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Lead and cadmium accumulation in anuran amphibians of a permanent water body in arid Midwestern Argentina

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Abstract

Purpose Heavy metals have been detected in water and sediments from the Embalse La Florida, an artificial lake in the arid region of San Luis province, Argentina, representing one of the few sources of permanent water for reproduction of native anuran species. This study assesses lead (Pb) and cadmium (Cd) concentrations in the anuran species found in this water reservoir as well as differences between compounds, species and sites of collection.

Methods Adult anuran amphibians were collected on the north and south shores of the Embalse La Florida and Pb and Cd concentrations were measured in whole body homogenates digested using wet ashing techniques.

Results All individuals of the six species assayed had detectable levels of Pb and Cd that ranged from 1.19 to 5.57 $\mu\text{g/g}$ dry mass and from 1.09 to 6.86 $\mu\text{g/g}$ dry mass, respectively. Anuran amphibians collected in the more contaminated south shore accumulated 21% more Cd and 40% more Pb than individuals from the less altered north shore. Cd and Pb accumulation was not significantly correlated with the concentration in water at the site of collection.

Conclusions Amphibians of the Embalse La Florida accumulate Cd and Pb. Between and within species, differences were detected in Cd and Pb concentrations. Differences in metal concentrations between species, metals, and individuals collected on shores of the Embalse La Florida with different contamination, were detected. Therefore, it is crucial to implement adequate policies to protect amphibians from the accelerated urban development experienced in this location.

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1 Introduction

Heavy metals are stable and persistent contaminants released into the environment by mining industries, motorcar exhaust fumes, fertilizers, and other human activities and by-products (Cid and Caviedes-Vidal 2010; Järup 2003; Norton 2007). Heavy metals tend to accumulate in soils and sediments because they cannot be degraded or destroyed, resulting in the striking environmental pollution that has been a matter of growing concern over the last decades (Loumbourdis et al. 2007). The hazardous effects on vertebrates include teratogenesis and anomalies in reproduction (Loumbourdis and Wray 1998). Availability of chemical species, delivery route, and delivery rate of individual metals are influenced by many

factors (Bochenek et al. 2008; John and Leventhal 1995; Peijnenburg and Vijver 2007) and play crucial roles in their uptake by an organism (Linder and Grillitsch 2000). In aquatic environments, Cd is generally adsorbed to organic materials and easily accumulated by organisms. Pb on the other hand, is transferred into solid phases and has lower transportation capacity and low solubility (Bochenek et al. 2008; Dong et al. 2007).

Some studies have shown that amphibians accumulate Pb and Cd proportionally to concentrations in their surrounding environment (Berzins and Bundy 2002; Birdsall et al. 1986) and are adversely affected by these heavy metals either in natural or experimental exposures. Bullfrog tadpoles inhabiting a coal ash-polluted habitat had high body burdens of several trace elements (Rowe et al. 1996), impaired swimming performance and predator avoidance (Raimondo et al. 1998), and altered oral morphology (Rowe et al. 1996). Heavy metals (cadmium (Cd), zinc, and lead (Pb) and contaminated soil) exerted harmful effects on growth, development, survival to metamorphosis, and predator avoidance behavior of *Rana luteiventris* tadpoles, exposed in outdoor artificial mini-ecosystems (Lefcort et al. 1998). Exposure to Pb and Cd increased mortality, malformations, and behavioral disorders in *Bufo arenarum* embryos (Herkovits et al. 1997; Pérez-Coll and Herkovits 1990). Survival and metamorphosis decreased in American toad (*Bufo americanus*)

tadpoles exposed to Cd from shortly after hatching through metamorphosis (James and Little 2003). This effect of delayed or incomplete metamorphosis was also observed in *Xenopus laevis* tadpoles (Sharma and Patiño 2009). Other effects documented for Pb in amphibians are skeletal malformations (Sparling et al. 2006) and reproductive toxicity (Wang and Jia 2009).

Dry environments challenge amphibians and therefore natural or artificial water reservoirs in these regions are important to the ecology of these species (Jofré et al. 2010). The Embalse La Florida is an artificial lake, built 60 years ago, situated within a mountain system that divides the San Luis province (Argentina) longitudinally into a dry region to the west, and more humid regions to the east (Fig. 1). This water reservoir is an important site for amphibians, because it is one of the few sources of permanent water available for reproduction and early development of eight anuran species described to date in this semi-desert area of the Argentinean Midwest (Jofré et al. 2010). The majority of the anuran species from Embalse La Florida are categorized as not threatened and with stable population trends (Lavilla 2008). Only *Melanophryniscus stelzneri* could be considered to be of conservation concern since it has been categorized as vulnerable with conservation threatened by extractive practices (Lavilla 2008). Conservation statuses of these species are referred to Argentina in general.

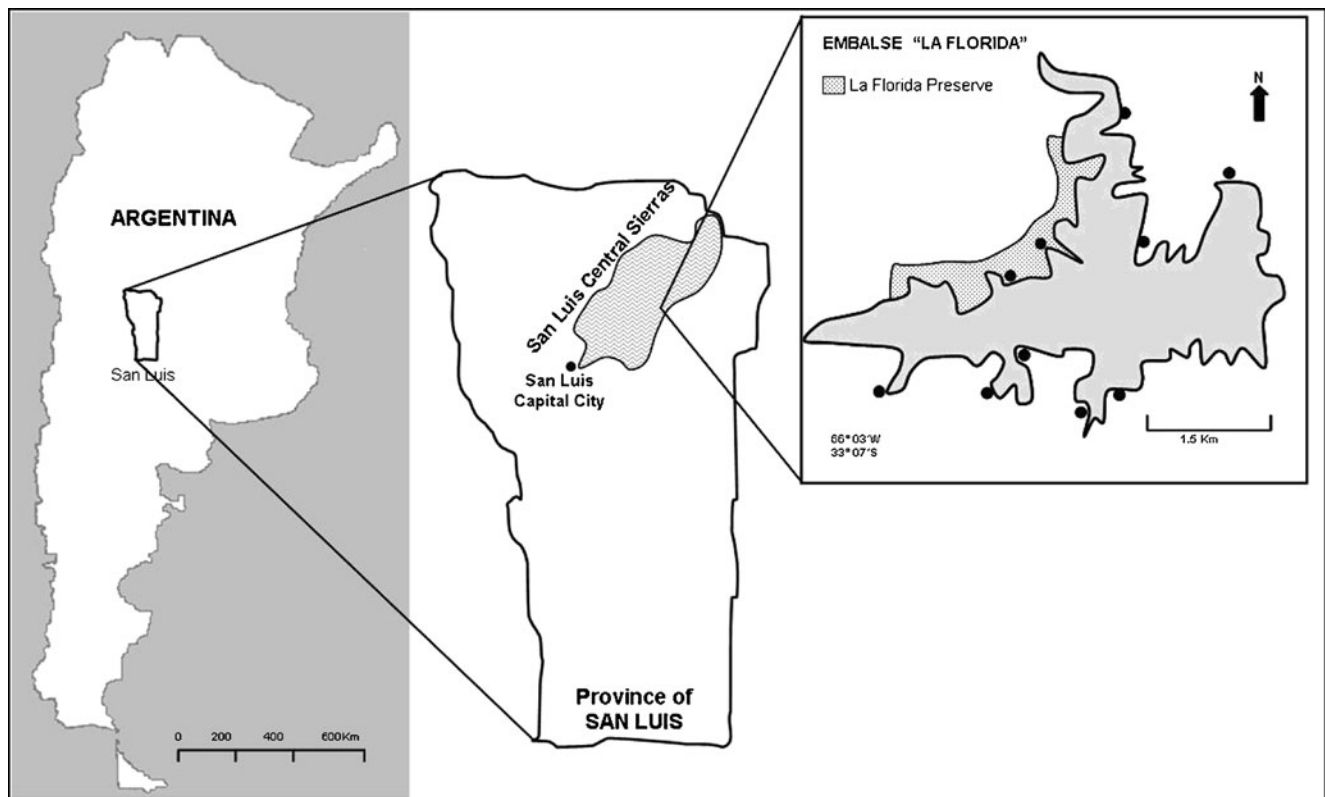


Fig. 1 Location of the Embalse La Florida water reservoir (San Luis, Argentina). *Black dots* indicate collection sites

To our knowledge, no amphibian species preservation studies are available to date in the Midwest of Argentina.

Cd and Pb have been previously detected in water (ranging between not detected to $6.25 \mu\text{g L}^{-1}$ and between 0.25 and $386.40 \mu\text{g L}^{-1}$, respectively), sediments, and fauna samples from Embalse La Florida (Antón et al. 2003; Cid et al. 2009, 2011). Observed metal contamination along the length of the water reservoir is not constant (Table 1), the north shore exhibits low metal contamination and best water quality and conversely, the south shore, is more contaminated (Antón et al. 2003; Cid et al. 2011).

Therefore, given the significant role of the Embalse La Florida for amphibian ecology and its reported metal contamination, we conducted this study to assess Pb and Cd concentrations in the anuran species found around this water reservoir. We tested three predictions. First, adult amphibians from La Florida accumulate Pb and Cd and body burdens are different between compounds (Cd and Pb) and anuran species. Second, individuals collected in the north shore have lower burden concentrations than individuals from the more contaminated south shore. Third, accumulation of heavy metals is directly proportional to respective concentrations found in water.

2 Materials and methods

The Embalse La Florida ($33^{\circ}07'S$, $66^{\circ}02'W$; 1,030 m.a.s.l.) is located 46 km Northeast of San Luis city, its surface area covers about 6.52 km^2 and has two tributary rivers: Río Grande and Río Trapiche. The Embalse La Florida is connected, through the Río Grande, to a small (1.07 km^2 of surface area) recently constructed (2001) permanent reservoir, the Embalse Antonio Esteban Agüero located 8 km away from La Florida, higher up in the sierras. The spillway of the Embalse La Florida originates the Río Quinto river, which connects it downstream with the reservoir Embalse Paso de las Carretas (7.56 km^2 of surface area), located 26 km away. A few other not connected permanent reservoirs are located more than 20 km away from Embalse La Florida. This water body is located in a region where water

Table 1 Concentrations of cadmium and lead (mean \pm 1 standard error) detected at two depths in water from Embalse La Florida during the summer season (Antón et al. 2003)

Metal	Depth (cm)	Heavy metal concentration in water ($\mu\text{g/L}$)	
		North shore	South shore
Cadmium	30	0.139 \pm 0.043	0.424 \pm 0.235
	500	0.216 \pm 0.123	1.216 \pm 1.590
Lead	30	18.837 \pm 4.957	69.110 \pm 27.077
	500	25.875 \pm 3.429	90.450 \pm 64,106

availability fluctuates throughout the year and rains, concentrated in summer, increase water availability, and generate flooding and water runoff that discharges into the lake. In addition, population around the lake is dramatically increased during summer due to vacation tourism. Both these factors boost the introduction of contaminants, organic matter, suspended solids, and wastes from upstream human activities to the water body during summer (Cid et al. 2011). In addition to higher concentrations of metals in water samples from the south shore of this reservoir (Antón et al. 2003), the highest levels of physicochemical parameters indicating organic/anthropogenic pollution (ammonium, nitrate, nitrite, phosphate, sulfide, viable anaerobic bacteria, total coliform, COD, and BOD) were also found in the bays of the south shoreline, heavily exploited for recreational activities (i.e. fishing, boating, and swimming) during spring–summer, due to the presence of recreational areas and small water flow because of the orographic configuration of the coast (Cid et al. 2011). The water from areas in the south shore can be also considered as polluted because some of the quality parameters such as Pb, ammonium, and total coliforms, have values higher than the maximum admitted values in drinking water (Cid et al. 2011). The less organic polluted areas were located in the central zone of the reservoir and in the north shore, where a State Natural Preserve and the Río Grande river inflow, with low human presence, are located. (Cid et al. 2011). Consequently, collection sites located on the south shore of the reservoir were selected because they represent a potential high exposure to contaminants and poor water quality to amphibians, and those situated at the north shore, would exemplify a low contaminant exposure and better water quality.

Adult anuran amphibians ($n=16$) were collected at sites on the north and south shores of the reservoir (Fig. 1) on three sampling nights between October and December of 2001 and two sampling nights between November and December 2002. All locations were chosen because they were thought to have habitat structure suitable for amphibian breeding. Individuals from six native species were trapped: *Rhinella arenarum* ($n=6$), *Leptodactylus mystacinus* ($n=4$), *Hypsiboas cordobae* ($n=2$), *Odontophrynus occidentalis* ($n=2$), *M. stelzneri* ($n=1$), and *Pleurodema tucumanum* ($n=1$) using nets or directly by hand on the shores, on streams that flows into of the embalse, and in nearby temporary ponds. Individuals were identified to species and transported to the laboratory where weight and morphometrics measurements were recorded.

Processing of samples, extraction and quantification of Cd and Pb were performed following the analytical procedure applied by Cid et al. (2009). Wet samples were weighed and then dried at $90\text{--}100^{\circ}\text{C}$ for about 24 h, until constant mass. Dry samples were then digested in a 2:1 (v/v) mixture of nitric (Merck, Ultrapur) and perchloric acid

(Merck, Suprapur). Each (about 0.1 g) was placed in a digestion tube and treated with 2 ml of concentrated nitric acid. Next, it was heated progressively at 150°C for 1 h, after which 1 ml of perchloric acid was added, and it was heated at 200°C. Lastly, we added 1 ml of oxygenated water (Merck, Ultrapur). The digestion was finalized when no fumes were observed and the mixture was pale and without sediments. After decomposition, the solution was transferred quantitatively to a 5-ml volumetric flask. Method 200.9 revision 1.2 4/91 (determinations of trace elements by stabilized temperature graphite furnace atomic absorption spectrometry) was used to determine Cd and Pb in samples in a Perkin-Elmer graphite furnace atomic absorption spectrometry model AAnalyst 200 (GFAAS-GF 900). Detection limit was approximately 0.01 µg metal/g dry weight. The concentrations of the heavy metals tested in whole body tissue were expressed in micrograms per gram of dry mass. Validation was carried out on a synthetic sample (cow liver homogenate) with the addition of Cd and Pb standard solutions traceable to standard reference material from National Institute of Standards and Technology: stock standard solution of Cd Merck of 1,003±2 mg/l, lot OC 105824, Cd (NO₃)₂ in HNO₃ 0.5 mol/l; stock standard solution of Pb Merck of 1,000±2 mg/l, lot OC186400, Pb (NO₃)₂ in HNO₃ 0.5 mol/l. Blanks, reference material, and spiked samples were always run with samples. Percent recoveries of Cd and Pb in reference material ranged from 98.0% to 99.5%. Spike recoveries accepted ranged from 95% to 105%.

Values of metal concentrations are expressed as the mean±1 standard error of the mean. One-way ANCOVA was used to test for differences between body concentrations of Cd and Pb for all anurans analyzed, including body mass as a covariate. Log-transformed values were used to meet ANOVA requirements. The same test was used to assess differences between compounds within species for *R. arenarum* ($n=6$) and *L. mystacinus* ($n=4$), the only two species with enough data points to perform statistics. The other four species lacked sufficient data ($n\leq 2$) to be included in a by species comparison. Body levels of Cd and Pb were compared between *R. arenarum* and *L. mystacinus* using an independent samples t test. The same analysis was performed to compare concentrations of Cd and Pb in whole bodies between shores (north and south) for all individuals assayed and between individuals of *Rhinella arenarum* collected in the north ($n=3$) and south ($n=3$) shores. Correlations between metal concentrations in whole bodies and concentrations of Cd and Pb in water at the location of collection were performed by Pearson correlation test. Data of Cd and Pb concentrations in water were from water samples collected on January 2000 for colleagues of our laboratory studying metal contamination on eight sub-zones of Embalse La Florida, each one having four or five sampling sites (Antón et al. 2003; Cid et al. 2011).

3 Results

All anuran amphibians exhibited detectable concentrations of Cd and Pb (Table 2). Differences between Cd and Pb (micrograms per gram) on a dry mass basis were not significant when testing all individuals together ($n=16$; $F_{1, 29}=0.009$; $p=0.924$); body mass (range, 6.88–100 g) was not a significant covariant in this analysis ($F_{1, 29}=1.301$; $p=0.263$).

Significant differences between Cd and Pb concentrations were observed for individuals of *R. arenarum* ($F_{1, 10}=5.343$; $p<0.05$) but not for *L. mystacinus* ($F_{1, 6}=3.424$; $p=0.114$) (Table 2). In both species, body mass was not a significant covariate in the comparison between metals ($F_{1, 9}=0.628$; $p=0.449$ for *R. arenarum*; $F_{1, 5}=0.827$; $p=0.405$ for *L. mystacinus*).

The pattern of Cd and Pb accumulation was different between *R. arenarum* and *L. mystacinus*. *R. arenarum* accumulated significantly higher amounts of Pb than Cd ($F_{1, 9}=5.343$; $p<0.05$). *L. mystacinus* show a tendency to accumulate more Cd than Pb, although the difference between metals was not significant ($F_{1, 5}=3.424$; $p=0.114$). Differences in accumulation of Cd between both species were not significant ($t=-1.058$; $p=0.326$), but individuals of *R. arenarum* accumulated significantly higher amounts of Pb than *L. mystacinus* ($t=4.145$; $p<0.05$) (Table 2).

Anuran amphibians collected in the south shore grouped all together exhibited a tendency (not statistically significant) for higher concentrations of Cd and Pb (21% and 40%, respectively) in their body tissues ($t=-1.049$; $p=0.314$ for Cd; $t=-1.530$; $p=0.174$ for Pb) (Table 2). Similarly, no differences between shores were observed for Cd in *R. arenarum* whole bodies ($t=-1.103$; $p=0.340$) (Table 2). Nevertheless, differences in Pb accumulation were significantly different between shores, higher for *R. arenarum* collected in the south shore compared with individuals captured in the north of the Embalse La Florida water reservoir ($t=-3.661$; $p<0.05$) (Table 2).

Cd and Pb accumulation in whole bodies of anuran amphibians from La Florida was not significantly correlated with the concentration in water of the reservoir at the location where they were collected (Pearson correlation coefficient (r)=0.328; $p=0.213$ for Cd; $r=-0.081$; $p=0.766$ for Pb) (Fig. 2).

4 Discussion

4.1 Heavy metals in amphibians: concentrations and possible effects

All the individuals ($n=16$) of the anuran amphibian species assayed (*R. arenarum*, *H. cordobae*, *L. mystacinus*, *M.*

Table 2 Body masses (mean±1 SEM) and whole body metal concentration (mean±1 SEM and range) of amphibians in the study

Species	Common name	Number	Body mass (g)	Cadmium ($\mu\text{g g}^{-1}$ dry mass)	Lead ($\mu\text{g g}^{-1}$ dry mass)
<i>Rhinella arenarum</i>	Common toad	6	54±11.28	1.82±0.49 ^a	3.13±0.31 ^a A
				3.18±1.36 ^b	5.08±0.60 ^b B
				2.50±0.67 ^c a	4.11±0.54 ^c b, a
				1.09–5.26	2.65–5.57
<i>Leptodactylus mystacinus</i>	Moustached frog	4	12.7±1.1	3.56±1.28	1.88±0.29 b
				2.15–6.86	1.42–2.51
<i>Hypsiboas cordobae</i>	Cordoba tree frog	2	8.1±1.7	3.45±1.11	3.29±1.83
				2.66–4.23	2.00–4.59
<i>Odontophrynus occidentalis</i>	Lesser escuerzo	2	21.7±13.9	3.88±0.66	1.65±0.64
				3.42–4.35	1.19–2.1
<i>Melanophryniscus stelzneri</i>	Redbelly toad	1	1.46	2.06	1.78
<i>Pleurodema tucumanum</i>	Spotted-flanks four-eyed frog	1	2.83	1.21	2.04
Mean concentration for all individuals studied		16		2.73±0.57 ^a	2.29±0.23 ^a
				3.31±1.32 ^b	3.84±0.82 ^b
				2.94±0.36 ^c	2.86±0.37 ^c

Different uppercase letter (A, B), different lowercase letter (a, b) or different italic bold lowercase letter (**a**, **b**) indicate significant differences between metal concentrations by ANOVA or by independent samples *t* test ($p < 0.05$). Body samples dried at 90–100°C

^a Individuals collected in the north shore of Embalse La Florida

^b Individuals collected in the south shore of Embalse La Florida

^c All individuals

stelzneri, *O. occidentalis*, and *P. tucumanum*) had detectable concentrations of Pb and Cd; therefore, the first part of the first prediction of this study was supported. To date, most of the studies about Pb and Cd poisoning in anuran amphibians in Argentina are focused on the evaluation of their effects in

laboratory exposure experiments (Chiesa et al. 2006; Fink and Salibián 2005; Fridman et al. 2004; Natale et al. 2006). We are aware of only one other study that reported concentrations of heavy metals in wild captured anuran amphibians from Argentina. In that work Arrieta et al. (2001) found between 1.99 and 4.66 mg Pb /dl of blood in *R. arenarum* (*B. arenarum*) adults (body mass of 138.6±2.4 g) collected in the surroundings of La Plata city, Buenos Aires. No comparison can be established between these data and ours because a relationship has not been established between blood and whole body concentrations for Cd and Pb in anuran amphibian species studied. Few studies report heavy metal concentrations in other groups of animals from Argentina. Whole body metal concentrations in amphibians were lower than concentrations detected in organs of bird species from the same ecosystem (Cid et al. 2009). Amphibians from La Florida had Cd and Pb concentrations similar to those detected in invertebrates of the coastal Patagonia in the south of Argentina (Pb, 1.64 to 13.20 $\mu\text{g/g}$ dry weight and Cd, 1.12 to 6.64 $\mu\text{g/g}$ dry weight) and Cd concentrations below those detected in the zooplankton of Bahía Blanca estuary, a coastal plain located on the southeastern coast of Buenos Aires province (Fernández Severini et al. 2009; Gil et al. 2006). When contrasted to mammals and birds from Patagonia (Pb, 0.12 to 5.5 $\mu\text{g/g}$ and Cd, 0.04 to 491 $\mu\text{g/g}$), La Florida anurans had similar concentrations of Pb and lower concentrations of Cd (Gil et al. 2006).

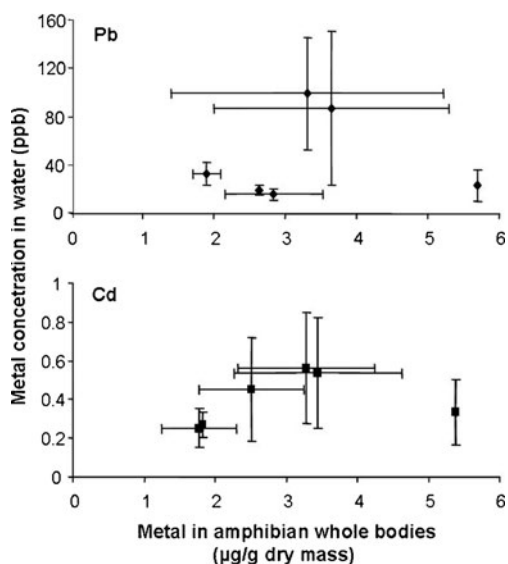


Fig. 2 Relationship between concentrations of metals in amphibian whole bodies (samples dried at 90–100°C) and concentrations in water of the Embalse La Florida water reservoir. Vertical and horizontal error bars are standard errors of metal concentrations in amphibians (x-axis) and in water (y-axis)

Cd concentrations in water samples of the Embalse La Florida reservoir are below those reported to cause significant effects (on survival, eclosion, growth, or development) on amphibians in laboratory studies (Gross et al. 2007; Herkovits et al. 1997; Lefcort et al. 1998; Loumbourdis et al. 1999; Sharma and Patiño 2009). The exposure of *R. arenarum* embryos to waterborne Cd concentrations between 0.5 and 4 mg/l resulted in mortality and retarded development (Perez Coll and Herkovits 1996). While it is unknown at which whole body concentration Cd becomes lethal to amphibians, it has been suggested that concentrations over 5 µg/g Cd wet weight in any vertebrate are life threatening (Eisler 1985).

Concentrations of Pb detected in water of the Embalse La Florida reservoir are also below those related to lethal effects on amphibians reported from laboratory experiments (Horne and Dunson 1995; Pérez-Coll et al. 1998). However, these concentrations are near or above concentrations reported to produce sublethal effects in laboratory exposures (Mouchet et al. 2007; Zhang et al. 2007). Lethal concentrations (LC₅₀) between 470 and 950 µg/L of Pb were estimated for different larval stages of *R. arenarum* (Pérez-Coll et al. 1998) and for adult individuals the acute 120-h lethal dose (LD₅₀) of injected Pb was 89.2 mg/100 g of body weight (Arrieta et al. 2000). The effects of Pb poisoning in wildlife are associated with organ tissue concentrations over 10 µg/g (Scheuhammer 1991). Berzins and Bundy (2002) reported reduced weight and delayed development in *X. laevis* tadpoles containing body concentrations of Pb of 3 µg/g. Delta-aminolevulinic acid de-hydratase inhibition, free erythrocyte protoporphyrin increase, and reduced hematocrit were observed in adult *R. arenarum* injected weekly with 50 mg/kg of body that accumulated over 1,158 µg/g of Pb in organ tissues (Arrieta et al. 2004).

Metal concentrations for all anuran amphibians collected in La Florida reservoir were similar to accumulation values detected in amphibians from other moderately contaminated or reference sites (Loumbourdis and Wray 1998; Roe et al. 2005) and lower than concentrations found in amphibians from contaminated wetlands (Snodgrass et al. 2003).

Even though concentrations of Cd and Pb in water and amphibians of the Embalse La Florida are below those reported as harmful, we cannot discard possible synergistic effects due to the exposure to mixtures of heavy metals (Cd and Pb) or metals and organochlorine pesticides, also detected in amphibian from this ecosystem (Jofré et al. 2008).

4.2 Comparison between metals and species

The second part (differences between metals and species) of the first prediction was partially supported. Average body burdens of Cd and Pb were not different when all the individuals from the different species assayed were

compared. However, when heavy metals concentrations were analyzed within species, *R. arenarum* showed higher amounts of Pb than Cd and higher burdens of Pb than *L. mystacinus* as well. A variety of factors determines the accumulation of any contaminant into an organism, including diet, habitat use, duration of exposure, and individual physiological traits (Freda 1991; Sparling and Lowe 1996). *R. arenarum* has long reproductive cycles, in some regions breeding opportunistically almost year-round and has been registered vocalizing in La Florida for longer periods than *L. mystacinus* (Jofré et al. 2010; Leynaud et al. 2006; Vaira 2002). The first species, therefore, might be more exposed to metal contamination either from the water or food. Another possible explanation is that while *R. arenarum* lays eggs in coiled strings on shallow waters (Sanabria et al. 2007) where they are directly exposed to contaminants, *L. mystacinus* embryos and larvae are less exposed to water contaminants, because it oviposits mainly in dry lands into subterranean foam nests, where tadpoles are able to develop without water (Condez et al. 2009; De-Carvalho et al. 2008).

Bioavailability of individual metals plays a crucial role in their uptake by an organism (Linder and Grillitsch 2000). Heavy metals, that have latent harmful effects while they are bound to bottom sediments in aquatic environments, become available to organisms when they are released to soluble fractions as a result of changes on physical, chemical or biological conditions (Bochenek et al. 2008). Many factors, that include the total concentration and speciation of metals, mineralogy, ionic composition, pH, redox potential, temperature, organic matter content, and water flow rate will determine the chemical species available (e.g., as free metal ion or complex by organic or inorganic ligands), delivery route, and delivery rate to biological receptors (Bochenek et al. 2008; John and Leventhal 1995; Peijnenburg and Vijver 2007).

Both Cd and Pb are bound to sediments in lake and fluvial environments and may be mobilized again to water when chemical and physical changes occur (Schintu et al. 1991). Adsorptive and mobilization properties differ between both metals, thus differences in availability to organisms are expected. Cd is mainly present as exchangeable reducible forms that are easily mobilized and mainly adsorbed to organic materials that facilitate their accumulation in aquatic organisms. Pb on the other hand, is easier to transfer into solid phases from water and has lower transportation capacity; moreover, inorganic Pb compounds are commonly abundant in sediment, but have low solubilities in natural water where they are mainly adsorbed to metal oxides (Bochenek et al. 2008; Dong et al. 2007). In a river polluted by base-metal mining, Cd was the most mobile and potentially bioavailable metal (Prusty et al. 1994). These differences in adsorption and mobilization between Cd and Pb may explain the observed accumulation of Cd in anurans from La Florida, despite its low concentration in water.

4.3 Bioaccumulation

Our second prediction, regarding differences in metal concentrations between individuals collected on shores with dissimilar metal contamination in water, was not supported. We fail to find differences on metal concentrations between all anuran individuals grouped by coasts collection site. Potential explanations are that differences are masked by interspecific differences (e.g., body size, microhabitat, or dietary habits). Individuals of *R. arenarum* collected in the south shore of the Embalse La Florida showed amounts of Pb in their body 1.6 times higher than those collected in the north coast, matching Pb concentrations reported for water and sediments. Therefore, our second prediction received support at the intraspecific level. Studies including more individuals of each species are needed in order to prove the same pattern visualized for *R. arenarum* in the other anuran amphibians dwelling in La Florida.

When we related metal body burdens (for all individuals assayed) to concentration in water of the reservoir at the location where each anuran was collected, no apparent relationship was found, therefore our third prediction was not supported either. A possible reason for the absence of relationship is that amphibians accumulate body burdens of metals than enter not only through absorption across their semipermeable skin but also through their diet. (Burger and Snodgrass 2001). However, concentrations of organochlorine pesticides in the same amphibians were significantly correlated to the level of each compound in water (Jofré et al. 2008). It was not possible to perform a within species relationship between metal concentration in water and body tissues, due to insufficient number of individuals of each species for most collection points.

If we estimate bioaccumulation factors (BAF=concentration of metal in amphibian whole body/concentration of metal in water (Ivanciuc et al. 2006)), using our amphibian whole body concentrations and Cd and Pb concentrations detected in water samples collected on January 2000 on eight sub-zones of Embalse La Florida (Antón et al. 2003; Cid et al. 2011), BAF of Cd (7.11 ± 3.65) are higher than BAF of Pb (0.09 ± 0.07). The ability to accumulate Cd, despite its very low concentration in the surrounding water, may be an indication of intermittent exposure to various contaminants and of the continuous accumulation of heavy metals (Vogiatzis and Loumbourdis 1998). The complex life cycles of many amphibians may make them important for transferring contaminants from aquatic to terrestrial food chains; amphibian larvae that accumulate high concentrations of elements may transfer these elements within aquatic communities and also, as metamorphs, to terrestrial communities and uncontaminated terrestrial food webs (Snodgrass et al. 2003; Roe et al. 2005; Urine et al. 2007)

5 Conclusions

Amphibians that inhabit shallow waters near or at the shores of the Embalse La Florida accumulate Cd and Pb obtained from their surrounding environment. Some differences in metal concentrations between species, metals, and individuals collected on shores of the embalse with different impact of contamination, were detected. More studies, tending to answer the real effect of contaminants at a population level are needed in order to adequately protect amphibians in this ecosystem that is experiencing an accelerated urban growth and human impact and holds the highest richness of anuran amphibians detected in the semiarid region of San Luis (Jofré et al. 2010).

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