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Original article [1]

Lead levels in *Prochilodus lineatus* from the Pilcomayo River: comparison of historical and current data and implications for human health risk

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ABSTRACT

The Pilcomayo River has been historically affected by metal contamination associated with mining activities in the Andean headwaters, raising concerns about metal accumulation in fish and potential risks for human consumption. This study assessed current lead concentrations in muscle, liver, and opercular tissues of *Prochilodus lineatus* collected in 2024 in the middle basin of the Pilcomayo River (Villa Montes, Bolivia), and compared them with historical data to depict trends in Pb contamination. Lead levels differed significantly among tissues, with the highest concentrations in operculum (0.799 ± 0.535 mg kg^{-1} ww), followed by liver (0.099 ± 0.111 mg kg^{-1} ww), and muscle (0.013 ± 0.012 mg kg^{-1} ww). Human exposure, evaluated through estimated daily intake (EDI), ranged from 8.67 x 10^{-7} to 4.26 x 10^{-5} mg kg^{-1} day^{-1} depending on the population group and consumption scenario, and are below exposure levels associated with adverse health effects reported by World Health Organization.

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RESUMEN

Niveles de plomo en Prochilodus lineatus en el río Pilcomayo: comparación de datos históricos y actuales e implicaciones para el riesgo para la salud humana. El río Pilcomayo ha estado históricamente impactado por la contaminación metálica originada en actividades mineras andinas, lo que genera preocupación por la acumulación de plomo en peces y sus riesgos para la salud humana. Este estudio evaluó las concentraciones actuales de Pb en músculo, hígado y opérculo de *Prochilodus lineatus* recolectados en 2024 en la cuenca media del Pilcomayo (Villa Montes, Bolivia), comparándolas con datos previos para identificar tendencias. Los resultados mostraron diferencias claras entre tejidos: el opérculo presentó los valores más altos (0.799 ± 0.535 mg kg^{-1}), seguido por el hígado (0.099 ± 0.111 mg kg^{-1} peso húmedo) y el músculo (0.013 ± 0.012 mg kg^{-1}). La exposición humana se estimó mediante la ingesta diaria (EDI), con rangos de 8.67 ×10^{-7} a 4.26 ×10^{-5} mg kg^{-1} día^{-1}, cifras inferiores a los umbrales de riesgo definidos por la Organización Mundial de la Salud.

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INTRODUCTION

Mining-related contamination of freshwater ecosystems represents an environmental issue worldwide, due to its long-term impacts on water and sediment, biota and human populations that depend directly on riverine resources.[1] In mining-affected watersheds, metals and metalloids released during ore extraction and processing are transported downstream mostly through the mobilization and deposition of contaminated particles, possibly generating persistent contamination even far from the original source areas[1]. Metals derived from mining activities can also be transferred to aquatic food webs and accumulate in fish tissues. Actually, elevated concentrations of lead (Pb) and other trace elements were reported in fish from river basins impacted by polymetallic mining in different regions worldwide[2 3 4]. Such accumulation may induce sublethal and pathological effects in fish including tissue damage, physiological impairment and altered reproductive performance[5 6]. In addition, in regions where fisheries contribute to the subsistence of riverine communities, the presence of potentially toxic metals in edible fish tissues can represent a critical pathway of chronic human exposure to metallic contaminants[7 8 9].

Among metal contaminants, Pb is a non-essential element whose toxicity is widely recognized even at very low levels of exposure, affecting neurological development and being associated with increased risk of chronic kidney and cardiovascular diseases in adults as well as cognitive and behavioral impairments in children[10]. In aquatic environments, Pb can be incorporated into fish from both water and sediments, accumulating preferentially in metabolically active organs such as liver, kidney, gills and brain, as well as in mineralized structures including bones, where it may be retained for long periods[11 12 13]. The magnitude of Pb accumulation in fish tissues depends on environmental factors including Pb concentration and bioavailability, water characteristics (pH, temperature, electrical conductivity, organic matter content and other parameters), as well as fish biological characteristics such as species, age and feeding behavior, with higher burdens often observed in benthic and sediment-feeding species[12 14]. Unlike mercury, however, Pb is not consistently biomagnified along aquatic food chains, as its accumulation is more strongly influenced by direct environmental exposure than by progressive amplification across trophic levels[15 16].

In South America, several Andean river basins have been impacted by historical and ongoing metal mining activities[17 18]. The upper reaches of the Pilcomayo River, located in the Bolivian Eastern Cordillera at elevations exceeding 5000 m a.s.l (above sea level), drains areas characterized by continuous mining activity since pre-Hispanic times. Mining activities intensified from the sixteenth century onwards with Spanish colonization and the exploitation of the Cerro Rico located in Potosí, Bolivia[19 20 21]. Along its course, the Pilcomayo River transports large volumes of sediments, including mining residues enriched in potentially toxic metals released from mining waste erosion and ore processing plant effluent discharges, which are deposited in the riverbed and the alluvial plains and can be remobilized during flood events[20 22 23 24]. Previous studies have reported elevated concentrations of metals and metalloids in water, sediments, and alluvial soils of the upper Pilcomayo basin, frequently exceeding international guideline values for various environmental uses[19 21]. Although dissolved metal concentrations decrease rapidly downstream the mining areas due to adsorption and precipitation onto particulate matter, the remobilization of contaminated sediments represents a potential risk of contamination for the downstream basin, where numerous riverside communities are established and depend on riverine resources.

Since the late 1990s, several studies have reported the presence of potentially toxic metals in fish from the Pilcomayo River, with Pb consistently identified as a contaminant of environmental and human health concern; concentrations in edible tissues vary across species and organs, and can exceed guideline values for human consumption[25 26 27]. These studies have linked Pb occurrence to historical mining activities in the upper basin, remobilization of contaminated sediments during flood events, and the long-term storage of metal-rich material within the river system. Among the studied species, *Prochilodus lineatus* (sábalo) represents the principal fishery resource of the region and constitutes a fundamental source of protein and income for riverside communities in the middle and lower sections of the Pilcomayo River[28 29]. This potamodromous and benthopelagic species inhabits large river systems of the Paraná–Paraguay basin and performs medium- to long-distance reproductive migrations[30 31]. *P. lineatus* is primarily detritivorous, grazing on periphytic algae and ingesting fine organic particles associated with bottom sediments, a feeding strategy that may enhance its exposure to sediment-bound contaminants such as Pb.[31] However, available data of metal in fish tissues mostly correspond to measurements conducted in the 1990s and early 2000s; the lack of recent data limits the assessment of the potential current impact of mining activities on the contamination of fishery resources in the Pilcomayo River and exposure of human population.

In this context, the aim of the present study was to evaluate current Pb levels in three tissues (muscle, liver and operculum) in specimens of *P. lineatus* collected in 2024 in Villa Montes (Bolivia), a city located in the middle basin of the Pilcomayo River. These results are compared with historical data from the literature to assess long-term variations in Pb concentration in *P. lineatus*. Finally, a risk assessment related to lead exposure through the consumption of *P. lineatus* is presented to evaluate potential implications for human health.

EXPERIMENTAL

Study area

The Pilcomayo River basin is one of the major transboundary river systems in South America. This basin covers an area of approximately 272000 km^2 located in the south of Bolivia, north of Argentina and west of Paraguay[22 24]. The river originates in the Eastern Cordillera of the Bolivian Andes, at elevations over 5000 m a. s. l., draining high altitude regions of eastern Potosi Department, where intensive metal mining has been carried out for several centuries. From these headwater areas, the Pilcomayo River flows southeastward across western Chuquisaca Department and eastern Tarija Department, progressively descending until the lowlands of the Chaco region at Villa Montes, southern Bolivia, located approximately at 390 m a.s.l. at the foot of the Andes. The Pilcomayo river reaches elevations of about 200 m a.s.l. at the Bolivian – Argentine - Paraguay border[22].

The present study focuses on the middle basin of the Pilcomayo River, in the region of Villa Montes (Figure 1). In this sector, the river exhibits pronounced hydrological variability, with mean monthly discharge values over the period 2005-2017 ranging from 37 m^3 s^{-1} in September at the end of the low flow period to around 771 m^3 s^{-1} in February at the peak of the high-water period.[32] However, variability extends well beyond these typical ranges, as mean daily discharges can fall below 10 m^3 s^{-1} during the dry season, while instantaneous peak flows during extreme flood events may exceed 7000 m^3 s^{-1} [32].The Pilcomayo is characterized by high sediment loads, transporting an estimated 80 – 100 million tons of suspended material per year, which are largely derived from natural erosion processes in the upper basin and subsequently redistributed across the Chaco plain[22 24]. At Villa Montes, the river exhibits strong seasonal contrasts in suspended solid concentrations. At the beginning of the 21th century, during the rainy season (January–April), suspended sediment concentrations were extremely high, with mean values reported around 23.6 g L^{-1} (range 10.7–56.0 g L^{-1}), whereas during the dry season (May–July), suspended solids decrease markedly to 0.011 g L^{-1} on average[23]. According to Smolders *et al*. (2002)[23], this seasonal pattern reflected intense erosion and sediment inputs during high discharge periods, resulting in substantial dilution of mining-derived materials by large volumes of uncontaminated sediments with an average Pb concentration of 35.3 mg kg^{-1}. In contrast, during the dry season, overall sediment load was much lower due to reduced erosion, although a higher proportion of the suspended material may originate from mining residues with a Pb concentration ranging from 352 to 3345 mg kg^{-1}.[23] The extremely high sediment load of the river has been suggested to limit the Pb contamination levels of downstream floodplains by mining waste through dilution effects.

In addition to this seasonal sediment load variability, the Pilcomayo River experiences episodic flood events locally known as "borrachera". These events occur at the onset of the rainy season and are characterized by acute fish mortality. They take place when the first rains and resulting flood pulses mobilize large amounts of fine sediments previously accumulated in the riverbed, dramatically increasing suspended solid concentrations. The extreme turbidity levels (up to 23.6 g L^{-1}) can impair gill function by clogging the respiratory surfaces, thereby reducing oxygen exchange efficiency[24]. The resulting hypoxic stress leads to fish disorientation, physiological collapse, and, in severe cases, mass mortality events.

Sample collection

Fish sampling was carried out in October 2024 during a "borrachera" event, where eleven dead *P. lineatus* specimens were collected from the riverbank a few hours after stranding during -this event. Muscle and bone (operculum) tissues were obtained from all individuals, whereas liver samples were collected only from five specimens due to strong tissue deterioration observed in the others specimens. Biometric parameters were recorded for all specimens, including total length (LT, from the tip of the snout to the end of the caudal fin), standard length (SL, from the snout to the base of the caudal fin), and the body mass. Additionally, sex was determined.

Prior to dissection, all working surfaces and surgical instruments (scalpels, scissors, and forceps) were thoroughly washed with detergent, rinsed with deionized water and ethanol in order to avoid potential contamination. Muscle samples were obtained from the dorsal region below the dorsal fin. At first, scales were removed and the area rinsed with deionized water. A portion of white muscle tissue was excised after removing the skin, avoiding contact with blood and bone tissue. Liver and operculum samples were extracted, ensuring no contact with other organs to prevent cross-contamination. All tissue samples were placed in hermetically sealed plastic bags and stored at –20 °C until analysis.

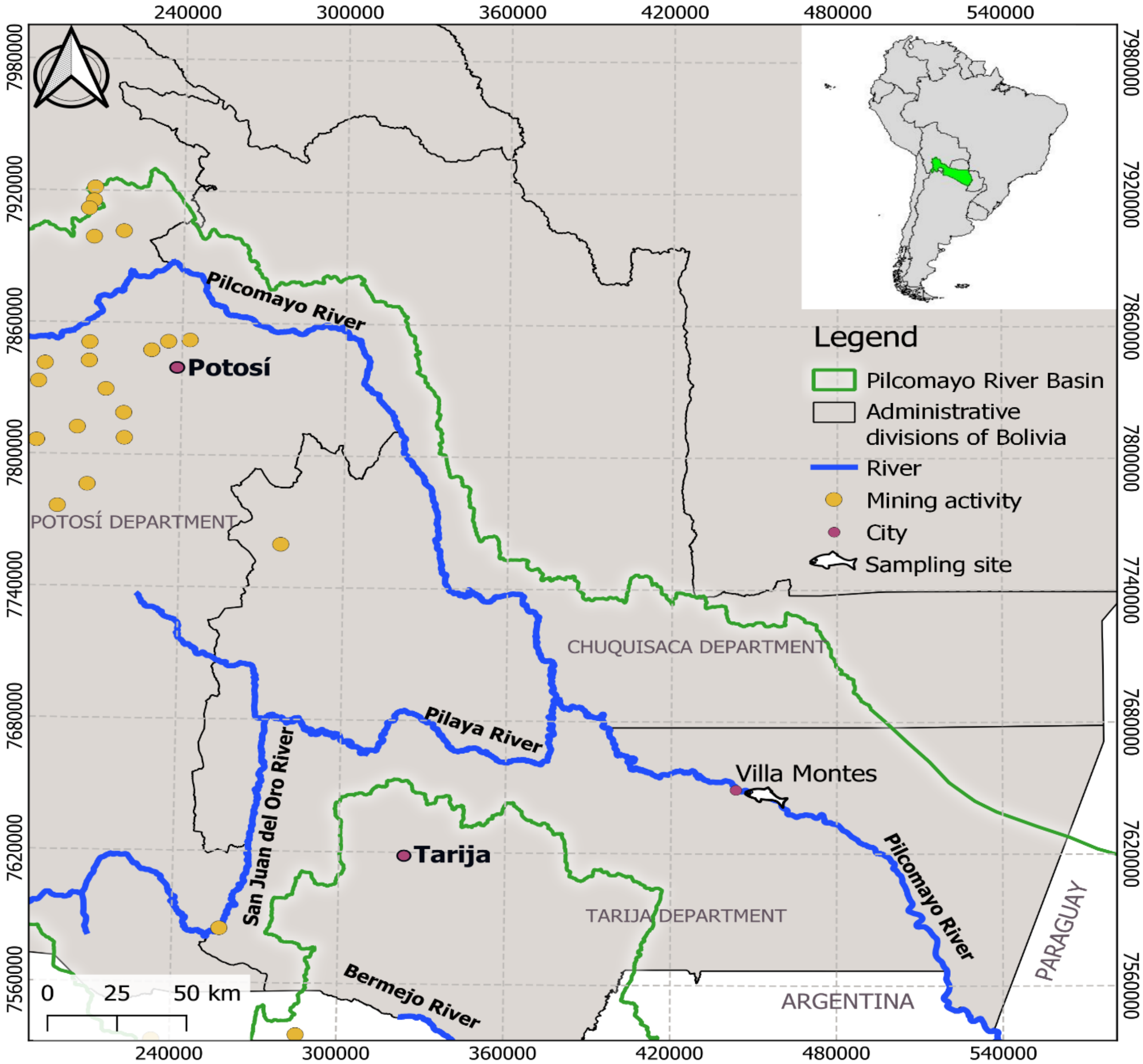

Figure 1. Map of the Pilcomayo River basin and sampling location in the Villa Montes area (Bolivia, South America)

Sample preparation and lead quantification

Fresh tissue samples were individually weighed to determine their initial wet mass. Samples were subsequently freeze-dried and reweighed to obtain dry mass. Moisture content (%) was calculated as the percentage of mass loss relative to the initial wet weight, according to the following equation:

$$\%\,moisture = \frac{W_{wet} - W_{dry}}{W_{wet}} * 100 \quad \text{(Equation 1)}$$

Where W_{wet} is the initial weight of the fresh sample and W_{dry} is the weight of the sample after drying. This calculation represents the proportion of water lost during the drying process.

After freeze-drying, samples were homogenized by fine grinding using a Retsch Mixer Mill MM 400 equipment. A required amount of dried tissue was placed in the milling jar and milled until a homogeneous fine powder was obtained. Between samples, the milling jars were thoroughly cleaned in order to prevent cross-contamination. The resulting powdered samples were stored in acid-cleaned polyethylene containers.

For sample acid digestion and analysis, all tubes, vials and micropipette tips were pre-cleaned by soaking in 10% (m/v) HNO_3 for 24 h and rinsed three times with ultrapure water (resistivity > 18.2 MΩ·cm, MilliQ, Millipore). Tissue preparation and processing were performed under controlled conditions in a class 10,000 clean laboratory at HydroSciences Montpellier (Université de Montpellier, France), using high-purity reagents: 15 M HNO_3 (Analpure, Analytika®) and 22.6 M HF (Suprapur, Merck®). For each batch of sample digestion, a procedural blank and a certified reference material (CRM) were included. The CRM used were DORM-2 and DORM-5 (fish muscle) and DOLT-2 and DOLT- 5 (fish liver) from the National Research Council of Canada (NRC-CNRC).

Approximately 0.1 g of each freeze-dried milled tissue (muscle, liver and operculum) and certified reference materials were weighed into PTFE digestion vessels. Samples were digested with 5.0 mL of HNO_3 (15 M) and 0.5 mL of HF (22.6 M) using a microwave-assisted digestion system (ultraWAVE, Milestone®), with a temperature ramp up to 200 °C. After cooling, digested solutions were transferred to PFA (perfluoroalkoxy) vials and evaporated to dryness at 80 °C. The residues were dissolved in 10 mL of 1.5 M HNO_3 and subjected to ultrasonic treatment for 30 min to ensure complete dissolution. The digested solutions were appropriately diluted in 0.15 M HNO_3 prior to analysis by inductively coupled plasma–mass spectrometry (ICP-MS).

Lead concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS, iCAP TQ, Thermo Scientific) at the AETE-ISO platform (OSU-OREME, Université de Montpellier, France). Quantification was performed using an external multi-element calibration approach. Calibration curves were considered acceptable when correlation coefficients (R^2) were ≥0.995. Instrumental drift and potential matrix effects were corrected through the use of internal standards (Be, Sc, Ge, Rh, Ir) continuously introduced on-line during analysis. Instrument stability and accuracy were verified by periodic analysis of a certified reference material of water (SLRS-6 from the National Research Council of Canada) throughout the analytical sequence.

Quality assurance and quality control

Instrumental performance was first verified using the certified reference material SLRS-6 (river water), for which measured Pb concentrations (0.16 ± 0.001 µg L^{-1}, n=3) were within the certified uncertainty range (0.17 ± 0.03 µg L^{-1}), corresponding to a recovery of 95 % (Table 1), confirming satisfactory instrumental accuracy.

Procedural blanks were included in each digestion batch to assess that no contamination occurred during the entire protocol. Lead concentrations in blanks were below the limit of detection (LOD = 0.0017 µg L^{-1}), estimated as three times the standard deviation of the measurements in blanks (0.15 M HNO_3). Considering the detection limit as the maximum possible blank concentration, the potential contribution of blanks would represent less than 2.3 % of the Pb concentrations measured in samples, and was therefore considered negligible.

The accuracy of Pb determination in fish tissues was evaluated through the analysis of certified reference materials DORM-2 and DORM-5 (fish muscle) and DOLT-2 and DOLT-5 (fish liver), and subjected to the same digestion and analytical procedure as for the samples. Measured Pb concentrations showed recoveries between 90 and 100 % compared to the certified values (Table 1), except for the CRM DORM-2, which value was close to the certified uncertainty but presented a slightly low recovery (79 %) as already observed in other studies[33]. Overall, these results confirm the reliability of the method for the determination of Pb in fish tissues.

Estimated Daily Intake (EDI)

The potential human exposure to Pb through fish consumption was assessed by calculating the Estimated Daily Intake (EDI) using the following equation[34]:

$$EDI = \frac{C_{Pb} \times IR}{BW} \qquad \text{(Equation 2)}$$

Where C_{Pb} is the mean Pb concentration in edible tissue (mg kg^{-1} , wet weight), IR is the average ingestion rate of fish (g day^{-1}) and BW is the body weight of the consumer (kg).

Fish consumption in Bolivia is relatively low at the national scale, with estimates ranging between approximately 1.4 and 2.3 kg per person per year according to reports from the Food Agriculture Organization of the United Nations[35]. However substantially higher consumption rates have been documented in riverine and indigenous communities of the Pilcomayo River where fish constitutes an important component of local diets and food security[36]. In communities located near Villa Montes in the Pilcomayo river River basin, fish consumption has been estimated between 40 and 60 kg person^{-1} year^{-1},[36] corresponding to approximately 110 – 164 g day^{-1}. For children, fish consumption has been assumed to represent 40 % of the adult ingestion rate[29 37], resulting in a fish ingestion rate of 44 – 65.6 g day^{-1}. Average body weights of 60 kg for adults and 20 kg for children were used in the calculations. These values are consistent with anthropogenic data reported for the Bolivian population in national demographic and health surveys[38].

Table 1. Certified and measured Pb concentrations for the CRM of water (SLRS-6) and fish tissues (DORM-2, DOLT- 2, DORM-5 and DOLT-5) expressed as mean ± standard deviation (SD).

CRM	n	Matrix	Certified Pb value	Measured Pb value	Recovery (%)
SLRS-6 (µg L^{-1})	3	River water	0.17 ± 0.03	0.16 ± 0.001	95
DORM-2 (mg kg^{-1})	6	Fish muscle	0.065 ± 0.007	0.051 ± 0.006	79
DOLT-2 (mg kg^{-1})	2	Fish liver	0.22 ± 0.02	0.20 ± 0.04	90
DORM-5 (mg kg^{-1})	2	Fish muscle	0.058 ± 0.006	0.058 ± 0.001	100
DOLT-5 (mg kg^{-1})	2	Fish liver	0.162 ± 0.032	0.145 ± 0.008	90

Statistical treatment of data
Distribution of Pb concentrations in muscle, liver and operculum tissues were assessed for normality using the "Shapiro test" function. As normal distribution assumptions were not satisfied, non-parametric analyses were applied. Since all tissues were collected from the same individuals of *P. lineatus*, paired comparisons were performed using the Wilcoxon signed-rank test. This test evaluates whether the distribution of two differences between paired observations is centered around zero and does not require normally distributed data, making it appropriate for small sizes and asymmetric distributions[39]. Statistical significance was set at $p < 0.05$. All analyses we|re carried out using RStudio software[40].

RESULTS AND DISCUSSION

Concentrations of lead in the different tissues
The standard length of sampled *P. lineatus* varied only slightly (24.7 ± 0.6 cm) and was not correlated with Pb concentration in the three tissues (R^2<0.05). Lead concentrations varied markedly among tissues of *P. lineatus*. In muscle, concentrations ranged from 0.006 to 0.046 mg kg^{-1} wet weight (ww) (mean ± SD: 0.013 ± 0.012 mg kg^{-1} , n = 11). In liver, concentrations ranged from 0.009 to 0.293 mg kg^{-1} ww (mean ± SD: 0.099 ± 0.111 mg kg^{-1} , n = 5). Operculum showed the highest Pb levels, ranging from 0.278 to 1.90 mg kg^{-1} (mean ± SD: 0.799 ± 0.535 mg kg^{-1} , n = 7) (Figure 2).

Normality tests (Shapiro–Wilk) indicated that Pb concentrations did not follow a normal distribution in muscle, liver, and operculum tissues ($p < 0.05$). Therefore, paired comparisons were performed using the non-parametric Wilcoxon signed-rank test, considering a directional hypothesis of higher Pb concentrations in operculum compared to liver and muscle, and in liver compared to muscle, based on average concentrations. Significant differences were observed among tissues: operculum showed higher Pb concentrations than liver ($p = 0.0313$) and muscle ($p = 0.008$), and liver exhibited higher concentrations than muscle ($p = 0.0313$).

This pattern of accumulation (operculum > liver > muscle) is consistent with physiological differences among tissues. Liver is generally expected to accumulate higher Pb concentrations due to the presence of metal binding proteins such as metallothioneins[16 28.] The markedly elevated Pb levels observed in opercular tissue is also generally observed in fish and is associated with the accumution of Pb in bone due to its chemical similarity with calcium[41 42]. In contrast, the comparatively low concentrations detected in muscle suggest limited translocation to edible tissue.

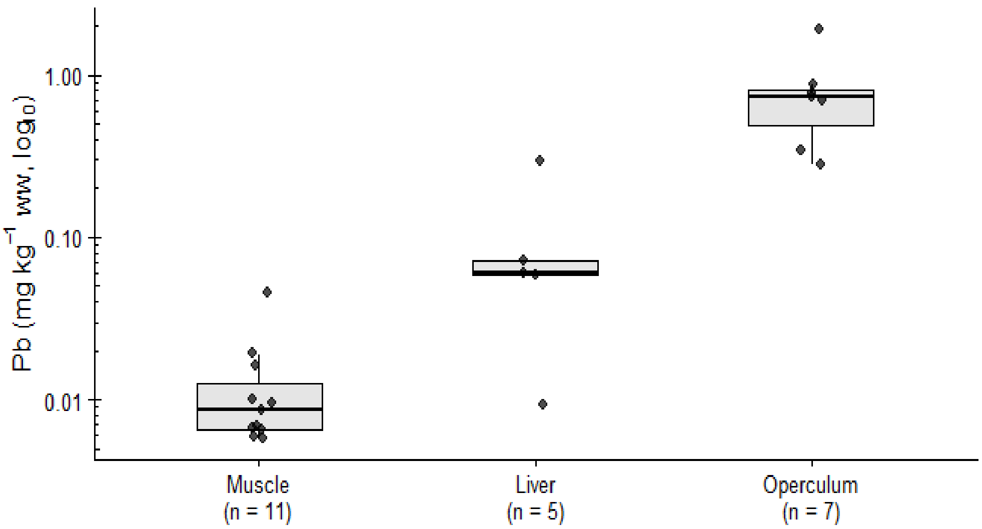

Figure 2. Lead concentrations (mg kg^{-1} ww) in muscle, liver, and operculum of the analyzed fish specimens, data are shown on a $\log_{10}$ scale. Boxes represent interquartile range, horizontal lines indicate medians, and whiskers show the data range; individual points correspond to each specimen.

It is noteworthy that the highest outlier values observed in liver and operculum correspond to the same individual, suggesting enhanced Pb accumulation in this specimen, potentially related to factors such as age, exposure history, or habitat variability. In contrast, the outlier observed in muscle corresponds to a different individual, for which liver data were not available, preventing further comparison across tissues.

Comparison with previous studies in the Pilcomayo River (Villa Montes region)

Lead concentrations measured in *P. lineatus* from the middle Pilcomayo River in the present study were markedly lower than those reported in historical records from the same basin (Table 2). Muscle concentration in fish collected in Villa Montes in 2024 averaged 0.013 mg kg^{-1} ww, whereas previous studies reported substantially higher values during the late 1990s and the early 2000s, ranging from 0.06 to 1.27 mg kg^{-1} ww in muscle tissues of *P. lineatus*[27 42 43]. Even higher concentrations were reported upstream in the middle basin, with Pb levels in whole fish exceeding 5 mg kg^{-1} at Zapaterambia and Yukimbia in 1996[25]. This marked decline suggests a substantial reduction in Pb exposure of *P. lineatus* in the middle Pilcomayo River over the last three decades.

From a spatial perspective, the concentrations measured in the present study are comparable to those reported in other sectors of the Pilcomayo basin and nearby river systems. For instance, similar muscle Pb concentrations were reported for *P. lineatus* sampled in 2023 in the lower Pilcomayo River (0.013 mg kg^{-1} ww) and in the Bañado la Estrella wetland (0.014 mg kg^{-1} ww)[29]. In contrast, higher Pb levels have been reported in other South American aquatic systems such as Chascomús Lake (Argentina), a shallow lake influenced by agricultural runoff and urban wastewater inputs, where muscle concentrations averaged 0.76 mg kg^{-1} ww[33] . Studies conducted in the Paraná River basin generally report very low concentrations in muscle tissues (<0.007 mg kg^{-1} ww), although higher values occur in organs such as liver (0.028 – 0.9 mg kg^{-1} ww)[28]. Together, these comparisons indicate that current Pb levels in fish from the middle Pilcomayo River fall within the lower range reported for regional freshwater ecosystems (Table 2).

Elevated Pb concentrations observed in historical studies of the Pilcomayo River have often been associated with an impact of mining activities located in the Andean headwaters in Bolivia, which have historically released metal rich sediments into the river system[20]. Because the Pilcomayo River transports high sediment loads across the Andean - Chaco transition, metal contamination can be redistributed over long distances. However, the much lower concentrations observed in recent sediment samples may reflect changes in mining practices (e.g. tailing dam construction),[29 42] natural sediment dilution processes, or shifts in metal bioavailability within the river system. The approximately 500 km distance between Villa Montes and major mining districts in the Andean region may also contribute to reduced exposure due to progressive metal-bearing sediment deposition and dilution along the downstream course of the river.

Table 2. Lead concentrations (mg kg^{-1} wet weight) in tissues of *Prochilodus lineatus* from the Middle Pilcomayo River basin (Bolivia) and other South American freshwater systems based on historical records and the present study

Location	Year of sampling	Sampling size (n)	Muscle	Liver	Operculum	Environmental context	Reference
Middle Pilcomayo River basin							
Middle Pilcomayo River (Villa Montes)	2024	11	0.013 ± 0.012	0.099 ± 0.111	0.799 ± 0.535	Downstream, Andean-Chaco transition; aprox. 500 km from mining areas	This paper
	2007	32	1.27	1.32	NS		[27]
	2006	NS	0.12	0.3	0.63		[42]
	2003	NS	0.16	0.36	0.78		
	1999	NS	0.06	0.09	0.49		
	1998	NS	0.77	0.54	1.5		
	1997	NS	0.19	0.4	2.3		
	1997	NS	0.23	1.60	NS		[43]
Other sites							
Lower Pilcomayo	2023	10	0.013 ± 0.008	NS	NS	Lower Pilcomayo basin, Andean–Chaco transition; sediment-rich river affected by historical Andean mining inputs	[29]
Bañado la Estrella		9	0.014±0.005	NS	NS	Floodplain wetland associated with the Pilcomayo River in the Chaco plain	
High Paraná River	2017	13	<0.007	0.028±0.003	NS	Argentina-Paraguay international boundary	[28]
Middle Paraná River		13	<0.007	0.11±0.68	NS		
La Plata River		15	<0.007	0.9±0.70	NS	Argentina-Uruguay international boundary	
Chascomus Lake, Argentina	2011	5	0.76±0.80	NS	NS	Shallow Pampean lake in the Slado River basin (Chaco-Pampa plain); alkaline, turbid system with high suspended particulate matter	[33]
Bermejo River	1998	NS	0.13	0.25	0.76	Adjacent Andean-Chaco basin used as a relatively unpolluted reference area comparable to the Pilcomayo	[28]
	1997	NS	0.67	0.17	0.93		

Notes: NS: not specified in the original source

Human health risk assessment associated with the Pb exposure through *P. lineatus* consumption

Two consumption scenarios were considered in the present assessment. The low consumption scenario represents the potential exposure associated with occasional consumption of this species through its commercialization using the estimated fish ingestion rate in Bolivia[35], whereas the high consumption scenario reflects the dietary habits of riverine indigenous communities for whom fish constitutes an important component of local diets[36] In both scenarios, exposure estimates were calculated separately for adults and children in order to account for differences in body weight and potential vulnerability.

The estimated daily intake (EDI) values for Pb exposure through the consumption of *P. lineatus* varied depending on the assumed consumption scenario and population group (Table 3). Under the low consumption scenario, EDI values were 8.67×10^{-7} to 1.30×10^{-6} mg kg^{-1} day^{-1} for adults and from 2.60×10^{-6} to 3.90×10^{-6} mg kg^{-1} day^{-1} for children. In contrast, under the high consumption scenario representative of riverine communities in the basin of the Pilcomayo River, EDI values increased to $2.38 \times 10^{-5} – 3.55 \times 10^{-5}$ mg kg^{-1} day^{-1} for adults and $2.86 \times 10^{-5} – 4.26 \times 10^{-}$

^{5} mg kg^{-1} day^{-1} for children (Table 3). In all scenarios, children presented higher estimated exposure levels than adults due to their lower body weight despite a relative lower amount of fish consumed.

Table 3. Estimated daily intake (EDI) of Pb associated with the consumption of *Prochilodus lineatus* under different fish consumption scenarios for adults and children in the middle basin of the Pilcomayo River (Villa Montes, Bolivia)

Scenario	Population	Pb concentration (mg kg^{-1} ww)	Average fish ingestion rate IR (g day^{-1})	Body weight BW (kg)	EDI (mg kg^{-1} day^{-1})
Low consumption	Adults	0.013	4 - 6	60	$8.67.10^{-7}$ - $1.30.10^{-6}$
Low consumption	Children	0.013	4 - 6	20	$2.60.10^{-6}$ - $3.90.10^{-6}$
High consumption	Adults	0.013	110 - 164	60	$2.38.10^{-5}$ - $3.55.10^{-5}$
High consumption	Children	0.013	44 – 65.6	20	$2.86.10^{-5}$ - $4.26.10^{-5}$

To evaluate the potential health risk associated with these exposure levels estimated through *P. lineatus* consumption, the calculated EDI values are compared with reference values for Pb intake established by the World Health Organization (WHO). The WHO does not provide a guideline value for tolerable intake of Pb as no safe level of exposure to Pb has been identified due to its toxicity observed even at very low level of exposure[44]. Nevertheless, an exposure to 6×10^{-5} mg kg^{-1} day^{-1} of Pb in children and 1.2×10^{-3} mg kg^{-1} day^{-1} of Pb in adult has been associated with minimal but adverse health effect corresponding to a loss of 1 IQ point in children and an increase of 1 mmHg in blood pressure in adults, respectively[44].

Overall, the calculated EDI values remained below the Pb exposure levels associated with small but adverse health effects reported by the World Health Organization. These results indicate that the consumption of *P. lineatus* from the middle basin of the Pilcomayo River does not represent a major pathway of Pb exposure for the general bolivian population with a low fish-consumption diet. Even under the high consumption scenario, which reflects riverine communities with frequent fish consumption, the estimated Pb intake for adults remained very low relative to the WHO reference value. These results indicate that the potential health risk association with Pb exposure through *P. lineatus* consumption is likely minimal in the general and local populations. However, it should be noted that Pb intake in children with a high fish-consumtion diet ($2.86.10^{-5}$ - $4.26.10^{-5}$ mg kg^{-1} day^{-1}) are lower but close to potentially detrimental dose for Pb established by the WHO for children (6.10^{-5} mg kg^{-1} day^{-1}).

However, this exposure assessment should be interpreted considering several limitations. The calculations were based on mean Pb concentrations measured in muscle tissue of few (n=11) *P. lineatus* specimens. A larger set of data including a number > to 30 specimens is generally recommended to obtain a more robust statiscal conclusion. On another hand, fishes were sampled at one sampling date in Villa Montes, and therefore do not account for possible spatial or temporal variability in contamination levels. Furthermore, fish consumption rates may vary considerably among individuals and communities depending on fishing success, cultural dietary practices, seasonal availability, and consumption patterns, including the intake of other tissues such as liver or bone. The consumption of larger quantities of fish even with moderate concentrations of Pb could result in health risks in adults and children due to excessive intake of the metal. Finally, this assessment considers only Pb exposure through *P. lineatus* consumption, while other dietary sources and environmental pathways may also contribute to the total Pb exposure of local populations.

CONCLUSIONS

Lead concentrations in *P. lineatus* from the middle Pilcomayo River showed clear differences among tissues, with the highest levels detected in opercular tissue, followed by liver, while muscle presented the lowest concentrations. These results suggest limited translocation of Pb to edible tissues. When compared with historical records from the Pilcomayo basin, Pb concentrations measured in fish collected in Villa Montes in 2024 were substantially lower than those reported during the late 1990s and early 2000s, suggesting a marked decline in Pb contamination in fish in the middle basin over the last decades. The estimated daily intake (EDI) values calculated for two fish consumption scenarios indicate that Pb exposure associated with the consumption of *P. lineatus* is very low for both adults and children. These findings suggest that the consumption of this species from the middle Pilcomayo River is unlikely to represent a significant pathway of Pb exposure for local populations with a high fish-consumption diet except for children for whom the EDI remained lower but close to Pb intake value associated with small but adverse effect as established by the WHO. Overall, the results provide updated information on Pb contamination in an historically impacted river system and highlight the importance of continued environmental monitoring in the Pilcomayo River basin and other mining-impact rivers.

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