Heavy metal concentration in Neotropical Aquatic Snakes (Helicops pastazae) and its potential as a bioindicator of water pollution

María Jose Hurtado-Morales (mj.hurtado10@uniandes.edu.co)
Universidad de Los Andes Facultad de Ingenieria https://orcid.org/0000-0001-7472-3509

Manuel Rodríguez
Universidad de Los Andes

Adolfo Amezquita
Universidad de Los Andes

Research Article

Keywords: Heavy metals, bioaccumulation, tissues, biological indicator, water quality, Helicops pastazae

Posted Date: March 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-291191/v1

Abstract

The present study aimed at testing the potential role of the aquatic snake *Helicops pastazae* as indicators of water pollution by heavy metals. In particular, we tested whether the total heavy metal concentration is related to (1) the distance and position (upstream vs downstream) of the sampling point with the discharge of wastewater; (2) the taxonomic group studied and its place in the trophic chain: piscivorous snakes vs characid fish that co-occur with them; and (3) the organ or tissue examined: snake liver versus muscle. We quantified cadmium (Cd), chromium (Cr) and lead (Pb) by using atomic absorption spectrophotometry with electrothermal atomization. Significant differences were found between some of the sampling points, particularly high metal concentrations were detected upstream on point 1, but no clear spatial pattern was found. There were no significant differences in the concentration of any metal between fish and snake muscle, suggesting potential mechanisms of metal excretion in snakes. With regard to interactions, the snake liver had the highest concentrations of cadmium and the muscle of lead and chromium, which may indicate tissue affinity differences for certain metals. Altogether, our results indicate that *H. pastazae* differentially accumulates contaminants, depending on the tissue and location, which render them useful bioindicators of water contamination.

Introduction

The discharge of domestic or industrial wastewater has severe impacts on communities of aquatic organisms, leading to a decrease in water quality, and potentially affecting public health, fauna and flora. One of the main environmental problems related to aquatic ecosystems is pollution by heavy metals, due to point discharges or diffuse sources to bodies of water, mineralization of rocks, or mining activities upstream of a water system (Macías 2015).

Heavy metals are natural elements of the earth's crust, which in trace quantities play a positive role on the life of organisms; in very high concentrations, however, they are usually toxic. Because of their high toxicity and difficult transformation, they undergo bioaccumulation processes in aquatic organisms, which negatively affects physiological performance and ecological interactions (Zorrilla 2011). Initially they can be assimilated by phytoplankton and filter organisms and subsequently incorporated into the trophic chain, increasing their concentration as they are ingested by higher trophic organisms (Márquez et al. 2008). Due to their chemical stability, living beings cannot metabolize heavy metals, which further explains their bioaccumulation until reaching high levels of toxicity. Additionally they can be efficiently absorbed through biological membranes due to their high chemical affinity with the sulfhydryl group (-SH) present in proteins (Mancera-Rodríguez and Álvarez-León 2006).

The heavy metals of higher ecotoxicological importance in aquatic environments are mercury, arsenic, chromium, lead, cadmium, nickel and zinc; their ionic forms enter the cell through metal cation transport systems. Their toxicity and uptake depend on concentration, the exposure time and various environmental factors (Zorrilla 2011). One of the main causes of its chemical toxicity is based on its ability to bind with organic molecules, based on reactions with specific ligands, namely sulfhydryl groups, and amino, phosphate, carboxyl and hydroxyl radicals. The resulting organometallic complexes may inhibit protein structural changes, and thereby affect the transport of essential elements and the metabolism of reactive oxygen species or of free radicals related to oxidative stress, among others (Zorrilla 2011).

Snakes may serve as biological indicators of environmental pollution, given their position as primary or secondary predators, their restricted migration, and their long life cycle (Heinz et al. 1980; Campbell and Campbell 2001; Burger et al. 2005, 2007, 2017; Albrecht et al. 2007). Still, snakes are not commonly used in environmental pollution studies, despite the potential value of aquatic snakes as bioindicators (Burger 2005, 2007; Campbell and Campbell 2001, 2005; Heydari and Riyahi 2015). *Helicops pastazae* (Colubridae) is distributed from Colombia to Argentina (Uetz et al. 2019). They have semi-aquatic habits, as evidenced in adaptations such as nostrils and eyes in dorsal position and keeled scales (Segall et al. 2016). Snakes in this genus are considered to be top predators of the food chain and feed on fish, anurans, lizards and other snakes. Although the specific diet of *H. pastazae* is poorly known, it is thought to feed on fish and in any case an opportunistic predator (Almendáriz et al. 2017).

To assess its potential as bioindicator, the present study aimed at estimating the concentration of heavy metals in different tissues of *H. pastazae*, and to compare the bioaccumulation in this predator and the fish species it potentially feeds on. The sampling site covered a section of the Batá river (eastern Colombian Andes), which receives residual water from the sewage system of the municipality of Santa María, through the streams of La Argentina, El Toro and Caño Cangrejo; there is no liquid waste treatment and the networks are in poor condition (Corpochivor 2003). Upstream this section, the river receives water from the Chivor reservoir, which probably concentrates the contamination by sediments, residues and dissolved nutrients (Corporación Autónoma Regional de Cundinamarca et al. 2006). Given that the Batá river has a varied fauna composition, that there are no studies of heavy metals in this area, and that snakes have potential as bioindicators, a study of total quantification of cadmium, lead and chromium concentrations was carried out in liver and muscle of the snakes of the *Helicops pastazae* species, and in fish muscle.

The following hypotheses were tested: 1) The total heavy metal concentration depends on the distance and position (upstream or downstream) of the sampling point with the discharge of wastewater; it is expected to be greater downstream of it. 2) The total heavy metal concentration depends on the taxonomic group studied and its place in the trophic chain: it will be higher in the piscivorous snakes than in fish. And 3) it also depends on the organ or tissue examined: presumably higher in the liver compared to the muscle.

Methods

Study site

Four sampling points were selected, two upstream of the wastewater discharge (known as Caño Cangrejo), and two downstream the municipality of Santa María (Fig. 1 and Fig. 2). The sampling was carried out in the months of January and February.

The procedures for euthanasia and manipulation of individuals were approved on October 18, 2018 by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) by analyzing the animal use format COR_C.FUA_18 – 016.
Individuals were collected under the permit: "Permiso Marco de Recolección de Especímenes de Especies Silvestres de la Diversidad Biológica con Fines de Investigación Científica No Comercial", certified under the research project PR.6.2018.4967 "Integración de rasgos funcionales y moleculares en estudios evolutivos de comportamiento y fisiología", with mobilization permit P04967S3591_N0004.

Figure 1 Location of the study site on the map of Colombia. The department of Boyacá and the location of the municipality of Santa María within this department, where the sampling was carried out, are presented in detail

Figure 2 Sampling design, number of snakes captured and detailed map of the sampling points in the Bata river, located in the municipality of Santa María: Point 1 (4º51'41"N, 73º16'03"W), point 2 (4º51'32"N, 73º15'57"W), point 3 (4º51'19"N, 73º15'57"W) and point 4 (4º51'04"N, 73º16'03"W)

Snakes were found and collected under rocks along the rivers' edge, during the day. Most individuals were adult (length greater than 30 cm), but juveniles were also collected where finding adults became difficult. Individuals were euthanized by intracardiac injection of 3–5 mL of 2% xylocaine anesthetic for adults, and a 1–2 mL dose for juveniles. This euthanasia process is approved by the AVMA (American Veterinary Medical Association) and guarantees a non-traumatic death. Subsequently, tissues were extracted and later preserved in a refrigerator with ice until arrival to the laboratory, always within 48 h.

An unidentified fish species was abundant in the study area, belonging to the family Characidae; because of their size and distribution along the river edges, they are probably part of the snake diet. They were collected during the day by a net on the banks of the river, and euthanasia was performed by freezing, to be preserved until they reached the laboratory.

We chose to sample three metals of ecotoxicological importance (Henze et al. 2002): chromium, lead and cadmium. The geological baseline of the municipality of Santa María reveals the presence of clays, sandstones, gypsum, limolites, among others, which generate mostly iron and aluminum derivatives (Corpochivor 1996).

Sample preparation and laboratory procedures

Sample preparation and digestion was carried out using a modification of EPA method 3052 (EPA 1996), adjusted on the basis of preliminary tests. Each individual was washed with deionized water to remove dirt and external contamination on the skin (Burger 1992). To obtain the sample of the snakes, muscle and liver tissue were extracted from the individual doing a 2 cm longitudinal incision made ventrally in the middle of the body, using a ceramic scalpel and stainless-steel tweezers to avoid traces of metals and pollution (FAO/SIDA 1983). In the case of fish, muscle tissue was removed by making an incision above the lateral line, between the dorsal and caudal fins (Queensland Government 2018). The material was stored in plastic containers previously washed and sterilized with 5% nitric acid and deionized water; subsequently it was refrigerated at -17°C until digestion and respective analysis (FAO/SIDA 1983).

We proceeded to weigh each of the samples in the vials to carry out the digestion, and to later estimate the concentration of metal per unit mass. Afterwards, each sample was predigested for 1 hour by adding 10 mL of concentrated nitric acid, allowing the vessels to release gases. Subsequently the samples were put in the microwave, completely sealing the vials. The temperature of each sample rose to 180°C in approximately 5.5 minutes and remained at this temperature for 9.5 minutes until complete decomposition, followed by a cooling step. At the end of the microwave program the samples were allowed to cool for 5 minutes before extraction. When the seals were removed from each vial the sample was filtered, and 5% nitric acid was added to complete 10 mL in case of volume loss (EPA 1996). All metal concentrations are expressed in μg/g of wet tissue weight. The measurement of heavy metals was carried out through the ContrAA® atomic absorption spectrometer, with electrothermal atomization, taking into account the measurement of standard samples of known concentration and blanks to be able to estimate the specific calibration curve (Burger 1992). For the quantification, a dilution of 25 μL of sample in 1 mL of deionized water (25:1000) was carried out, and after the measurement, each concentration obtained had to be multiplied by 40. The detection limits, calculated using at least 10 targets, were (in μg/g) 0.00009 for chromium, 0.0000008 for cadmium and 0.000042 for lead.

Statistical analysis

All statistical analyses and graphics were carried out using the R software (R Core Team, 2013), as implemented in RStudio. Multivariate and univariate normality tests were performed for each set of continuous data. When they did not meet the assumptions of normality and homogeneity of variances and could not be transformed to achieve a normal distribution, non-parametric tests were used.

To assess the correlation between the concentration of the analyzed metals, the Spearman non-parametric correlation test was performed. On the other hand, to model the eventual relationship between the concentration of metals in each tissue and the body mass of the snakes we built generalized linear models (GLM) with a Poisson link, since the output variable (concentration) has a lower distribution limit of 0.

To test whether the concentration of each metal was predictable from the type of organism (snake or fish), the type of snake tissue (liver or muscle), and the sampling point (the position with respect to the discharge of residual water in the river), we built linear models within a Bayesian approach. A Gamma Hurdle distribution was assumed, which allows analyzing continuous data that includes a high number of zeros (i.e. zero-inflated data).

Results

A total of 28 individuals of *Helicops pastazae* and 28 individuals of fish from the Characidae family were collected; 7 individuals of each species at each sampling point (Fig. 2). Female snakes weighed on average 78.71 ± 44 g and measured on average 43.4 ± 11 cm SVL, while the males weighed on average 39.28 ± 21 g and measured on average 33.7 ± 6 cm SVL. Juveniles (total length less than 30 cm, estimate from the smaller female that could be sexed) weighed on average 5.42 ± 0.5 g and measured on average 16.3 ± 0.6 cm SVL.

Snake body mass was inversely correlated with chromium concentrations ($Z=-2.411$, $p = 0.0159$, $n = 83$ samples, correlation coefficient=-0.02995) (Fig. 3c), but not with lead or cadmium. On the other hand, the pairwise correlation among the concentration of heavy metals was far from significant (-0.0826 < Spearman correlation coefficient < 0.0995 and $P > 0.37$ in all cases), which means that individuals with a high concentration of a particular metal do not necessarily have a high concentration of the other metals.

Table 1
Heavy metal concentration in different tissues of fish and snakes of the Bata river (arithmetic mean ± standard deviation in µg/g wet weight).

	n	Cadmium	Lead	Cromium
Fish				
Muscle	28	0.012 ± 0.038	0.492 ± 0.790	1.168 ± 1.536
Snake				
Muscle	27	0.001 ± 0.002	0.601 ± 1.970	0.779 ± 0.728
Liver	28	0.006 ± 0.010	0.127 ± 0.189	0.517 ± 0.824

Table 2
Heavy metal concentration in different tissues of fish and snakes at different points of the Bata river (arithmetic mean ± standard deviation in μg/g wet weight).

	Sampling point	n	Cadmium	Lead	Cromium
Fish					
Muscle	1	7	0.003 ± 0.004	0.469 ± 0.358	0.387 ± 0.406
	2	7	0.010 ± 0.025	0.343 ± 0.385	1.714 ± 1.531
	3	7	0.034 ± 0.071	0.986 ± 1.438	2.199 ± 2.208
	4	7	0.001 ± 0.003	0.170 ± 0.171	0.373 ± 0.436
Snake					
Muscle	1	6	0.0013 ± 0.0031	0.210 ± 0.211	0.749 ± 0.828
	2	7	0.0005 ± 0.0015	1.808 ± 3.798	0.628 ± 0.784
	3	7	0.0003 ± 0.0008	0.209 ± 0.106	0.521 ± 0.453
	4	7	0.0006 ± 0.0015	0.120 ± 0.220	1.292 ± 0.703
Liver	1	7	0.008 ± 0.018	0.312 ± 0.243	0.206 ± 0.949
	2	7	0.004 ± 0.004	0.071 ± 0.138	0.271 ± 0.238
	3	7	0.011 ± 0.005	0.048 ± 0.046	0.082 ± 0.122
	4	7	0.002 ± 0.004	0.077 ± 0.166	0.909 ± 1.232

Considering each sampling point, significant differences were only observed for chromium at point 4, where the concentrations were higher in the snakes with respect to the fish (Fig. 4). Fish muscle revealed a higher average concentration of the analyzed metals, compared to the snake muscle. Cadmium, chromium and lead do not show significant differences between the muscle of fish and snakes (Fig. 5).

Significant differences could not be appreciated between the concentration of different metals in the same tissue (Table 1). For cadmium, significantly higher concentrations were found in the liver in comparison with the snake muscle (Fig. 6, Fig. 7a), and lead and chromium concentrations were significantly higher in the muscle (Fig. 6, Fig. 7b, Fig. 7c). These patterns remain when comparing the average concentrations of metals within each of the river points (Table 2).

Regarding the comparison between sampling points, upstream and downstream the water discharge, we found higher chromium and cadmium concentrations at point 3 in comparison to point 1, and higher lead concentrations at point 2 in comparison to points 1 and 4, when considering fish and snake muscle (Fig. 5). On the other hand, point 1 had higher concentrations for all metals in comparison to at least one of the other points when considering snake tissues (Fig. 7). This reveals that no clear spatial pattern was found.

Figure 3 Relationship between body mass and **(A)** levels of cadmium, **(B)** levels of lead, and **(C)** levels of chromium, in different snake tissue of the species *Helicops pastazae*

Figure 4 Heavy metal concentration in different sampling points of the Bata river in fish and snake muscle. Asterisk (*): Significant differences between organisms, based on the Bayesian credibility interval of 95% (boxes)

Figure 5 Magnitude of the effect, according to Bayesian models, of the organism (muscle of fish and snakes) and the sampling point on the concentrations of **(A)** Cadmium; **(B)** Lead; and **(C)** Chromium (Reference line: fish muscle and point 1).

Figure 6 Heavy metal concentration in different sampling points of the Bata river in snake liver and muscle. Asterisk (*): Significant differences between organisms, based on the Bayesian credibility interval of 95% (boxes)

Figure 7 Magnitude of the effect, according to Bayesian models, of the tissue (snake liver and muscle) and the sampling point on the concentration of **(A)** Cadmium; **(B)** Lead; and **(C)** Chromium (Reference line: liver and point 1).

Discussion

The frequency of females, males and juveniles was variable at each sampling point (Fig. 2). An attempt was made to strictly collect adult individuals but given that in some points not enough were found it was necessary to capture juveniles. Although the juvenile condition could imply less bioaccumulation of metals, this pattern did not appear in the analyzes. There was no evidence of umbilical cord and some of the juveniles were shedding their skin. This evidence of growth is an indicator that food intake has been carried out and taking into account that the diet is a route of exposure to metals (Lemaire et al. 2018; Hopkins et al. 2001), it could be concluded that even with few ingested animals metal bioaccumulation takes place.

Comparison between sampling points

Two statistical models were developed to assess whether there were differences between sampling points: one considering the organism and another considering the type of tissue, in order to compare between organisms, snake tissues and sample points. In the first model, significant differences were obtained for chromium and cadmium concentration, which were higher at point 3 than point 1 (Fig. 5). In the second model lower concentrations were observed near the water discharge zone, particularly in point 3 for lead and chromium (Fig. 7). This may indicate that both the liver and the muscle of snakes are tissues that allow to observe differences along the river. It can be noted as a general trend that metal concentrations are higher in point 1 when analyzing snake tissue.

The absence of an spatial gradient in the concentration of metals, expected to be lower upstream of the discharge of wastewater and higher downstream, may be due to the Bata river being downstream of the

Chivor reservoir, that receives sewage from the basin and has dammed waters with high contamination by sediments, residues and dissolved nutrients (Corporación Autónoma Regional de Cundinamarca et al. 2006). This would cause the upstream points to show higher metal concentrations in comparison to some of the other points, which was the general tendency for the second Bayesian linear model (Fig. 7). Similarly, according to conversations with the residents of the municipality, the Chivor dam discharges very high flows in the Bata river, approximately once a year and for several days, and also during periods of long inflows to the reservoir. The sudden increase in the flow would cause the river to be contaminated in its entirety with the dammed waters by transporting different pollutants and eroded material that comes from the dam and the river basin (MAVDT 2005). Additionally, the increase in river flow could drag some organisms downstream, possibly disrupting the expected pattern due to spatial migration. However, it is not possible to stablish the potential source of higher concentration of metals in the river with the current data, given that no predictable results where found at a spatial level.

In addition, since the sampling points are not far from each other, the snakes could migrate from one point to another, perhaps obscuring the expected spatial pattern. *Helicops pastazae* is a species of aquatic snake little studied, and nothing is known about its movement or migration patterns. In *Nerodia sipedon*, a neartic aquatic snake, different movement patterns were found depending on the time of the year, presenting changes in its range of activity without occupying an established or permanent home range (Macartney et al. 1988). However, other studies indicate that aquatic snakes have limited home ranges (Campbell and Campbell 2001; Hopkins et al. 1999; Heinz et al. 1980). Then the possibility that *H. pastazae* presents migration along the river is possible, and not having relatively fixed home ranges would dilute the expected spatial patterns.

Interspecific comparisons

Fish from the Characidae family and *H. pastazae* aquatic snakes have marked differences in diet, occupying different positions in the food chain. The snakes studied are top predators of this ecosystem and are considered opportunistic predators (Almendáriz et al. 2017). It was expected that predatory snakes had a higher concentration of heavy metals with respect to fish, since there is bioaccumulation along the food chain (Heydari and Riyahi 2015). Even so, there were no significant differences in any metal concentrations between the fish and snake muscle (Fig. 5). It cannot be ruled out that there are different chemical and kinetic dynamics between species, since several researchers have found that different aquatic snakes from the same place of study had different levels of metals in their tissues (Heydari and Riyahi 2015). In fact, a study conducted on a snake of the species *Thamnophis sauritus* and anura larvae, found that snakes had significantly lower levels of lead and cadmium, compared to larvae, indicating that these metals were not biomagnifying to superior trophic levels (Albrecht et al. 2007). Similarly, it has been established that lead and cadmium concentrations do not increase along the trophic chain in surface water ecosystems, even finding that the levels of these metals were lower in tissues of predatory fish and higher in lower levels of the food chain (Jezierska and Witeska 2006; Kenšová et al. 2010).

Likewise, although there are studies that indicate a generalist diet for the genus *Helicops*, there are no studies that determine the diet of *H. pastazae*, except for a study by Almendáriz, Barriga and Rivadeneira (2017), which states that possibly it feeds on fish of the species *Hypostomus pyrineusi*. Without knowing with certainty the diet of this species of aquatic snake in the study area, it is possible that the prey-predator comparisons bring up an unexpected result.

On the other hand, obtaining statistically equal concentrations between fish and snakes, may indicate that they have some mechanism to get rid of heavy metals. Various studies infer that snakes can excrete metals through different mechanisms, such as cesium through feces, skin shedding, and egg production (Campbell and Campbell 2001). Also, it was found that aquatic snakes of the *Nerodia sipedon* species can sequester chromium, lead, manganese and mercury in their skin, due to the high concentrations found in comparison with all body tissues, which suggests that through frequent shedding of skin they can excrete metals and decrease the pollutant load (Burger 1992; Campbell et al. 2005). Similarly, when comparing the levels of heavy metals between males and females of some snake species, it was concluded that there may be a transfer of metals from the female to the eggshell (Campbell et al. 2005; Burger et al. 2017).

Comparison between tissues

Significant differences could not be appreciated between the concentration of different metals in the same tissue (Table 1). Even so, different tissues of *H. pastazae* did bioaccumulate metals differentially (Fig. 7). Cadmium was significantly more concentrated in the liver when compared to muscle, and lead and chromium had significantly higher concentrations in muscle. This corroborates that tissues have different affinity for certain metals, and that not all tissues are useful for evaluating traces of a particular metal. It has been observed that cadmium is accumulated mainly in the kidney and liver of fish (Jezierska and Witeska 2006; Panchanathan and Vattapparumbil 2006). Also, fish muscle is usually the tissue with lower levels of metals (Jezierska and Witeska 2006). The higher concentration of metals in the liver may be due to the fact that it is a metal storage and detoxification organ. However, the high concentrations of lead and chromium found in muscle are not explained. There are studies that show that the concentration of cadmium is higher in the liver of fish, while that of lead is homogeneous in the sampled tissues (Zorrilla 2011).

Regarding studies in snakes (*Pituophis melanoleucus*), it has been found that metals have a greater affinity with the skin, especially lead, because higher concentrations were found with respect to other body tissues (Burger 1992). In contrast, in alligators (*Alligator mississippiensis*) the liver had the highest concentrations of cadmium, arsenic, manganese, mercury and selenium, and muscle the highest concentrations of lead and chromium (Burger et al. 2000). The highest concentrations of cadmium in liver were found in aquatic snakes (*Nerodia fasciata*) fed with contaminated and uncontaminated prey (Hopkins et al. 1999), as in *Nerodia spp.*, where chromium and lead were higher in the skin (Burger et al. 2007), which is consistent with other studies conducted in *Nerodia sipedon* (Campbell et al. 2005). A study in marine snakes (*Lapemis curtus*) showed no differences in concentration between muscle and liver for lead and cadmium. In the present study, higher concentrations of cadmium were found in the liver compared to muscle, in accordance with other studies in aquatic snakes (Burger et al. 2007; Hopkins et al. 1999; Campbell et al. 2005). Likewise, higher concentrations of lead and chromium were found in the muscle compared to the liver, which is consistent with other studies in aquatic snakes and other reptiles (Burger et al. 2000; Burger et al. 2007; Campbell et al. 2005). The diversity of results in the literature shows that it is difficult to predict the tissue where each type of metal will accumulate.

It has been found in several studies that the liver accumulates high concentrations of metals, regardless of the route of incorporation, and is considered a good indicator of water contamination, since the concentrations that accumulate in this organ are proportional to the found in the environment, especially for the cases of copper and cadmium (Jezierska and Witeska 2006). The differential concentration of metals in each tissue could be explained given the differences in metal affinity to sulfhydryl, amino, phosphate, carboxyl and hydroxyl groups, functional groups that can vary between tissues generating different chemical reactions for the formation of an organometallic compound (Zorrilla 2011). Lead has a high capacity for erythrocyte binding and can easily be substituted with divalent cations (Zuluaga, Gallego and Ramírez 2015), so it is possible that it has high affinity with tissues that have high blood flow, as most heavy metals (van der Brink 2004). High affinity of cadmium with kidneys, liver, and bones has been found (Zuluaga, Gallego and Ramírez 2015). Because of these affinity differences, it can be inferred that tissues vary in their value as a bioindicator of different metals.

Snakes as bioindicators

The present study reflects high levels of metals compared to similar studies, especially lead and chromium, found in different tissues of aquatic snakes. There were no differences between the concentrations of metals found in fish and snakes, which shows that aquatic snakes can be a good indicator of water pollution. For a species to be a useful bioindicator, certain conditions must be taken into account: 1) there is a relationship between tissue contamination levels and dietary exposure (Hopkins et al. 2001); 2) there is a relationship between the levels of contamination in the tissues and the levels in the ecosystem, that is, that the contaminants found in the ecosystem are concentrated at detectable levels in the tissues, and 3) the species should reflect the levels of contamination in a specific area (Heinz et al.,1980).

Regarding the first condition, a relationship between the levels of heavy metals in the tissues in comparison to the levels of metals in their diet was not found, because, as discussed, it is not known with certainty whether the sampled fish are part of the snake diet. Likewise, several studies have shown that lead and cadmium have lower concentrations in predators than in prey (Jezierska and Witeska 2006; Kenšová et al. 2010). Even so, finding detectable levels of metals in the tissues of snakes, can help us conclude that they are obtaining contaminants from their diet, even though the concentrations are not higher with respect to those of fish. The second requirement is met, since detectable heavy metal levels were observed in the snake tissues, which reflect the contamination present in the ecosystem in which they live. Beyond this, the differences in concentration in each tissue and organism may depend on toxicodynamics (Burger et al. 2007), and on the different rates of incorporation and excretion of metals, environmental factors, among others. Regarding the third condition, the present study did not show the expected results with respect to each sampling point in the Bata river, perhaps due to the possibility of snake migration or wastewater discharges, but aquatic snakes did reflect the heavy metal pollution differentially at different sampling points of this river.

Additionally, aquatic snakes are believed to be relatively sedentary, and may be adequate indicators of local contamination (Heinz et al. 1980), although specific migration patterns for *H. pastazae* are unknown. On the other hand, the abundance of this species of snake in the Bata river and its long life cycle makes them good indicators of contamination at long temporal scales (Burger 1992), and that they can be monitored at different points of the river without threatening their population. Apart from this, aquatic snakes are primary or secondary predators in the trophic chain, and may be susceptible to bioaccumulation of environmental pollutants, making them useful for evaluating compounds that can be transferred by trophic mechanisms (Campbell and Campbell 2001). Taking into account all of the above, aquatic snakes of the *Helicops pastazae* species can be useful bioindicators of pollutant accumulation, in this case of heavy metals, at different temporal and spatial scales.

Declarations

Funding

Department of Biological Sciences, Department of Civil and Environmental Engineering, and Deparment of Chemistry in Universidad de los Andes contributed with their financial support for the development of this research.

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethics approval

The procedures for euthanasia and manipulation of individuals were approved on October 18, 2018 by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) by analyzing the animal use format COR_C.FUA_18-016. Individuals were collected under the permit: “Permiso Marco de Recolección de Especímenes de Especies Silvestres de la Diversidad Biológica con Fines de Investigación Científica No Comercial”, certified under the research project PR.6.2018.4967 “Integración de rasgos funcionales y moleculares en estudios evolutivos de comportamiento y fisiología”, with mobilization permit P04967S3591_N0004.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available as a file in the Electronic Supplementary Material and from the corresponding author on reasonable request.

Code availability

The code generated and used during the current study are available from the corresponding author on reasonable request.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

References

1. Albrecht J, Abalos M, Rice TM (2007) Heavy metal levels in ribbon snakes (Thamnophis sauritus) and Anuran larvae from the Mobile-Tensaw River Delta, Alabama, USA. Arch Environ Contam Toxicol 53:647-654. https://doi.org/10.1007/s00244-006-0175-3
2. Almendáriz A, Barriga R, Rivadeneira D (2017) Feeding behavior of Helicops pastazae Shreve 1934 (Serpentes, Colubridae, Dipsadinae) in the Ecuadorian Amazon. Herpetol Notes 10:449-451.
3. Burger J (1992) Trace element levels in Pine Snake hatchlings: tissue and temporal differences. Arch Environ Contam Toxicol 22:209-213. https://doi.org/10.1007/BF00213287
4. Burger J, Campbell KR, Campbell TS, Shukla T, Jeitner C, Gochfeld M (2005) Use of skin and blood as nonlethal indicators of heavy metal contamination in Northern Water Snakes (Nerodia sipedon). Arch Environ Contam Toxicol 49:232-238. https://doi.org/10.1007/s00244-004-0098-9
5. Burger J, Campbell KR, Murray S, Campbell TS, Gaines KF, Jeitner C, Shukla T, Burke S, Gochfeld M (2007) Metal levels in blood, muscle and liver of Water Snakes (Nerodia spp.) from New Jersey, Tennessee and South Carolina. Sci Total Environ 373:556-563. https://doi.org/10.1016/j.scitotenv.2006.06.018
6. Burger J, Gochfeld M, Jeitner C, Zappalorti R, Pittfield T, DeVito E (2017) Arsenic, cadmium, chromium, lead, mercury and selenium concentrations in Pine Snakes (Pituophis melanoleucus) from the New Jersey Pine Barrens. Arch Environ Contam Toxicol 72:586-595. https://doi.org/10.1007/s00244-017-0398-5
7. Burger J, Gochfeld M, Rooney AA, Orlando EF, Woodward AR, Guillette LJ (2000) Metals and metalloids in tissues of American Alligators in three Florida Lakes. Arch Environ Contam Toxicol 38:501-508. https://doi.org/10.1007/s002440010066
8. Campbell KR, Campbell TS (2001) The accumulation and effects of environmental contaminants on snakes: a review. Environ Monit Assess 70:253-301. https://doi.org/10.1023/A:1010731409732
9. Campbell KR, Campbell TS, Burger J (2005) Heavy metal concentrations in Northern Water Snakes (Nerodia sipedon) from East Fork Poplar Creek and the Little River, East Tennessee, USA. Arch

Environ Contam Toxicol 49:239-248. https://doi.org/10.1007/s00244-004-0200-3

10. Corpochivor (1996) Plan Ambiental para el municipio de Santa María, Boyacá. https://1library.co/document/q2nd01jq-plan-ambiental-municipio-santa-maria-boyaca.html. Accessed 20 November 2018.
11. Corpochivor (2003) Municipio de Santa María. Esquema de Ordenamiento Territorial: Documento resumen. Santa María. http://www.corpochivor.gov.co/wp-content/uploads/2015/11/EOT-Santa-Mar%C3%ADa.pdf. Accessed 20 November 2018.
12. Corporación Autónoma Regional de Cundinamarca, Corpochivor, Corpoboyacá, Universidad Nacional de Colombia (2006) Plan de ordenación y manejo ambiental de la cuenca del río Garagoa – subcuenca río Machetá en la juridiccion CAR. Corporación Autónoma Regional. https://www.car.gov.co/uploads/files/5ac674d6e8eed.pdf. Accessed 20 November 2018.
13. EPA (1996) Method 3052: Microwave assisted acid digestion of siliceous and organically based matrices. https://www.epa.gov/sites/production/files/2015-12/documents/3052.pdf. Accessed 20 November 2018.
14. FAO/SIDA (1983) Part 9. Analyses of metals and organochlorines in fish. In: FAO/SIDA Manual of methods in aquatic environment research. Roma, pp 33.
15. Heinz GH, Haseltine SD, Hall RJ, Krynitsky AJ (1980) Organochlorine and mercury residues in snakes from Pilot and Spider Islands, Lake Michigan—1978. Bull Environ Contam Toxicol 25:738-743. https://doi.org/10.1007/BF01985601
16. Henze M, Harremoes P, Jansen JLC, Arvin E (2002) Wastewater Treatment: Biological and Chemical Processes. Berlin.
17. Heydari Z, Riyahi A (2015) Concentrations of trace elements in the kidney, liver, muscle, and skin of short sea snake (Lapemis curtus) from the Strait of Hormuz Persian Gulf. Environ Sci Pollut Res 22:15781-15787. https://doi.org/10.1007/s11356-015-4631-3
18. Hopkins WA., Roe JH, Snodgrass JW, Jackson BP, Kling DE, Rowe CL, Congdon JD (2001) Nondestructive indices of trace element exposure in squamate reptiles. Environ Pollut 115:1-7. https://doi.org/10.1016/S0269-7491(01)00098-7
19. Hopkins, WA, Rowe CL, Congdon JD (1999) Elevated trace element concentrations and standard metabolic rate in Banded Water Snakes (Nerodia fasciata) exposed to coal combustion wastes. Environ Toxicol Chem 18:1258-1263. https://doi.org/10.1002/etc.5620180627
20. Jezierska B, Witeska M (2006) The metal uptake and accumulation in fish living in polluted waters. Soil and Water Pollution Monitoring, Protection and Remediation 69:107–114. https://doi.org/10.1007/978-1-4020-4728-2_6
21. Kenšová R, Čelechovská O, Doubravová J, Svobodová Z (2010) Concentrations of metals in tissues of fish from the Věstonice Reservoir. Acta Vet Brno, 79:335-345. https://doi.org/10.2754/avb201079020335
22. Lemaire J, Bustamante P, Olivier A, Lourdais O, Michaud B, Boissinot A, Galán P, Brischoux F (2018) Determinants of mercury contamination in viperine snakes, Natrix maura, in Western Europe. Sci

Total Environ 635:20-25. https://doi.org/10.1016/j.scitotenv.2018.04.029

23. Macartney JM, Gregory PT, Larsen KW (1988) A tabular survey of data on movements and home ranges of snakes. J Herpetol 22:61-73. https://doi.org/10.2307/1564357
24. Macías PG (2015). Determinación de metales pesados (Pb, Cd, Cr) en agua y sedimentos de la zona estuarina del río Tuxpan, Veracruz. Universidad Veracruzana. https://cdigital.uv.mx/bitstream/123456789/41940/1/MaciasHernandezPatricia.pdf. Accessed 16 April 2018.
25. Mancera-Rodríguez N, Alvarez-León R (2006) Estado del conocimiento de las concentraciones de mercurio y otros metales pesados en peces dulceacuícolas de Colombia. Acta Biol Colomb 11:3-23.
26. Márquez A, Senior W, Fermin I, Martínez G, Castañeda J, González A (2008) Cuantificación de las concentraciones de metales pesados en tejidos de peces y crustáceos de la laguna de Unare, Estado Anzoátegui, Venezuela. Rev Cient 18:73-86. https://zenodo.org/record/160421#.X-PCHapKhQI
27. MAVDT (2005) Resolución número 1066: Por la cual se establece un plan de manejo ambiental y se toman otras determinaciones. Ministerio de Ambiente, Vivienda y Desarrollo Territorial. http://www.mamacoca.org/docs_de_base/Legislacion_tematica/res_1066_050805.pdf. Accessed 5 May 2019.
28. Panchanathan J, Vattapparumbil IP (2006) Patterns of cadmium accumulation in selected tissues of the catfish Clarias batrachus (Linn.) exposed to sublethal concentration of cadmium chloride. Vet Arh 76:167-177.
29. Queensland Government (2018) Environmental Protection Water Policy 2009- Monitoring and Sampling Manual. Queensland Government. https://environment.des.qld.gov.au/water/monitoring/sampling-manual/pdf/biological-assessment-fish-collection-and-the-dissection-for-the-purpose-of-chemical-analysis-of-tissues.pdf. Accessed 20 November 2018.
30. R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.
31. Segall M, Cornette R, Fabre AC, Godoy-Diana R, Herrel A (2016) Does aquatic foraging impact head shape evolution in snakes? Proc R Soc B-Biol Sci 283:20161645.
32. Uetz P, Freed P, Hošek J (eds.) (2019) The Reptile Database. http://www.reptile-database.org. Accessed 5 May 2019.
33. Van der Brink N (2004) Soil and higher organisms: from bottom-up relations to top-down monitoring. In: P. Doelman, & H. Eijsackers Vital soil: Function, Value and Properties. pp 235.
34. Zorrilla MF (2011) Estado del arte sobre la presencia de metales pesados en tejidos y agallas de peces. Universidad Autónoma de Occidente. https://red.uao.edu.co/bitstream/10614/1637/1/TAA00771.pdf. Accessed 16 April 2018.
35. Zuluaga J, Gallego S, Ramírez CM (2015) Content of Hg, Cd, Pb and As in fish species: a review. Rev Vit 22:148-149. http://dx.doi.org/10.17533/udea.vitae.v22n2a09.

Figures

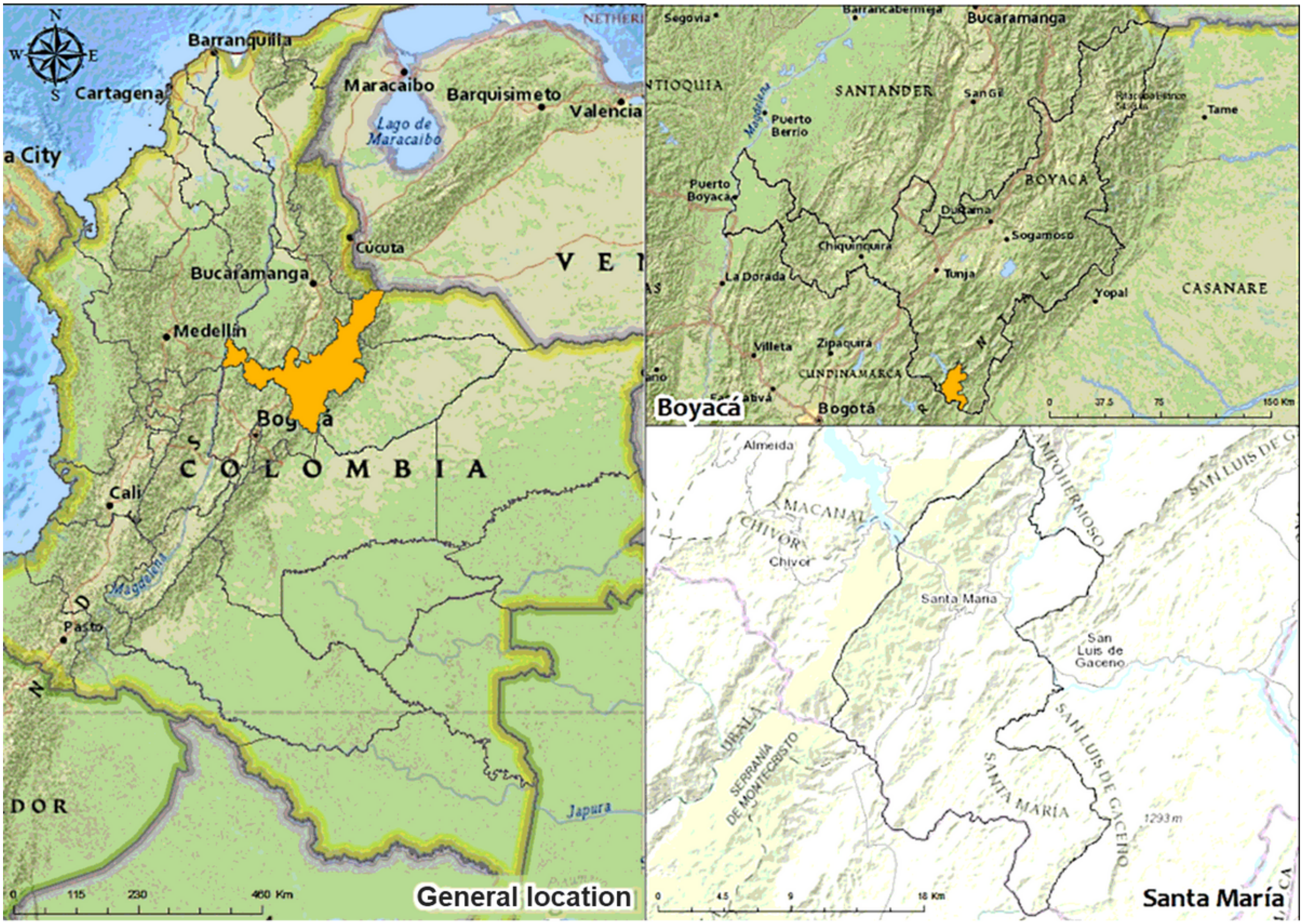

Figure 1

Location of the study site on the map of Colombia. The department of Boyacá and the location of the municipality of Santa María within this department, where the sampling was carried out, are presented in detail

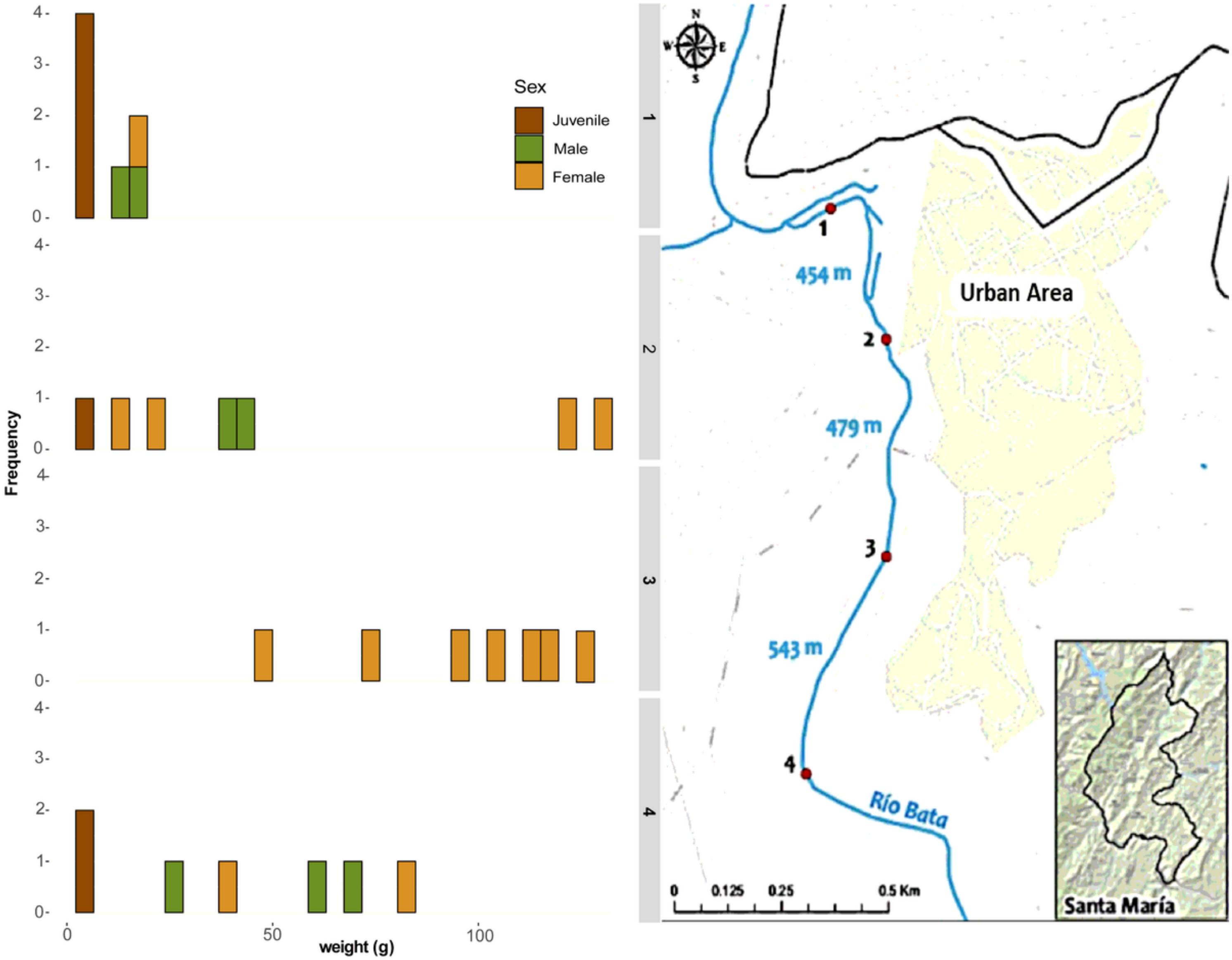

Figure 2

Sampling design, number of snakes captured and detailed map of the sampling points in the Bata river, located in the municipality of Santa María: Point 1 (4º51’41”N, 73º16’03”W), point 2 (4º51’32”N, 73º15’57”W), point 3 (4º51’19”N, 73º15’57”W) and point 4 (4º51’04”N, 73º16’03”W)

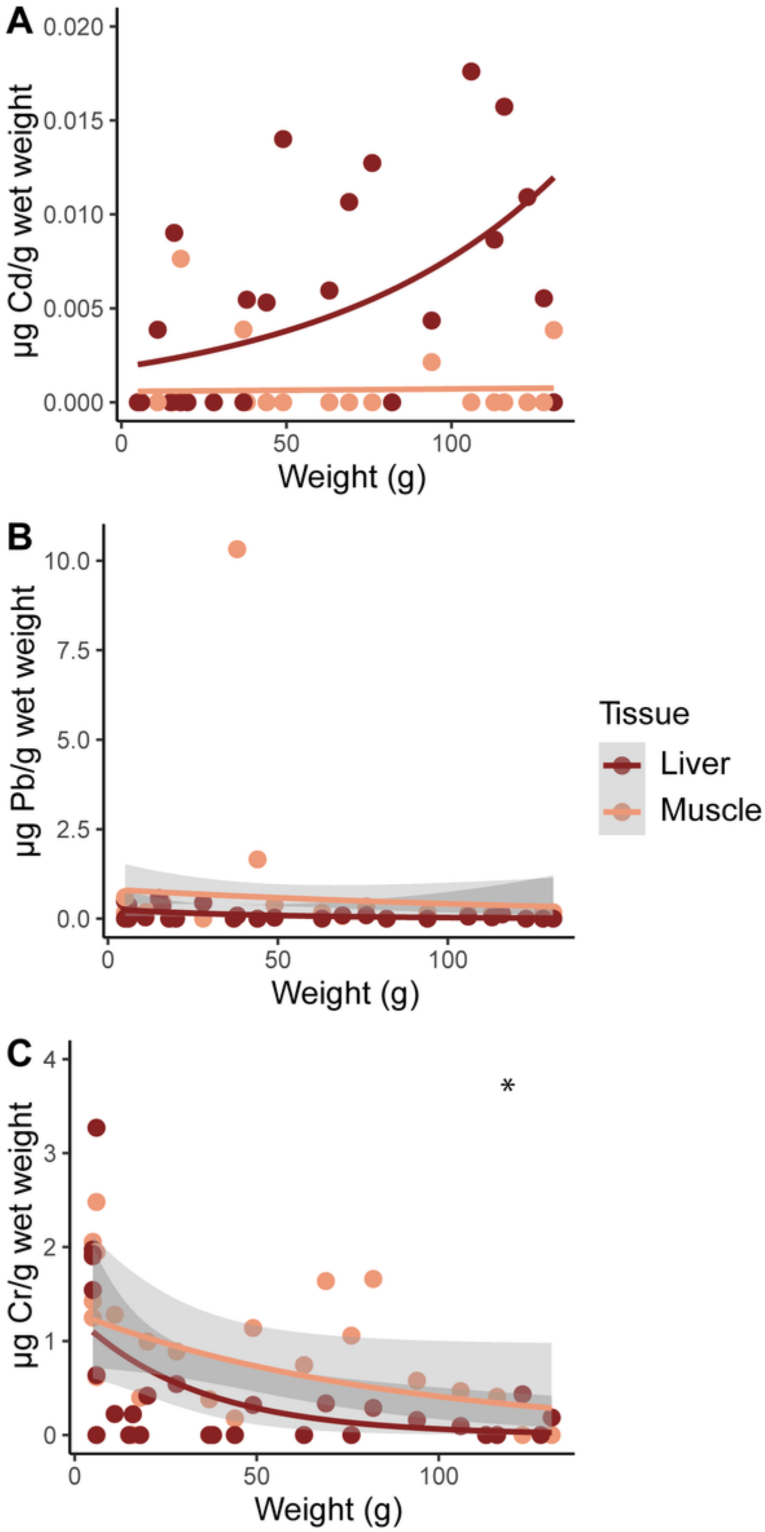

Figure 3

Relationship between body mass and (A) levels of cadmium, (B) levels of lead, and (C) levels of chromium, in different snake tissue of the species Helicops pastazae

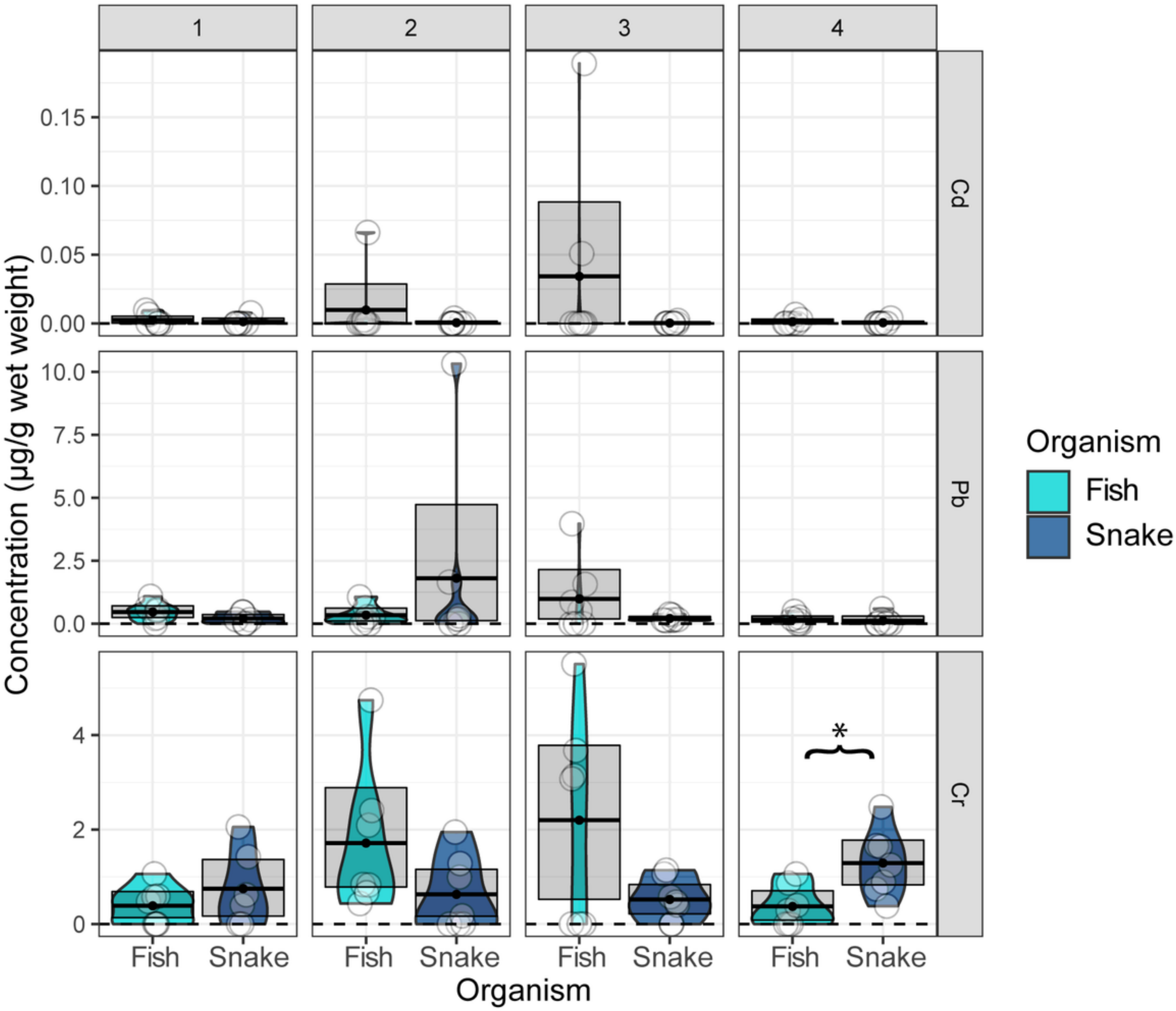

Figure 4

Heavy metal concentration in different sampling points of the Bata river in fish and snake muscle. Asterisk (*): Significant differences between organisms, based on the Bayesian credibility interval of 95% (boxes)

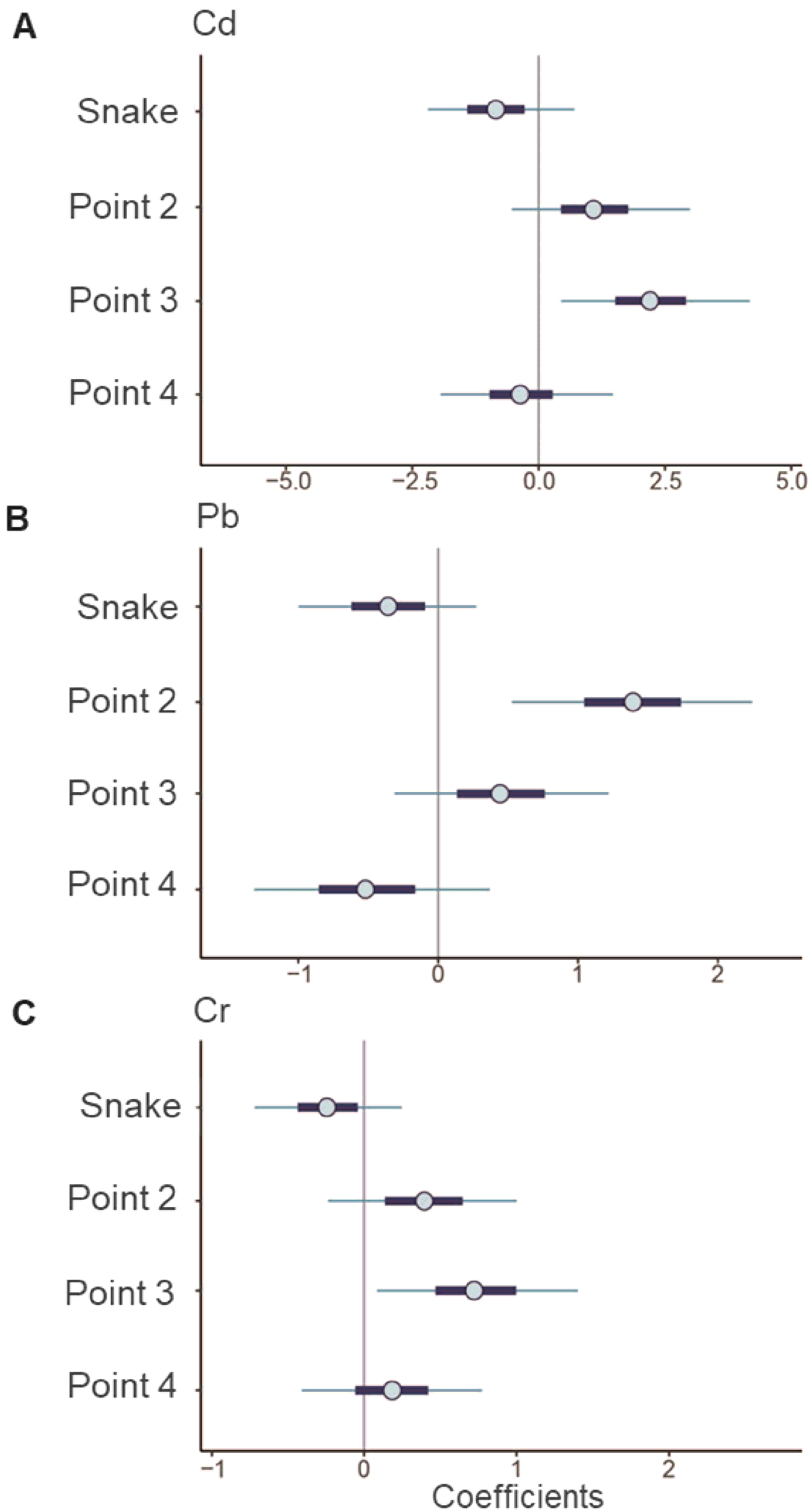

Figure 5

Magnitude of the effect, according to Bayesian models, of the organism (muscle of fish and snakes) and the sampling point on the concentrations of (A) Cadmium; (B) Lead; and (C) Chromium (Reference line: fish muscle and point 1).

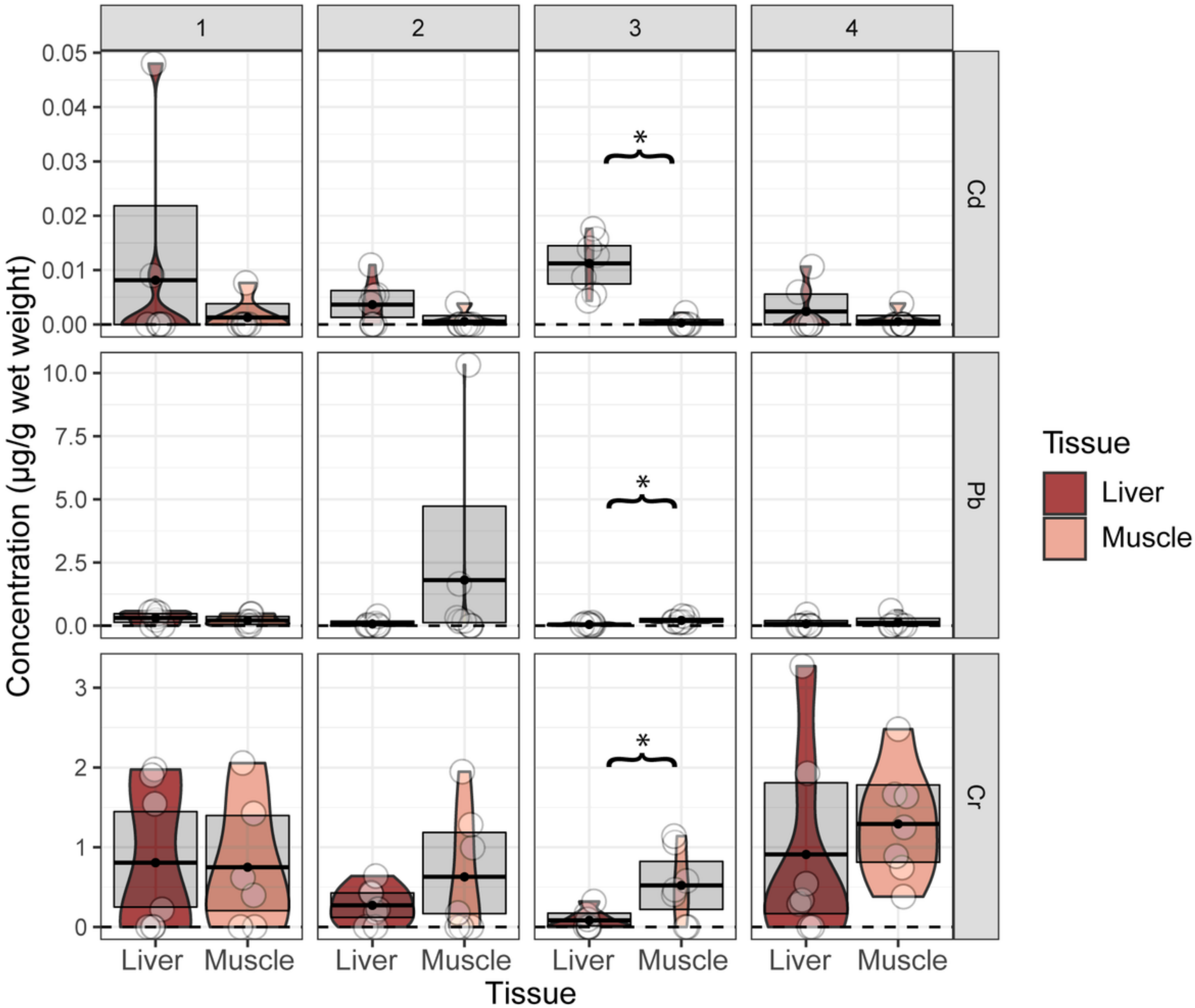

Figure 6

Heavy metal concentration in different sampling points of the Bata river in snake liver and muscle. Asterisk (*): Significant differences between organisms, based on the Bayesian credibility interval of 95% (boxes)

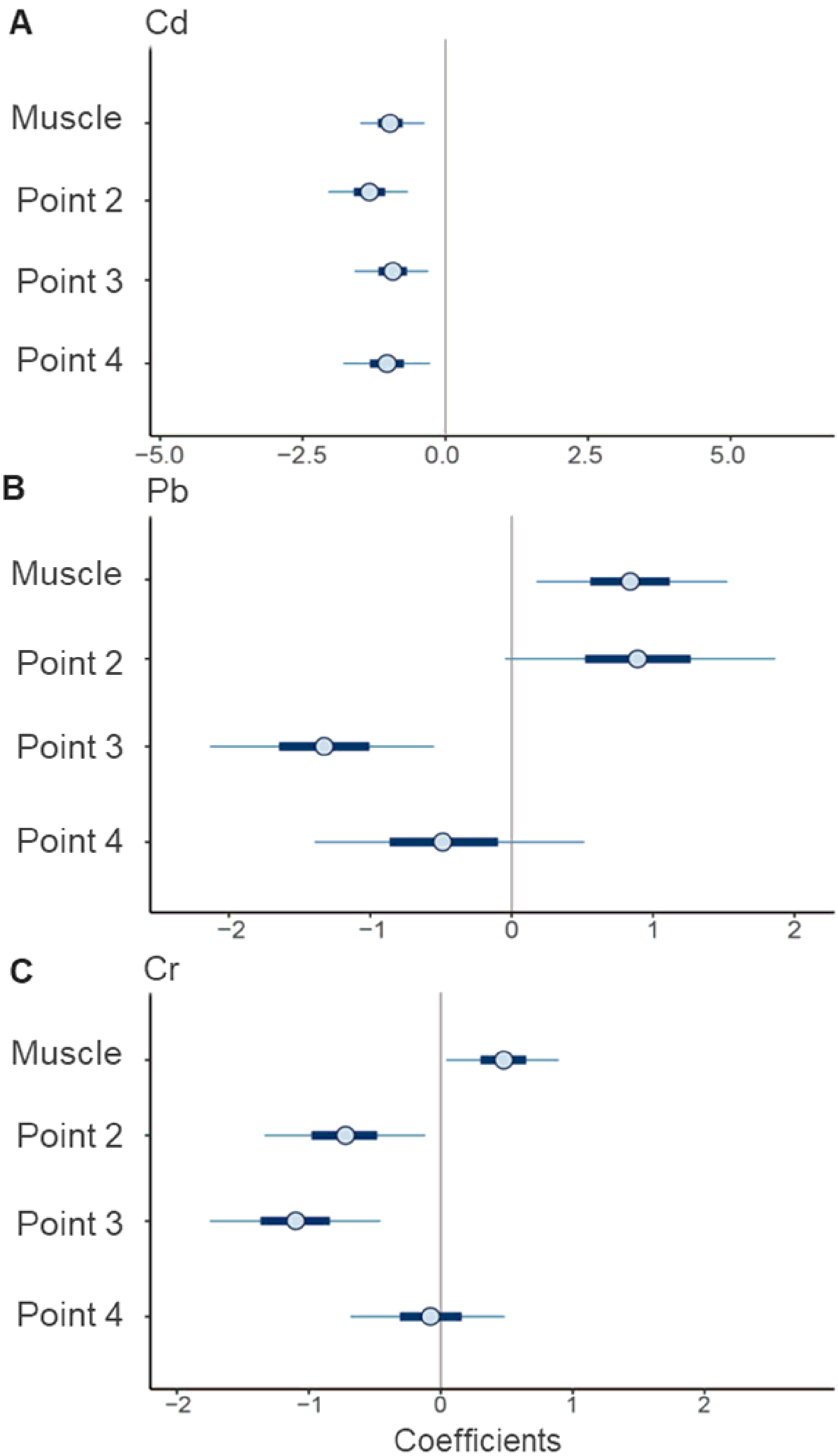

Figure 7

Magnitude of the effect, according to Bayesian models, of the tissue (snake liver and muscle) and the sampling point on the concentration of (A) Cadmium; (B) Lead; and (C) Chromium (Reference line: liver and point 1).