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Bioaccumulation of metals and metalloids in caudal tips of snakes from the Southern Pantanal

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Abstract

The Pantanal is one of the largest wetlands in the world and is of great importance for biodiversity and the socioeconomics of central South America. Nevertheless, the Pantanal is currently facing severe anthropogenic impacts, including chemical disturbances. Herein we quantified the levels of arsenic (As), silver (Ag), aluminum (Al), barium (Ba), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), manganese (Mn), molybdenum (Mo), lead (Pb), selenium (Se), vanadium (V), and zinc (Zn) in caudal tips of snakes from Southern Pantanal. Except for Zn, an essential element naturally found in elevated concentrations in snakes and other vertebrates, the highest levels were found for Al, Ba, and Mn. Notably, impure iron and manganiferous ores intensively explored in the studied region present elevated Al, Ba, and Mn content. Concentrations of As were positively correlated to the size of the yellow anaconda *Eunectes notaeus* while Ba levels were negatively correlated to size in this species. Levels of Mn and V were negatively correlated with the size of the water snake *Helicops leopardinus*. Higher burdens in species that feed on higher trophic levels were not verified for any element. Significant intraspecific differences in species commonly sampled in two sites with distinct degrees of anthropogenic disturbance were detected only for the yellow-bellied snake *Erythrolamprus poecilogyrus*. Our study suggests that mining activity could lead to mobilization and bioaccumulation of metals and metalloids in snakes from Southern Pantanal, but allochthonous sources from agricultural, silvicultural, and urban areas from the adjacent Brazilian Central Plateau should also be considered.

Keywords Aluminium · Barium · Environmental contamination · Manganese · Reptiles · Wetlands

Introduction

The Pantanal is one of the largest wetlands in the world, ranging from Central-Western Brazil (states of Mato Grosso and Mato Grosso do Sul) to parts of Eastern Bolivia and Paraguay. During periods of elevated precipitation, the Pantanal floodplain can reach an area of about 200,000 km² (Vieira et al. 2011). The plains, associated with the rivers that overflow and seasonally flood them, have crucial socioeconomic and local importance, considering the subsistence, commercial, and tourism/sporting fishing practiced in those systems. Moreover, Pantanal wetlands provide essential ecosystem services, which include flood control, carbon storage, aquifer recharge, and the maintenance of biodiversity (Alho and Reis 2017).

The Brazilian Pantanal has suffered significant human interference due to intensive mining, agriculture, and silviculture expansion, an increase in large-scale fluvial navigation due to the implementation of the Paraguay-Paraná Waterway, and increasing urbanization (Tomas et al. 2019). These activities and conditions of use of the territory generate pollutants that are released directly (from rivers and floodable areas of the Pantanal Plain) or indirectly (from areas of the plateau surrounding the Pantanal Plain) into the Pantanal ecosystems (Alho and Vieira 1997; Gottgens et al. 2001). Thus, each action aiming to investigate the

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occurrence of contaminants in Pantanal's ecosystems is crucial for its conservation. In this context, the use of varied biomonitors is useful for evaluating pollutant occurrence in various biological compartments of the Pantanal, considering their specificity for the use of habitats and trophic relationships (Parmar et al. 2016).

Snakes are recognized bioindicators of environmental contamination (Campbell and Campbell 2001; Lettoof et al. 2021). More than 4,000 species (Uetz et al. 2025) are distributed in various habitats, from high elevations to the sea and estuaries. Snakes present carnivorous habits, occupying intermediate and top trophic levels in their ecosystems. Except for some aquatic/semiaquatic species, which can disperse over long distances, most species have restricted home ranges. Snakes also present relatively long lifespans and continuous growth (Campbell and Campbell 2001; Hurtado-Morales et al. 2022). An increasing number of studies using snakes to evaluate the pollution status by a range of elements and organic and inorganic compounds in both aquatic and terrestrial environments have been published worldwide (e.g., Burger et al. 2007, 2017; Quintela et al. 2019, 2024a; Lettoof et al. 2020a; Hurtado-Morales et al. 2022). Those studies have contributed to monitoring the contamination of several areas of high environmental relevance. Considering the elevated diversity of snakes in the Pantanal, with approximately 84 species (Piatti 2017), and the relatively high abundance of some species, they make a good model for local ecotoxicological approaches.

In the present study, we determined the concentrations of arsenic (As), silver (Ag), aluminum (Al), barium (Ba), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), manganese (Mn), molybdenum (Mo), lead (Pb), selenium (Se), vanadium (V), and zinc (Zn) in caudal tips of semiaquatic and terrestrial snakes from two areas under distinct conditions of land/water use and ecosystems conservation in Brazilian Southern Pantanal (hereinafter BSP). The use of caudal tips is a reliable non-lethal method for ecotoxicological analyses in snakes (Hopkins et al. 2001; Burger et al. 2006; Wylie et al. 2009). We hypothesize that: (1) snakes from an area subjected to severe anthropogenic interventions would present higher concentrations of the analyzed elements when compared to individuals from a more conserved area; (2) intraspecific concentrations would be higher in individuals with larger sizes (older individuals), corroborating a pattern of increased bioaccumulation along the lifetime; (3) species that occupy higher trophic levels would show higher element burdens.

Materials and methods

Study area

We sampled the snakes in two BSP transects established in the municipalities of Corumbá and Ladário, Mato Grosso do Sul state. The Paraguay-Urucum transect (PU; 18º59'33" S −57º39'53" W, 19º13'39" S −57º26'38" W; 36.6 km on Western Estrada Park and BR-262 roads) comprised a stretch of the Paraguay River and respective floodplains, the peri-urban zones of Corumbá and Ladário, and the Urucum and Santa Cruz Massifs. This region is currently under severe anthropogenic disturbance. The intense navigation in the sampled Paraguay River stretch is made by motorized boats of varied sizes, including large (about 120 m-long) barge convoys. Six urban effluent discharge points were identified in the eastern margin. A harbor located on the eastern margin is used mainly for the flow of iron ore. Intensive mining of iron and manganese ores occurs in the Urucum and Santa Cruz massifs. The BR-262 road presents intense traffic, mainly trucks carrying ores to the harbor facilities. Corumbá County is facing an increasing process of urbanization. The Miranda-Abobral transect (MA; 19º26'00" S −57º03'10" W, 9º34'33" S −57º10'09" W; 41.2 km on Eastern Estrada Park and BR-262 roads) comprises the main channels and flooding plains of the Miranda and Abobral rivers. The region presents well-preserved floodplains and forest patches. The Miranda and Abobral show no sign of eutrophication; navigation mainly involves low-power stern-motorized boats. The main economic activities developed in the area are extensive livestock farming, tourism, and commercial and sport fishing (Fig. 1).

Biological sampling

We determined the concentrations of As, Ag, Al, Ba, Cd, Co, Cr, Cu, Hg, Mn, Mo, Pb, Se, V, and Zn in the caudal tips of 91 individuals distributed in 22 snake species (Table 1). Between July 2023 and January 2024, the transects established in the two sampled sites were intensively traveled by car at low speed (20–30 km/h), while stretches of the rivers Paraguay, Miranda, and Abobral were also traveled by kayak. All the snakes found were manually captured (a snake gripper was used for venomous species), photographed, identified to species, and measured (snout-vent length). The identity of the species was made through analysis of external morphology. A small sample of caudal tips (between 15 and 35 mm long) was extracted with sterile scalpel blades from each encountered individual. After the procedure, the individuals were released at the location of capture; some individuals were vouchered for ongoing studies on anatomy and systematics. The samples were washed with MilliQ

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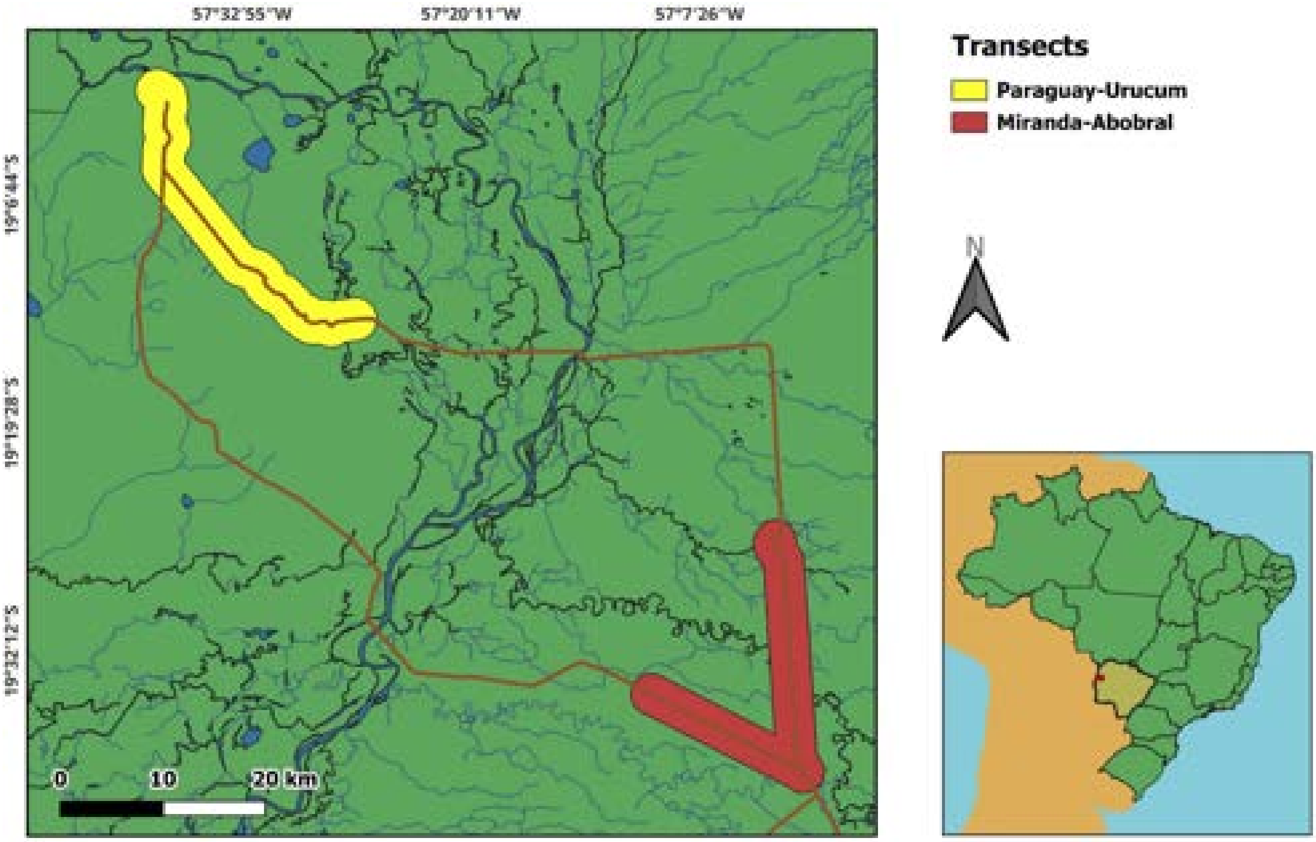

Fig. 1 Location of the study area and the two sampled stretches in the Brazilian Southern Pantanal

water, packed in tagged sterile Eppendorf tubes, and kept in thermic boxes. In the laboratory, samples were kept in a freezer at -18 °C. Collecting was authorized by the Brazilian environmental agency Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (process nº 62966-4). All procedures adopted follow institutional and international ethical protocols in animal use in scientific research.

Laboratory procedures

The samples were digested in a Multiwave GO Anton Paar microwave oven, using the sample preparation method for food and other organic samples developed by the microwave oven manufacturer (Digestion of Food and Other Organic Samples for Element Analysis with ICP, Anton Paar). This method was verified by the manufacturer by the digestion of three certified reference materials (CRM): NIST 1570a (spinach), NIST 1566b (oyster tissue), and BCR-414 (plankton).

The samples were weighed and transferred to digestion flasks containing 4 mL HNO3 and 2 mL HCl (both concentrated reagents). The samples were then placed in a rotor (model 12HVT50) and subjected to a heating and cooling program. The digests were collected, adjusted to 25 mL, and stored in polypropylene flasks. The element determinations were performed by inductively coupled plasma optical emission spectrometry (ICP-OES, model ICPE 9820, Shimadzu). To determine the analytes, all samples were filtered with a 0.45 µm filter (Chromafil, Xtra PTFE 45/25). The ICP-OES was calibrated using multielement standards with concentrations ranging from 10 to 200 µg L^{-1}. The limits of detection (LODs) were estimated after the determination of the analytes of 11 analytical blanks (4 mL HNO3 and 2 mL of HCl, calibrated to 25 mL). The LODs were calculated according to the IUPAC recommendation (LOD = 3 σ + Blank) and are shown in Table 2.

Data analysis

The concentrations obtained were expressed in µg.g^{-1} dry weight (d.w.). Means and standard deviations were calculated for the concentrations of each element in each species. The dataset comprised a minimum of four individuals analyzed per sampling site. In these datasets, values below the limit of detection were considered null values.

All datasets were checked for normality through a Shapiro-Wilk test, which detected normal distribution for 62 datasets and non-normal distribution for 65 datasets. The

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Table 1 Snake species analysed for metals and metalloid concentrations in the Brazilian Southern Pantanal and their respective number of individuals sampled (n), range of snout-vent length (SVL), habits, and diet

Species	Miranda-Abobral		Paraguay-Urucum		Habits	Diet (most common prey)
	N	SVL (mm)	*N*	SVL (mm)		
Boa constrictor			3	599-1,686	terrestrial, arboreal	birds, lizards, mammals [1,2]
Epicrates crassus			7	571-1,145	Terrestrial	birds, small mammals[1,2]
Eunectes notaeus	5	930-2,111	1	1,615	semiaquatic, terrestrial	fish, reptiles, birds, mammals [1,3,4]
Bothrops mattogrossensis	4	400–931	4	488–989	terrestrial	anurans, small mammals[1,4]
Crotalus durissus			8	565-1,082	terrestrial	small mammals[1,5]
Drymarchon corais			1	1,588	terrestrial	anurans, snakes[1,4]
Leptophis marginatus			1	928	arboreal, terrestrial	anurans, lizards[1]
Palusophis bifossatus	3	1,124-1,390			terrestrial, semiaquatic	anurans, lizards, snakes, small mammals[1,6]
Dipsas turgida			1	425	terrestrial	molusks[1]
Leptodeira pulchriceps	1	591			terrestrial, arboreal	anurans[1]
Hydrodynastes gigas	1	1,625			semiaquatic, terrestrial	fish, anurans, snakes, mammals[1,4,7]
Helicops leopardinus	14	152–486			semiaquatic	fish, anurans[1,4]
Philodryas olfersii			3	407–499	arboreal, terrestrial	anurans, birds, lizards, small mammals[1,8]
Philodryas patagoniensis	1	782	2	792–809	terrestrial, arboreal	anurans, lizards, snakes, small mammals[1,9]
Clelia clelia	1	842			Terrestrial	lizards, snakes, small mammals[1,10]
Mussurana bicolor	4	353–636	2	495–911	Terrestrial	amphibians, lizards, snakes[1,10]
Oxyrhopus rhombifer			4	283–612	Terrestrial	small mammals, lizards[1,10]
Pseudoboa nigra			1	878	Terrestrial	lizards[1,10]
Dryophylax chaquensis	1	410			semiaquatic	anurans[11]
Erythrolamprus poecilogyrus	7	389–545	8	335–530	terrestrial	anurans[1]
Erythrolamprus typhlus			2	417–640	terrestrial	anurans[1]
Lygophis dilepis			1	393	terrestrial	anurans[1]

[1]Marques et al. (2005), [2]Pizzatto et al. (2009), [3]Barros et al. (2011), [4]Strüssmann and Sazima (1993), [5]Sawaya et al. (2008), [6]Marques and Muriel (2007), [7]López and Giraudo (2004), [8]Hartmann and Marques (2005), [9]Quintela and Loebmann (2019), [10]Gaiarsa et al. (2013), [11]Bellini et al. (2013)

Table 2 Limits of detection (LOD (μg.g-1)) of the analyzed elements in snakes from the Brazilian Southern Pantanal

Element	LOD (μg.g-1)
As	0.007
Ag	0.018
Al	1.00
Ba	0.002
Cd	0.012
Co	0.036
Cr	0.018
Cu	0.030
Hg	0.126
Mn	0.002
Mo	0.029
Pb	0.142
Se	0.364
V	0.006
Zn	0.890

existence of a significant correlation between each element concentration and the individual's size (snout-vent length) was verified for the species with at least four individual concentrations above the limit of detection (>LOD), through Pearson's simple linear correlation test for the normally distributed data set and Spearman's correlation for the non-normally distributed data set (very strong correlation: r=0.90–1.00 or r = -0.90 – -1.00; strong correlation: r=0.70–0.89 or r = -0.70 – -0.89; moderate correlation: r=0.40–0.69 or r = -0.40 – -0.69; weak correlation: r=0.10–0.39 or r = -0.10 – -0.39; negligible correlation: r=0.01–0.09 or r = -0.01 – -0.09) (Schober et al. 2018).

The existence of intraspecific differences in the concentrations of each element between the two sampling sites was verified by a *t*-test (normally distributed data) and Mann-Whitney (non-normally distributed data), for the species with at least four individual concentrations>LOD from each site. At each sampling site, the existence of interspecific differences in the concentrations of each element was examined using ANOVA (normally distributed data) or Kruskal-Wallis (non-normally distributed data) for species with at least four individual concentrations>LOD. Then, pairwise tests (Dunn or Tukey pairwise) were applied to identify pairs of species with significant differences in their concentrations. Mann-Whitney tests were applied for the elements with only two specific datasets per sampling site. A significance level of $p<0.5$ was adopted.

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Results

Means, standard deviations, and ranges of concentrations of all elements are presented in Table 3. Arsenic concentrations>LOD were found in 85 samples (93.4% of total samples). The highest and lowest As mean concentrations were detected in *O. rhombifer* and *E. crassus* from PU, respectively; the highest individual concentrations were found in *D. chaquensis* from MA, while the lowest were found in *E. notaeus* and *P. bifossatus* from MA. A total of 29 samples (31.8%) presented Ag concentrations>LOD; the highest and lowest concentrations were detected in *O. rhombifer* from PU and *P. bifossatus* from MA, respectively. Concentrations of Al>LOD were found in 60 samples (65.9%). The highest and lowest Al concentrations were found in *E. poecilogyrus* from MA and *C. durissus* from PU, respectively; the highest and lowest individual concentrations were determined in *E. poecilogyrus* and *E. notaeus* from MA, respectively. Ba levels>LOD were found in all samples. The highest and lowest Ba mean concentrations were found in *H. leopardinus* from MA and *O. rhombifer* from PU, respectively; the highest and lowest individual concentrations were detected in *E. typhlus* from PU and *E. notaeus* from MA, respectively. Cd levels>LOD were found in 57 samples (62.6%). The highest and lowest Cd mean concentrations were detected in *O. rhombifer* and *C. durissus* from PU, respectively; The highest and lowest individual concentrations were determined in *D. hypoconia* from MA and *B. mattogrossensis* from PU. Concentrations of Co>LOD were found only in two samples (2.2%), corresponding to *B. mattogrossensis* and *E. poecilogyrus* from MA, the highest value detected in the former. Levels of Cr>LOD were found in three samples (3.3%) corresponding to *O. rhombifer* and *C. durissus* from PU, and *H. leopardinus* from MA; the highest concentration was detected in *O. rhombifer*. Cu concentrations>LOD were found in 76 samples (83.5%). The highest and lowest Cu mean concentrations were detected in *E. poecilogyrus* from MA and PU, respectively; the highest and lowest individual concentrations were detected in *E. poecilogyrus* and *H. leopardinus* from MA, respectively.

Levels of Hg>LOD were found in eight samples (8.8%), with both the highest and lowest individual concentrations detected in *E. crassus* from PU. Mn concentrations>LOD were found in all samples. The highest and lowest mean Mn concentrations were found in *E. poecilogyrus* and *E. notaeus* from MA, respectively; the highest and lowest individual concentrations were detected in *P. nigra* from PU and *E. notaeus* from MA, respectively. Levels of Mo>LOD were detected in three samples (3.3%) corresponding to *B. mattogrossensis* and *E. poecilogyrus* from MA, and *C. durissus* from PU; the highest burden was determined in *E. poecilogyrus*. Concentrations of Pb>LOD were found in six samples (6.6%); the highest and lowest individual levels were determined in *E. crassus* and *P. patagoniensis* from PU, respectively. Burdens of Se>LOD were detected in 43 samples (47.2%). The highest and lowest Se concentrations were found in *E. poecilogyrus* and *H. leopardinus* from MA, respectively; the highest and lowest individual concentrations were both detected in individuals of *E. poecilogyrus* from MA. Levels of V>LOD were found in 48 samples (52.7%). The highest and lowest mean V levels were found in *E. poecilogyrus* from MA and *C. durissus* from PU, respectively; the highest and lowest individual levels were determined in *E. poecilogyrus* from MA and *B. mattogrossensis* from PU, respectively. Concentrations of Zn>LOD were found in all samples. The highest and lowest Zn mean concentrations were detected in *E. poecilogyrus* and *E. notaeus* from MA, respectively; the highest and lowest individual burdens were determined in *D. hyponia* from MA and *B. constrictor* from PU, respectively.

A significant ($p=0.043$) and very strong positive correlation was detected between As concentrations and size in *E. notaeus*; a significant ($p=0.040$) strong negative correlation was detected between Ba concentrations and size in *E. notaeus*. A significant ($p=0.031$) moderate negative correlation was observed between Mn concentrations and size in *H. leopardinus*. A significant ($p=0.027$) strong negative correlation was detected between V levels and size in *H. leopardinus*. A barely significant ($p=0.050$) strong correlation was detected between Cu levels and size in *C. durissus*. A barely significant ($p=0.057$) moderate negative correlation was observed between Cd concentrations and size in *H. leopardinus*. The results of element concentrations and size correlation tests are shown in Table 4.

Analyses on intraspecific differences between sampling sites were performed for *B. mattogrossensis* (As, Ba, Mn, Zn) and *E. poecilogyrus* (As, Al, Ba, Cu, Mn, Se, V, Zn). Significant differences ($p=0.040$) were found only for Mn in *E. poecilogyrus* (Table 5). Significant interspecific differences were found for Ba in PU ($p=0.045$) and for Mn ($p=0.030$) and Zn ($p=0.016$) in MA (Table 6). In PU, significant differences in element concentrations between species pairs were detected for Ba (*C. durissus* and *E. poecilogyrus* [$p=0.024$], *E. crassus* and *O. rhombifer* [$p=0.036$], and *E. poecilogyrus* and *O. rhombifer* [$p=0.012$] and Cd (*C. durissus* and *O. rhombifer* [$p=0.018$]). In MA, significant differences in element concentrations were found for Ba (*E. notaeus* and *E. poecilogyrus* [$p=0.026$], *E. notaeus* and *H. leopardinus* [$p=0.012$], *E. notaeus* and *M. bicolor* [$p=0.031$]), Mn (*E. notaeus* and *E. poecilogyrus* [$p=0.003$], *E. notaeus* and *H. leopardinus* [$p=0.003$]), and Zn (*E. notaeus* and *E. poecilogyrus* [$p=0.002$], *E. notaeus* and *H. leopardinus* [$p=0.001$], *E. notaeus* and *M. bicolor* [$p=0.022$]).

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Table 3 Concentrations (μg.g^{-1} dry weight; mean ± one standard deviation (range)) of arsenic (As), silver (Ag), aluminum (Al), barium (Ba), cadmium (Cd), Cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), manganese (Mn), molybdenum (Mo), lead (Pb), selenium (Se), vanadium (V), and zinc (Zn) in caudal tips of snakes from Brazilian Southern Pantanal

Species	Site (nº of samples)	As	Ag	Al	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Pb	Se	V	Zn
Eunectes notaeus	Miranda - Abobral (*n* = 5)	(<LOD −0.039)3	(<LOD −0.049)1	(<LOD −1.980)2	2.692 ± 3.056 (0.096–7.572)5	(<LOD −0.034)1	< LOD	< LOD	(<LOD −0.143)3	< LOD	0.704 ± 0.663 (0.048–1.479)5	< LOD	(<LOD −0.225)1	< LOD	(<LOD −0.010)1	6.796 ± 4.597 (1.82–13.72)5
	Paraguay - Urucum (*n* = 1)	0.032	0.022	< LOD	2.391	0.021	< LOD	< LOD	< LOD	< LOD	0.890	< LOD	< LOD	< LOD	< LOD	2.89
Boa constrictor	Paraguay - Urucum (*n* = 3)	(0.012–0.112)3	< LOD	(<LOD −2.620)1	(2.116–19.107)3	(<LOD −0.049)1	< LOD	< LOD	(0.075–0.448)3	< LOD	(0.190–12.003)3	< LOD	< LOD	(<LOD −1.486)2	< LOD	(11.54–76.60)3
Epicrates crassus	Paraguay - Urucum (*n* = 7)	0.017 ± 0.049 (<LOD −0.135)6	(<LOD −0.038)1	21.636 ± 38.924 (<LOD −91.16)5	18.405 ± 21.129 (1.159–62.516)7	0.034 ± 0.034 (<LOD −0.098)5	< LOD	< LOD	0.634 ± 0.639 (0.111–1.996)7	(<LOD −6.667)2	5.024 ± 5.576 (0.214–17.022)7	< LOD	(<LOD −0.385)1	1.606 ± 2.363 (<LOD −6.818)5	(<LOD −0.176)2	77.901 ± 75.712 (20.16–234.22)7
Bothrops mattogrossensis	Paraguay - Urucum (*n* = 4)	0.078 ± 0.059 (0.018–0.153)4	(<LOD −0.038)2	(<LOD −6.93)2	9.361 ± 9.464 (3.305–23.333)4	(<LOD −0.106)2	< LOD	< LOD	(<LOD −0.444)2	(<LOD −0.791)1	3.122 ± 2.101 (0.249–4.829)4	< LOD	< LOD	(<LOD −5.463)1	(<LOD −0.045)2	62.85 ± 72.933 (7.83–170.33)4
	Miranda - Abobral (*n* = 4)	0.087 ± 0.068 (0.034–0.188)4	(<LOD −0.057)2	(<LOD −172.95)2	10.103 ± 8.591 (2.347–22.182)4	0.041 ± 0.014 (0.025–0.061)4	(<LOD −0.102)1	< LOD	(<LOD −3.201)2	< LOD	6.567 ± 9.387 (0.20–20.523)4	(<LOD −0.038)1	< LOD	(<LOD −2.775)1	(<LOD −0.214)1	71.892 ± 104.559 (13.21–228.15)4
Crotalus durissus	Paraguay - Urucum (*n* = 8)	0.039 ± 0.031 (<LOD −0.089)7	(<LOD −0.041)1	13.21 ± 12.958 (<LOD −40.83)7	8.922 ± 15.560 (0.916–46.142)8	0.013 ± 0.021 (<LOD −0.063)4	< LOD	(<LOD −0.044)1	0.776 ± 0.622 (0.042–2.104)8	< LOD	3.445 ± 4.176 (0.375–13.124)8	(<LOD −0.029)1	(<LOD −0.227)1	1.069 ± 1.002 (<LOD −3.08)6	0.041 ± 0.052 (<LOD −0.153)5	40.63 ± 40.636 (12.19–125.77)8
Clelia clelia	Miranda - Abobral (*n* = 1)	0.063	< LOD	3.881	5.655	0.049	< LOD	< LOD	0.444	< LOD	5.704	< LOD	< LOD	< LOD	< LOD	24.793
Dipsas turgidus	Paraguay - Urucum (*n* = 1)	0.036	< LOD	< LOD	7.699	0.021	< LOD	< LOD	< LOD	< LOD	4,901	< LOD	< LOD	< LOD	< LOD	25.667
Drymarchon corais	Paraguay - Urucum (*n* = 1)	0.031	< LOD	3.881	11.104	< LOD	< LOD	< LOD	< LOD	< LOD	8.401	< LOD	< LOD	0.744	0.023	47.124
Dryophylax chaquensis	Miranda - Abobral (*n* = 1)	0.314	< LOD	5.301	130.578	0.423	< LOD	< LOD	0.398	< LOD	1.773	< LOD	< LOD	< LOD	0.122	197.934
Erythrolamprus poecilogyrus	Paraguay - Urucum (*n* = 8)	0.046 ± 0.031 (<LOD −0.085)7	(<LOD −0.069)1	25.896 ± 50.886 (<LOD −145.38)6	12.667 ± 5.887 (4.97–20.367)8	(<LOD −0.060)3	< LOD	< LOD	0.522 ± 0.253 (<LOD −2.100)7	< LOD	3.699 ± 3.077 (1.001–8.751)8	< LOD	< LOD	0.967 ± 1.073 (<LOD −3.169)5	0.063 ± 0.122 (<LOD −0.363)5	32.05 ± 17.762 (7.83–59.75)8
	Miranda - Abobral (*n* = 7)	0.105 ± 0.083 (0.024–0.249)7	(<LOD −0.080)2	163.2 ± 375.101 (<LOD −1,011.29)4	14.208 ± 14.688 (5.911–47.069)7	0.033 ± 0.058 (<LOD −0.164)4	(<LOD −0.067)1	< LOD	2.804 ± 5.329 (<LOD −14.652)6	< LOD	10.090 ± 7.987 (2.309–24.778)7	(<LOD −0.090)1	< LOD	3.373 ± 4.494 (<LOD −10.131)6	0.365 ± 0.705 (<LOD −1.94)5	126.468 ± 131.141 (7.78–316.26)7
Erythrolamprus typhlus	Paraguay - Urucum (*n* = 2)	(0.080–0.160)2	< LOD	(8.38–13.90)2	(4.648–582.450)2	(<LOD −0.046)1	< LOD	< LOD	(0.176–0.226)2	(<LOD −2.888)1	(5.551–8.601)2	< LOD	< LOD	(<LOD −1.845)1	(<LOD −0.089)1	(105.42–110.51)2
Helicops leopardinus	Miranda - Abobral (*n* = 14)	0.085 ± 0.078 (0.02–0.314)14	0.049 ± 0.049 (<LOD −0.147)8	41.903 ± 124.633 (<LOD −472.1)9	19.051 ± 24.241 (0.831–89.866)14	0.057 ± 0.048 (<LOD −0.16)11	< LOD	0.057 ± 0.048 (<LOD −0.16)11	0.795 ± 1.187 (<LOD −4.543)12	(0.717–1.440)2	9.938 ± 10.383 (0.303–34.541)14	< LOD	< LOD	0.373 ± 0.171 (<LOD −1.668)4	0.093 ± 0.062 (<LOD −0.884)8	60.029 ± 73.302 (14.66–300.55)14
Hydrodynastes gigas	Miranda - Abobral (*n* = 1)	0.022	< LOD	4.677	13.533	< LOD	< LOD	< LOD	0.186	< LOD	0.331	< LOD	< LOD	< LOD	0.032	54.187
Leptodeira pulchriceps	Miranda - Abobral (*n* = 1)	0.106	< LOD	< LOD	13.094	0.070	< LOD	< LOD	0.299	1.147	0.455	< LOD	< LOD	< LOD	0.044	78.651
Leptophis marginatus	Paraguay - Urucum (*n* = 1)	0.047	0.031	3.269	1.048	0.043	< LOD	< LOD	0.141	< LOD	13.574	< LOD	0.202	0.490	0.007	12.866
Lygophis dilepis	Paraguay - Urucum (*n* = 1)	0.064	< LOD	< LOD	15.170	0.025	< LOD	< LOD	0.153	0.719	13.904	< LOD	< LOD	< LOD	0.015	75.325

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Table 3 (continued)

Species	Site (nº of samples)	As	Ag	Al	Ba	Cd	Co	Cr	Cu	Hg	Mn	Mo	Pb	Se	V	Zn
Mussurana bicolor	Paraguay - Urucum (*n* = 2)	(0.048–0.095)2	(<LOD −0.067)1	(<LOD −21.58)1	(2.788–89.666)2	(0.046–0.069)2	< LOD	< LOD	(0.111–0.396)2	< LOD	(4.447–12.729)2	< LOD	< LOD	(<LOD −2.219)1	(<LOD −0.032)1	(13.87–100.85)2
	Miranda - Abobral (*n* = 4)	0.059 ± 0.043 (0.017–0.105)4	(<LOD −0.071)2	(<LOD −66.39)3	13.222 ± 9.269 (6.556–26.946)4	0.063 ± 0.056 (0.013–0.139)4	< LOD	< LOD	0.742 ± 0.833 (0.112–1.898)4	< LOD	5.870 ± 6.152 (1.399–14.899)4	< LOD	< LOD	(<LOD −0.757)1	(<LOD −0.205)3	35.127 ± 14.127 (14.29–48.25)4
Oxyrhopus rhombifer	Paraguay - Urucum (*n* = 4)	0.109 ± 0.113 (0.026–0.268)4	(<LOD −0.196)3	(<LOD −37.42)2	2.497 ± 1.484 (0.804–4.402)4	0.101 ± 0.076 (0.028–0.204)4	< LOD	2.817	(<LOD −0.163)3	< LOD	8.180 ± 7.480 (1.188–15.344)4	< LOD	< LOD	(<LOD −1.600)1	(<LOD −0.112)2	22.387 ± 18.082 (3.95–46.40)4
Palusophis bifossatus	Miranda - Abobral (*n* = 3)	(0.010–0.026)3	(<LOD −0.046)1	(1.77–8.32)3	(0.303–1.265)3	< LOD	< LOD	< LOD	(0.094–0.248)3	< LOD	(0.248–1.076)3	< LOD	< LOD	(<LOD −0.478)1	(<LOD −0.027)2	(4.86–14.16)3
Philodryas olfersii	Paraguay - Urucum (*n* = 3)	(<LOD −0.171)2	(<LOD −0.046)1	(8.48–194.99)3	(3.029–35.960)3	(<LOD −0.092)1	< LOD	< LOD	(0.296–2.825)3	< LOD	(5.511–13.458)3	< LOD	< LOD	(1.725–4.020)3	(<LOD −0.205)2	(34.34–149.02)3
Philodryas patagoniensis	Paraguay - Urucum (*n* = 2)	(<LOD −0.047)1	< LOD	(<LOD − 5.81)1	(1.415–17.695)2	(<LOD −0.042)1	< LOD	< LOD	(<LOD −0.166)1	< LOD	(15.101–41.825)2	< LOD	(<LOD −0.143)1	(<LOD −1.008)1	(<LOD −0.082)1	(14.73–55.71)2
	Miranda - Abobral (*n* = 1)	0.026	< LOD	8.70	6.485	0.020	< LOD	< LOD	0.076	< LOD	0.723	< LOD	< LOD	< LOD	< LOD	39.02
Pseudoboa nigra	Paraguay - Urucum (*n* = 1)	0.138	0.044	45.163	5.197	0.068	< LOD	< LOD	1.194	< LOD	41.825	< LOD	0.243	1.654	0.060	21.562

Discussion

Previous investigations conducted in the BSP have detected alarming levels of potentially toxic elements in tissues of the aquatic and semiaquatic vertebrates. These studies pointed to high burdens of Pb, Cd, and Al in tissues of fish (Viana et al. 2022; Quintela et al. 2024b), As in the Pantanal alligator *Caiman yacare* (Quintela et al. 2020), and Hg in the giant otter *Pteronura brasiliensis* (Soresini et al. 2021). The present study, the first to use snake tissues as matrices for ecotoxicological analysis in BSP, broadens the spectrum of the vertebrate taxa with indications for the status of contamination of biological compartments in the region, emphasizing the metals Al, Ba, and Mn.

It is worth noting that, despite shedding light on the magnitude of contamination levels, comparisons with other studies presented here are limited due to methodological differences, mainly regarding sample preparation (wet or dry samples). Notwithstanding, Burger et al. (2017) conducted experiments for the conversion of metal levels from wet weight (w.w.) to dry weight (d.w.) in muscle, liver, and kidney samples of the pine snake *Pituophis melanoleucus*, and found conversion factors ranging from 3.72 to 4.31. To provide better comparisons, conversion factors (CF) of Burger et al. (2017) were applied to the data of the comparative studies herein accessed.

Another point is the structural difference between the tail clip and the tissues analysed in comparative studies. Tail clips are mainly composed of muscle, skin, and caudal vertebrae, and Burger et al. (2006) considered this segment 'representative of whole body tissue'. Still, there are studies in which the positive correlations in metals and metalloids between tail clips and tissue samples of liver, muscle, heart, and kidney were verified (Hopkins et al. 2001; Wylie et al. 2009; Haskins et al. 2021), so that tail clips reflect the elements' bioaccumulation in these organs.

Metal and metalloid concentrations in snakes from the BSP

More than half of the samples contained Al levels > LOD. Aluminum is nonessential to the metabolism of reptiles (Grillitsch and Schiesari 2010). Among the non-function elements, this had the highest mean and maximum concentrations in samples of some species associated with both drier (*E. crassus*, *C. durissus*, *P. nigra*, *B. mattogrossensis*, *P. olfersii*) and wet habitats (*H. leopardinus*, *E. poecilogyrus*) of the two sampling sites. Thus, it is presumable that Al could represent an element of concern regarding its bioaccumulation in biological compartments BSP, also considering its highlighted occurrence in the local fish (Viana et al. 2022; Quintela et al. 2024a). Grillitsch and Schiesari (2010)

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pointed out endocrine disruption and neurotoxic disorders as potential adverse effects of Al in reptiles, but to our best knowledge, there is no information specific to snakes. Few studies have evaluated the levels of Al in free-ranging snakes inhabiting environments under anthropogenic disturbance. Wylie et al. (2009) found mean and maximum concentrations of 23.8 and 375 $\mu g.g^{-1}$ d.w. in liver samples of the aquatic giant garter snake (*Thamnophis gigas*) from agricultural areas/wetlands mosaics in northern California (Wylie et al. 2009). Liver, kidney, and muscle samples of terrestrial saw-scaled viper (*Echis pyramidum*) and Kenyan sand boa (*Eryx colubrinus*) from soil-contaminated El-Faiy desert, Egypt, showed Al mean levels between 48.06 and 132.8 $\mu g.g^{-1}$ (Sleem et al. 2019; not specified if d.w. or w.w.). Muscle of banded water snake (*Nerodia fasciata*), brown water snake (*N. taxispilota*), and cottonmouth (*Akistrodon piscivorous*) from waterbodies contaminated by nuclear waste in Savannah River, South Carolina, USA, presented meam Al concentrations between 93 and 431 $\mu g.g^{-1}$ w.w (Burger et al. 2006; 25 to 116 $\mu g.g^{-1}$ d.w when applying the CF of Burger et al. 2017]). The burdens found in many of the species analyzed in our study fell within the range reported in those previous investigations. The high concentration of 1,011 $\mu g.g^{-1}$ determined in an *E. poecilogyrus* from MA deserves mention. Therefore, Al represents an element of major concern for the BSP wildlife.

Aluminum comprises about 8% of the Earth's crust and is mobilized to the environment by both natural and anthropogenic processes. One major way of anthropogenic Al mobilization is rock fragmentation through mining processes (WHO 1997). Lithogeochemical analyses of clastic and chemical sedimentary rocks of Urucum and Santa Cruz massifs revealed high levels of Al_2O_3 in these matrices (Viehmann et al. 2016; Saldanha 2017). Clastic and chemical sedimentary layers are associated with "pure" iron and manganese ores in the Urucum and Santa Cruz formations (Viehmann et al. 2016). Therefore, during the extraction of Fe and Mn ore, a considerable amount of Al-rich clastic/chemical rocks is fragmented, which contributes to the release of Al to terrestrial and aquatic compartments of the region. Although mining could be considered a potential source, the Al environmental enrichment may also occur naturally through erosion and dissolution of sedimentary rocks.

Barium was highly important in our analyses, present in levels > LOD in all samples. There is no evidence for the physiological role of Ba in reptiles, and no information on the potential deleterious effects of the element in any representative of this vertebrate group is available (Grillitsch and Schiesari 2010). When excluding the essential Zn, Ba represented the element with higher individual concentrations in *B. constrictor*, *B. mattogrossensis*, *C. durissus*, *E. typhlus*, and *M. bicolor* from PU and *E. notaeus* from MA. Scarce data on Ba concentrations in snakes from impacted areas indicated ranges of 1.39 to 7.97 $\mu g.g^{-1}$ .d.w. in liver of *T. gigas* from northern California (Wylie et al. 2009), 25 to 64 $\mu g.g^{-1}$ in muscle of *N. fasciata*, *N. taxispilota*, and *A. piscivorous* from Savannah River (Burger et al. 2006; 6.7 to 17.2 $\mu g.g^{-1}$ .d.w. when applying the CF of Burger et al. 2017]), and 0.025 to 1.40 $\mu g.g^{-1}$ w.w. in liver of western tiger snake *Notechis scutatus occidentalis* from urban and periurban lakes of Perth, Australia (Lettoof et al. 2020a; 0.006 to 0.35 $\mu g.g^{-1}$ .d.w. when applying the CF of Burger et al. 2017]). The levels found in our samples are within the range reported for these species, but the burden 582.5 $\mu g.g^{-1}$ detected in an *E. typhlus* individual is noteworthy. Barium is a little representative element in Earth's crust, comprising only 0.05% of its composition (ATSDR 2007). In ferriferous and sedimentary rocks of the Urucum and Santa Cruz formations, Ba comprised one of the most representative trace elements, along with strontium and titanium (Viehmann et al. 2016; Saldanha 2017). Rocky matrices, however, may represent the primary source of Ba environmental enrichment in the study area, and the mobilization of the element may be greatly enhanced by intensive mining activity.

Manganese is an essential element, but high levels in reptiles are associated with endocrine disruption and neurotoxic disorders (Grillitsch and Schiesari 2010). Maximum Mn concentrations in *H. leopardinus*, *P. patagoniensis* from PU, and *E. poecilogyrus* from MA were above the highest levels reported in previous analyses on snake tissues from impacted regions, including blood, muscle, and liver of water snakes *Nerodia fasciata* and *N. sipedon* from urban/suburban and rural areas of New Jersey, Tennessee, and South Carolina (20.44 $\mu g.g^{-1}$ w.w.; Burger et al. 2007; 5.4 $\mu g.g^{-1}$ d.w when applying the CF of Burger et al. 2017]), and the already mentioned studies which examined *T. gigas* (19.7 $\mu g.g^{-1}$ d.w.; Wylie et al. 2009)d *scutatus occidentalis* (0.94 $\mu g.g^{-1}$ w.w.; Lettoof et al. 2020a; 0.28 $\mu g.g^{-1}$ d.w when applying the CF of Burger et al. 2017]). The Mn levels detected in *H. leopardinus*, *P. patagoniensis*, and *E. poecilogyrus* were also higher than those found in muscle, liver, and kidney samples of a snake assemblage from Lagoa Santa Karst, which is highly impacted by mining, industry, and urbanization (Quintela et al. 2024b). Manganese is a principal constituent of the Urucum-Santa Cruz complex's lithology (Viehmann et al. 2016), and the Mn ore is a major mineral resource exploited in the region (Brito 2011). The high Mn burdens detected in our samples, therefore, may reflect the high input of the element into the environmental compartments of the study area via mining fragmentation. Still, the role of erosion and weathering of rocks should not be ruled out.

Due to their toxicity, As, Cd, Hg, and Pb are non-essential elements of outstanding importance (Grillitsch and

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Table 4 Pearson and spearman values for the correlation between body size (snout-vent length) and element concentrations in snakes from the Brazilian Southern Pantanal

Species	As	Ag	Al	Ba	Cd	Cu	Mn	Se	V	Zn
Eunectes notaeus	$r=0.956$ **$p=0.043$**	-	-	$r=-0.830$ **$p=0.040$**	-	-	$r=-0.647$ $p=0.164$	-	-	$r=-0.102$ $p=0.846$
Epicrates crassus	$r=-0.759$ $p=0.141$	-	$r=-0.753$ $p=0.141$	$rs=-0.178$ $p=0.713$	$r=-0.381$ $p=0.526$	$rs=-0.428$ $p=0.353$	$rs=-0.535$ $p=0.235$	$rs=-0.600$ $p=0.291$	-	$rs=-0.107$ $p=0.839$
Bothrops matogrossensis	$r=-0.458$ $p=0.253$	$r=-0.275$ $p=0.724$	$rs=-0.400$ $p=0.583$	$rs=-0.190$ $p=0.664$	$r=-0.369$ $p=0.470$	$rs=-0.200$ $p=0.833$	$rs=0.095$ $p=0.816$	-	-	$r=-0.500$ $p=0.216$
Crotalus durissus	$r = -0.399$ $p=0.374$	-	$r=0.205$ $p=0.659$	$rs=-0.333$ $p=0.427$	$rs=0.632$ $p=0.500$	$r=0.703$ $p=0.051$	$rs=0.109$ $p=0.796$	$rs=-0.371$ $p=0.497$	$r=-0.502$ $p=0.388$	$rs=-0.500$ $p=0.216$
Erythrolamprus poecilogyrus	$rs=-0.210$ $p=0.470$	-	$rs=0.164$ $p=0.650$	$rs=-0.008$ $p=0.974$	$rs=-0.468$ $p=0.289$	$rs=0.035$ $p=0.907$	$rs=0.023$ $p=0.934$	$rs=0.246$ $p=0.465$	$rs=-0.024$ $p=0.946$	$rs=0.017$ $p=0.949$
Helicops leopardinus	$rs=-0.340$ $p=0.233$	$rs=-0.119$ $p=0.793$	$rs=-0.266$ $p=0.493$	$rs=-0.340$ $p=0.233$	$r=-0.586$ $p=0.057$	$rs=-0.538$ $p=0.070$	$rs=-0.573$ **$p=0.031$**	$r=-0.177$ $p=0.822$	$rs=-0.785$ **$p=0.027$**	$rs=-0.481$ $p=0.081$
Mussurana bicolor	$r=-0.005$ $p=0.991$	-	$r=-0.505$ $p=0.494$	$r=0.142$ $p=0.758$	$r=-0.477$ $p=0.338$	$rs=-0.028$ $p=0.959$	$r=0.148$ $p=0.778$	-	$r=-0.550$ $p=0.449$	$r=0.616$ $p=0.192$
Oxyrhopus rhombifer	$r=0.675$ $p=0.324$	-	-	$r=-0.496$ $p=0.503$	$r=0.767$ $p=0.232$	-	$r=0.406$ $p=0.593$	-	-	$r=0.025$ $p=0.974$

Significant correlations are marked in bold; *p* indicates the probability of significance; *r* indicates the value of pearson's correlation; *Rs* indicates the value of spearman's correlation

Table 5 Results of tests for intraspecific differences in element concentrations in the snakes' caudal tips between the two sampling sites in the Brazilian Southern Pantanal

Species	As	Al	Ba	Cu	Mn	Se	V	Zn
Bothrops matogrossensis	$t=0.197$ $p=0.830$	-	$t=0.116$ $p=0.816$	-	$U=7$ $p=0.885$	-	-	$U=7$ $p=0.788$
Erythrolamprus poecilogyrus	$t=1.571$ $p=0.135$	$U=7$ $p=0.171$	$U=24$ $p=0.694$	$U=12$ $p=0.234$	$U=10$ **$p=0.040$**	$U=13$ $p=0.273$	$U=9$ $p=0.626$	$U=20$ $p=0.396$

Significant correlations are marked in bold; *p* indicates the probability of significance; *t* indicates the value of *t*-tests; *U* indicates the value of Mann-Whitney tests

Table 6 Results of tests for interspecific differences in element concentrations in the snakes' caudal tips between the two sampling sites in the Brazilian Southern Pantanal

Species	As	Al	Ba	Cd	Cu	Mn	Se	V	Zn
Paraguay - Urucum	$F=1.025$ $p=0.419$	$H=0.911$ $p=0.634$	$H=9.696$ **$p=0.045$**	$H=5.57$ $p=0.061$	$H=0.956$ $p=0.619$	$H=1.508$ $p=0.852$	$H=3.851$ $p=0.145$	$H=3.851$ $p=0.888$	$H=4.087$ $p=0.392$
Miranda - Abobral	$H=0.936$ $p=0.816$	$H=8$ $p=0.148$	$H=7.362$ $p=0.117$	$H=1.967$ $p=0.578$	$H=0.347$ $p=0.840$	$H=10.66$ **$p=0.030$**	$H=12$ $p=0.915$	$H=12$ $p=0.284$	$H=12.18$ **$p=0.016$**

Significant correlations are marked in bold; *p* indicates the probability of significance; *F* indicates the value of ANOVA tests; *U* indicates the value of Kruskal-Wallis tests

Schiesari 2010). The levels of As detected in our study were markedly lower than those considered alarming in previous studies (e.g., Hopkins et al. 1999; Wylie et al. 2009; Quintela et al. 2024). Despite low concentrations, As was detected in more than 90% of our samples, indicating that this element could be widespread in the local ophidiofauna. Arsenic was found in high frequency and higher concentrations in other vertebrate groups in BSP, which includes demersal and benthopelagic fish (Viana et al. 2022) and the Pantanal alligator *Caiman yacare* (Quintela et al. 2020). Arsenic, therefore, should be considered a major concern for the BSP wildlife, taking into account that exposure to this element could be severely hazardous to organisms (Jamwal et al. 2023).

Levels of Cd > LOD were found in more than half of our samples. Maximum levels of Cd found in *E. poecilogyrus* and *H. leopardinus* from MA, and *O. rhombifer* from PU were similar to the highest means and maximum concentrations found in water-related snakes from polluted sites such as wetlands and rivers of New Jersey, Tennessee, and South Carolina (Campbell et al. 2001; Burger et al. 2007), northern California (Wylie et al. 2009), and Colombia (Hurtado-Morales et al. 2022). The Cd levels detected in our samples were, therefore, markedly lower than those found in both aquatic and terrestrial snakes from Minas Gerais, a region severely impacted by mining activity in southeastern Brazil (Quintela et al. 2024b; Toledo et al. 2024), which may imply

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that snakes from BSP are not severely contaminated. In our study area, Cd was found in comparatively lower concentrations in *C. yacare* (Quintela et al. 2020) and higher levels in fish (Viana et al. 2022; Quintela et al. 2024a). Due to its presumably ample distribution, studies using other bioindicators and the continuous monitoring of Cd levels in BSP are recommended.

Although infrequent in our samples (only eight individuals presented levels > LOD), Hg concentrations detected in BSP were within the ranges reported in snakes from areas known to be contaminated by the element (Burger et al. 2005, 2007; Campbell et al. 2001; Drewett et al. 2013; Haskins et al. 2021; Quintela et al. 2024b). Mercury is certainly the most investigated element in the BSP biota. Mercury was absent in fish of distinct habitats from the Miranda River (Quintela et al. 2024a). *Caiman yacare* from the Paraguay River and Nhecolândia lakes presented Hg levels lower than the burdens recovery in our study (Vieira et al. 2011). Still, levels in giant otters from Miranda and Rio Negro basins were similar to or higher than those in our samples (Fonseca et al. 2005; Soresini et al. 2021). The occurrence of Hg in tissues of the giant otter was attributed by Soresini et al. (2021) to long-distance dispersal from gold mines located in the Northern Pantanal, about 470 km from the Miranda River and Rio Negro stretches sampled by the authors. In this trend, snakes from the Southern Pantanal may also have been bioaccumulating Hg from allochthonous sources. Lead was even less represented in our samples, detected in low concentrations > LOD in only six individuals. The Pb burdens found in our samples were similar to those found in snakes inhabiting pristine and rural areas (Wylie et al. 2009; Burger et al. 2017), but lower than concentrations detected in snakes from periurban polluted sites and areas impacted by intensive mining (Hurtado-Morales et al. 2022; Quintela et al. 2024b; Toledo et al. 2024). Lead levels in snakes were lower than concentrations determined in fish (Quintela et al. 2024a) and *C. yacare* (Quintela et al. 2020). Therefore, Pb does not currently represent a major concern for BSP snakes, but the monitoring of this element is recommended considering its high toxicity and occurrence in three vertebrate groups investigated so far in the region (Quintela et al. 2020, 2024a; present study).

Silver is a non-essential heavy metal of high ecotoxicological relevance (Grillitsch and Schiesari 2010), and studies related to its effect on snakes are limited; however, Ag content in snake tissues was assessed for *N. s. occidentalis* from periurban wetlands of Perth (Leetoof et al. 2020a). The Ag concentrations in BSP snakes were higher than those found by Leetoof et al. (2020a), but still markedly lower than levels of other heavy metals considered concerning in our study (e.g., Mn, Cu). Further, Ag levels > LOD were not determined in fish from the Miranda River (Quintela et al. 2024a), which suggests that this element currently does not represent a threat, at least to ectotherm vertebrates in the Pantanal territory between Paraguay and the middle Miranda rivers. However, due to its high toxicity and considerable frequency in snakes (32% of the samples with levels > LOD), the monitoring of Ag in BSP wildlife is recommended.

Selenium, V, and Zn are essential elements in vertebrate metabolism (Anke et al. 2000; Grillitsch and Schiesari 2010) and were well-representative in our sample. Selenium was well-accessed in previous studies on levels of inorganic contaminants in free-range snakes. The concentrations found in *E. crassus*, *B. mattogrossensis* from PU, and *E. poecilogyrus* from MA were remarkable and comparable to the levels determined in snakes from impacted wetlands of Northern California (Wylie et al. 2009) and periurban Perth (Leetoof et al. 2020a), but conspicuously lower than coal ash-polluted swamps of Savannah River Site (Hopkins et al. 1999). Vanadium was scarcely investigated in ecotoxicological approaches on snakes. Our study found low levels, except for the maximum level in *E. poecilogyrus* from MA. The concentrations in our samples were lower than those found in *T. gigas* from Northern California (Wylie et al. 2009), but similar to the burdens detected in semiaquatic snakes from the Savannah River Site (Burger et al. 2006). Zinc was present in elevated concentrations, emphasizing the highest levels in *E. crassus*, *H. leopardinus*, *B. mattogrossensis*, and *E. poecilogyrus* from MA. Those levels were higher when compared to the levels found in tissues from polluted ecosystems (Burger et al. 2006; Sleem et al. 2019; Quintela et al. 2024b; Toledo et al. 2024). Therefore, it is important to highlight that snakes are apparently tolerant to high Zn burdens, considering Zn plasmatic levels from 5 to 50 times higher than those of birds and mammals, and the higher concentrations even when compared to other reptiles such as crocodilians, turtles, and lizards (Lance et al. 1995).

Despite being metabolically essential, high levels of Cu and Cr in reptiles can promote immune, hepatic, and renal disorders, the development of cancer, and reproductive dysfunction (Cr) (Grillitsch and Schiesari 2010). Mean Cu concentrations in *C. durissus*, *E. poecilogyrus*, *H. leopardinus*, and *P. olfersii* were similar to mean levels in snakes from areas exposed to chemical disturbance worldwide (Burger et al. 2006; Albrecht et al. 2007; Sleem et al. 2019; Leetoof et al. 2021; Quintela et al. 2024b; Toledo et al. 2024). Chromium, in turn, was recovered in very low levels except in an individual of *O. rhombifer*. Concentrations > LOD of the essential metals Co and Mo were also scarce in our samples.

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The relationship between snake size and metal and metalloid concentrations

Significant relationships between body size and element concentrations in the present study were detected only for the semiaquatic species *E. notaeus* and *H. leopardinus*. In this context, our hypothesis that intraspecific concentrations would be higher in larger individuals was corroborated only for As in *E. notaeus.* There is no evidence of As increased bioaccumulation along the individual's development in snakes. In contrast, other authors who examined the correlation between As concentrations and body size (Burger et al. 2007, 2017; Quintela et al. 2019, 2024b) found no evidence of increased concentration in larger individuals. Hopkins et al. (1999) used body mass as a measurement of individual development and also found no positive correlation between this variable and As concentrations in liver samples of *N. fasciata*. The patterns of As bioaccumulation must be further investigated, but the contrasting results obtained by the few studies so far indicate that specific intrinsic factors may be involved in the balance between As absorption, metabolization, and excretion.

The negative correlation between body length and Ba levels in *E. notaeus* suggests the existence of mechanisms of excretion of this metal along the individual's development. This may also occur in relation to Mn and V, whose levels were negatively related to the length of *H. leopardinus* individuals. We did not find any study that had examined the relationships between Ba and V and measurable parameters of aging in snakes. In relation to Mn, Burger et al. (2007) detected a significant weak negative correlation between the concentrations of this metal in skin samples and the body size of representatives of the genus *Nerodia*. Quintela et al. (2024b), in turn, found no significant correlations between body length and Mn burdens in the muscle, liver, and kidney of semiaquatic *Helicops modestus* and four terrestrial species, including a considerable sample of the rattlesnake *C. durissus*, a species also investigated in our study.

Silver, Al, Cd, Cu, Se, and Zn were the other elements tested for the relationships between individual development and bioaccumulation patterns in our work, and all lacked significant positive or negative correlations. Previous studies also found no significant correlations between Cd levels and snakes' body length (Albrecht et al. 2007; Burger et al. 2007, 2017; Quintela et al. 2024b) and body mass (Hopkins et al. 1999; Hurtado-Morales et al. 2022). Levels of Cu in whole-body samples of semiaquatic *Thamnophis saurita* were negatively correlated with body length (Albrecht et al. 2007). Species of *Nerodia* lacked significant correlations between Se concentrations in skin and individuals' length (Burger et al. 2007). None of the species sampled by Quintela et al. (2024b) showed evidence of interrelationships between size and the burdens of Cu and Zn. No reference to the bioaccumulation of Ag and Al during the development of snake individuals in wild populations was found.

The pattern of lower concentration of a given element in larger individuals of a population submitted to a balanced spatiotemporal condition of exposure indicates the occurrence of metabolic mechanisms of excretion of the element. Hopkins et al. (2001) treated *N. fasciata* with As, Cd, Cu, Se, and V contaminated prey and found higher levels of all these elements in shed skin when compared to blood and tail clips. Skin shedding, therefore, is a functional method for elimination of elements examined by Hopkins et al. (2001), and this property may also extend to Ba, Mn (negatively correlated with *E. notaeus* and *H. leopardinus* in our study), and other metals and metalloids. Another way that snakes eliminate metals and metalloids (females) is maternal transfer, a process in which elements are allocated to follicles, eggs, and embryos. There is evidence of vertical transfer of As, Mn, Hg, Mo, and Zn in viviparous *N. S. occidentalis* (Lettoof et al. 2020b), Hg in viviparous *N. sipedon* (Chin et al. 2013; Cusaac et al. 2016), and Se in oviparous *Boaedon fuliginosus* (Hopkins et al. 2004). The mechanism of excretion via maternal transfer, therefore, can also occur in the species addressed in our study.

Intra- and interspecific differences in metal and metalloid concentrations

Due to the limited samples, it was possible to examine only two species for intraspecific differences in element concentrations between PU and MA. From these, the only significant difference was detected for the Mn in *E. poecilogyrus*, whose concentrations were higher in MA. When considering the means and maximum levels, higher values occurred in samples from MA for both *E. poecilogyrus* and *B. mattogrossensis*, except for the maximum Ba individual level in the latter. These results were counter to our expectations of more elevated burdens in individuals from the Paraguay-Urucum, the sampling site apparently under a higher influence of pollution sources generated by anthropogenic disturbance. The human activities of intensive mining, fluvial and road transport of ores, and urbanization in Paraguay-Urucum are plainly in contrast with the predominantly livestock activity and the extensive areas of preserved ecosystems in the Miranda-Abobral stretch. Similar to our study, the burdens of As, Cd, and Pb in caudal crests of *C. yacare* population from Miranda-Abobral were higher than those detected in the population of Poconé, a region under severe impact of gold mining and agriculture (Quintela et al. 2020). These findings, in addition to other studies that had identified high Hg levels in organisms from sites located hundreds of kilometers from Hg pollution sources

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(Vieira et al. 2011; Soresini et al. 2021), suggest that metals and metalloids as contaminants are largely diffused in Pantanal. Thus, even territories characterized by low-impact human activities and a high degree of ecosystem conservation in the biome are subjected to environmental contamination induced by external pollutant sources.

When analyzing the aspects of metal and metalloid pollution in biotic and abiotic compartments of Pantanal, two main factors should be considered: (1) dispersion of elements mobilized and introduced by local sources (e.g., mining activity at Urucum and Santa Cruz massifs, road traffic, fluvial ore transport); (2) dispersion of elements from external sources (e.g., agricultural and silvicultural areas, industries) located in the surrounding plateaus of Mato Grosso and Mato Grosso do Sul. The lower altitude and the configuration of rivers that originate in plateaus and flow to the floodplain are geomorphological characteristics favorable to the dispersion of allochthonous pollutants to the Pantanal. The plateaus surrounding the Pantanal are subjected to severe chemical disturbance due to pesticides largely used in agriculture, mining, industries, and increasing urbanization (Alho and Vieira 1997; Viana et al. 2023; Quintela et al. 2024a). Currently, large portions of the plateau of Mato Grosso do Sul, originally covered by the Cerrado, (Brazilian savannah) are being converted to silviculture. Metals and metalloids present in pesticides, wood preservatives, industrial processes, urban waste, and other sources are accumulated in the soil and water compartments of plateau areas by runoff, leaching, and volatilization-precipitation, and enter the Pantanal lowlands via catchments. The annual flooding cyclic events diffuse contaminants to the Pantanal plains (Alho and Vieira 1997; Dores 2016). Flooding and dynamics of superficial water may represent a factor associated with a trend for homogenized conditions of organisms' exposure to metals and metalloids in BSP, where the local characteristics of land use are not solely related to the status of local environmental contamination.

Ingesting contaminated prey is the main route of metal and metalloid bioaccumulation in snakes (Hopkins et al. 2001). Our study, however, found no evidence of higher element burdens in species feeding on higher trophic levels. Still, it is presumable that significant interspecific differences in Ba, Mn, and Zn, and a barely significant difference in Cd levels, may be a reflection of distinct feeding habits, considering that distinct prey accumulate metals and metalloids at different rates due to differences in exposure conditions (Hopkins et al. 2001; Albrecht et al. 2007; Quintela et al. 2024b). In the pairwise comparisons of samples from PU, significant differences between Ba, Mn, and Zn concentrations in the yellow anaconda *E. notaeus* and the considerably smaller species *E. poecilogyrus*, *H. leopardinus*, and *M. bicolor* were remarkable. The yellow anaconda is the second-largest snake in the Brazilian Pantanal (after only the green anaconda *Eunectes murinus*), achieving up to 4 m in length (Strüssmann and Sazima 1993). From the species herein examined, this is the one that feeds on the highest trophic levels, preying on large animals such as adult caimans and large semiaquatic snakes (including canibalism) (Strüssmann and Sazima 1993; Barros et al. 2011; Camera and Prudente 2023). Nonetheless, Ba, Mn, and Zn concentrations were significantly lower in *E. notaeus* in relation to *E. poecilogyrus*, *H. leopardinus*, and *M. bicolor*, indicating that higher trophic levels do not imply higher bioaccumulation of these elements in the local snake assemblage. Still, there is no evidence of Ba, Mn, and Zn biomagnification in both aquatic and terrestrial systems (ATSDR 2007, 2012; Grillitsch and Schiesari 2010; Mann et al. 2011; Cardwell et al. 2013). Regarding samples from PU, significant differences were found in Ba concentrations between wetland-dwelling and anurophagous *E. poecilogyrus* and terrestrial *C. durissus* and *O. rhombifer*, both predators of small mammals and lizards (Sawaya et al. 2008; Gaiarsa et al. 2013).

Cadmium levels were significantly higher in *O. rhombifer* than in *C. durissus*. Interestingly, the species present similar diets, feeding on small mammals and lizards. Consumption of lizards by *C. durissus* is occasional, while this represents a main prey of *O. rhombifer* (Sawaya et al. 2008; Gaiarsa et al. 2013). Still, the two species were found in syntopy in the seasonal forests of PU, and small mammals are supposed to be a dietary component under similar exposure to metals and metalloids in the diet of both species. Lizards may bioaccumulate Cd at higher rates than small mammals, reflecting the more elevated burdens in *O. rhombifer*. The low sample size, therefore, does not allow for further conclusions. A larger sample associated with the use of stable isotopes indicative of trophic positions would be more enlightening about the relationship between diet and bioaccumulation of metals and metalloids in snakes and other representatives of the rich reptilian fauna of Pantanal.

Conclusion

The present study adds to the knowledge on the contamination of the wildlife of Pantanal by potentially toxic metals and metalloids. The Southern Pantanal is facing severe anthropogenic impacts, and chemical disturbances must be considered of major relevance, as a growing number of studies have detected the contamination in tissues of distinct vertebrate groups (Fonseca et al. 2005; Soresini et al. 2021; Viana et al. 2022, 2023; Quintela et al. 2020, 2024a; present study). The high levels of Al, Ba, and Mn in snakes reflect the local geological background and elements from rocky matrices through intensive mining. Currently, mining

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activity in Urucum and other massifs of the Southern Pantanal is a vector of socioenvironmental impacts (Tomas et al. 2010; EJAtlas 2023), and our study suggests that this could be extended to contamination of wildlife by the elements abundantly present in the composition of the explored rock formations. Intensive agriculture, the increasing silviculture, and the urbanization of the adjacent plateau should also be considered sources of allochthonous pollutants for BSP wildlife.

Data availability

Data will be available upon request to the corresponding author.

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Author contributions Study conception and manuscript writing were performed by FMQ; Field sampling was performed by FMQ and VM; Laboratory procedures were provided by DG; Substantial contributions to the text were made by DG, DJS, and UG.

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Declarations

Conflict of interest The authors declare no competing interests.

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