



Study of metals transfer from environment using teeth as biomonitor

J.D.T. Arruda-Neto^{a,b,h,*}, L.P. Geraldo^c, G.R. Prado^a, F. Garcia^d, M.C. Bittencourt-Oliveira^e, J.E.S. Sarkis^f, F. Martinez-Lusardo^g, L. Lima-Cazorla^g, D. Rosa-Medero^g, T.E. Rodrigues^a, G.C. Genofre^h

^a Physics Institute, University of São Paulo, São Paulo-SP, Brazil

^b FESP, São Paulo Engineering School, São Paulo-SP, Brazil

^c Catholic University/UNISANTOS, Santos-SP, Brazil

^d Santa Cruz State University, Ilhéus-Ba, Brazil

^e Agriculture School/ESALQ, University of São Paulo, São Paulo-SP, Brazil

^f Institute for Energy and Nuclear Research/IPEN, São Paulo-SP, Brazil

^g Instituto Superior de Tecnología y Ciencias Aplicadas, Havana, Cuba

^h Grajau General Hospital and CENEPEs, São Paulo-SP, Brazil

ARTICLE INFO

Article history:

Received 15 October 2009

Accepted 9 December 2009

Available online 6 January 2010

Keywords:

Urban environment
Heavy metals burden
Human exposure
Teeth as biomonitors
Lead transfer to bones
Biokinetics model

ABSTRACT

We investigated chronic incorporation of metals in individuals from poor families, living in a small, restrict and allegedly contaminated area in São Paulo city, the surroundings of the Guarapiranga dam, responsible for water supply to 25% of the city population. A total of 59 teeth from individuals 7 to 60 years old were collected. The average concentrations of Pb, Cd, Fe, Zn, Mn, Ni and Cr were determined with an Atomic Absorption Spectrophotometer. The concentrations of all metals as function of the individuals' age exhibited a remarkable similarity: peaks between 7 and 10 years and sharply decreasing at higher ages, which could be attributed to alimentary habits and persistence to metals exposure all along the individuals' life span. From all the measured metals, lead and cadmium were a matter of much more concern since their measured values are close to the upper limits of the world wide averages.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

The occurrence of toxic metals in the environment is, by one hand, high in the agenda of plant stress physiology studies and, on the other hand, quite important in evaluating potential risks for human health when present in the food chain.

In fact, metals are certainly the most ancient toxic elements known by men. Anthropocentric 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).

The south region of Sao Paulo city has been subject to intense and irregular occupation for more than 20 years, associated with lack of basic infrastructure. This region hosts the Guarapiranga dam (G-dam), covering an area of 229 km² inside the limits of Sao Paulo city, and is responsible for water supply to 25% of the city population, nearly 3.7 millions of inhabitants (Maglio, 1994). The fringe surrounding the dam is irregularly occupied by 60,000 individuals, approximately, which use the water of this dam for recreation and leisure. Also, this

population consumes fish from the dam and utilizes its water for irrigation of domestic plantations.

Measurements performed on sediment and particulate materials in the dam revealed concentrations of lead, copper, zinc and cadmium, among others, above internationally accepted limits (Patella and Mozeto, 1999). In this context, lead and cadmium, distributed throughout the environment, are the two most concerning metals from a toxicological point of view. However, information concerning environmental contamination and risk assessment was so far available mostly with the use of animals as biomonitors (Schärer et al., 1991).

In this work we deal with chronic incorporation of several heavy metals by individuals from families belonging to low socioeconomic classes, living in a small, restrict and allegedly contaminated area in Sao Paulo city – the G-dam region.

Since chronic incorporation is likely occurring, we have used indicators working as contaminant dumps through long periods of time (biomonitors for long-range exposures). Actually, teeth fulfill nearly all of our investigation needs since: (1) they are easily obtainable; (2) present the same structure of bone and, therefore, they have the same metal affinity, and (3) their remodeling is slow and, 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). In this regard,

* Corresponding author. Physics Institute, University of São Paulo, São Paulo-SP, Brazil.
E-mail address: arruda@if.usp.br (J.D.T. Arruda-Neto).

Table 1

Concentration of metals in teeth expressed in relative values (see text for details) as a function of the individual's age. Measurement errors (not shown) are in between 10% and 15%.

Age (years)	Mn	Fe	Zn	Cr	Cd	Pb	Ni
7	1	1	1	1	1	1	1
10	0.8113	0.4049	1.0174	0.8925	0.6666	1.1367	0.5314
18	0.3045	0.1158	0.2203	0.8785	0.4259	0.4772	0.7692
28	0.3719	0.2433	0.2914	0.8691	0.4444	0.5486	0.7062
52	0.3638	0.2341	0.3094	0.7710	0.4444	0.5388	0.7272
64	0.2991	0.2205	0.1269	0.3177	0.3333	0.3257	0.4265

we have recently carried out a similar study in the G-dam region, but focused only on the incorporation of lead. It was found that the amount of lead in children living in the surroundings of this water body, was approximately 40% higher than those from the control region (Arruda-Neto et al., 2009).

2. Materials and methods

The sampling area was the surroundings of the G-dam, from its rim up to a couple of kilometers inland. 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. As the control region we chose a large district far from the surroundings of the G-dam.

A total of 59 human tooth samples were collected plus 35 from the control region. To remove the residues of soft tissues before grinding, teeth were washed with 30% v/v H₂O₂ 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 ground with a cryogenic mill Model 6800 Freezer/mill (SPEX, Metuchen, NJ, USA) and prepared in triplicate. About 100 mg of dried and ground material were accurately weighed in Polypropylene vessels and adapted into the TFM[®] vessels, and then 2.5 ml of 50% v/v HNO₃ and 0.5 of H₂O₂ were added. After digestion, the TFM[®] microwave vessels were cooled, the digestate transferred to 10 ml volumetric flasks and the volume adjusted with Milli-Q water. Quantification of Mn, Fe, Zn, Cr, Cd, Pb and Ni was carried out with an Atomic Absorption Spectrophotometer (Buck Scientific 210 VGP). Typical operation conditions for e.g. Pb were: current of 2.8 mA, wave length equal to 283.3 nm, spectral band steps of 7.0 nm and a calibration curve fitted by the straight line equation $y = 0.0252x + 0.0003$ ($R^2 = 0.9996$), where y is the absorbance and x is the metal concentration (mg/l).

3. Results

The results are expressed as averages of the triplicate measurements. They are represented in Table 1 as fractions of the values measured for the lowest age (7 years old children), that is, all values were divided by those corresponding to 7 years old children. In this sense, results for all the investigated metals are equal to 1 at this age. As we will see below, this data presentation format allows for a direct comparison of all results as function of the individuals age. However, in Table 2 we show absolute value

Table 2

Concentration of lead and cadmium in teeth expressed in absolute values (µg-metal/g-teeth) as a function of the individual's age (years). Measurement errors (not shown) are in between 10% and 15%.

Age (years)	Pb (µg/g-teeth)	Cd (µg/g-teeth)
7	1.60	12.3
10	1.19	8.20
18	0.76	5.24
28	0.88	5.47
52	0.86	6.63
64	0.52	4.10

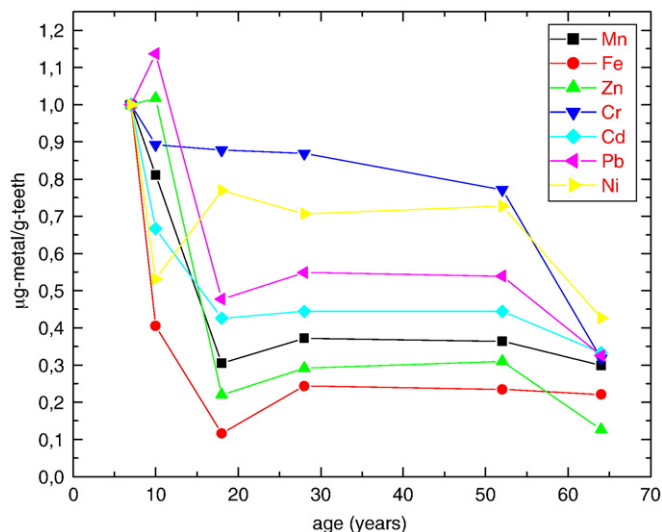


Fig. 1. Relative concentrations of metals in teeth as a function of the individual's age. The concentrations for each metal were divided by the concentration corresponding to the lowest age (7 years).

results for the two most significant metals present as contaminants in the waters of the G-dam, Pb and Cd.

All results are also shown in Fig. 1, and in Fig. 2 we make salient the two most important contaminating metals, Pb and Cd. Results obtained in the control region are systematically 40% to 60% lower than those corresponding to the G-dam region.

We have developed, implemented and tested at this Laboratory an approach (the BOKINETICS code) based on a biokinetics model recommended by the ICRP-69 (ICRP, 1995; Leggett, 1993). The BOKINETICS code was used and validated in the case of uranium chronic intake (Garcia et al., 2006; Prado, 2007). In this biokinetic 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 the individual's ages.

With the use of our BOKINETICS code plus the incorporation of a set of transfer rate parameters (ICRP-69), we calculated the accumulation of lead in the skeleton as a function of the individuals' age. The obtaining of quantitative results requires the inputting into the code of 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, we could use our results to infer the daily intake of lead. In fact, in Fig. 3 we show the output of the BOKINETICS 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 7–10 years. The average chronic lead incorporation which best fitted our results was equal to 2.3 µg of Pb per day.

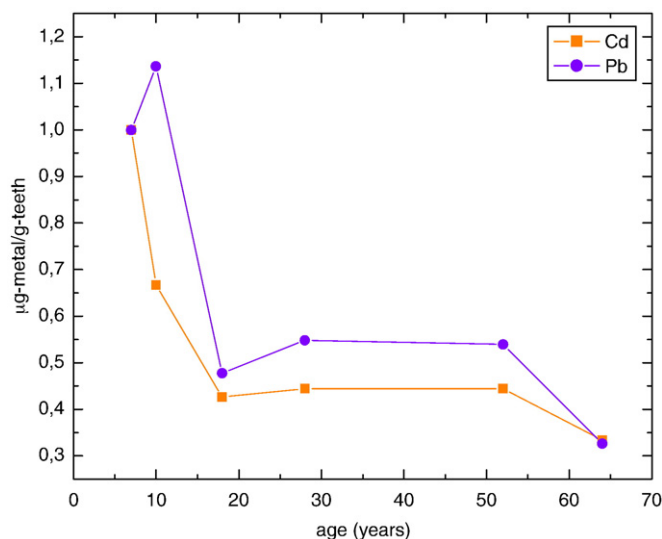


Fig. 2. The same as in Fig. 1, but only for Pb and Cd.

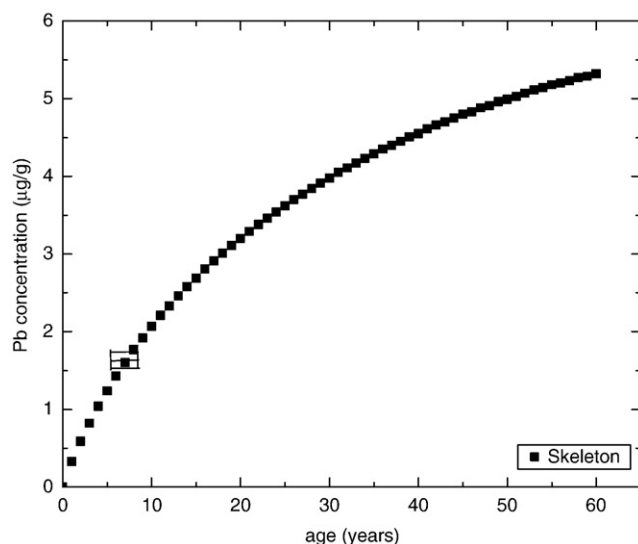


Fig. 3. Lead concentration in skeleton as function of the age, obtained from the BOKINETICS routine (see text for details). The box traversed by the curve represents the average of our experimental results for teeth in 7 years old individuals. The height of this box is the dispersion of the average value.

4. Discussion and conclusions

The most intriguing aspect of these results is the strong correlation verified between individual age and bioaccumulation for all metals investigated, that is,

- It was observed for ages between 7 and 10 years accumulation peaks for all metals, and
- Metal concentrations sharply decrease for ages between 10 and 20 years, followed by the formation of a plateau from 20 to 50 years, and another decrease for ages over 60 years.

Although the number of teeth we sampled in a wide interval of individuals age (7–60 years old) is limited, it should be stressed that the strong correlation observed for all seven metals greatly compensates this shortcoming.

4.1. Clearance aspects of Cr, Ni and Fe bioaccumulation

The overall trend of all metals indicates that bioaccumulation is more intense in the childhood period followed by a sharp decrease (clearance) and the formation of a plateau at ages between 20 and 50 years old (Fig. 1). However, this sharp decrease is not verified particularly for Chromium; its plateau is only 10% lower than the bioaccumulation peak, indicating that clearance of this metal is negligible. Nickel also has small clearance. Fortunately, Cr and Ni are not as harmful as Pb and Cd.

On the other hand, clearance of Iron is quite effective, since its plateau is 80% lower than the bioaccumulation peak.

4.2. Lead and Cadmium concentration levels

We would like to make salient the following aspects of our results:

- The concentrations found for the toxic metals lead and cadmium is a matter of much more concern in comparison to other metals, since their measured values are close to the upper limits of the world wide averages.
- The highest concentrations for all metals were found, ironically, in individuals under 15 years old. In fact, the most vulnerable individuals are children, particularly from the neonatal period

until the pre-puberty. For lead, in particular, learning difficulties, lack of concentration and memorization, and aggressive behavior have been reported (Wakefield, 2002).

4.3. Long-term metal biokinetics

From these results obtained with teeth it is possible to estimate the metal content in several other organs, as well as the average daily incorporation by means of our BOKINETICS code. We performed an application for the case of lead accumulation in the skeleton (Fig. 3), with procedures similar to those undertaken elsewhere (Arruda-Neto et al., 2009). It is important pointing out that the skeleton works as a reservoir for metals, which are available to other organs via the blood stream. This kind of data is very important for epidemiological studies in large populations.

From the output of our BOKINETICS code for long-term and daily ingestion of lead (Fig. 3), normalized by our experimental data at 7–10 years old (1.60 µg-lead/g-teeth), we observe that the accumulation of lead in the skeleton is monotonically increasing with age. Such a calculation assumes that the individuals were subject to a daily intake of lead (equal to 2.3 µg per day) starting at their neo-natal stage. Now, by observing the results displayed in Fig. 2 we are led to the conclusion that the donors of the teeth samples, particularly those over 10 years old, did not start lead intake at the earliest periods of their lives.

From the cross checking of experimental results and biokinetics inferences, as shown in Figs. 2 and 3, respectively, it would be possible to draw a picture of alimentary habits and persistence to metals exposure all along the individuals' life span.

4.4. Children targeting

It is quite revealing the observation that for ages between 7 and 10 years the accumulations exhibit peaks for all metals. Such a circumstance could be explained by the fact that children play outdoors often, thus increasing their chances of exposure.

Therefore, nutrition and playing habits, contact with the water, as well as other forms of exposure to metal sources, in children and adults, give rise to differentiated exposure regimes.

Also, the accumulation peaks observed in children for all metals are 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 and cadmium. As verified in animal experiments with bone seekers other than lead and cadmium, as uranium, it is very likely that young individuals are often in positive metal balance (between uptake and excretion) due to a build up of metals in the growing skeleton (Arruda-Neto et al., 2004 and references therein).

References

- Arruda-Neto JD, et al. Long-term accumulation and microdistribution of uranium in the bone and marrow of beagle dog. *Int J Radiat Biol* 2004;80(8):567–75.
- Arruda-Neto JD, de Oliveira MCC, Sarkis JES, Bordini P, Manso-Guevara MV, Garcia F, Prado GR, Krug FJ, Mesa J, Bittencourt-Oliveira MC, Garcia C, Rodrigues TE, Shtejler K, Genofre GC. Study of environmental burden of lead in children using teeth as bioindicator. *Environ Int* 2009;35(3):614–8.
- Garcia F, et al. Uranium levels in the diet of São Paulo City residents. *Environ Int* 2006;32:697–703.
- Goyer RA, Clarkson TW. Toxic effects of metals. In: Klaassen CD, editor. Casarett and Doull's Toxicology: the basic science of poisons. New York: McGraw-Hill; 2001. p. 811–37.
- ICRP-69. Age-dependent doses to members of the public from intake of radionuclides: Part 3 ingestion dose coefficient. ICRP Publication 69. Oxford: Elsevier Science Ltd; 1995.
- Leggett RW. An age-specific kinetic model of lead metabolism in humans. *Environ Health Perspect* 1993;101(7):598–616.
- Maglio IC. Strategies for the management of hydrographic basins. The Guarapiranga basin program (in Portuguese). Impacts evaluation, São Paulo – International Association for Impact Assessment; 1994. p. 63–77.

- Patella E, Mozeto A. Biogeochemical characterization of the heavy metals content in sediments of the Guarapiranga reservoir. *Proceedings of the Brazilian Congress on Sanitary and Environmental Engineering* (in Portuguese). Brazil: Rio de Janeiro; 1999, p. 2191–6.
- Prado, G.R., 2007. Study of environmental contamination by uranium in the municipality of Caetité-Ba, using human teeth as bioindicators (in Portuguese, abstract in English). Master Degree Dissertation, Santa Cruz State University, Bahia, Brazil (unpublished). Available at: http://www.uesc.br/cursos/pos_graduacao/mestrado/mdrma/teses/dissertacao_georgia.pdf.
- Rabinowitz MB, et al. Relationships between serial blood lead levels and exfoliated tooth dentin levels: models of tooth lead kinetics. *Calcif Tissue Int* 1993;53:338–41.
- Schärer K, et al. High lead content of deciduous teeth in chronic renal failure. *Pediatr Nephrol* 1991;5:704–7.
- Tsuji LJS, et al. Elevated dentine lead levels in adult teeth of first nation people from an isolated region of northern Ontario, Canada. *Bull Environ Contam Toxicol* 1997;59:854–60.
- Wakefield J. The lead effect. *Environ Health Perspect* 2002;110:A574–80.