10.5005/jp-journals-10029-1019

RESEARCH ARTICLE

Mercury Accumulation in Fish: The Potential Effect of Dental Amalgam Wastewater

Tiago Novaes Pinheiro, Alberto Consolaro, Alice Sakuma, Marcia Liane Buzzo, Marcia Sena de Carvalho, Maria Cristina Duran

ABSTRACT

Objectives: Dental amalgam discharge in the wastewater stream is a potential source of mercury (Hg) that may contaminate the soil, water and wildlife, once released without safety measures. This study aimed the total Hg accumulation in fish *Carassius auratus* comparing different water environments: Water from the Tietê river basin close to the metropolitan area of Bauru, Brazil; tap water contaminated with 0.5 gm/l load of dental amalgam and tap water was used as control.

Study design: Fish were kept in glass aquaria, each water environment consisted of 25 specimens. The inner organs and muscles were sampled at 1 hour and 28 days for total Hg accumulation analysis. Also, water samples were analyzed at 1 hour, 7, 14, 21 and 28 days through cold vapor atomic absorption spectrometry.

Results: Only the fish kept at water from the Tietê river and contaminated tap water had Hg detected on its sampled tissues. The highest median levels were found in inner organs: 12.36 mg/kg–Tietê river and 262.64 mg/kg–contaminated tap water. The lowest median concentrations were found in muscles: 0.13 mg/kg–Tietê river and 0.54 mg/kg–contaminated tap water.

Conclusion: These findings alert to the potential environmental risks of dental amalgam waste discharge.

Keywords: Dental amalgam, Mercury accumulation, Fish, Water environment, Cold vapor atomic absorption spectrometry.

How to cite this article: Pinheiro TN, Consolaro A, Sakuma A, Buzzo ML, de Carvalho MS, Duran MC. Mercury Accumulation in Fish: The Potential Effect of Dental Amalgam Wastewater. Int J Experiment Dent Sci 2012;1(2):75-80.

Source of support: Nil

Conflict of interest: None declared

INTRODUCTION

The basic composition of dental amalgam, by weight, are silver (20-34%), tin (8-15%), copper (1-15%), other metals such as palladium and indium (0-5%) and mercury (Hg), which constitutes 42 to 52% of the total mass.[1] Environmental aspects of Hg have become an important issue in Dentistry. Recently, it has been shown that Hg from an environmental exposure to representative samples of dental amalgam typically found within the dental wastewater discharge stream is bioavailable to fish and may accumulate in internal tissues.[2]

It was estimated that the total population of practicing dentists in the United States may generate annually 18,159 kg of recyclable noncontact Hg. This Hg is originated from remnants of amalgam prepared for dental procedures but not applied in the oral cavity. Other 2,763 kg of contact Hg generated by dental operations may be discarded in the environment, in a form of a heterogeneous mixture of amalgam, dentine, enamel and other particulate matter.[3] Also, residual Hg content and leaching Hg from used amalgam capsules have the potential to become equally significant.[4] The identification of sources of heavy metals in urban wastewater in Stockholm found that the most Hg dominant emission source was from dental amalgam.[1] Furthermore, a study prepared for the Association of Metropolitan Sewerage Agencies in the United States[5] estimated that dental waste contributes approximately 35% of the Hg entering wastewater treatment.

According to the American Dental Association (ADA), 'dental amalgam is considered a safe, affordable and durable material that has been used to restore the teeth of more than 100 million Americans'.[6] Dental amalgam is still a restorative material with widespread utilization in the world, coexisting with new restorative materials as a therapeutic alternative. Intensive researches have tried to find other restorative materials to replace dental amalgam. Composites are widely used for adult patients, but they are not considered as durable as amalgam. For the primary dentition, glass ionomer fillings have provided a suitable alternative. Gold restorations are considered to be of good quality but too expensive for widespread use. Ceramic materials are becoming more popular, are under further development and can be a promising alternative in the future.

Some authorities have shown restrictions against the use of dental amalgam due to health or environmental reasons. Health Canada recommended that dental amalgam should be avoided in people allergic to Hg; if possible, its placement or removal in teeth of pregnant women should be avoided; also, utilization of alternative options for treatment of primary teeth has been suggested.[7] In Denmark, the authorities had decided to ban the use of amalgam by 1999 for environmental reasons, but this was postponed because no satisfactory alternative to amalgam in all situations had been found by 1998.[8]

This scenario is different in other countries as Brazil. In fact, there is no official Brazilian legislation regarding a proper discharge of heavy metals by health care institutions, such as dental clinics, hospitals and laboratories.[9] The Brazilian National Environmental Council (CONAMA)

establishes different maximum Hg levels for each class of water bodies according to their use, varying from 0.0001 to 0.002 mg/l, and a maximum Hg level of 0.01 mg/l for wastewater from a single source.[10] The problem is that most dental wastewater is directly released into the water bodies with sewage water. To worsen this situation, there is a lack of wastewater treatment plants that actually remove this type of heavy metal in Brazil.

The present study aimed at evaluating the bioavailability and accumulation of Hg from dental amalgam in fish following its release into the environment. Three different environmental conditions were selected: Water collected from the Tietê river basin neighboring the metropolitan area of the city of Bauru, state of São Paulo (Brazil) in a location close to where all sewage water from this city is discharged; tap water contaminated with 0.5 gm/l load of dental amalgam, consistent with that of waste before it is diluted within the wastewater stream and tap water used as control. The fish *C. auratus* was chosen to compare the experimental findings with a previous study.[2]

MATERIALS AND METHODS

Consent for developing this study was obtained with a research ethics committee (process nº-21/2004) and all the procedures conducted during the experiment obeyed international recommendations.

Fish

Seventy-five specimens of *C. auratus* were used as a model teleost species weighing 3 to 5 gm of net weight. Fish were obtained from a local supplier, belonged from the same genetic stock, age and had similar size. Fish were housed in three glasses, frameless, covered 30-L aquaria. Fish were acclimated in the laboratory for 1 week and fed once a day with commercial goldfish food (Alcon Ltd.) until the end of the experiment.

Water Environments

Three different sources of water were chosen for exposure in the present study:

- *Group A:* dechlorinated municipal tap water, used as control.
- *Group B:* water from the Tietê river basin collected in the location were the Bauru Creek, a water stream that receive the discharge of all sewage water from the metropolitan area of Bauru, confluences with the Tietê river (Fig. 1).
- *Group C:* dechlorinated municipal tap water contaminated with 0.5 gm/l load of dental amalgam, following the procedures suggested by Kennedy[2] (Fig. 2).

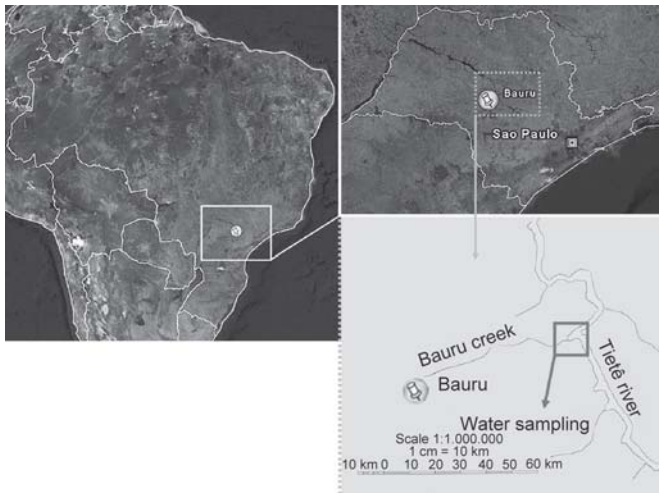

Fig. 1: Map location of the confluence of the Bauru Creek and the Tietê river, close to the metropolitan area of Bauru, state of São Paulo. The water sampling point is highlighted

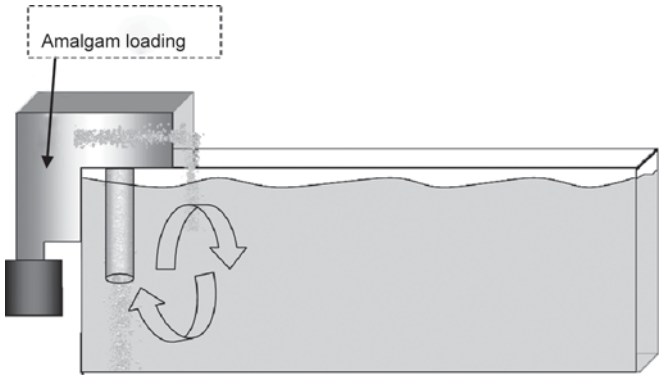

Fig. 2: Schematic representation of the study model applied in the present study. The 30L aquarium had an external filter attached where was kept a nylon mesh. In group C, with dental amalgam loading, the amalgam particles remained within the nylon mesh. The remaining groups had empty nylon meshes. Inspired in the material and methods described by Kennedy[2]

Exposure Periods

At the beginning of the experiment, each aquarium had 25 fish. After 1 hour of exposure, 10 fishes from each tank were randomly sampled (T1), followed by the remaining 15 fishes from each tank at 28 days (T28). During the experiment, water samples were collected from each tank at 1 hour, 7, 14, 21 and 28 days. The water samples were collected in polyethylene flasks previously decontaminated with nitric acid and sodium chloride preservative solution.[11]

Total Hg Analysis

Sampled fishes were dissected with removal of scales, the cranial part with the gills and all fins. The inner organs were collected and the remaining carcass was considered as muscle and weighed separately. The selected samples were grouped by exposure periods (T1 and T28) and classified by tissue type (inner organs and muscle). Each group was

than homogenized and digested. The total Hg was determined by cold vapor atomic absorption spectrometry (CV AAS) using a PerkinElmer flow-injection system for atomic spectroscopy FIAS 400, in accordance with the method previously described by Akagi et al.[12] Certified reference material, dogfish muscle, DORM-2, National Research Council of Canada, Institute for Environmental Research and Technology was used for quality control and validation.

The limit of quantification for total Hg was 0.06 mg/kg in the inner organs and muscle and 0.0001 mg/l in water samples.

Statistics

Statistical analysis was performed using nonparametric Kruskal-Wallis one-way analysis of variance test. It was used to determine if there were significant differences in median tissue Hg concentrations between fish exposed to different water environments at either T1 or T28. Differences were considered significant at $p < 0.05$.

RESULTS

The aquaria were kept clean during the experimental period; there was minimum accumulation of algae and/or organic material. Every uneaten food excess was removed by the aquarium filter. The temperature range was 14 to 16°C. The nylon mesh inside the filters remained intact in all study groups, without any loss of bulk amalgam particles out of the exposure system for group C. Great accumulation of organic matter was observed in the nylon mesh of group B compared to the other groups.

Survival of fish was 100% at T1, but during the remaining 28 days of experiment, dead fish were recovered from all tanks, namely one specimen in group A, one in group B and three in group C. By the time the experimental period T28 ended, the fish in group C presented loss of mobility, special disorientation and scale color alteration. This finding did not occur in fish from other tanks.

Tables 1 and 2 show total Hg concentrations in water, muscle and internal organs related to its experimental periods. The results regarding total Hg concentration in water are graphically represented in Figure 3.

The fish from group A were considered as control group and revealed very low concentrations of Hg, with total Hg levels below the limit of quantification. Moreover, the maintenance system and testing methods used did not introduce any Hg from sources other than dental amalgam

Table 1: Total mercury mean concentration in water samples in the different groups according to the experimental periods

Period	*Total Hg concentration (10^{-3} mg/l)*		
	Group A	*Group B*	*Group C*
1 hour	0.1	0.8	2.3
7 days	0.1	0.8	6.6
14 days	0.1	0.9	2.4
21 days	0.1	0.6	2.1
28 days	0.0	0.6	2.8

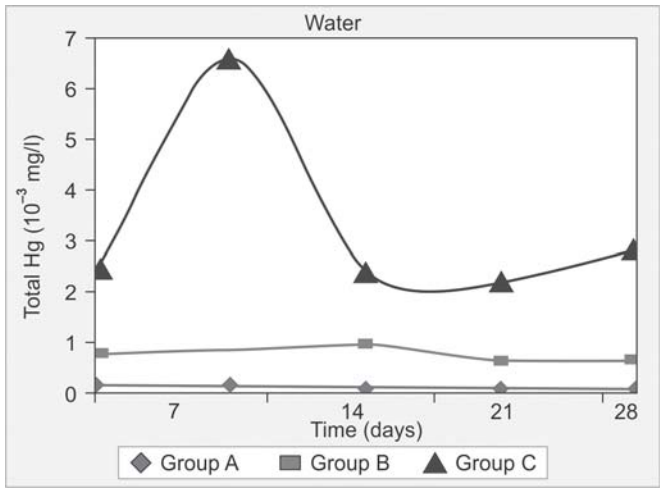

Fig. 3: The total Hg concentration in water of the three tested environments. Hg levels were significantly higher only when compared to group C—amalgam contaminated water, with group A—control

Table 2: Descriptive statistics of total Hg concentration in fish muscle and internal organs (mg/kg) from the three examined groups according to T1 and T28 assay periods

Period	*Descriptive statistics*	*Group A*		*Group B*		*Group C*	
		Muscle	*IO*	*Muscle*	*IO*	*Muscle*	*IO*
1 hour	Mean	ND	ND	0.08	17.48	0.07	301.88
	Medium	ND	ND	0.07	10.20	0.07	256.00
	Minimum	ND	ND	ND	2.08	ND	222.72
	Maximum	ND	ND	0.17	42.68	0.10	468.37
	Standard deviation	ND	ND	0.046	14.120	0.013	102.458
28 days	Mean	ND	ND	0.13	14.84	0.67	276.72
	Medium	ND	ND	0.13	12.36	0.54	262.64
	Minimum	ND	ND	0.13	4.74	0.41	192.24
	Maximum	ND	ND	0.14	37.12	1.29	418.47
	Standard deviation	ND	ND	0.004	10.484	0.296	69.657

ND: Non detected; IO: Internal organs

in group C. Fish from group B were exposed only to the Hg present in water collected from the Tietê river basin.

Total Hg concentrations in muscle and in the inner organs during the experimental periods are represented in Figures 4 and 5. The medians of total Hg concentrations in fish from group B at T1 was 0.07 mg/kg in muscle and 10.20 mg/kg in inner organs. Analysis of fish at T28 showed medians of total Hg of 0.13 mg/kg in muscle and 12.36 mg/kg in inner organs. Hence, at T1, muscle samples from group B did not present significant difference in relation to group A, while at T28 the medians of total Hg concentrations in muscles almost doubled in relation to T1 ($p = 0.012$). However, Hg levels in inner organs of fish from group B did not present significant statistical differences between the two experimental periods ($p = 0.728$).

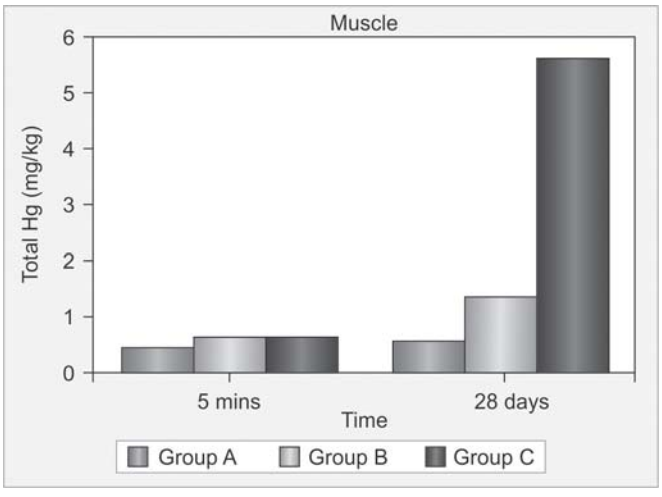

Fig. 4: Mean concentrations for muscles (A) control—Hg levels remained practically unchanged, (B) Tietê river—total Hg uptake doubled during the experiment and (C) dental amalgam contaminated water—significant increase of Hg levels, when compared to group A

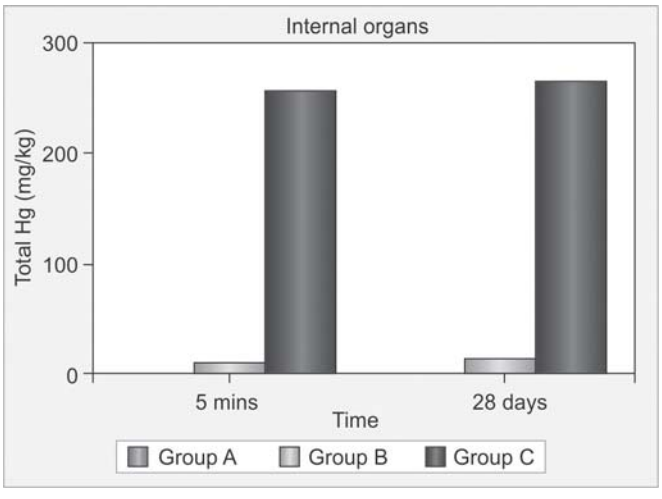

Fig. 5: Mean concentrations for the internal organs (A) control—under the quantification limit, (B) Tietê river—no significant increase of total Hg levels and (C) dental amalgam contaminated water—very high concentrations at T1 and T28

Regarding group C, there was a significant increase in medians of total Hg concentrations in muscles comparing T1 and T28 ($p = 0.01$), while in inner organs there was a great increase, yet without significant statistical difference ($p = 0.935$). Therefore, it might be assumed that total Hg uptake speed in the musculature is lower than observed in inner organs of the study samples.

DISCUSSION

The Brazilian Council of Dentistry (CFO) has 209,643 licensed dental practitioners,[13] 1,115 of them are registered in Bauru.[14] Although the amount of Hg from dental wastewater released from dental practice have not been estimated yet in Brazil in g/dentist/day way, dental amalgam is still a very popular dental material, widely applied on restorative dentistry, mostly in public health care. Considering that all sewage water from Bauru metropolitan area is discharged in the Bauru Creek, it is reasonable to assume the significance of dental wastewater as a major Hg source in this specific water body.

Several studies have previously addressed the establishment of Hg concentrations in tissue samples of fish relating its levels with the water environment, feeding habits and with different species samples.[15-18] The present study is concerned with the dental wastewater effect in freshwater environments that receive Hg contaminated sewage water discharge. It has been inspired by a previous study that revealed that fish can accumulate Hg from an environmental exposure to dental amalgam.[2] The need of further studies focusing on the identification and understanding of Hg uptake from this source under various environmental conditions, as reported by Kennedy,[2] encouraged the present study.

The results obtained in this study for total Hg levels in muscle at T28 are similar to those previously reported by Kennedy[2] in samples maintained in aquarium containing 1 gm/l amalgam load. This finding suggests that amalgam solubility was quite higher in the present study. Moreover, this particularity can be directly related with methodological differences between both studies. The modifications performed to the method suggested by Kennedy[2] aimed to make the exposure system adequate for total Hg cold vapor atomic absorption spectrometry analysis. These modifications comprised increasing fish samples with higher weight, continuous feeding during the experiment, grouping the inner organs instead of analyzing only the liver and brain, and finally process the muscle with the body carcass. Therefore, the modifications increased the volume of samples leading to substantial values for statistical purposes. The technique employed in the present study for total Hg

CV AAS analysis has a high sensitivity and followed the procedures for sample preparation described by Akagi et al.[12]

Periodical water sampling in the exposure systems during the experiment is a novel procedure that distinguishes the present study. It was established to understand the Hg uptake within the exposure system and compare with Hg accumulation in the fish from the experimental model. A gradual decrease of total Hg concentration in water was observed in all study groups, demonstrating that Hg available in the experimental environment is really incorporated by fish and probably by other components of the exposure system such as aquarium walls, aquarium filter, biofilms, etc.

The total Hg levels in tissue samples of fish from group C exceeded 0.5 mg/Kg which is the maximum amount established by Brazilian legislation.[19] The observations regarding fish physical and behavioral alterations noted on group C might be related to mercurialism, since fish from other groups did not present any signs of distress. The welfare measures provided to all sampled fish also directs the comprehension of Hg contamination of the fish from group C at T28. Methyl Hg analysis could contribute a lot for the comprehension of these facts, maybe although the total Hg levels in the water decreases along the experiment, methyl Hg concentrations increases. Further studies should aim to respond this question.

Total Hg analysis of water collected at the Tietê river revealed Hg level below the permissible limit (0.0002 mg/l), according to Brazilian law.[10] Total Hg levels in the studied water samples were similar to those found on check points at adjacent areas from the Company of Technology of Environmental Sanitation.[20] The point selected for water collection in the present study aimed an area with minimal Hg sources other than from urban sewage water. Besides dental wastewater, other possible Hg sources acting in Bauru metropolitan region could be industrial activities; storm water inflow, improper disposal of Hg thermometers, vehicle service facilities, household products and laundry gray water. Moreover, even if these sources could influence Hg levels as potential pollutants, the dental wastewater discharged in Bauru Creek without an affective regulation just increase the need of a public health procedure aiming this potential environmental problem.

The experimental treatment using dental amalgam does not provide an accurate measure of the exposure of fish to dental waste, since the dental amalgam concentration is much higher than fish in natural systems would encounter. This study is part of an effort to establish a reliable exposure model. The experimental group B, with Tietê River water, tried to get as close as possible of an environment in which fish are exposed to the Hg influenced by dental wastewater discharge. Further studies should aim to develop this exposure system in order simulate natural profiles.

ACKNOWLEDGMENT

The present study was supported by grants of Fundación MAPFRE, though MAPFRE-Public Health School at University of São Paulo research support award.

REFERENCES

1. Sörne L, Lagerkvist R. Sources of heavy metals in urban wastewater in Stockholm. Sci Tot Environ 2002;298:131-45.
2. Kennedy CJ. Uptake and accumulation of mercury from dental amalgam in the common goldfish, Carassius auratus. Environ Pollut 2003;121:321-26.
3. Drumond JL, Cilas MD, Croke K. Mercury generation potential from dental waste amalgam. J Dent 2003;31:493-501.
4. Stone ME, Pederson ED, Cohen ME, Ragain JC, Karaway RS, Auxer RA, et al. Residual mercury content and leaching of mercury and silver from used amalgam capsules. Dent Mater 2002;18:289-94.
5. Mercury source control and pollution prevention program evaluation: Final report. Prepared for: Association of Metropolitan Sewerage Agencies (AMSA). Washington: Larry Walker Associates; 2002. Available from: http://archive.nacwa.org/getfileb882.pdf?fn=finalreport.pdf.
6. Dental amalgam: Update on safety concerns. ADA council on scientific affairs. J Am Dent Assoc 1998;129:494-503.
7. Health Canada. The safety of dental amalgam: Health Canada states position. 1996. Available from: http://www.hc-sc.gc.ca/ahc-asc/media/nr-cp/1996/1996_63_e.html.
8. Ylinen K., Löfroth G. Nordic dentists' knowledge and attitudes on dental amalgam from health and environmental perspectives. Acta Odontol Scand 2000;60:315-20.
9. Brazil. Ministry of Health. Safety manual of the hospitalar environment: Department of Technical Standards. Brasília 1995;1:195.
10. Brazil. CONAMA. National Council on the Environment. CONAMA Resolution n° 357, March 17, 2005: Provides for the classification of water bodies and environmental guidelines for your environment, as well establishes the conditions and effluent discharge standards, and other matters. Available from: http://www.mma.gov.br/port/conama/legi.cfm.
11. Knechtel JR. Utilization of the mercury (II) chloride complex for the long-term storage of samples containing part per 10^9 levels of mercury. Analyst 1980;105:826-29.
12. Akagi H, Malm O, Branches FJP, Kinjo Y, Kashima Y, Guimarães JRD, et al. Human exposure to mercury due to gold mining in the Tapajos River basin, Amazon, Brazil: Speciation of mercury in human hair, blood and urine. Water Air Soil Pollut 1995;80:85-94.
13. Federal Board of Dentistry. Search by CRO total professional category Dental Surgeon. Brasília, 2006. Available from: http://www.cfo.org.br/busca_dados/totais/tot_prof_cro.asp.

14. Regional Council of Dentistry of São Paulo. Distribution of Dental Surgeons in the state of São Paulo. São Paulo, 2006. Available from: http://www.crosp.org.br/CROSPBUSCA/MACROZONAS.aspx.
15. Durrieu G, Maury-Brachet R, Boudou A. Goldmining and mercury contamination of the piscivorous fish Hoplias aimara in French Guiana (Amazon basin). Drug Chem Toxicol 2005;23:41-54.
16. Dusek L, Svobodova Z, Janouskova D, Vykusova B, Jarkovsky J, Smid R, et al. Bioaccumulation of mercury in muscle tissue of fish in the Elbe River (Czech Republic): Multispecies monitoring study 1991-1996. Ecotoxicol Environ Saf 2005;61:256-67.
17. Kainz M, Mazumder A. Effect of algal and bacterial diet on methyl mercury concentrations in zooplankton. Environ Sci Technol 2005;15:1666-72.
18. Holsbeek L, Das HK, Joiris CR. Mercury speciation and accumulation in Bangladesh freshwater and anadromous fish. Sci Total Environ 1997;30:201-10.
19. Brazil. Sanitary surveillance secretariat of the ministry of health decree Nº 685, August 27th, 1998. General principles for the establishment of maximum tolerance levels for inorganic contaminants. Available from: http://www.anvisa.gov.br/e-legis/.
20. CETESB. Report quality of inland waters of the state of São Paulo. São Paulo. 2002. Available from: http://www.cetesb.sp.gov.br/Agua/rios/relatorios.asp.

ABOUT THE AUTHORS

Tiago Novaes Pinheiro (Corresponding Author)

Professor, Department of Dentistry, Amazonas State University, Brazil
e-mail: tiagonpinheiro@yahoo.com.br

Alberto Consolaro

Chairman, Department of Estomatologia, Bauru Dental School São Paulo, Brazil

Alice Sakuma

Researcher, Department of Bromatologia, Adolfo Lutz Institute São Paulo, Brazil

Marcia Liane Buzzo

Researcher, Department of Bromatologia, Adolfo Lutz Institute São Paulo, Brazil

Marcia Sena de Carvalho

Researcher, Department of Bromatologia, Adolfo Lutz Institute São Paulo, Brazil

Maria Cristina Duran

Researcher, Department of Bromatologia, Adolfo Lutz Institute São Paulo, Brazil