efficient contamination pathway for lead, resulting in concentrations 70% higher than in the control region. We also inferred the average concentrations of lead in other organs of these children, by making use of our BIOKINETIC code.

1. Introduction

Metals are certainly the most ancient toxic elements known by men. Anthropic intervention has changed their potential effects on health, particularly by means of transportation among several regions on earth through air, water, soil and food stuff (Goyer and Clarkson, 2001). In this work we deal with chronic incorporation of lead, a quite important, highly toxic and harmful metal. The data taking was focused on children from families belonging to low socioeconomics classes, living in a small, restrict and allegedly contaminated area in Sao Paulo city.

1.1. Lead

Gasoline in Brazil is unleaded since 1992. However, lack of rigorous control on the disposal to the environment of domestic and industrial waste, associated with the low recycling rate of batteries, makes lead an ever present contaminant and at non-negligible amounts (Franco-Netto

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et al., 2003). Additionally, lead is used in more than 200 distinct industrial processes.

Epidemiological and toxicological studies undertaken in individuals exhibiting lead concentration in blood, near or below the maximum recommended by the World Health Organization (10 µg dl^{-1}), surprisingly revealed that toxic effects are more intense in individuals belonging to low socioeconomics classes. In particular, learning difficulties, lack of concentration and memorization, and aggressive behavior have been reported (Smith et al., 1983; Canfield et al., 2003; Needleman et al., 1990; Wakefield, 2002). The most vulnerable individuals are children, particularly from the neonatal period until the pre-puberty.

These findings upgrade the importance of studying chronic contamination scenarios with low, non-critical lead quantities. In Brazil, as in many others countries, the great majority of studies refer to regions where acute and occupational contamination has been verified.

1.2. Sao Paulo city and the Guarapiranga dam — case study

The south region of Sao Paulo city has been subject to intense and irregular populational occupation for more than 20 years, associated with lack of basic infrastructure. This region hosts the Guarapiranga dam, which belongs to a huge hydrographical basin located south of Sao Paulo city, covering an area of 643 km^2 encompassing Sao Paulo city itself plus six neighboring villages. About 36% of the basin area (229 km^2) with the dam inside is in Sao Paulo city. The Guarapiranga dam (G-dam, from now on) is responsible for water supply to 25% of Sao Paulo population, nearly 3.7 millions of inhabitants. The fringe surrounding the dam is irregularly occupied by 60,000 souls.

Measurements undertaken on sediment and particulate materials in the dam revealed concentrations of lead, copper, zinc and cadmium above internationally accepted limits. For lead in particular, although its content in water is within normal limits, the concentration in sediment is very high (Patella and Mozeto, 1999).

1.3. Tooth: a reliable long-range bioindicator

Humans' exposure to metals is commonly monitored through the analysis of urine, blood, hair and saliva. These bioindicators are easy to sample, and existing measuring techniques and methodologies are well established. However, blood and urine data provide information on recent exposures, that is, those occurring at times close to the sampling date. Furthermore, appreciable amounts of the contaminant could be gradually incorporated from a steady source, without an alarming increase of its level in blood (Fremlin and Edmonds, 1980). Hair and fingernail are considered medium-range bioindicators, associated with exposure times from a few months to years and providing, in the case of hair, more precise information on acute exposures (Aitio and Kallio, 1999).

According to the goals of the present study, i.e. chronic incorporation, it is necessary the use of indicators working as contaminant dumps through long periods of time (bioindicators for long-range exposures). In this regard, teeth fulfill nearly all of our investigation needs since: (1) they are easily obtainable from children; (2) present the same structure of bone and, therefore, they have the same metal affinity, and (3) their remodeling is slow; as a consequence, the contaminants clearance is much smaller *vis-à-vis* other organs (Rabinowitz et al., 1993). Therefore, teeth allow for both an exposure longstanding record and inferences on the contaminant content of the skeleton (Tsuji et al., 1997).

2. Materials and methods

2.1. Apparatus

A Finnigan Mat (Bremen, Germany) model Element 1 high-resolution inductively coupled plasma mass spectrometer (ICP-MS) was used throughout. Teeth samples were ground with a cryogenic mill Model 6800 Freezer/mill (SPEX, Metuchen, NJ, USA). A laser particle size analyzer Model Mastersizer E (Malvern, Worcestershire, UK) was used for particle size measurements. For sample decomposition a closed vessel microwave oven, MARS 5 (CEM) was used.

2.2. Reagents, materials and solutions

We have used high purity de-ionized water (resistivity 18.2 mΩ cm) obtained by a Milli-Q® water purification, and an analytical reagent grade HNO3. All solutions were stored in polyethylene bottles. Plastic bottles, polypropylene tubes and glassware were cleaned by soaking in 10% v/v HNOO3 for 24 h, rinsing at least tree times with Milli-Q water and dried and stored in a class 100 laminar flow hood.

Stock standard solutions were prepared from 10 mg/l stock solutions of Pb (Spex Industries Inc., Edson. New Jersey, USA). In all standard solutions we used 2% HNO_3. High pure reagents were used and the water was produced in a Millipore Super-Q apparatus (Millipore, Milford, MA, USA).

2.3. Samples and wet digestion

A total of 74 human tooth samples were collected from children by local dentists from Sao Paulo city in Brazil. To remove the residues of soft tissues before grinding, teeth were washed with 30% v/v H_2O_2 during 2 h. After removing the soft tissue, the whole teeth were washed with Milli-Q water, dried in a class 100 laminar flow hood and oven dried for 1 h at 105 °C.

Teeth samples were prepared in triplicate. About 100 mg of dried and ground material were accurately weight in Polypropylene vessels and adapted into the TFM® vessels, and then 2.5 ml of 50% v/v HNO_3 and 0.5 of H_2O_2 were added. After digestion, the TFM® microwave vessels were cooled, the digestate was transferred to 10 ml volumetric flasks and the volume was adjusted with Milli-Q water.

A Certified Reference Material (H-5 animal bone) from the International Atomic Energy Agency (IAEA) was analyzed in five ICP-MS runs to obtain method validation for lead. Results for the concentrations of uranium in teeth samples were then obtained by comparison with sample standards. The evaluation of the uncertainties was accomplished with a routine on-line with the ICP-MS, which take into account only the accuracy of measurements.

3. Results and discussion

The sampling area corresponded to the surroundings of the Guarapiranga dam, from its rim up to a couple of kilometers inland, presenting a dense and irregular demographic occupation by families belonging to the lowest socioeconomics classes. Teeth were collected with the help of the Faculty of Dentistry, University of Santo Amaro, which maintains and operates an Odonto-pediatric office inside the studied area, offering gratuitous odontological services to its population.

The control area was Osasco, a developed village west of Sao Paulo city and about 60 km far from the Guarapiranga hydrographical basin, where its population uses only treated water supplied by official and authorized companies.

A total of 74 deciduous teeth were collected and analyzed, 50 from children living in the G-dam region and 24 far out of this region (the control region). Some teeth collected at the G-dam region were discarded because of their very bad carious condition. The results are shown in Table 1, where the concentrations are expressed as µg of lead per g of tooth mass (dry), and they agree with the results from similar studies carried out in many other densely populated areas in Europe and USA (Schärer et al., 1991; Karakaya et al., 1996; Tvinnereim et al., 2000). In Table 2 the results are discriminated according to teeth type and condition.

3.1. Comparing results between regions

We started by calculating the mean weighed lead concentration in teeth for the whole set of samples, that is, for both boys and girls, and we got (1.28 ± 0.11) µg g^{-1} and (0.91 ± 0.12) µg g^{-1} for children from the G-dam surroundings and the control region, respectively (see Table 1).

Expressing the uncertainties as percentages we obtain 8.6% (for the G-dam — 0.11) and 13% (for the control region — 0.12). Thus, the mean lead concentration in teeth from the G-dam surroundings is (41 ± 9) % higher than in the control region, corresponding to a quite statistically significant difference.

Table 1
Lead concentration in tooth from the studied regions

Characteristics	Dam's surroundings		Control region	
Number of teeth	50		24	
Concentration of lead in teeth (μg g^{-1})	1.28±0.11		0.91±0.12	
Sex	(%)	Lead concentration (μg g^{-1}+SD)	(%)	Lead concentration (μg g^{-1}+SD)
Boys	38.5	1.36±0.20 (n=24)	47.8	1.00±0.2 (n=12)
Girls	61.5	1.14±0.14 (n=15)	52.2	0.82±0.15 (n=11)
Age range (years)	4–12		4–13	
Teeth condition	(%)		(%)	
Carious	22		29.2	
Non carious	78		70.8	

The whole set of data also has internal consistency, since when the results are separated by gender nearly the same difference are found. In fact, the mean lead concentration in teeth of boys from the G-dam surroundings is 36% higher than in boys from the control region, while for girls the difference is 39%, and both results agree with the mean lead concentration for the whole set (boys plus girls) within the uncertainties. This very nice consistency casts a great deal of confidence on the measurements we carried out.

Thus, people living in the G-dam region is appreciably more exposed to lead when compared to the control region, and by the same amount for both genders. This finding strongly indicates that environmental lead is more abundant in the Guarapiranga dam region, and/or that its population is more intensely targeted by this contaminant.

Now, when results are compared between boys and girls from the same region, we note that boys are more exposed to lead than girls, and by the same amount of approximately 20% in both regions. Such a circumstance could be explained by the fact that boys play out-door more often than girls, increasing therefore their chances of exposure.

3.2. Influence of teeth type and conservation condition

There are also substantial differences between lead concentrations measured in teeth when they are analyzed according to their type and conservation conditions (carious and non-carious), as shown in Table 2. In fact, there is a statistically significant difference of approximately 30% between incisors/molars and canines, while the comparison with pre-molars is inconclusive because of their poor statistics.

The condition of teeth also influenced significantly the absorption of lead. Table 2 (results from the G-dam region only) indicates that in carious teeth lead absorption is 33% higher. This could well be an indication that carious teeth absorb more lead in an attempt to compensate the demineralization process. Quite interesting, however, when results of carious and non-carious molars from boys are averaged, the absorption difference jumps to 76%. It is quite compelling, therefore, to conclude that a carious molar is a much more efficient contamination pathway for lead. It is important pointing out that such a contamination scenario through carious molars could be increasingly more harmful when dealing with populations belonging to low socioeconomics classes (see discussion above).

3.3. The children age issue

We show in Fig. 1 a plot of the "relative difference" (R) between the lead concentrations measured at the G-dam (C_G) and control (C_C) regions, as function of children ages, here defined as

$$R=[C_G-C_C]/C_C \tag{1}$$

Examining the plot in Fig. 1 for 7 year old children we observe that $R\approx0.7$, meaning that the concentration difference $[C_G-C_C]$ is equal to 70% of the concentration measured in the control region, which is therefore a huge difference. For 6 year old children, on the other hand, we obtained $R\approx0.2$, that is, the difference $[C_G-C_C]$ is equal only to 20% of the concentration measured in the control region. Interestingly, there is nearly no difference between results from the two regions for 9 year old children, because we obtained $R\approx0$.

Table 2
Samples from Guarapiranga's region

Type of teeth	(%)		
Incisor	12	1.26±0.25 (n=6)	
Molar	70	1.26±0.16 (n=35)	
Canine	14	0.98±0.11 (n=7)	
Pre molar	4	0.75±0.67 (n=2)	
Teeth condition	(%)	All samples	
Carious	22	1. 29±0.08 (n=11)	1.32±0.29 (n=9)
Non carious	78	0.97±0.16 (n=39)	0.75±0.06 (n=8)

The results shown in Fig. 1 are both appealing and intriguing. We note in this regard that,

1. C_C (control region) has an almost flat behavior as function of the children age. In this sense, the resonant behavior observed for R should be solely attributed to a bump in C_G (G-dam region).
2. It could be argued that such a bump in C_G reflects a differentiated exposure regime. But this is unlikely since all teeth came from children living in the same area and, therefore, exposed in average to the same environmental lead sources.
3. Nutrition and playing habits, contact with the water, as well as other forms of exposure to lead sources are similar. In this way, they cannot explain the huge variations observed in C_G as function of the children age. For instance, lead concentrations in teeth of 7–8 year old children are about 4 times higher than those for 6 and 9 year old children (see Fig. 1).

Given the limited nature of our results these observations suggest, although do not prove, that this lead resonance is *metabolic-like*. This issue deserves further investigation, because the similarity between teeth and bones would lead us to conjecture that the nature of such characteristics should reside in the bioaccumulation process of bones.

Such conjecture is consistent with the likely possibility that nutrition deficiencies, associated with the needy way of life of children living in the G-dam region, could promote metabolic substitution of essential elements (as e.g. calcium) by bone seeker contaminants as lead. As verified in animal experiments with bone seekers other than lead, as uranium, it is very likely that young individuals were often in positive lead balance (between uptake and excretion) due to a *build up of lead* in the growing skeleton (Arruda-Neto et al., 2004). On the other hand, it would be also important knowing the amount of lead which is incorporated by other organs, as discussed below.

3.4. Lead biokinetics in the skeleton and other organs

In this regard, we developed, implemented and tested at this Laboratory an approach (the BIOKINETICS code) based on a biokinetics model for Pb recommended by the ICRP67 (ICRP, 1993; Leggett, 1993). The BIOKINETICS code was used and validated in the case of uranium chronic intake (Garcia et al., 2006). It uses two alternative methods: the Hamilton–Caley algebraic method (Birchall and James, 1989) and the 4th order Runge–Kutta numerical method (Press et al., 1992), for mathematical solution of differential equation systems.

In this biokinetical approach, and in other biokinetics models, bone and teeth are nearly indistinguishable regarding metal incorporation (as e.g. lead and uranium) within 10% to 20%, according to individuals' ages. The younger the individual is the greater the similarity between teeth and bones. In fact, the contents of lead experimentally measured in teeth (Al-Naimi et al., 1980; Hernández-Guerrero et al., 2004) and in bones (Barry, 1981; Farias et al., 1998), from individuals with ages ranging from 1 to 21 years, were similar within the experimental uncertainties.

With the use of our BIOKINETICS code plus the incorporation of a set of transfer rate parameters (ICRP-67), we calculated the accumulation of lead in the skeleton as function of the individuals' age. The obtaining of quantitative results requires to input into the code the daily amount of incorporated lead which, in the case of our study (the G-dam region), is unknown. However, by assuming that the accumulation of lead in the skeleton is similar to the one in teeth (as discussed above), we could use our results to infer the daily intake of lead. In fact, in Fig. 2 we show the output of the BIOKINETICS code for skeleton, where the daily amount of incorporated lead was adjusted in order to fit our results for teeth averaged in the age interval of 5–10 years. The average chronic lead incorporation which best fitted our results was equal to 2 μg of Pb per day.

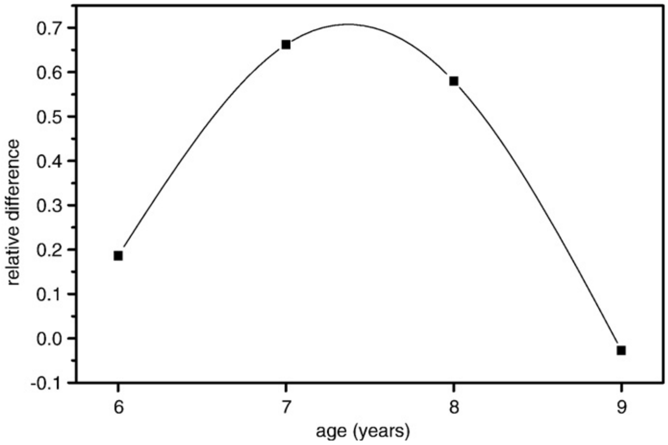

Fig. 1. Relative difference (R) between the lead concentrations measured at the G-dam and at the control region, as function of children ages (see Eq. (1)).

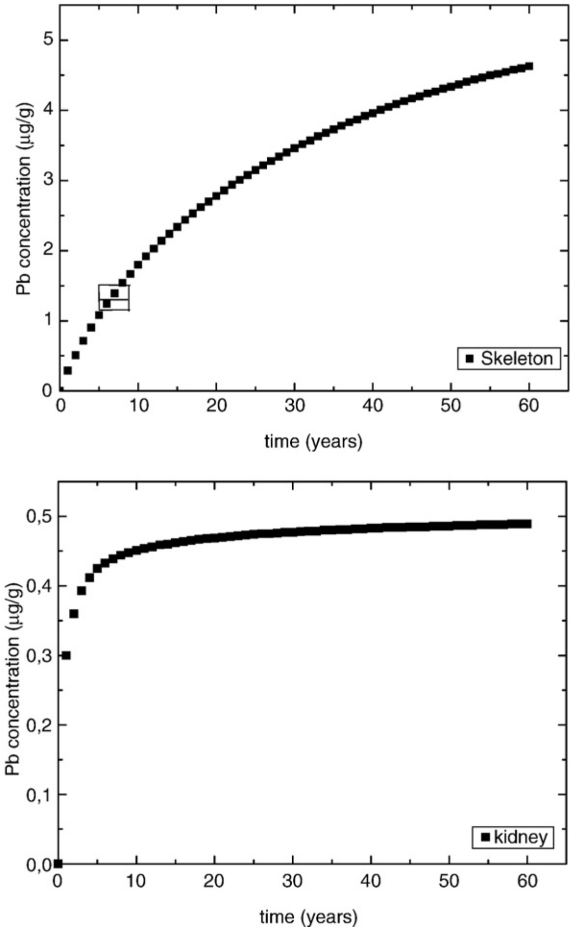

Fig. 2. Results for lead concentration in the skeleton and kidneys as function of the age, as obtained from the output of our BIOKINETICS routine (dotted curves). The box traversed by the curve represents the average of our experimental results for teeth, in the interval 7–10 years old. The height of this box is the dispersion of the average value.

Thus, by considering a chronic ingestion regime at a rate of 2.0 μg Pb/day, and for an uninterrupted exposure over a period of 60 years, we also obtained results for a few other organs (Figs. 2 and 3). They correspond to estimates of the average lead burden in organs of those children living close to the Guarapiranga dam, which donated their deciduous teeth to this study. We observe in these figures that liver, kidney and soft tissue reach the saturation plateau within only after the first 10 years of uninterrupted exposure, approximately. Bone, without turnover, does not reach the steady equilibrium saturation plateau.

Also from Figs. 2 and 3 we note that liver, kidneys and soft-tissues, present lead concentrations equivalent to 80%, 25% and 2%, respectively, of the one calculated for the skeleton of individuals 10 years old. This would allow us to estimate that the lead burden in these same organs, and belonging to the children living close to the Guarapiranga dam are,

Skeleton = 1.2–1.4 μg g^{-1}
Liver = 1.0–1.1 μg g^{-1}
Kidneys = 0.3–0.4 μg g^{-1}
Soft-tissues = 0.02–0.03 μg g^{-1}

4. Conclusions and final remarks

1 The average concentration of lead in teeth from children living in the surroundings of the Guarapiranga dam (our case study) is approximately 40% higher than the one corresponding to the control region.
2 Considering gender, boys absorb 20% more lead than girls.
3 Lead concentrations are higher in molar and carious teeth.
4 Grouping the results in terms of gender, tooth type and condition, it is concluded that a carious molar of boys is a much more efficient contamination pathway for lead, resulting in concentrations 70% higher than in the control region.
5 The average concentration of lead in teeth of children 5 to 10 years old, living in the surroundings of the Guarapiranga dam, is equal to 1.3 μg g^{-1} approximately (Table 1).
6 From this concentration value, plus the BIOKINETIC code, it was possible to tentatively infer a daily amount of incorporated lead equal to 2 μg per day.
7 From this 2 μg of Pb per day input into the BIOKINETIC code, it was possible estimating the lead burdens in other organs, such as liver, kidneys and soft-tissues.
8 When concentrations are discriminated by children age, the results suggest the occurrence of a “metabolic resonance” in the lead absorption by teeth from 7 and 8 year old children (Fig. 1). Given the limited statistical nature of our results, this could be regarded only as an educated guess.

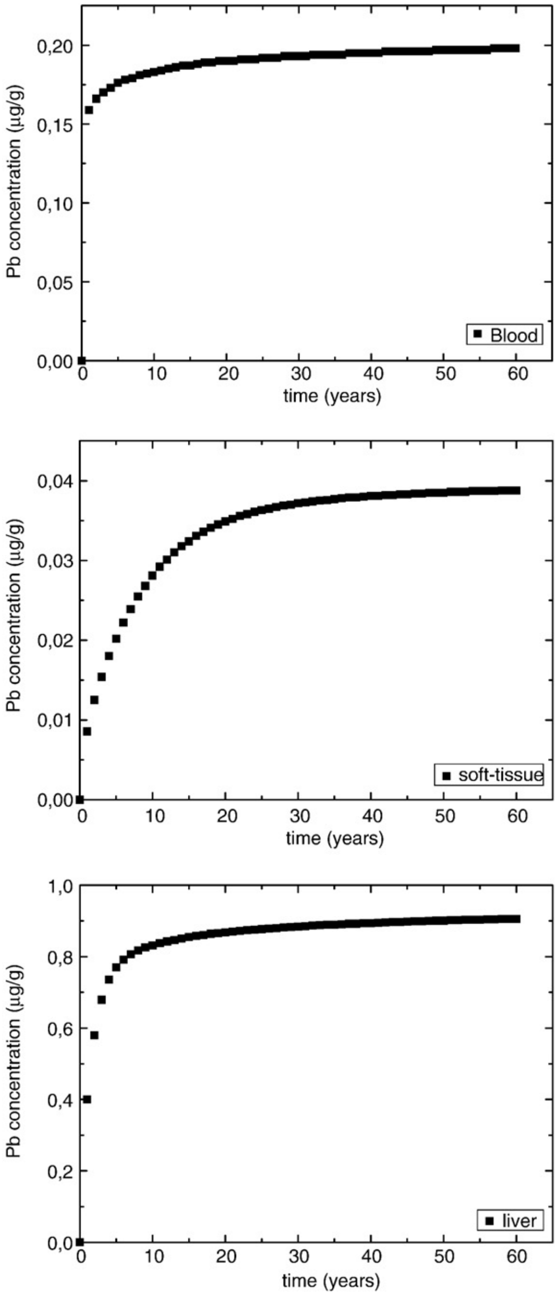

Fig. 3. Results for lead concentration in blood, liver and soft tissue as function of the age, as obtained from the output of our BIOKINETICS routine.

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