



Mercury content in the fur of jaguars (*Panthera onca*) from two areas under different levels of gold mining impact in the Brazilian Pantanal

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ABSTRACT

The Pantanal is the largest inland wetland in the world and is under increasing anthropogenic threats, including long-term regionally intensive gold mining practices. Gold mining activities are known to cause the release of harmful pollutants such as mercury (Hg) to the surrounding environment. Jaguars (*Panthera onca* (Linnaeus, 1758)) are apex predators, and therefore show great potential to accumulate Hg by biomagnification. We hypothesize that total Hg content in the fur of jaguars from two sites within the Brazilian Pantanal would be significantly different as a function of distance from active gold mining operations. The Hg content was determined by fluorescence spectrometry. The mean \pm SD Hg content in jaguars from the study site influenced by gold mining (SB) was compared to jaguars sampled in the area free of gold mining activities (CA) using a one-way ANOVA. The mean Hg content in jaguars from SB ($673.0 \pm 916.8 \mu\text{g g}^{-1}$) is significantly different from jaguars sampled in CA ($29.7 \pm 23.3 \mu\text{g g}^{-1}$), $p = 0.03$. The maximum recorded content of Hg was $2,010.4 \pm 150.5 \mu\text{g g}^{-1}$, highest level ever recorded in a wild animal. The data indicate that Hg is an important threat to jaguars within at-risk regions of the Pantanal.

Key words: biomagnification, heavy metals, mammal, wetland.

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INTRODUCTION

Mercury (Hg) is a rare element found in deposits within the earth's crust, with significant environmental distribution due to anthropogenic activities such as mining (Ehrlich and Newman 2008, Kirby et al. 2013). The wide-spread use of Hg in intensive gold mining practices transforms the surrounding environments into pollution hotspots (Vieira et al. 2011). Most forms of Hg are toxic to biotic life (Lacerda and Fitzgerald 2001, Risher et al. 2002), and methylmercury (MeHg) is neurotoxic (Leady and Gottgens 2001, Risher et al. 2002, Akira et al. 2004). For organisms, the primary route of entry of organic Hg is the ingestion of MeHg-contaminated food (Callil and Junk 2001, Risher et al. 2002), although the ingestion of MeHg-contaminated water may be another significant contributor.

The presence of Hg in the environment affects species within the food chain through the processes of bioaccumulation and biomagnification, as the pollutants accumulate in tissues of organisms and are made biologically available (Gutleb et al. 1997, Callil and Junk 2001). MeHg is typically found in higher concentrations in adult fishes and in larger, long-lived predators of aquatic organisms (Callil and Junk 2001, Risher et al. 2002, Chan et al. 2003, Fonseca et al. 2005). In mammals, MeHg is rapidly absorbed through the gastrointestinal tract and over time is redistributed to other organs and body parts, including fur (Wobeser and Swift 1976, Wobeser et al. 1976, Akira et al. 2004, Nuttall 2006). High concentrations of Hg has been documented in several free-living mammalian species, including: *Lutra canadensis* (Wren 1985, Wren et al. 1986, Halbrook et al. 1994, Evans et al. 1998, 2000, Yates et al. 2005, Sleeman et al. 2010); *Lutra lutra* (Mason 1988, Mason and Madsen 1992, Hyvärinen et al. 2003); *Mustela vison* (Wobeser et al. 1976, Evans et al. 2000, Yates et al. 2005); *Neovison vison* (Wobeser et al. 1976, Basu et al.

2007); *Puma concolor coryi* (Roelke 1990, Roelke et al. 1991, Dunbar 1994, Barron et al. 2004); and *Pteronura braziliensis* (Gutleb et al. 1997, Fonseca et al. 2005).

The jaguar (*Panthera onca*) is the largest felid in the Neotropics, and the third largest worldwide (Hoogesteijn and Mondolfi 1992, Soisalo and Cavalcanti 2006, Campos Neto et al. 2011). Categorized as a Near Threatened species by the IUCN (Caso et al. 2008), the jaguar has historically ranged from the southwestern US through northern Argentina, with current range reduced by nearly 55% (Sanderson et al. 2002, Caso et al. 2008). The Pantanal population is considered one of the remaining strongholds of wild jaguars, whose density ranges from 6.5 – 6.6 individuals per 100 km² (Soisalo and Cavalcanti 2006). The authors point out yet, that a large portion of the biome faces augmented anthropogenic pressures such as ranching, agriculture and development.

Previous studies have assessed the large-scale environmental impacts of Hg in a variety of ecosystems, including in wetlands such as the Brazilian Pantanal (Hylander et al. 1994, 2000a, b, Callil and Junk 2001, Lacerda and Fitzgerald 2001, Leady and Gottgens 2001, Fonseca et al. 2005, Vieira et al. 2011, Alho and Sabino 2012). About 200 years before this present study, metallic Hg was introduced in the town of Poconé in the northern Pantanal (Mato Grosso state, Brazil), associated with gold mining activities to separate gold from ore (Callil and Junk 2001). Prior research indicates that the floral and faunal diversity of the Pantanal is severely threatened by anthropogenic activities, including Hg emissions from gold mining areas (Alho et al. 1988, Lacerda and Salomons 1998, Hylander et al. 2000b, Callil and Junk 2001, Leady and Gottgens 2001, Fonseca et al. 2005, Alho and Sabino 2012).

The prey base of jaguars includes over 85 species (Weckel et al. 2006), and includes aquatic mammalian, crocodylian, and fish species (Hayward

et al. 2016). As apex predators, jaguars are at risk of accumulating high concentrations of pollutants that are biomagnified through the food chain. To our knowledge, only one other study examined Hg content in jaguars; concentrations from the teeth of two deceased individuals in Colombia were analyzed and found that concentrations were significantly lower than the minimum legal threshold (Racero-Casarrubia et al. 2012).

In the Brazilian Pantanal, jaguars may experience higher levels of Hg bioaccumulation and biomagnification as a result of extensive gold mining activities. Considering the toxic properties of Hg and its pervasive use at the upstream gold mining areas in the Pantanal floodplain, we hypothesize that Hg levels will be significantly higher in jaguars residing in the region of nearest proximity to gold mining operations, versus individuals residing in the site farther removed from the influence of gold mining.

MATERIALS AND METHODS

STUDY SITES

The Pantanal is a vast 140,000 km² wetland located in central-western Brazil (Alho et al. 1988, Alho and Sabino 2012), where the elevation ranges from 50 to 150 m above sea level (Alho and Vieira 1997). Individual jaguars were captured in two areas in the Pantanal (Fig. 1). The first site, Fazenda São Bento – SB (17°20'35.79"S, 56°43'39.33"W), is a privately owned cattle ranch and research headquarters located in the Itiquira River Basin (Mato Grosso state), 118.6 km downstream of Poconé (16°16'1.97"S, 56°37'35.51"W) in a region influenced by gold mining and Hg contamination. The second site, Refúgio Ecológico Caiman – CA (19°57'15.58"S, 56°18'15.20"W), is a privately-owned refuge dedicated to ecotourism and livestock use. Located in the Miranda River Basin (municipality of Miranda, Mato Grosso do Sul state), CA is a region free of gold mining

activities, found 408.2 km from Poconé and 289.6 km from SB.

The Pantanal is characterized by distinct seasonality, with the rainy season averaging about 160 mm of rainfall per month (October – March) and dry season averaging 50 mm of rainfall per month (April – September; Soisalo and Cavalcanti 2006). The seasonal nature of the Pantanal has significant impact on floral and faunal life, where water levels can rise by up to 5 m (Junk and da Silva 1995, Gottgens et al. 2001), with maximum inundation in March and minimum in October (Hamilton et al. 1996, Guimarães et al. 2000).

The wetland is comprised of three distinct regions: the high (Alto); middle (Medio); and low (Baixo) (Hamilton et al. 1996, Guimarães et al. 2000). The Alto Pantanal possesses numerous veins of gold within clay layers; as a result, about 60 gold mining sites operate within this region alone (Nogueira et al. 1997, Hylander et al. 2000b). During extraction of gold from the clay Hg is used as an amalgamating agent, which results in Hg losses to the air, soil and water (Hylander et al. 1994, 2000b, Alho and Vieira 1997, Guimarães et al. 1998, 1999, Lacerda and Salomons 1998, Leady and Gottgens 2001). These losses impact the environment on local and regional scales, due to the elevated levels of Hg contamination in water and sediments (Alho and Vieira 1997, Lacerda and Salomons 1998, Guimarães et al. 1999, Callil and Junk 2001, Leady and Gottgens 2001, Alho and Sabino 2012).

DATA COLLECTION

Individual jaguars were captured using soft-hold foot-snares (Balme et al. 2007). The captures occurred in June 2013 in SB and in April through October 2013 in CA, during optimal dry season conditions. We monitored the snares every two hours through very high frequency (VHF) transmitters, with snares operational from 6:00pm

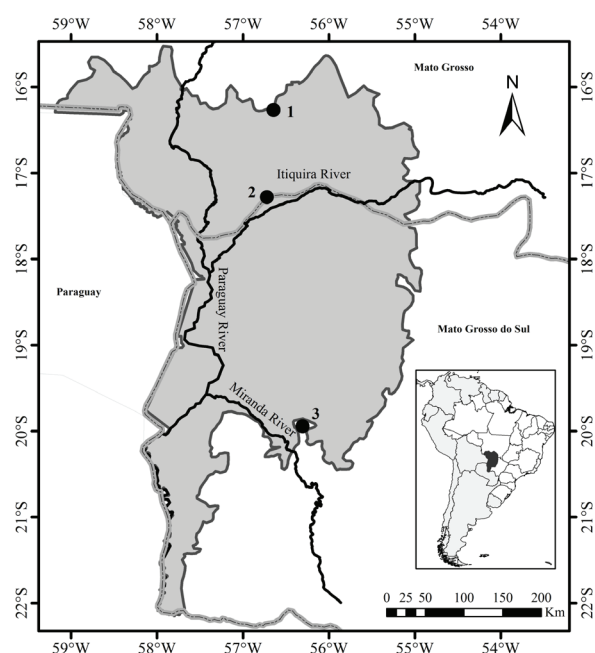


Figure 1 - Pantanal floodplain located in central-western Brazil, with the capture and gold mining sites, indicated by point location. Here, 1 - municipality of Poconé, Mato Grosso state (16°16'1.97"S, 56°37'35.51"W); 2 - Fazenda São Bento (17°20'35.79"S, 56°43'39.33"W); 3 - Refúgio Ecológico Caiman (19°57'15.58"S, 56°18'15.20"W), municipality of Miranda, Mato Grosso do Sul state.

to 7:00am. We immobilized the captured animals with a dissociative combination dosage of 5 mg/kg tiletamine hydrochloride and zolazepam hydrochloride administered via a dart fired from a CO₂ rifle. A unique identification number was assigned to each captured animal. Each individual was examined for general body condition, sex, weight, and age (adult >2 years; subadult; and juvenile) based on tooth wear. Fur samples were collected and stored in plastic bags. We performed animal handling and sample collection according to ethical procedures and animal welfare standards (Sikes et al. 2011), and in adherence to protocols of the Brazilian Environment Institute (ICMBIO; permit #42093-1).

MERCURY CONTENT ANALYSIS

Fur samples were triple washed with a mixture of diethyl ether : propanone (3:1, v/v), then submerged

in a solution of 5% EDTA (w/v) for 1 hour to remove superficial grease and dust to avoid external metal contamination. The samples were rinsed twice in ultra-purified water in a Milli-Q system (18 MΩ cm resistivity, oven-dried for 20 hours at 80°C, and then digested in a microwave oven. A mass of about 150 mg was directly weighed in Teflon jars, and then combined with 6 ml of nitric acid (HNO₃) sub-boiling bi-distilled and 2 ml of hydrogen peroxide (H₂O₂). Hg analysis was performed in triplicate, in the Laboratory of Mass and Atomic Spectrometry of Universidade Federal de Santa Catarina, using atomic fluorescence spectrometry coupled with chemical vapor generation (CVG AFS). Steam generation conditions were regulated to: 6% (v/v) of HCl; 4% (w/v) of SnCl₂; 0.08% (w/v) of KMnO₄; and 0.05% (v/v) of defoamer. The accuracy of the methods was evaluated by analyzing one certified reference material (BCR-397 Human hair – Community Bureau of Reference) and comparing using a t-test at 95% confidence level. The precision was evaluated using relative standard deviation of individual samples measured in triplicate.

STATISTICAL ANALYSIS (ANOVA)

We submitted the resulting Hg content values to the normality Shapiro-Wilk test and converted the data into base10 logarithmic scales in order to reach the normal range. The log-transformed data were used in a one-way analysis of variance (ANOVA) in program R (R Core Team 2016). Results were considered significantly different at $p < 0.05$, and are expressed in units of $\mu\text{g g}^{-1} \pm \text{SD}$ in dry-weight basis.

RESULTS

We captured nine individual jaguars in the two study areas (Table I), with four in SB (3 adult males; 1 juvenile male), and five in CA (2 adult females; 2 subadult females; 1 adult male). Body mass averages were 102.5 ± 10.6 kg ($n = 2$ females)

and 100.5 ± 16.9 kg ($n = 4$ males) for adult females and for adult males, respectively. The average value obtained for Hg in the reference sample BCR 397 Human hair was 11.9 ± 0.2 $\mu\text{g g}^{-1}$, which was not significantly different from the certified value of 12.0 ± 0.5 $\mu\text{g g}^{-1}$.

The CVG AFS measurements used to analyze the jaguar fur samples were determined satisfactory through external calibration with an aqueous standard (linear correlation coefficient, $R > 0.9999$). The analysis included reasonable precision as indicated by relative standard deviations (RSD) of less than 8% ($n = 3$). The limit of detection (LOD) was defined as three times the standard deviation of ten measurements of the blank sample, divided by the slope of the calibration curve. Using an aliquot of 0.15 g dried jaguar fur sample digested in a 50-mL final volume solution, we obtained an estimated LOD of 0.07 $\mu\text{g g}^{-1}$. The limit of quantification (LOQ) was defined as 3.3 times the LOD. The LOQ was estimated at 0.23 $\mu\text{g g}^{-1}$ and considered adequate for the fur samples.

The mean content of Hg in individual jaguar fur samples ($n = 3$ per animal) varied widely among individuals from the SB site, ranging from 26.1 ± 5.1 $\mu\text{g g}^{-1}$ in a juvenile male, to $2,010.4 \pm 150.5$ $\mu\text{g g}^{-1}$.

The mean individual values in the CA site ranged from 11.3 ± 2.4 to 70.0 ± 7.2 $\mu\text{g g}^{-1}$ (Table I). The untransformed mean Hg content in jaguars from the SB area (673.0 ± 916.8 $\mu\text{g g}^{-1}$) was compared to jaguars from CA area (29.7 ± 23.1 $\mu\text{g g}^{-1}$; Fig. 2). Hg concentrations were not normally distributed (Shapiro-Wilk p -value = $1.989e^{-05}$). The ANOVA was therefore performed on log-transformed data and indicated a significant difference between sites ($p = 0.03$).

DISCUSSION

This is the first study to evaluate Hg content in jaguars from the Brazilian Pantanal, and the second to report Hg content for this species (Racero-Casarrubia et al. 2012). In the present study, results from the log-transformed one-way ANOVA indicate that the mean content of Hg in the fur of SB jaguars was significantly different from those in CA.

The SB site is in close proximity to the city of Poconé (linear distance of 118.6 km), which has high levels of human activity - including historically intensive gold mining during the 1980s to 1990s, through the present day. Nearly 2 tons of Hg are sequestered in gold mine deposits around

TABLE I
Biological data and Hg levels ($\mu\text{g g}^{-1}$ dry weight) in the fur of individual jaguars captured in two areas (SB; CA). Hg content is presented as mean \pm SD ($\mu\text{g g}^{-1}$).

Animal ID	Sex	Age	Weight (kg)	Date of Capture	Hg content ($\mu\text{g g}^{-1}$)	
					SB	CA
SB396	Male	Adult	105	90 June 2013	133.2 ± 27.1	---
SB434	Male	Juvenile	10	19 June 2013	26.1 ± 5.1	---
SB414	Male	Adult	106	20 June 2013	522.1 ± 27.9	---
SB435	Male	Adult	76	21 June 2013	2010.4 ± 150.5	---
CA432	Female	Subadult	61	14 April 2013	---	70.3 ± 7.2
CA433	Female	Adult	95	16 April 2013	---	20.5 ± 2.9
CA439	Male	Adult	115	18 October 2013	---	11.3 ± 2.4
CA440	Female	Adult	110	21 October 2013	---	25.8 ± 6.3
CA445	Female	Subadult	81	27 October 2013	---	20.8 ± 4.6

SB = Fazenda São Bento; CA = Refúgio Ecológico Caiman.

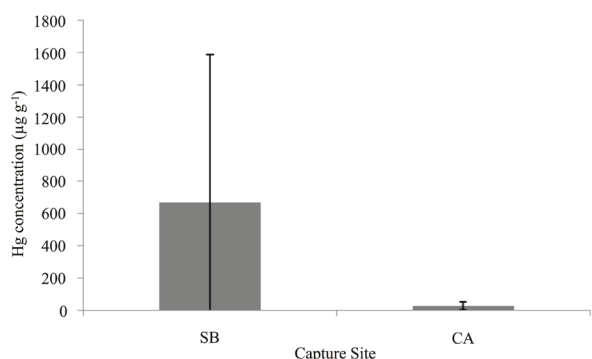


Figure 2 - Total Hg content ($\mu\text{g g}^{-1}$ dry weight \pm SD) in the fur of jaguars by capture site. Here, SB = Fazenda São Bento (n = 4 individuals); CA = Refúgio Ecológico Caiman (n = 5 individuals).

the Poconé region (Lacerda et al. 1991a, Callil and Junk 2001). The process of amalgamation in gold mining results in environmental degradation ranging from the direct release of Hg into the water, and the erosion of riverbanks due to high-pressure water jets (Vieira et al. 2011). Rainfall, coupled with anthropogenically-induced high levels of erosion (e.g., agriculture; clear-cutting along riverbanks), causes Hg to percolate through the water table and into the lowland plateau (Hylander et al. 2000a). This percolation effect may result in the overall higher concentrations of Hg observed in the sampled individuals of SB versus CA. Although the CA study site is free from direct gold mining influence, we recorded Hg contents in the fur of jaguars that ranged from 11.3 – 70.3 $\mu\text{g g}^{-1}$. This may be due to the latent percolation effects, where Hg is carried by floodwaters from the northern to the southern regions of the Pantanal.

Maximum concentrations of Hg were previously recorded in the teeth of jaguars in Colombia ($0.0887 \pm 0.013 \mu\text{g g}^{-1}$ fresh weight; Racero-Casarrubia et al. 2012) and were considered below harmful biotic limits ($0.05 \mu\text{g g}^{-1}$; Allen 1989, WHO 1991). As there is a current absence of further literature on Hg levels in jaguars, we draw further comparisons to a diverse base of existing

studies which reported Hg concentrations in other mammalian species (Table II). For example, in North America, published studies of *Puma c. coryi* in the Everglades reported maximal Hg contents of $90 \mu\text{g g}^{-1}$ (fur) and $110 \mu\text{g g}^{-1}$ (liver) dry weights (Roelke 1990, Roelke et al. 1991, Dunbar 1994, Barron et al. 2004). The next highest recorded Hg value of $183 \mu\text{g g}^{-1}$ (fresh weight) was reported in the fur of *L. canadensis* (Sleeman et al. 2010). With a conversion of 3:1 of dry : wet weight (Puls 1994), the value detected by Sleeman et al. (2010) increases to $549 \mu\text{g g}^{-1}$. HgTot in human hair collected from villagers living near Poconé ranged from 0.3 to $3.11 \mu\text{g g}^{-1}$ (Nogueira et al. 1997). Such concentrations are in stark contrast to the World Health Organization Hg limit of $0.05 \mu\text{g g}^{-1}$ (Allen 1989, WHO 1991). Our data suggest that the Hg content in the fur of jaguars from SB is the highest ever recorded in wild mammals.

The food web within the Pantanal is complex and includes diverse communities of both aquatic and terrestrial biota (Junk et al. 2006). Aquatic predators can accumulate Hg through the consumption of contaminated prey (Callil and Junk 2001), resulting in high concentrations of Hg in the aquatic food chain (Leady and Gottgens 2001). Lacerda et al. (1991b) recorded Hg concentrations of about $0.91 \pm 0.06 \mu\text{g g}^{-1}$ in *Pomacea caniculata* near gold mines in the northern Pantanal. Within the same region, Callil and Junk (2001) recorded values as high as $2.04 \pm 1.27 \mu\text{g g}^{-1}$ of Hg in *Pomacea scalaris*. Hylander et al. (2000b) recorded total mercury (HgTot) content as high as $2.05 \mu\text{g g}^{-1}$ fresh weight in a predatory fish (*Serrasalmus* spp.) in a creek in Alto Pantanal, near Poconé, that ranged from 0.04 to $2.05 \mu\text{g g}^{-1}$ (fresh weight). High values of Hg were also detected in *Serrasalmus spiroleura* ($0.15 \mu\text{g g}^{-1}$; dry weight) and *Pygocentris nattereri* ($0.30 \mu\text{g g}^{-1}$; dry weight) from Hg-impacted regions in the northern Pantanal (Leady and Gottgens 2001).

TABLE II
Maximum Hg level ($\mu\text{g g}^{-1}$ dry weight) detected in the fur of jaguars (*Panthera onca*) from the Brazilian Pantanal in comparison to Hg contents ($\mu\text{g g}^{-1}$ dry or wet weight) reported in previously published mammalian studies.

Species	Location	Tissue	Maximal Content($\mu\text{g g}^{-1}$)		Reference
			Dry Weight	Wet Weight	
<i>N. vison</i>	Saskatoon, Canada	Liver	58.2	---	Wobeser and Swift (1976)
		Kidney	34.9	---	
		Fur	31.9	---	
		Muscle	15.2	---	
		Brain	13.4	---	
<i>L. canadensis</i>	Ontario, Canada	Liver	96.0	---	Wren (1985)
		Kidney	58.0	---	
		Muscle	36.0	---	
		Brain	30.0	---	
<i>P. c. coryi</i>	Florida, USA	Liver	---	110	Dunbar (1994)
		Fur	---	90.0	
<i>P. Braziliensis</i>	Brazilian Pantanal	Kidney	---	4.59	Fonseca et al. (2005)
		Liver	---	4.30	
		Fur	---	3.68	
		Muscle	---	0.17	
<i>L. canadensis</i>	Virginia, USA	Kidney	353.0	---	Sleeman et al. (2010)
		Liver	221.0	---	
		Fur	---	183.0	
		Brain	151.0	---	
		Muscle	121.0	---	
<i>P. onca</i>	Colombia	Teeth	---	0.0887	Racero-Casarrubia et al. (2012)
<i>P. onca</i>	Brazilian Pantanal	Fur	2,010.04	---	Present study

Hg has also been reported in crocodylian species (Yanochko et al. 1997, Elsey et al. 1999, Burger et al. 2000, Rumbold et al. 2002), including *Caiman c. yacare* from areas of intensive human activity in the Brazilian Pantanal (0.02 to 0.36 μg^{-1} wet weight; Vieira et al. 2011). The relatively high Hg values recorded in different levels of the ecosystem corroborate the presence of an elevated rate of biomagnification in the Hg-impacted Pantanal region.

Previous research indicates that fish and apex predators within the aquatic community tend to store Hg in readily-digestible muscle, whereas avian and mammalian species store Hg in feathers and fur (Hylander et al. 2000b, Leady and Gottgens 2001, Risher et al. 2002, Davis

et al. 2003). The accumulation of Hg within the food chain is greatest in long-lived, apex species (Roelke 1991, Callil and Junk 2001, Leady and Gottgens 2001, Chan et al. 2003). Jaguars are generally opportunistic top predators with flexible diets (Emmons 1987, Cavalcanti and Gese 2010, Da Silveira et al. 2010), and will often prey upon *Hydrochaeris hydrochaeris* and *Caiman c. yacare* when available, but will also consume small (<1 kg), medium (1-15 kg), and large terrestrial prey (>15 kg; Polisar et al. 2003, Weckel et al. 2006, Azevedo and Murray 2007, Da Silveira et al. 2010, Perilli et al. 2016).

With the Brazilian Pantanal's remarkable biodiversity, complex food web, and human population that relies on high per capita fish

consumption, understanding Hg dynamics and the risks of exposure is therefore particularly urgent (Leady and Gottgens 2001) for informing wildlife conservation plans and human health programs in the greater Pantanal region. The jaguar may serve as a useful indicator species for the continued monitoring of Hg concentrations within this environment.

CONCLUSIONS

Contamination of Hg in the biotic and abiotic factors of the ecosystem is well documented in the Brazilian Pantanal, and appears to bioaccumulate through aquatic, carnivorous links in the food web. With the highest body burdens observed in jaguars from the northern Pantanal region of SB, where gold mining activities were most intensive, our data suggest that the Hg content in an individual jaguar from the Brazilian Pantanal is the highest ever recorded in wild animals. Chronic exposure of jaguars to Hg may potentially compromise relative individual health and productivity. Thus, Hg should be considered a threat to this key species. The jaguar may serve as a sentinel species for short- and long-term study and monitoring. Further research is needed to further quantify the degree of Hg contamination within the greater Pantanal region.

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