Microchemical Journal 94 (2010) 171–174

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Determination of heavy metals by inductively coupled plasma-optical emission spectrometry in fish from the Piracicaba River in Southern Brazil

Andrew Meche [a], Manoela C. Martins [b], Bruna E.S.N. Lofrano [b], Carey J. Hardaway [a], Mark Merchant [a,*], Luciano Verdade [b]

[a] Department of Chemistry, McNeese State University, Lake Charles, Louisiana, USA
[b] Animal Ecology Lab, University of São Paulo–Piracicaba SP 13418-900, Brazil

ARTICLE INFO

Article history:
Received 17 October 2009
Received in revised form 28 October 2009
Accepted 28 October 2009
Available online 13 November 2009

Keywords:
Brazil
Effluent
Heavy metals
ICP-OES
Piracicaba River

ABSTRACT

A total of 202 fish, representing 16 species, were collected during 2008 (March–October) in the Tanquan region of the Piracicaba River using nets. Flesh samples were collected and analyzed, using inductively coupled plasma-optical emission spectroscopy for Al, As, Cd, Co Cr, Cu, Mn, Mo, Ni, Pb, Se, Sn, Sr, and Zn. The results showed that the flesh of these fish all contained extremely high levels of Al and Sr, and moderately high levels of Cr, As, Zn, Ni, Mn and Pb. The metals were higher in these fish during rainy season, with fish collected during the months of March and October being the highest. In addition, the accumulation of metals was species-dependent. Cascudos (*Hypostomus punctatus*) and piranhas (*Serrasalmus spilopleura*) exhibited high levels of almost all of the metals, while curimbata (*Prochilodus lineatus*) had moderate levels. A few species, including pacu (*Piaractus mesopotamicus*) and dourado (*Salminus maxillosus*), had very low levels of most metals. The results show that the Piracicaba River Basin is widely contaminated with high levels of many toxic heavy metals, and that human consumption of some fish species is a human health concern.

1. Introduction

The Piracicaba River Basin, located in the State of Sao Paulo, is considered to be one of the most polluted areas in Brazil [1]. The surrounding area is utilized heavily for the commercial production of sugar cane and eucalyptus trees, and agricultural runoff is a major source of pollution in the river system [2,3]. The heavy usage of fertilizers with high concentrations of metals also, undoubtedly, contributes to the heavy metal burden of this ecosystem [4]. In addition, some areas of the basin are heavily populated and, as recently as 1999, only 16% of the sewage was treated, thus causing additional acute problems for the water quality of the Piracicaba River [5].

The Piracicaba River has also been reported to contain large and heavy metal loads from industrial effluents in the populated and industrialized regions, and heavy metals have been detected throughout the food chain [6,7]. Recent studies by Piña et al. [8] showed that Geoffroy's side-necked turtles (*Phrynops geoffroanus*) from this river basin contained extremely high levels of many different heavy metals. Some authors have suggested that the heavy metal content of the fish in the Piracicaba River serve as a biomonitor for the health of the river basin ecosystem, and an indicator for health concerns to the human populations that consume fish from the river [9,10]. This study was conducted to determine the heavy metal contamination in fish collected from the Tanquan region of the Piracicaba River, and to determine if humans that consume fish from this river system might be at risk for heavy metal-related health issues.

2. Materials and methods

2.1. Study area

The tissue samples were collected from fresh commercial fish at Vila do Tanquan (20°31′S, 48°32′W), a small village of fishermen at Piracicaba River, upstream from Barra Bonita Hydroelectric Reservoir in São Paulo, Southeastern Brazil. Eight monthly sample collections were conducted from March 2008 to October 2008. However, no collections were conducted from November to February because of reproductive seasonal legal ban of commercial fisheries [11].

2.2. Capture of fish

All fish sampled were collected from a single fisherman in order to assure regularity in fishing methods. Net fishing took place from 17:00 to 07:00. The fresh fish were acquired straight from the fisherman before any processing and then trasferred to the laboratory on ice (40 min). Muscle samples (approximately 2 g, 1 × 1 cm) were collected

* Corresponding author. Department of Chemistry, McNeese State University, 450 Beauregard, Kirkman 221A, Lake Charles, LA 70609, USA. Tel.: +1 337 475 5773.
E-mail address: mmerchan@mcneese.edu (M. Merchant).

0026-265X/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.microc.2009.10.018

by scalpel incisions in the middle portion of the body, distal from opperculae, and stored at −20 °C. Time from net-capture to the tissue sample storage at the freezer was between 3 and 17 h.

2.3. Preparation of samples

Fish samples were dried in an oven at 80–85 °C overnight. The samples were removed from the oven, allowed to cool, and ground in a clean mortar and pestle. Approximately 0.5 g of each sample was placed in a teflon microwave digestion bomb with 10 mL of concentrated HNO_3. The samples were allowed to ramp to 180 °C for 5 min, digest at 180 °C for 9.5 min, and cool down for 5 min in a MARS 5 microwave digestion system (CEM, Matthews, NC). The samples were then transferred to clean volumetric flasks, and diluted with H_20 to 10 mL. The samples were stored at 5 °C until ready for analysis of metals.

2.4. Analysis of metals

The samples were compared to a multi-element standard curve to determine the ppm of each analyte in the digested solution. The samples were analyzed three independent times on a Varian 715 ES ICP optical emission spectrometer. The wavelengths used for the detection and measurement of Al, As, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Se, Sn, Sr, and Zn were 396.152, 188.980, 214.439, 238.892, 267.716, 327.395, 257.610, 202.032, 231.604, 220.353, 196.026, 189.925, 407.771, and 213.857 nm, respectively.

2.5. Statistics and controls

To ensure analytical accuracy and prevent inaccurate measurements due to instrumental drift, the standard curve was analyzed every 50 samples. In addition, a 1 ppm standard for all metals was analyzed every 20 samples. Analysis of the 1 ppm standard produced results from 0.93 to 1.04 ppm during the entire period of sample analysis. Quality control/quality assurance (QC/QC) has been determined in previous studies from this laboratory [12,13].

3. Results and discussion

The results from ICP-OES analysis for the sixteen different species of fish are shown in Table 1. Analyses of all of the fish species ($n = 16$) examined revealed extremely elevated levels of Al, with the mean Al concentrations of all samples 8.38 ppm ($n = 201$). Thirteen of the seventeen species had Al concentrations above 5.0 ppm (Table 1). The soils in the southern Brazilian savannas are generally high in Al [14]. In addition, Krusche et al. [15] reported that the soils in the Piracicaba River Basin are high in Al, and that the Al can be liberated from the soil by the common acid rains in the region. There is enough aluminum in the soil that sugar cane burning is a concern due to the amount of Al released and the risk of human pulmonary disease [16].

Elevated levels of Cr were found in nearly all fish species tested (Table 1), while two of the species, the cascudo (*Hypostomus punctatus*) and the pirambeba (*Serrasalmus spilopleura*) having tissue levels in excess of 1 ppm. In addition, eight of seventeen species of fish showed levels of Ni in excess of 1 ppm (Table 1), with *Hoplosternum littorale* samples showing an extremely elevated Ni mean concentration of 13.5 ppm ($n = 6$). Eight of sixteen species of fish showed levels of Ni in excess of 1 ppm (Table 1), with *H. littorale* samples showing an extremely elevated Ni mean concentration of 13.5 ppm ($n = 6$). High levels of Cr have been reported in the Piracicaba soil sediment [17]. Jordao et al. [18,19] reported high concentrations of Cr in sediment, plants, and fish collected from the Piracicaba River. These high Cr concentrations were reportedly due to the tannery industry effluent into the Piracicaba River. The Cr in the fish tissues was more than 35 times the Brazilian government's recommended limit of human consumption.

All but three species of the fish tested exhibited levels of Ni above the detection limit. Eight of the species contained Ni levels more than 1.0 ppm. Furthermore, the caborja (*H. littorale*) exhibited mean levels of 13.5 ppm Ni. As recently as 2006, De Vive et al. [10] reported high concentrations of Ni in fish from the Piracicaba River. Jordão et al. [20] showed that suspended particles, plants, and water samples collected from different locations in the Piracicaba River contained elevated levels of Ni. It is known that relatively low levels of nickel exposure can cause immunological and carcinogenic effects in humans [21].

There were also elevated levels of Zn in the fish tissue samples. These results are supported by a study [22] which reported that the Piracicaba River water had elevated levels of Zn, and that these high concentrations appeared to be due to industrial effluent. Zn is an important endogenous metal which has been shown to have important physiological functions in the areas of immune function [23], protein synthesis [24], wound healing [25], DNA synthesis [26], and a variety of other general biochemical functions. Although zinc is an important dietary supplement for humans, excessive zinc intake can cause a broad spectrum of physiological problems [27].

There was an obvious seasonal pattern to metal contamination in the fish samples. The levels of 12 of the 14 metals analyzed (Al, As, Cd, Co, Cr,

Table 1
Heavy metal residues in different species of fish from the Piracicaba River. Fish samples were obtained from the Piracicaba River during March–October, 2008. Samples of flesh were digested in nitric acid and 14 different heavy metals were determined by ICP-OES. The values in the table are expressed as ppm and represent the mean for each metal in each species. ND = not detectable.

	Al	As	Cd	Co	Cr	Cu	Mn	Mo	Ni	Pb	Se	Sn	Sr	Zn
Total ($n = 202$)	8.38	0.17	0.06	0.06	0.35	0.98	0.62	0.08	1.25	0.77	0.24	0.23	1.98	7.24
Pimelodus maculatus ($n = 32$)	7.05	0.23	0.00	0.02	0.21	0.76	0.28	0.05	0.34	3.25	0.78	ND	3.18	6.88
Tilápia rendalli ($n = 47$)	8.87	0.23	0.05	0.03	0.37	1.15	0.70	0.08	1.66	0.36	0.07	0.08	0.85	7.05
Prochilodus lineatus ($n = 30$)	12.0	0.19	0.09	0.09	0.76	1.80	0.96	0.09	1.53	0.57	0.12	0.26	2.93	7.61
Triportheus sp. ($n = 2$)	6.99	ND	ND	ND	0.19	0.41	0.60	ND	1.16	0.69	0.00	ND	2.11	7.15
Plagioscion squamosissimus ($n = 35$)	6.61	0.11	ND	ND	0.16	0.57	0.36	ND	0.34	0.08	ND	0.28	0.45	6.84
Salminus maxillosus ($n = 8$)	0.72	ND	ND	ND	0.05	0.25	0.06	ND	ND	ND	ND	1.65	0.24	3.08
Hypostomus punctatus ($n = 8$)	8.72	0.93	0.92	1.40	1.52	1.72	3.24	1.48	1.27	1.41	1.61	1.60	2.00	9.25
Geophagus brasiliensis ($n = 6$)	4.98	ND	0.02	ND	0.23	0.19	0.44	ND	0.11	ND	ND	0.35	0.26	8.39
Leporinus elongatus ($n = 3$)	12.1	ND	ND	ND	0.25	0.88	0.84	ND	0.16	2.69	ND	ND	2.74	8.37
Leporinus sp. ($n = 10$)	5.45	ND	ND	ND	0.24	0.36	0.69	ND	0.41	ND	0.60	0.16	5.68	5.71
Piaractus mesopotamicus ($n = 1$)	2.83	ND	ND	ND	ND	0.03	0.10	ND	ND	ND	ND	1.54	0.15	4.48
Geophagus brasiliensis ($n = 4$)	24.9	ND	ND	ND	0.20	0.52	0.14	ND	2.83	ND	ND	ND	11.9	3.31
Hoplias malabaricus ($n = 1$)	4.15	ND	ND	ND	0.22	0.37	0.49	ND	0.05	ND	ND	0.25	0.67	8.03
Serrasalmus spilopleura ($n = 7$)	8.11	0.73	0.72	0.74	1.11	4.18	1.67	0.82	1.33	0.66	1.22	1.38	3.31	16.3
Rhamdia quelen ($n = 2$)	11.9	ND	ND	ND	0.27	0.19	0.35	ND	ND	ND	ND	ND	0.35	5.48
Hoplosternum littorale ($n = 6$)	10.2	ND	ND	ND	0.21	0.43	0.85	0.04	13.5	ND	ND	ND	0.89	5.57

Table 2
Heavy metals in Piracicaba River fish from different months. Fish samples were obtained from the Piracicaba River during March–October, 2008. Samples of flesh were digested in nitric acid and 14 different heavy metals were determined by ICP-OES. The values in the table are expressed as ppm and represent the mean for each metal in during the months specified. ND = not detectable.

Month	Al	As	Cd	Co	Cr	Cu	Mn	Mo	Ni	Pb	Se	Sn	Sr	Zn
March (n = 40)	7.84	ND	ND	ND	0.46	1.56	0.50	ND	0.23	0.19	0.54	0.05	3.57	6.59
April (n = 35)	9.33	0.05	0.01	0.00	0.21	1.06	0.49	ND	0.31	3.41	0.00	0.09	1.11	6.41
May (n = 21)	8.06	0.09	0.09	0.13	0.33	0.46	0.85	0.15	4.02	0.23	0.29	0.28	1.07	6.41
July (n = 29)	7.93	0.01	ND	0.03	0.27	0.65	0.38	0.05	0.80	0.11	0.15	0.18	0.55	6.61
August (n = 37)	7.89	0.53	0.12	0.06	0.38	0.76	0.82	0.14	0.75	0.21	0.26	0.42	1.77	7.07
October (n = 18)	13.41	0.60	0.28	0.36	0.70	2.14	1.04	0.38	3.64	0.52	0.47	0.46	3.88	10.10

Cu, Mn, Mo, Sr, and Zn) were higher in October than any other month. There are distinct wet and dry seasons in southern Brazil, with the rainy season beginning during the months of September or October, and continuing through April or May. It is interesting to note that the metal residues in the fish samples are elevated during October, at the start of the wet season. We postulate that the beginning of the rainy season washes metal contamination from the Piracicaba River Basin soils into the river, thus elevating the exposure of the fish during this time. Alternatively, a more plausible explanation for the seasonal fluctuations could be the reservoirs created by the damming of the Piracicaba River. The Piracicaba River has been completely dammed for hydroelectric energy production since the 1970s. The water level in dammed water courses is usually kept low at the rainy season to prevent inundations and high at the dry seasons to assure water supply for human purposes (i.e., urban, agricultural and industrial consumption). On the other hand, the water level in the river itself (out of the influence zone of the reservoirs) tends to be maintained at in opposite pattern, closer to natural: high in the rainy season and low at the dry season. In such circumstance, the river environment can be affected by changes in flow, sediment load, temperature and water quality [28]. The seasonal fluctuations in the metals are in direct contrast to a report by Rodgher et al. [17] in which the Piracicaba River water levels of Zn were 120 times higher in July than February. However, this same study reported much higher levels of Cd in February than in July. We found that the levels of Pb and Ni show high spikes in April and May, respectively. These anomalous results could be due to seasonal effluent waste streams containing these contaminants.

It was interesting to note that several species of fish contained high levels of all metals tested. For instance, the piranha (*S. spilopleura*) had among the highest levels of nearly all of the 14 metals tested, relative to the other 15 species of fish. Similarly, the cascudo (*H. punctatus*) was very high in all metals tested, and the curimbata (*Prochilodus lineatus*) contained moderate–high levels of all metals determined. Curimbata (*P. lineatus*) and tilapia (*Tilápia rendalli*) contained detectable levels of all 14 metals, while the mandi (*Pimelodus maculates*) showed 13 of the 14 metals. In contrast, the bagre (*Rhamdia quelen*) and pacu (*Piaractus mesopotamicus*) contained measurable levels of only 6 of 14 metals. The cascudo and curimbata are both bottom dwellers that feed on detritus, while the piranha is a predatory fish, and thus the high levels of metals in these species cannot be explained simply by feeding behaviors. Other fish species, such as the predatory dourado and pacu, were very low in nearly all metals examined. However, both of these species were very high in Sn, indicating that Sn might be a metal that has more potential for bioaccumulation in this ecosystem.

4. Conclusions

The results from this study showed that fish in the Tanquan region of the Piracicaba River are contaminated with a broad spectrum of heavy metals. The metal content is species-dependent, with some species showing high concentrations of all metals, and some showing low concentrations. The metal concentrations in the fish tissues were also time-dependent, with residues much higher during months during the rainy season (Table 2). We conclude that the regular consumption of some of these fish species, even during the dry season (May–August), when metal concentrations are lower, could pose serious human health risks. These health risks have been delineated in studies of heavy metal contamination in fish in Taiwan [29].

Acknowledgements

The authors wish to acknowledge the contribution of the fishermen from Vila do Tanquan, who made this study possible. Prof. José Eurico P. Cyrino helped with fish identification. The authors would also like to thank Dr. Jayne Garno and Algernon Kelley, of the Louisiana State University Chemistry Department, for help in digesting samples. Funds for this study were provided by the Fundação de Amparo à Pesquisa do Estado de São Paulo—FAPESP (proc. no. 2006/60954-4) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq (proc. no. 300480/2008-3). In addition, this project was supported by a Research Commercialization/Educational Enhancement Program grant awarded by the Louisiana Board of Regents.

References

[1] R.A.M. Silvano, A. Begossi, Seasonal dynamics of fishery at the Piracicaba River (Brazil), Fish. Res. 51 (2001) 69–86.
[2] M.R. Williams, S. Filoso, L.A. Martinelli, L.B. Lara, P.B. Camargo, Precipitation and river water chemistry of the Piracicaba River Basin, Southeast Brazil, J. Environ. Qual. 30 (2001) 967–981.
[3] S. Filoso, L.A. Maretinelli, M.R. Williams, L.B. Lara, A. Krushe, M. Ballester, R. Vicotira, P.B. de Camargo, Land use and nitrogen export in the Piracicaba River Basin, Southeast Brazil, Biogeochemistry 65 (2003) 275–294.
[4] J.J. Corbi, S. Trivinho-Strixino, A. dos Santos, Environmental evaluation of metals in sediments and dragonflies due to sugar cane cultivation in neotropical streams, Water Air Soil Pollut. 195 (2008) 325–333.
[5] L.A. Martinelli, A.V. Krusche, R.L. Victoria, P.B. Camargo, M. Bernardes, E.S. Ferraz, J.M. Moraes, M.V. Ballester, Impact of anthropogenic activity on the Hg concentrations in the Piracicaba River Basin (São Paulo State, Brazil), Water Air Soil Pollut. 110 (1999) 67–79.
[6] A.C. Tomazelli, L.A. Martinelli, W.E.P. Avelar, P.B. Camargo, A.H. Fostier, E.S.B. Ferraz, F.J. Krug, D. Santos Jr, Biomonitoring of Pb and Cd in two impacted watersheds in southeast Brazil, using the freshwater mussel *Anodontites trapesialis* (Lamarck, 1819) (Bivalvia: Mycetopodidade) as a biological monitor, Braz. Arch. Biol. Technol. 46 (2003) 671–682.
[7] A.C. Tomazelli, L.A. Martinelli, F.J. Krug, D. Santos, I. Rufini, P.B. Camargo, M. Horvat, Mercury distribution in medium-size rivers and reservoirs of the São Paulo State (Southeast Brazil), J. Environ. Qual. 36 (2007) 478–486.
[8] C.I. Piña, V.A. Lance, B.O. Ferronato, I. Guardia, T.S. Marques, L.M. Verdade, Heavy metal contamination in *Phrynops geoffroanus* (Schweigger, 1812) (Testudines: Chelidae) in a river basin, Sao Paulo, Brazil, Bull Environ. Contam. Toxicol. 83 (2009) 771–775.
[9] S. De Vives, B. Briuenza, S. Moreira, O.L. Zucchi, Heavy metals determination in fish using total reflection with synchrotron radiation, Xsen Bunseki Toronkai Koen Yoshishu 23 (2003) 123–124.
[10] S. De Vives, S. Moreira, B. Brienza, O.L. Zucchi, V.F.N. Filho, Analysis of fish samples for environmental monitoring and food safety assessment by synchrotron radiation total reflection x-ray fluorescence, J. Radioanal. Nucl. Chem. 270 (2006) 231–236.
[11] IBAMA - PORTARIA nº - 49, Portaria de defeso da bacia do Paraná, de 5 de novembro de 2007. http://www.ibama.gov.br/rec_pesqueiros/index.php?id_menu=86&id=7. Accessed in 11/11/2008.
[12] M.A. Hamilton, P.W. Rode, M.E. Merchant, J. Sneddon, Determination of selected heavy metals in seafood and fish in an industrial and a pristine waterway by inductively coupled plasma-optical emission spectrometry, Microchem. J. 88 (2008) 52–55.
[13] J.C. Richert, J. Sneddon, Determination of heavy metals in crawfish (*Procambrus clarki*) by inductively coupled plasma optical emission spectrometry ; a study over the season in southwest Louisiana, Anal. Lett. 44 (2008) 3198–3209.

[14] G.B. Nardato, M.C. Bustamante, Effects of fire on soil nitrogen dynamics and microbial biomass in savannas of Central Brazil, Pesqui. Agropecu. Bras. 38 (2003) 955–962.
[15] A.V. Krusche, P.B. de Camargo, C.E. Cerri, M.V. Ballester, L.B. Lara, R.L. Victoria, L.A. Martinelli, Acid rain and nitrogen deposition in a sub-tropical watershed (Piracicaba): ecosystem consequences, Environ. Pollut. 121 (2003) 389–399.
[16] J.E.D. Cancado, P.H.N. Saldiva, L.A.A. Pereira, L.B.L.S. Lara, P. Artxo, L.A. Martinelli, M.A. Arbex, A. Zanobetti, A.L.F. Braga, The impact of sugar cane-burning emissions on the respiratory system of children and the elderly, Environ. Health Persp. 114 (2006) 725–729.
[17] S. Rodgher, E.L.G. Espíndola, O. Rocha, R. Fracácio, R.H.G. Pereira, M.H.S. Rodrigues, Limnological and ecotoxicological studies in the cascade of reservoirs in the Tietê river (São Paulo, Brazil), Braz. J. Biol. 65 (2005) 697–710.
[18] C.P Jordão, J.L. Pereira, G.N. Jham, Chromium contamination in sediment, vegetation and fish caused by tanneries in the State of Minas Gerais, Brazil, Sci. Total Environ. 207 (1997) 1–11.
[19] C.P. Jordão, A.C. Silva, J.L. Pereira, J. Luiz, W. Brune, Chromium contamination in river waters caused by tanneries in the state of Minas Gerais, Brazil, Quim. Nova 22 (1999) 47–52.
[20] C.P. Jordão, M.G. Pereira, A.T. Matos, J.L. Pereira, Influence of domestic and industrial waste discharges on water quality at Minas Gerais State, Brazil, J. Braz. Chem. Soc. 16 (2005) 241–250.
[21] V. Ilic, V. Bojanic, B. Jovic, Epidemiological and pathogenetic effects of nickel poisoning, Acta Med. Median. 46 (2007) 37–44.
[22] C.P. Jordão, M.G. Pereira, J.L. Pereira, Metal contamination of river waters and sediments from effluents of kaolin processing in Brazil, Water Air Soil Pollut. 140 (2002) 199-138.
[23] N.W. Solomons, Mild human zinc deficiency produces an imbalance between cell-mediated and humoral immunity, Nutr. Rev. 56 (1998) 27–28.
[24] A.S. Prasad, Zinc: an overview, Nutrition 11 (1995) 93–99.
[25] W.J. Pories, J.H. Henzel, C.G. Rob, W.H. Strain, Acceleration of wound healing in man with zinc sulphate given by mouth, Lancet 1 (1967) 121–124.
[26] M. Ishido, T. Suzuki, T. Adachi, M. Kunimoto, Zinc stimulates DNA synthesis during its antiapoptotic action independently with increments of an anti-apoptotic protein, Bcl-2, in porcine kidney LLC-PK_1 cells, Pharmacol. Exp. Ther. 290 (1999) 923–928.
[27] G.J. Fosmire, Zinc toxicity, Amer. J. Clin. Nutr. 51 (1990) 225–227.
[28] J.D. Allan, M.M. Castillo, Stream Ecology: Structure and Function of Running Waters, Springer, Dordrecht, The Netherlands, 2007 436p.
[29] M.-P. Ling, H.-T. Hsu, R.-H. Shie, C.-C. Wu, Y.-S. Hong, Health risk of consuming heavy metals in farmed tilapia in central Taiwan, Bull. Environ. Contam. Toxicol. 83 (2009) 558–564.