

Heavy Metal Contamination in *Phrynops geoffroanus* (Schweigger, 1812) (Testudines: Chelidae) in a River Basin, São Paulo, Brazil

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Received: 25 February 2009 / Accepted: 9 September 2009 / Published online: 26 September 2009
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Abstract The Piracicaba River basin is considered the most disturbed river basin in the state of São Paulo. Considerable amounts of agricultural residues are seasonally drained into the river, and the region is also highly urbanized and industrialized with an incipient sewage treatment system. The presence of heavy metals has been previously reported for the water and riverbed in Piracicaba river basin. In this study we evaluated 13 heavy metals in the blood of 37 Geoffroy's side-necked turtles, *Phrynops geoffroanus*, from Piracicaba River and Piracicamirim Creek, one of its tributaries. Blood levels of As, Co, Cr, Se and Pb varied among sites, whereas Sn varied between males and females. However, no obvious pathology was detected. Serum level of Cu (2,194 ng g⁻¹) and Pb (1,150 ng g⁻¹) found in this study are the highest ever described for any reptile; however, no clinical symptoms have been detected in the present study. There is no information about the time scale of such contamination, which could be currently subclinical and yet lead to a breakdown in the population reproductive success in a few years. Based on the present study, legal enforcement is urged in order to locate and extirpate heavy metal sources

in the Piracicaba River basin. In addition, monitoring should include humans and commercial fish consumed in local markets.

Keywords Geoffroy's side-necked turtle · Piracicaba river · Piracicamirim creek

The Piracicaba River basin is considered the most disturbed river basin in the state of São Paulo in Southeastern Brazil (Martinelli et al. 2002; Fostier et al. 2005). Considerable amounts of agricultural residues (e.g., herbicides and fertilizers) are seasonally drained from the extensive sugar cane plantations into the river courses affecting their water quality. The region is also highly urbanized and industrialized with an incipient sewage treatment system that results in relatively high levels of both organic and inorganic pollution in the Piracicaba River and most of its tributaries (Ometo et al. 2000; Martinelli et al. 2002; Fostier et al. 2005). Heavy metals have been reported for the water (Falótico 2001) as well as the riverbed (Ferraz et al. 1996; Favaro et al. 2004), which is possibly related to the combination of non-point and point source pollution in the area.

Heavy metals contamination can cause severe damage in wildlife (Belskii et al. 2005; Spears et al. 2007; Márquez-Ferrando et al. 2009). Lead (Pb) can affect survivorship of waterfowl (Sileo et al. 2001). High levels of Pb, cadmium (Cd), copper (Cu) and zinc (Zn) resulted in reproductive failure in pied-flycatchers (Belskii et al. 2005), also high levels of mercury (Hg) suppress immune functions in sea turtles (Day et al. 2007). Even low levels of heavy metals are associated with a metabolic increase in snakes (Hopkins et al. 1999) and amphibians (Rowe et al. 1998), which can indirectly affect their reproduction and survivorship (Nagle et al. 2001).

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In previous studies on the Piracicaba River basin high levels of Pb and Cd in mollusks *Anodontites trapesalis* (Tomazelli et al. 2003) and of mercury (Hg) in fish were detected (Fostier et al. 2005). In this study we evaluated 13 heavy metals in the blood in the freshwater turtle *Phrynops geoffroanus* from the Piracicaba River and the Piracicamirim Creek, a tributary. Turtles are convenient organisms for contamination monitoring as they are long-lived, middle-positioned in the trophic chain (Nagle et al. 2001), and easily sampled.

This study was carried out in two locations of the Piracicaba River (Monte Alegre: 22°41'07"S, 47°33'58"W; and Tanquan: 22°40'11"S, 47°58'20"W), and in the Piracicamirim Creek (Campus: 22°42'51"S, 47°37'36"W). Monte Alegre is located approximately 10 km upstream from the city of Piracicaba and includes some areas of riparian forest along the original river border. Tanquan is located approximately 40 km downstream from Piracicaba City in the easternmost limit of Barra Bonita Reservoir. Piracicamirim Creek crosses Piracicaba City and meets Piracicaba River at the University of São Paulo Campus in the city suburb.

Piracicaba River basin (22°00' to 23°30'S, 45°45' to 48°30'W) spreads over 12,400 km² between Serra da Mantiqueira in the state of Minas Gerais and the Tietê River in the state of São Paulo. For a detailed area description see Lara et al. (2001), Martinelli et al. (2002) and Fostier et al. (2005). Piracicamirim Creek is a small tributary of the Piracicaba River. Its drainage covers an area of 132 km² (22°41'–22°52'S, 47°35'–47°43'W), over three municipalities (Saltinho, Rio das Pedras and Piracicaba). Its land use in 1995 included 61% of sugar cane plantations, 22% of exotic pastures, 9% of urban development, 4% of native forest fragments and 1.4% of eucalyptus/pine plantations (Toledo and Ballester 2001). In the mid 1980s, Piracicamirim Creek was considered highly polluted. The construction of a sewage treatment station in 1997 reduced pollution but did not solve the problem.

Materials and Methods

We used fish nets (15–30 m long × 2–3 m high) in Tanquan and Piracicamirim Creek and hand dip nets (0.4–0.5 m diameter) in Monte Alegre in order to capture the animals. These three sample sites can be considered as independent populations since during a year long radiotelemetry work with these turtles no animals have moved farther than eleven km, and those living in Piracicamirim Creek never did reach Piracicaba River, moreover they were stationary in some parts of the river (Marques et al. in preparation).

Turtles were transferred to the laboratory in order to be sexed, measured, weighted and marked. Blood (2 mL) was collected by the external branch of the jugular using 2 mL syringes fitted with 23 gauge needles (as described by Rogers and Booth 2004). One milliliter of blood was taken for heavy metal analysis and the remainder kept for genetic analysis. Animals were then released at capture site.

Blood samples were immediately diluted in distilled water (1:10) with 0.1% Triton X-100 to ensure lysis of the red cells, and frozen. The frozen samples were then shipped to San Diego for analysis. Samples were thawed then further diluted 1:10 in a weak acid solution (10 mL of concentrated HNO₃ + 5 mL concentrated HCl diluted to 1,000 mL with double distilled ultra pure water) and placed into 12 mL polyethylene screwcap tubes. The samples were then run on an ICP-MS 7500 instrument (Agilent Technologies, San Jose, CA). Standard curves for Al, Cr, Mn, Ni, Cu, Mo, and Sn (0, 0.1, 1, 10, 100 ng g⁻¹) and As, Cd, and Se (0, 0.05, 0.5, 5, and 50 ng g⁻¹) were run along with duplicate samples, spiked blanks, and spiked samples. Spike recovery ranged from 95% (Ni) to 108% (Cu, Mo, and Sn). An internal standard of Bi, Ge, Li 6, Rh, Sc, and Tb was run at a concentration of 100 ng g⁻¹ to determine consistency of analysis. The samples were tested for Al, Cr, Mn, Ni, Cu, As, Se, Mo, Cd, and Sn. The spike solution contained Al, B, Ba, Ca, Co, Cr, Cs, Cu, Fe, K, La, Mg, Mn, Na, Ni, Pb, Sr, V, Zn, Sn, Mo, As, Be, Cd, Se, and Tl. The instrument was tuned using a solution of Ce, Co, Li, Mg, Tl and Y (10 µg/mL). All internal standards and spike solutions were made in a weak acid solution containing 1% HNO₃ and 0.5 % HCl. Samples of quality control tissues from the National Institute of Standards consisting of powdered bovine liver and muscle (dissolved in concentrated nitric acid prior to analysis) were run at the same time as the blood samples. The results of analysis of quality control samples were greater than 95% accurate for the elements selected.

The heavy metals levels were analyzed by a factorial Analysis of Variance (ANOVA) where sex, site and its interaction were considered as grouping factors. Differences among sites were evaluated by Tukey Test (Zar 1996).

Results and Discussion

A total of 37 turtles were caught in the study sites as follows: Monte Alegre (N = 4), Tanquan (N = 14) and Piracicamirim Creek (N = 19). Seventeen turtles were male, 17 were females, and three were immature and could not be sexed. Animals from all sites had similar body sizes (Table 1).

Table 1 *Phrynops geoffroanus* carapace length (CSL), body mass (BM), sex (M:F) and serum level of heavy metals (ng g⁻¹) in three sites at Piracicaba river basin, Southeastern São Paulo, Brazil (mean ± standard deviation, *p*-value for ANOVA)

	Monte Alegre	Tanquan	Piracicamirim	<i>p</i>
CSL (mm)	248.5 ± 47.6	280.8 ± 39.3	284.6 ± 29.6	>0.05
BM (g)	1,500 ± 956	1,962 ± 885	2,287 ± 753	>0.05
Sex (m:f)	3:1	6:8	9:10	
Al	89.2 ± 2.6	111.3 ± 67.1	93.8 ± 14.0	>0.05
As	47.5 ± 25.1 A	5.1 ± 2.6 B	8 ± 4.3 B	<0.001
Cd	4.2 ± 0.5	3.4 ± 0.9	3.7 ± 0.9	>0.05
Co	17 ± 0.5 A	17.6 ± 4.9 A	13.2 ± 0.8 B	0.001
Cr	10.1 ± 2 AB	7.5 ± 1 B	11 ± 3.1 A	0.004
Cu	2,194.6 ± 234.8	1,154.4 ± 522.4	1,391.9 ± 611.2	>0.05
Mn	44.5 ± 2.8	35.1 ± 8.5	42.9 ± 13.6	>0.05
Mo	6.4 ± 0.9	7.9 ± 3.1	6.0 ± 1.8	>0.05
Ni	60.5 ± 12.2	53.6 ± 29.0	55.8 ± 25.5	>0.05
Pb	1,150.5 ± 173.9 A	535.2 ± 301.5 B	569.2 ± 300.5 B	0.016
Se	1,003.7 ± 173.6 A	270.7 ± 166.2 B	296.6 ± 151.3 B	<0.001
Sn	6 ± 0.3	6 ± 0.8	5.7 ± 0.7	>0.05
Sr	93.3 ± 5.5	85.1 ± 50.0	165.5 ± 147.1	>0.05
Zn	2,183.8 ± 1,017.2	1,166.0 ± 541.5	1,499.7 ± 1,044.6	>0.05

Different letters mean difference among groups

There was no difference between males and females, or among sites, on the plasma level of the following elements: Al, Mn, Ni, Cu, Zn, Sr, Mo, and Cd. There was no interaction between sex and site for those elements either (ANOVA: $0.067 < p < 0.993$). In addition, there was no difference among sites on the level of Sn (ANOVA: $p = 0.939$), but it was higher in males ($6.1 \pm 0.7 \text{ ng g}^{-1}$, $N = 17$) than in females ($5.5 \pm 0.4 \text{ ng g}^{-1}$, $N = 17$; ANOVA: $p = 0.049$). The level of As, Se and Pb were higher in Monte Alegre than in the other two sites (ANOVA: $p < 0.016$). The level of Cr was higher in Piracicamirim Creek than in Tanquan, but it was intermediate (i.e., similar) in Monte Alegre. The level of Co was lower in Piracicamirim than in the other two sites. There was also a difference between males and females in the level of As and Se, but this pattern may be due to sampling error as there was just one female and three males from Monte Alegre where those levels were higher.

Although the animals sampled had a relatively large range of body size (min = 330 g, max = 4,000 g), we did not find any ontogenetic influence on the serum level of heavy metals in *Phrynops geoffroanus*. Tin (Sn) was the only element that significantly varied between males and females, being higher in the former. A possible reason for this pattern is that females might eliminate it by the eggs (Hopkins et al. 2004; Campbell et al. 2005; Xu et al. 2006). Although selenium was the only element transferred from the females to the eggs in freshwater turtles *Trachemys scripta* (Nagle et al. 2001), Sn and Cd level in eggs was positively correlated with element concentration in blood (Guirlet et al. 2008). In captive reared alligators inadvertently fed lead shot, lead levels in yolk of ovarian follicles were extremely high (Lance et al.

2006), so elimination of a portion of a heavy metal load via the eggs is possible in reptiles.

There is no available information about the possible sources of heavy metals in the study area. However, we can possibly speculate whether they come from point or non-point sources. Levels of Al, Cd, Cu, Mn, Mo, Ni, Sr and Zn were similar among sites what could be due to a non-point source pollution. On the other hand, As, Pb, Se, Cr, and Co level varied among sites what could be due to a point source pollution possibly related to even a small amount of illegal industrial sewage.

Only six of the 14 elements evaluated in this study had lower levels than previous records from other polluted areas. Al, Co and Cr in this study were an order of magnitude lower than the levels reported to *Nerodia fasciata* (banded water-snake), *N. taxispilota* (brown water-snake) and *Akistrodon piscivorus* (cotton-mouth) in South Carolina, USA (Burger et al. 2006, 2007). Chromium had similar levels to what has been reported to *N. sipedon* (Northern water-snake) from an area that has received regular discharges from a thermonuclear weapon plant during the 1960s in eastern Tennessee, USA (Burger et al. 2005; Campbell et al. 2005). Recently, Gagneten et al. (2008) have showed that eggs from crabs (*Zilchiopsis collastinensis*) maintained in water with higher levels of Cr had higher concentration of this heavy metal, which could affect reproduction, than those in less polluted water. We also found levels lower by an order of magnitude for Mn, Ni and Zn than what has been reported to water snakes in USA (Burger et al. 2005, 2006; Campbell et al. 2005) and nestling *Nycticorax nycticorax* (black-crowned night herons) in Chesapeake and Delaware Bays (Golden et al. 2003).

Trachemys scripta (red-eared slider) presented serum levels of Zn as high as 5,000 ng g⁻¹ (Clark et al. 2000).

We found similar levels of As, Cd and Se to what has been previously described for *N. nyctocorax* (Golden et al. 2003) and water snakes in USA (Campbell et al. 2005; Burger et al. 2005, 2006, 2007), but higher levels of Se than freshwater turtles in Texas (Clark et al. 2000). Surprisingly, the serum level of Cu and Pb found in this study are the highest ever described for any other reptile. Former records for Cu are 650 ng g⁻¹ for *Nerodia fasciata* (Hopkins et al. 2001) and the red-eared slider (Clark et al. 2000) in Texas. Former records for Pb in reptiles are a fifth of the levels found in Tanquan and Piracicamirim Creek (Burger et al. 2005, 2006; Campbell et al. 2005). Only waterfowl presented higher levels of serum Pb (2,200 ng g⁻¹ in Lake Couer d'Alene, ID, USA), according to Spears et al. (2007). Pb serum level above 1,000 ng g⁻¹ is considered to cause clinical poisoning in waterfowl (Pain 1996). No clinical symptoms have been detected in the present study, but we expect at least an increase in the metabolic rate for *Phrynops geoffroanus*, as described by Hopkins et al. (1999) for other reptiles.

Reproduction in *Phrynops geoffroanus* does not seem to be affected in this study area as we could find both, nests and hatchlings apparently normal. This could suggest that the species is very tolerant to heavy metals. However, there are no data available about the time scale of such contamination, which could be currently subclinical and yet lead to a breakdown in the population reproductive success in a few years, even within a single generation timeframe. Cadmium contamination affects gonadal development in freshwater turtles resulting, possibly, in disruption of reproduction on later stages (Kitana and Callard 2008). In addition, as the species feed basically on Chironomidae larvae in similar polluted habitats (Souza and Abe 2000), we can expect even higher levels for carnivore fish due to trophic bioconcentration (Hopkins et al. 2001; Burger et al. 2006). Unfortunately, no comparative data is available for Mo, Sr, and Sn.

Based on the present results, legal enforcement is urged in order to locate and extirpate heavy metals sources in Piracicaba River basin. In addition, contamination monitoring should be extended to fish and people, as commercial fishing still occurs in the area (Silvano and Begossi 2001). It should be mentioned that heavy metal pollution in rivers produces a rapid increase in the concentration of these metal in the underground aquifers (Mendoza et al. 2008), possibly affecting a greater area than that at or surrounding the river or lake itself.

Acknowledgments The *Phrynops geoffroanus* ecology project was sponsored by FAPESP Research Grant. (n. 2005/00210-9). The animals were captured under IBAMA license (proc. 02010.000005/05-61).

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