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Impact of hydrocarbon extraction on heavy metal concentrations in lowland paca (*Cuniculus paca*) from the Peruvian Amazon

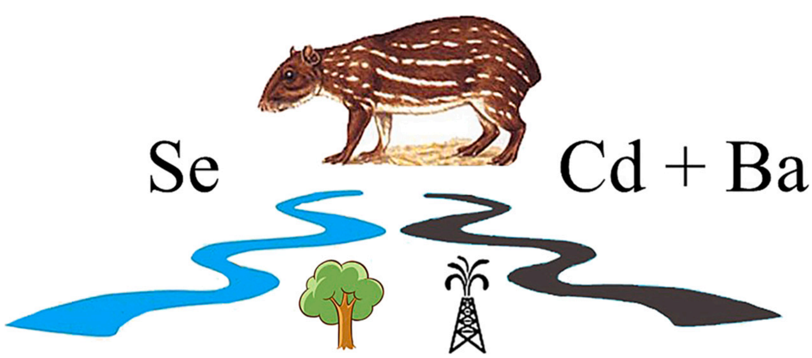
Pedro Mayor [a,b,*], Lucía Soliño [c], Mar Cartró-Sabaté [c], Martí Orta-Martínez [d,e,**]

[a] Dept. Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, Catalonia, Spain
[b] Museo de Culturas Indígenas Amazónicas, Fundamazonia, Iquitos, Peru
[c] Institut de Ciència i Tecnologia Ambientals, Universitat Autònoma de Barcelona, Catalonia, Spain
[d] Department of Evolutionary Biology, Ecology and Environmental Sciences, Universitat de Barcelona, Catalonia, Spain
[e] Institute de Recerca de la Biodiversitat, Universitat de Barcelona, Catalonia, Spain

HIGHLIGHTS

- Ba and Cd from oil pollution bioaccumulate in tropical wild lowland paca.
- Cd levels pose a health risk for indigenous people that rely on subsistence hunting.
- Oil activities are the source of high and unsafe blood Cd levels in indigenous people.
- Pacas in oil-polluted areas have low Se levels.

GRAPHICAL ABSTRACT



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ABSTRACT

Oil has been extracted from the Western Amazon since the 1920s, leading to severe environmental contamination due to frequent occurrence oil spills and the dumping of produced water. Local inhabitants, along with environmental and human rights organizations, have reported the adverse effects of oil-related pollution on their livelihoods and the ecosystems they depend on. Here, we study accumulation of oil-related heavy metals in wildlife, and its subsequent incorporation into the trophic chain. We analysed the concentration of 14 heavy metals (Cd, Cr, Hg, As, Ni, V, Ba, Se, Be, Fe, Cu, Zn, Mn, Al) in liver samples from 78 lowland pacas (*Cuniculus paca*) hunted for subsistence in an oil-polluted area from the northern Peruvian Amazon where oil has been extracted since the 1970s ($n = 38$), and two control areas, the Yavari-Mirín River basin ($n = 20$), and the Pucacuro River basin ($n = 20$). Pacas in the oil-polluted area have significantly higher concentrations of Cd ($P < 0.01$) and Ba ($P < 0.0001$) compared to those in control areas, suggesting bioaccumulation of oil-related pollution. Conversely, Se levels were significantly lower in the oil-polluted area ($P < 0.0001$), likely due to the sequestration of Se by other heavy metals, particularly Cd. Additionally, minor variations in other heavy

* Correspondence to: P. Mayor, Departament de Sanitat i d'Anatomia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB), Barcelona 08193, Spain.
** Correspondence to: M. Orta-Martínez, Department of Evolutionary Biology, Ecology and Environmental Sciences, Universitat de Barcelona, Catalonia, Spain.
E-mail addresses: pedrogines.mayor@uab.cat (P. Mayor), marti.orta@ub.edu (M. Orta-Martínez).

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metals, e.g., Fe and Zn, were observed in pacas from the oil-polluted area, whereas control areas showed higher concentrations of Ni and Cu. Mn and Al levels did not significantly differ between the study areas. These results underscore the impact of oil extraction on the absorption and assimilation of heavy metals in wildlife, point at oil activities as the source of the high and unsafe blood Cd levels reported for the indigenous population of the studied oil extraction area and raise concerns about the long-term health risks from oil extraction posed to local Indigenous People who rely on subsistence hunting.

1. Introduction

Oil and gas reserves overlap 33 % of the world's rainforests (Pellegrini et al., 2024). The impact of oil exploration and exploitation on tropical rainforest ecosystems has gained increasing recognition as a significant driver of environmental degradation (Azevedo-Santos et al., 2016; Butt et al., 2013; Finer et al., 2008; Harfoot et al., 2018; Rivera-Parra et al., 2020; Suárez et al., 2009, 2013).

Oil exploration in the western Amazon started in the 1920s, with a production boom arriving in the 1970s and the 2010s (Finer and Orta-Martínez, 2010). These prolonged oil extraction activities have caused significant environmental and social upheaval, impacting biodiversity and the indigenous communities residing in this region (Cartró-Sabaté et al., 2019; Orta-Martínez et al., 2007, 2018a, 2018b; Rosell-Melé et al., 2018). The consequences encompass deforestation due to the construction of oil infrastructure and roads, loss of biodiversity, fragmentation of ecosystems, and the proliferation of illegal logging and wildlife trade (Finer et al., 2008; Orta-Martínez et al., 2007; Suárez et al., 2009, 2013). It has also resulted in the degradation of natural resources for the indigenous populations and territorial conflicts (Orta-Martínez et al., 2007, 2018a). Recurring oil spills and inappropriate handling and discharge of the main wastewater produced during the process of oil extraction (i.e. produced or formation water), are a significant environmental concern (Jernelöv, 2010; Rivera-Parra et al., 2020). Produced water contains a mixture of harmful substances and its improper discharges into rivers, streams, or on soil has led to severe environmental contamination and ecological damage (Finer and Orta-Martínez, 2010; Ministerio del Ambiente, 2013a, 2013b, 2013c; Moquet et al., 2014; Rosell-Melé et al., 2018; Yusta-García et al., 2017). Produced water contain elevated levels of salts and mutagenic, carcinogenic, and bioaccumulative substances, such as radioactive isotopes, dispersed hydrocarbons, and heavy metals (Fakhru'l-Razi et al., 2009; Reátegui-Zirena et al., 2014; Vaikosen et al., 2014). Despite regulations and industry standards recommend produced water reinjection back into oil wells and discourage or ban the discharge of produced water onto land or surface waters, such practices have continued in the Peruvian Amazon until as recently as the early 2010s (Comisión de Pueblos Andinos Amazónicos y Afroperuanos, 2013; Orta-Martínez et al., 2018a).

In tropical regions, many of the oil and gas blocks overlap indigenous territories (Orta-Martínez and Finer, 2010). Rural and Indigenous Peoples heavily rely on subsistence activities and the utilization of wildlife as a primary source of protein and wild meat trade (El Bizri et al., 2020; Mayor et al., 2022). Consequently, the bioaccumulation of petrogenic heavy metals within the trophic chain carries significant implications for human health and poses a threat to the food security of these local populations.

Despite decades of intensive oil extraction activities in tropical rainforests (Orta-Martínez et al., 2022) and well-documented toxicological effects of certain oil-related pollutants (Monteiro et al., 2016; Vaikosen et al., 2014), there remains a lack of comprehensive data to thoroughly evaluate and address their potential impacts (Butt et al., 2013). One particular issue insufficiently investigated is the introduction of petrogenic compounds into the food web and its implications for biodiversity, food security, and the well-being of Amazonian communities.

While some oil-related heavy metals, present in crude oil and oil extraction by-products, naturally occur in trace amounts within the Earth's crust, oil pollution can significantly elevate their concentrations in the environment (Environmental Protection Agency - EPA, 2000). Certain oil-related heavy metals, such as copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), selenium (Se), and zinc (Zn) are essential elements required in small quantities for biological processes. However, exposure to high concentrations of these essential metals can lead to adverse effects (European Food Safety Authority - EFSA, 2006). Another group of oil-related heavy metals, including arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), mercury (Mg), and vanadium (V) (Fakhru'l-Razi et al., 2009), devoid of any recognized biological role, can be highly toxic even at minimal concentrations (Ali and Khan, 2019). This study assesses the assimilation of the most toxic heavy metals except lead (As, Cd, Cr, and Hg) and other metals associated with oil contamination (Ni, V, Ba, Se, Be, Fe, Cu, Zn, Mn, Al) in free-ranging populations of lowland pacas (*Cuniculus paca*, Linnaeus, 1766) within an oil extraction area of the northern Peruvian Amazon. Notably, Pb levels, although significant in oil-polluted areas, were previously studied in a separate scientific article (Cartró-Sabaté et al., 2019). Our investigation involves a comparison of heavy metal concentrations in the livers of lowland pacas from this oil extraction area and two control areas from the northern Peruvian Amazon.

2. Material and methods

2.1. Study area

The study area includes one oil-polluted area (the interfluvial area between the Pastaza and the Corrientes River basins) and two control sites, the Pucacuro National reserve and the Yavari-Mirín River basin in the northern Peruvian Amazon. These areas encompass the ancestral territories of the Achuar, Kichwa and Yagua Indigenous Peoples (Fig. 1). No oil extraction or industrial activities have taken place either in the Yavari-Mirín or the Pucacuro River basins. On the contrary, oil has been extracted from the interfluvial area between the Pastaza and the Corrientes River basins since the early 1970s, when the 192 oil block (former 1AB) was granted. The oil block 1AB/192 has been operated by different oil companies over the last 50 years (Occidental Petroleum Corporation, Pluspetrol Norte and Pacific Stratus Energy), and spans over 512,347 ha of the Tigre, Corrientes and Pastaza River basins.

Over four decades, until 2010, produced water from oil block 1AB/192 -up to 1 million barrels per day-was dumped onto land or surface waters from the area (Comisión de Pueblos Andinos Amazónicos y Afroperuanos, 2013; Orta-Martínez et al., 2018a), leading to severe contamination of soil and water (Orta-Martínez et al., 2007; Rosell-Melé et al., 2018; Yusta-García et al., 2017). As a consequence, in 2013 and 2014, the Peruvian government declared the environmental and health emergency in the study area (Ministerio del Ambiente, 2013a, 2013b, 2013c; Ministerio de Salud, 2014). Testimonies from local residents have consistently reported instances of oil-polluted fish and hunted animals (Dirección General de Epidemiología - DGE, 2006), and prior investigations have identified unsafe levels of As, Cd, Pb, and Hg in both children and adults living near the oil extraction facilities (O'Callaghan-Gordo et al., 2021, 2023).

Recent research conducted in the area has revealed that wildlife is ingesting oil-polluted soil, probably attracted by the high salinity of produced water (Orta-Martínez et al., 2018b). This new pathway of exposure to petrogenic compounds (i.e. re-directed geophagy), has resulted in elevated levels of Pb in these animals, exacerbating the problem of Pb pollution due to the use of lead-based ammunition for subsistence hunting (Cartró-Sabaté et al., 2019).

The three study areas are emplaced within the Amazonas Lowlands (average 210 m.a.s.l.) Freshwater Ecoregion of the World (FEOW-316), described in Abell et al. (2008). This continuous forest area combines lowlands and non-flooded upland forests. The climate in the region is typically equatorial with an annual temperature from 22 to 36 °C, a relative humidity from 80 % to 100 %, and an annual rainfall from 1500 to 3000 mm.

2.2. Sample collection

Between May 2013 and August 2015, local subsistence hunters from the three areas collected and preserved the livers of 78 hunted pacas in buffered 4 % formaldehyde solution (*v*/v) (see Table 1). Specifically, 38 pacas were sampled from the hunting grounds of 6 Achuar and Kichwa indigenous communities from the oil-polluted area: José Olaya and Jerusalén in the Corrientes River basin ($n = 17$), and Andoas Nuevo, Andoas Viejo, Titiyacu, and Los Jardines in the Pastaza River basin ($n = 21$). In addition, 40 pacas were collected from two control areas: 20 from the hunting grounds of the Yagua indigenous community of Nueva Esperanza in the Yavarí-Mirín River basin, and 20 individuals captured in the Pucacuro River basin by the environmental monitors of the Pucacuro National Reserve.

The choice of the paca as our biological indicator is grounded in its significance as: 1) the most commonly consumed and traded wild meat species in the Amazon (Peres, 2000), 2) a large rodent frequently found near water sources, where produced water is typically discharged, and 3) an effective indicator of local environmental conditions due to its limited territorial range, typically spanning from 0.7 to 3.4 ha (Beck-King et al., 1999; Marcus, 1984).

Importantly, it's worth noting that these samples were voluntarily donated by indigenous hunters, and no pacas were intentionally killed for the sole purpose of this study. All pacas included in the study were hunted using lead-based ammunition and were subsequently consumed by local inhabitants. Hunters were previously trained for the correct collection and preservation of the biological sample, and they recorded the date and location where each paca was captured. The research team evaluated the appropriate conservation status of the livers.

2.3. Sample preparation and heavy metal analysis

Before freeze-drying, liver samples were prepared by removing the outer layer, approximately 1 cm thick, using a ceramic knife. This procedure was carried out to mitigate potential surface contamination and the risk of underestimating heavy metal concentrations due to leaching

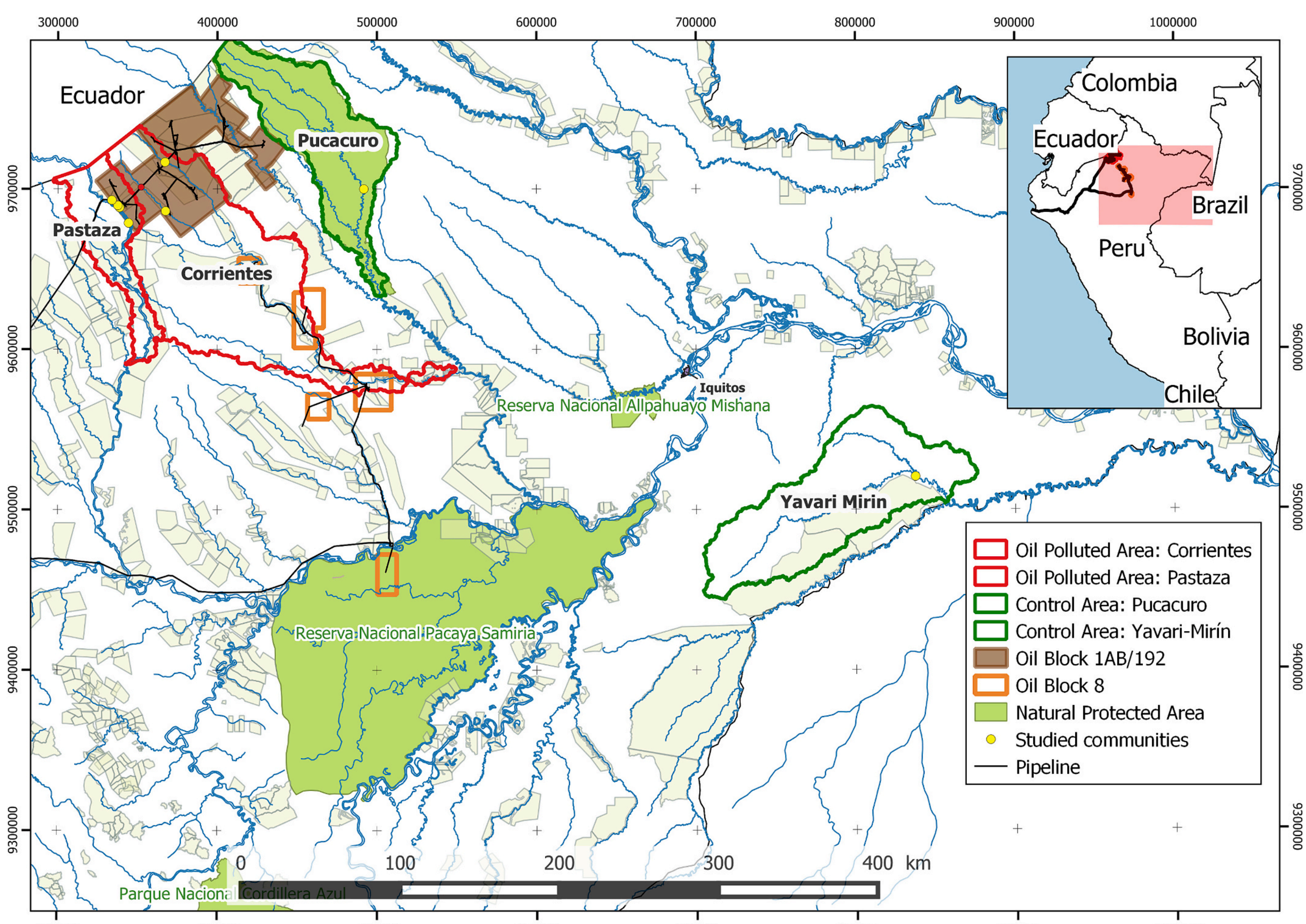

Fig. 1. Map of the study area. The Pastaza and Corrientes River basins —inhabited by Quechua and Achuar Indigenous People— are overlapped by the oil concession Block 192 (formerly 1AB). The Pucacuro National Reserve is a natural protected area inhabited by Kichwa Indigenous People. The Yavari-Mirín River basin is inhabited by only one Yagua indigenous community whose only economic activity is selective logging. There are no oil-extraction activities in the Pucacuro and Yavari-Mirin basins. Own elaboration based on open-source georeferenced data from Perupetro (oil concessions), Instituto del Bien Común -IBC- (indigenous titled lands) and the Peruvian Ministry of Environment (natural protected areas).

Table 1

Dry weight (DW) heavy metal concentrations (range, average, standard deviation, median, and Q1/Q3) in liver tissues of 78 pacas from oil-polluted (n=38) and control areas (n=40) in the Peruvian Amazon. The number and percentages of samples under the limit of quantification (LOQ) of the analytical method for each metal are displayed. NP=Not performed. The asterisk indicates heavy metals with significant differences between control and oil-concession areas.

Heavy metal concentration (DW)	LOQ	Samples from control areas (n=40)							Samples from oil-polluted area (n=38)							Test	P value
		n<LQ	n<LOQ (%)	Range	Average	SD	Median	Q1/Q3	n<LQ	n<LOQ (%)	Range	Average	SD	Median	Q1/Q3		
Se (μg/g)*	1.250	0	0	1.313-9.556	3.644	2.442	2.523	1.975/4.393	21	55	<LOQ-2.780	0.747	0.890	0.000	0.000/1.411	K-W	5.679×10^{-11}
Ba (μg/g)*	0.250	0	0	1.543-14.429	3.325	2.188	2.840	2.214/3.604	0	0	1.311-85.107	10.559	14.662	5.945	3.204/11.462	K-W	4.729×10^{-5}
Ni (μg/g)*	0.250	1	3	<LOQ-1.345	0.649	0.244	0.607	0.512/0.740	11	29	<LOQ-3.567	0.516	0.661	0.435	0.000/0.590	K-W	0.001
Cd (μg/g)*	0.250	1	3	<LOQ-1.814	0.621	0.267	0.583	0.503/0.680	3	8	<LOQ-3.695	1.074	0.889	0.734	0.576/1.530	K-W	0.008
Fe (mg/g)	0.025	0	0	0.264-1.556	0.603	0.261	0.556	0.425/0.718	0	0	0.254-1.531	0.719	0.275	0.721	0.527/0.864	t-test	0.051
Zn (μg/g)	25.000	0	0	52.009-88.489	73.145	8.626	74.005	67.060/78.880	0	0	39.625-111.291	79.012	17.082	80.680	70.020/89.980	t-test	0.063
Cu (μg/g)	1.250	0	0	6.335-16.158	10.556	2.409	10.538	8.506/11.749	0	0	6.013-14.482	9.605	2.110	9.462	7.884/10.815	t-test	0.067
Mn (μg/g)	0.250	0	0	10.11-112.593	38.318	23.100	33.579	23.370/47.520	0	0	7.493-83.360	30.755	16.475	30.264	18.996/35.561	t-test	0.176
Al (mg/g)	0.025	29	76	<LOQ-0.233	0.018	0.044	0.000	0.000/0.026	26	68	<LOQ-0.400	0.023	0.067	0.000	0.000/0.0269	K-W	0.738
As (μg/g)	0.250	40	100	<LOQ	<LOQ	<LOQ	<LOQ	0.000/0.000	37	97	<LOQ-264	0.007	0.043	0.000	0.000/0.000	NP	-
Be (μg/g)	0.250	40	100	<LOQ	<LOQ	<LOQ	<LOQ	0.000/0.000	38	100	<LOQ	<LOQ	<LOQ	<LOQ	0.000/0.000	NP	-
Cr (μg/g)	0.250	34	85	<LOQ-1.648	0.090	0.290	0.000	0.000/0.000	36	95	<LOQ-1.376	0.045	0.228	0.000	0.000/0.000	NP	-
Hg (μg/g)	0.250	38	95	<LOQ-1.383	0.011	0.049	0.000	0.000/0.000	38	100	<LOQ	<LOQ	<LOQ	<LOQ	0.000/0.000	NP	-
V (μg/g)	1.250	39	98	<LOQ-1.427	0.036	0.226	0.000	0.000/0.000	38	100	<LOQ	<LOQ	<LOQ	<LOQ	0.000/0.000	NP	-

effects in the outermost layer (Orta-Martínez et al., 2021; Sato et al., 2006). Freeze-dried samples were subsequently ground into a fine powder using a porcelain mortar and pestle. A 0.1 g aliquot of each homogenized sample was then digested using a mixture of HNO3, HCl, and HF in a microwave (Ultrawave, Milestone) at 240 °C for a duration of 15 min. To ensure the reliability of the analysis, digestion blanks were simultaneously created, and a sample of the formaldehyde solution (0.1 g aliquot) was also subjected to digestion to assess any potential heavy metal leaching. Subsequently, all samples were diluted with 1 % HCl (*v/v*) before being analysed by inductively coupled plasma mass spectrometry (ICP-MS) using a single quadrupole mass analyser (7500ce, Agilent Technologies) at the Analytical Chemistry Service of the Autonomous University of Barcelona (Catalonia, Spain; http://sct.uab.cat/saq), a laboratory with a valid ISO 9001:2015 certification, to quantify Al, As, Ba, Be, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Se, V, and Zn concentrations. In the sample blanks, the concentration of metals was below the limit of detection. Every 15 samples, a reference standard created in the laboratory was injected to measure reproducibility of the analyses (relative s.d. = 15 %). Accuracy and recovery checks were performed using a reference standard material of bovine liver NIST 1577c (National Institute of Standards Technology, US Department of Agriculture, Beltsville, MD, USA). Recovery (n = 5) was 94.06 ± 1.99 % (mean ± S.D.) for V, 96.74 ± 1.83 % for Cr, 92.29 ± 2.27 % for Mn, 94.25 ± 2.16 % for Fe, 97.18 ± 7.67 % for Ni, 94.49 ± 2.45 % for Cu, 94.20 ± 1.48 % for Zn, 100.34 ± 2.95 % for As, 100.00 ± 1.71 % for Se, and 94.42 ± 1.81 % for Cd. For Al, Be, Ba and Hg (Hg concentration was below to LOD), the accuracy of the analytical method was evaluated by spiking (i.e. adding a known amount of analyte to some of the samples at different concentrations), obtaining recovery values of 112 % for Be, 99 % for Ba, 84 % for Hg, and 100 % for Al. The limit of quantification (LOQ) for each compound is detailed in Table 1. Concentrations of heavy metals are reported as medians along with interquartile ranges (IQR). These concentration values are expressed on a dry weight (DW) basis, with the wet weight (WW) calculated by considering the weight ratio before and after freeze-drying.

2.4. Statistical analysis

Heavy metal concentrations below LOQ were computed as 0 for statistical analysis (Lubin et al., 2004). Because Cr, Be, V, As and Hg had a high percentage (>90 %) of samples below LOQ they were only considered for descriptive statistics. Normality of data was assessed with the Wilcoxon-Mann Whitney test and non-normal concentration values were transformed to the logarithm $\log(x + 1)$. We conducted four statistical analyses: 1) to examine differences for each heavy metal between the oil-polluted and control areas a non-paired T-student or Krustal-Wallis non-parametric test was performed; 2) to examine differences for each heavy metal according to the three study areas, an Anova or Krustal-Wallis non-parametric test was performed; 3) a Principal Component Analysis (PCA) was also run to assess the individual distribution of samples (observations) and nine heavy metals (variables) with <90 % of samples below the LOQ (Al, Fe, Mn, Ni, Cu, Zn, Se, Cd and Ba); and, 4) upper range of outliers, defined as values higher than the sum of the mean and one standard deviation, were compared for each heavy metal according with the origin of samples, oil-polluted and control areas, using a Chi-square test.

The R-studio interface, R version 3.6.2 (R Development Core Team, 2019) was used for statistical analysis. The package tidyverse was used for data manipulation and data tidying (Wickham et al., 2019), the FactoMineR, factoextra and cluster packages were employed for PCA (Kassambara and Mundt, 2020; Lê et al., 2008; Maechler et al., 2019), and the ggplot2 package was used for data visualization (Wickham, 2016).

3. Result

Liver levels for the 14 heavy metals assessed are shown in Table 1. Levels of Cd ($P = 0.008$) and Ba ($P < 0.0005$) were significantly higher in pacas from the oil-polluted area compared to those from the control areas, and a strong trend of higher Zn ($P = 0.063$) and Fe ($P = 0.05$) concentrations was also observed. Similarly, the oil-polluted area presented more samples with extreme values -upper range of outliers- of Cd ($P < 0.01$) and Zn ($P = 0.002$) and a strong trend with Ba ($P = 0.056$). Contrarily, the concentration of Se and Ni were significantly higher in the control areas ($P < 0.0005$ and 0.005, respectively), and presented a strong trend for Cu ($P = 0.067$). While the number of extreme values of Se was higher in control areas ($P = 0.003$), we did not find significant differences for Ni and Cu.

Although Mn levels were similar in both oil-polluted and control areas, we found significant differences ($P < 0.0001$) in the three study areas, being higher (in decreasing order) in the Pucacuro River basin, oil-contaminated area and Yavarí River basin. Al levels were not different between areas ($P > 0.15$). The number of extreme values of Al, Mn, Fe, and Cu did not show significant differences.

Fig. 2 shows the Principal Component Analysis (PCA) of heavy metal concentrations in pacas hunted in the oil-concession and the two control areas. The two first PCA dimensions explained 70.0 % of the distribution of individuals in the ordination space based on the presence of heavy metals (Dim 1: 61.3 %, Dim 2: 14.7 %). Considering both dimensions 1 and 2, the heavy metals providing the greatest explanation for the spatial distribution of individuals were Se (Dim 1: 5.9 %, Dim 2: 47.3 %), Cd (11.2 %, 16.4 %) and Ba (8.3 %, 17.3 %), and hence the most important to interpret the differences observed between the Corrientes, Pastaza, Pucacuro and Yavari-Mirín groups. The PCA shows a clear overlap within the samples from the control basins (Pucacuro and Yavari-Mirín River basins) and within the oil-polluted basins (Corrientes and Pastaza River basins); and, in turn, a clear separation of individuals is observed between both groups, oil-polluted and control areas. Fe (15.3 %) and Cu (14.4 %) have the highest correlation with Dimension 1, and are also correlated with Ni (12.4 %), Zn (11.8 %) and Al (9.72 %); thus, these elements can be considered redundant. Selenium (47.3 %) shows the maximum correlation with dimension 2, followed by Ba (16.4 %) and Cd (17.3 %). Samples from control areas were mainly characterized by the presence of Se and, to a lesser extent, Cu and Mn; while the oil-polluted areas were mainly differentiated by the presence of Ba and Cd.

4. Discussion

Our study has unveiled a concerning phenomenon: pacas inhabiting oil-polluted regions of the Peruvian Amazon are concurrently displaying higher levels of Cd and Ba. This suggests that the activities related to oil extraction are leading to the widespread accumulation of Cd and Ba, and probably, Zn and Fe, in paca populations. More importantly, these findings confirm that petrogenic pollutants are found within the trophic chain in the oil extraction areas and might be biomagnified at higher trophic levels.

Once these hazardous compounds enter the trophic chain, they have the potential to impact various trophic levels within the Amazon's ecosystem. This influence could be exacerbated from herbivores to apex predators, encompassing not only wildlife but also the local human populations. This is especially significant for Indigenous Peoples who rely on subsistence hunting as a primary source of daily protein. Pacas are a key source of protein for local communities in the Amazon, playing a critical role in ensuring food security and, could therefore, pose a health risk for the local indigenous population. Indeed, previous research has already identified elevated levels of Cd in blood and urine among children and adults residing in the same region and have associated these high levels with oil extraction activities (DIGESA, 2006; O'Callaghan-Gordo et al., 2023). The Ministry of Health of Peru

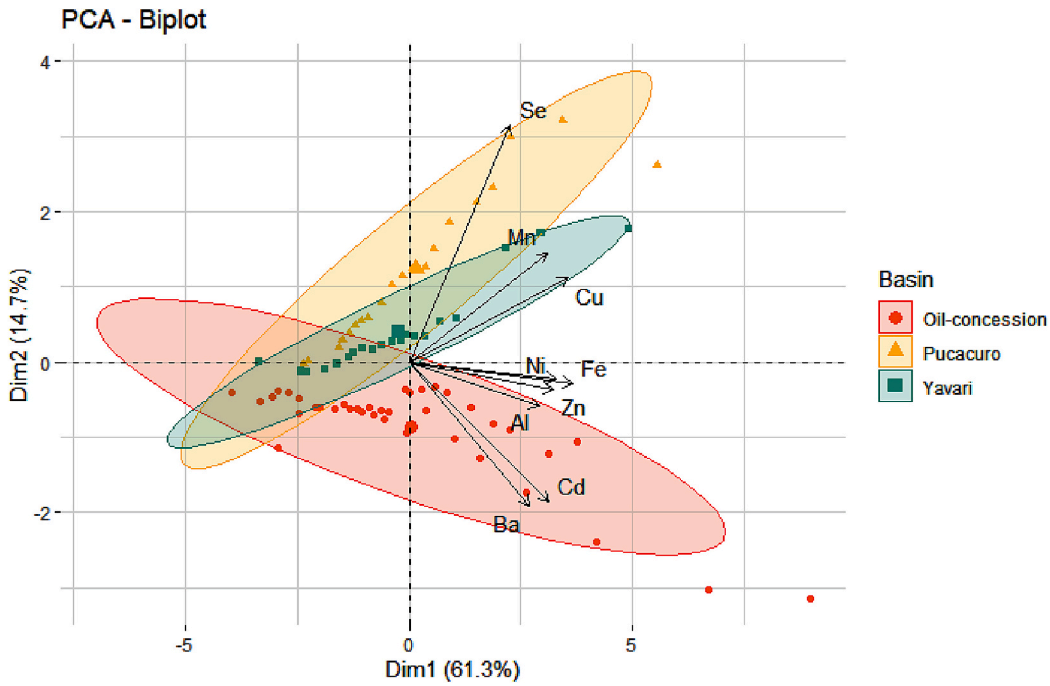

Fig. 2. Principal Component Analysis (PCA) of heavy metal concentrations in liver samples of 78 pacas hunted in the oil-polluted area of the Corrientes (n = 17) and Pastaza (n = 21) River basins and the control areas comprising the Pucacuro (n = 20) and Yavari-Mirín (n = 20) River basins.

reported that, in 2006, 98.6 % of children and 99.2 % of adults of the Achuar indigenous population exceeded the considered safe Cd blood levels (Orta-Martínez et al., 2007), and, according to O'Callaghan-Gordo et al. (2023), in 2016, 2 % of children and 13 % of adults of the indigenous population within the oil-block 1AB/192 had urine Cd levels above the reference values (2 μg/g) established by the Peruvian Ministry of Health.

Although there appear to be few putative sources for both elements other than the oil extraction industry in the oil-polluted area, other studies have identified other industrial activities that could contribute heavy metals, such as small-scale gold mines and landfills in the headwaters of the Pastaza River basin (Azevedo-Santos et al., 2021; Capparelli et al., 2020; González-Merizalde et al., 2016). However, it is unlikely that contamination from Ecuador transported by the Pastaza main stem could reach the upstream tributaries and the interfluve studied area between the Pastaza and Corrientes Rivers.

Cd and Ba have been widely linked to crude oil, produced waters, and drilling muds (Eccles et al., 2020; Fakhru'l-Razi et al., 2009; Lienemann et al., 2007; Neff, 2002). A published meta-analysis of water quality data collected by various Peruvian state institutions concluded that produced water from oil block 1AB/192 was a source of 0.16 and 179.48 Metric tons of Cd/year and Ba/year, respectively, in the Corrientes River, and 0.02 and 25.91 Metric tons of Cd/year and Ba/year, respectively, in the Pastaza River (Yusta-García et al., 2017).

Most developed countries have drawn up legal regulations to limit the transfer of heavy metals (As, Be, Cd, Cr, Hg, and V) that can be extremely toxic even in minute concentrations (Ali and Khan, 2019) to humans and the environment (Codex Alimentarius Comission, 1995; European Food Safety Authority - EFSA, 2011; European Commission, 2006; Sanidad Pesquera - SANIPES, 2016). Nevertheless, the intake limits from most heavy metals are highly variable depending on the legislation of each country, and there are even important variations in the recommendations of international health and food safety agencies.

If we compare our results with the acceptable limits of Cd in offal for human consumption set by the European Union Commission Regulation 1881/2006 (0.5 μg/g wet weight) and the Peruvian regulation for tuna (Sanidad Pesquera - SANIPES, 2016; 0.1 μg/g wet weight), 7.6 % and 79.5 % of the pacas studied, respectively, exceed these limits.

The reported concentrations indicate minimum contamination values, since the contamination in oil blocks 1AB/192 and 8 is extensive, but not homogeneous. The oil-polluted area presents a diversity of habitats including upland and lowland forests. Upland forests are less dependent on waterways, which serve as major transporters of heavy metals; consequently, they are likely to be less contaminated. Therefore, animals captured in oil-contaminated areas have been probably exposed to different levels of contamination and can be considered representative of the entire paca population.

Both acute and chronic Cd exposure triggers alterations at the molecular and tissue levels that induce disturbances in key organs and systems. Due to its extremely long biological half-life (16–33 years), chronic effects in humans are a serious concern, and long-term Cd exposure has been related to renal and hepatic impairment, bone demineralization (osteomalacia, osteoporosis, and the Itai-Itai disease), lung damage, alteration of endocrine system and reproduction, coronary-related issues (Genchi et al., 2020a; Geng and Wang, 2019; Kumar and Sharma, 2019; International Agency for Research on Cancer - IARC, 1993; Nordberg, 2004), and neurotoxicity related to Alzheimer's and Parkinson's diseases (Méndez-Armenta and Ríos, 2007). Moreover, Cd is listed in Group I of the International Agency for Research on Cancer classification as a human carcinogen (International Agency for Research on Cancer - IARC, 1993; International Agency for Research on Cancer - IARC, 2021).

In the control areas, we found higher concentrations of Se and Ni (although, in the case of Ni, the highest value was reported in the oil-polluted area), and trends of elevated Cu. The lower concentrations of Se in oil-polluted areas are in agreement with other studies which show a generalized Se deficiency in wild herbivores in polluted areas (Flueck et al., 2012). Indeed, average values in pacas from the oil-polluted area were close to those considered deficient (<0.15 mg/kg WW; Flueck et al., 2012) and even critically deficient (< 0.063 mg/kg WW; McDowell et al., 1995) for wild herbivores. These low Se levels may be due to low concentrations in soils, but also to exposure to other petrogenic heavy metals (i.e. Hg and Cd). Exposure to heavy metals has been proved to increase the physiological need for Se due to Se sequestration

(Sørmo et al., 2011). This essential micronutrient is a component of enzymes that scavenge oxidative free radicals that would otherwise degrade cell membranes (Shchedrina et al., 2010). Adequate levels of Se are necessary for proper bone metabolism, iodine metabolism, immune function (Kipp et al., 2012), and reproductive success (Flueck et al., 2012; Suttle, 2010). Although severe deficiency may result in obvious symptoms such as white muscle disease in ungulates, Se-deficient individuals display no obvious signs of malady, yet Se deficiency may often impede optimal population performance resulting in population decline without apparent cause (Flueck et al., 2012).

In contrast, samples from the Pucacuro and Yavarí-Mirín River basins had higher concentrations up to a levels potentially hazardous. In fact, elevated levels of Se can result in toxic and adverse effects, and prolonged exposure to excessive Se, known as chronic selenosis, can lead to various disorders including brittle nails, hair loss, gastrointestinal disturbances, skin rashes, the characteristic "garlic breath" odour, and neurological problems (Reilly, 2006). Severe intoxication may even cause critical conditions such as tubular necrosis and gastric ulcers or acute gastritis (Ullah et al., 2018).

Although Ni levels were significantly higher in the control areas (Fig. 3), we report the highest level of Ni (3.57 μg/g DW) in the oil-polluted area. Ni is a naturally abundant element (3 %) in the terrestrial crust (Rehman et al., 2018), and anthropogenic Ni exposure may result from industry, fossil fuel combustion, and waste incineration (Genchi et al., 2020b). Ni and vanadium (V) are the two most abundant metals in petroleum (not produced water) and, in fact, the Ni/V ratio is a useful oil classification parameter (Barwise, 1990). Extra-heavy oils also contain significant amounts Ni (Nguyen et al., 2022) and elevated levels of Ni may indicate locally polluted soils due to oil spills (not the discharge of produced water). However, Ni contamination in the remote Amazon would appear to have few other putative sources than oil extraction, and further soil studies are necessary to explain differences in Ni levels in the pacas from control areas. Although Ni is an important element for several vital functions, its increased exposure may lead to oxidative stress and mitochondrial dysfunction, hematotoxicity, and immune system toxicity, and can cause respiratory, renal, nervous, and cardiovascular diseases (Genchi et al., 2020b; Rehman et al., 2018; Tang et al., 2010). Finally, the IARC classified soluble and insoluble Ni compounds in Group 1 as human carcinogens (International Agency for Research on Cancer - IARC, 2012).

Higher Ba concentrations in pacas from the oil-polluted area, when compared to the control areas, also suggest the impact of oil pollution on wildlife. However, they may not represent itself a food safety issue. No regulations set the maximum permissible levels of Ba in meat in any legislation, but studies with experimental animals suggest that long-term oral exposure to Ba (200 mg barium/kg/day) can trigger renal lesions and detrimental health (Agency for Toxic Substances and Disease Registry - ATSDR, 2007). Barium sulfate ($BaSO_4$) is commonly used by the oil and gas upstream industry as a weighting agent in drilling muds to counteract pressure in the oil reservoirs drilled, preventing a blowout (Agency for Toxic Substances and Disease Registry - ATSDR, 2007; O'Rourke and Connolly, 2003). Barium sulfate is usually present in very high concentrations in produced water globally (Environmental Protection Agency - EPA, 2000; Fakhru'l-Razi et al., 2009). Due to its stability and low solubility in water, barium sulfate is not likely to disperse increasing its persistence in soils and in the ecosystem, but, in turn, this form causes less harmful health effects (Menzie et al., 2008). Ba does also occur in produced water and, it is worth to mention, however, that there is a strong correlation between concentrations of Ba and radium isotopes and naturally occurring radioactive materials (NORM) in produced water (Fakhru'l-Razi et al., 2009).

Fe and Zn levels in livers appeared to be related to the oil-concession area; conversely, Cu was associated with control areas. Although we found concentrations in livers slightly above those reported in meat

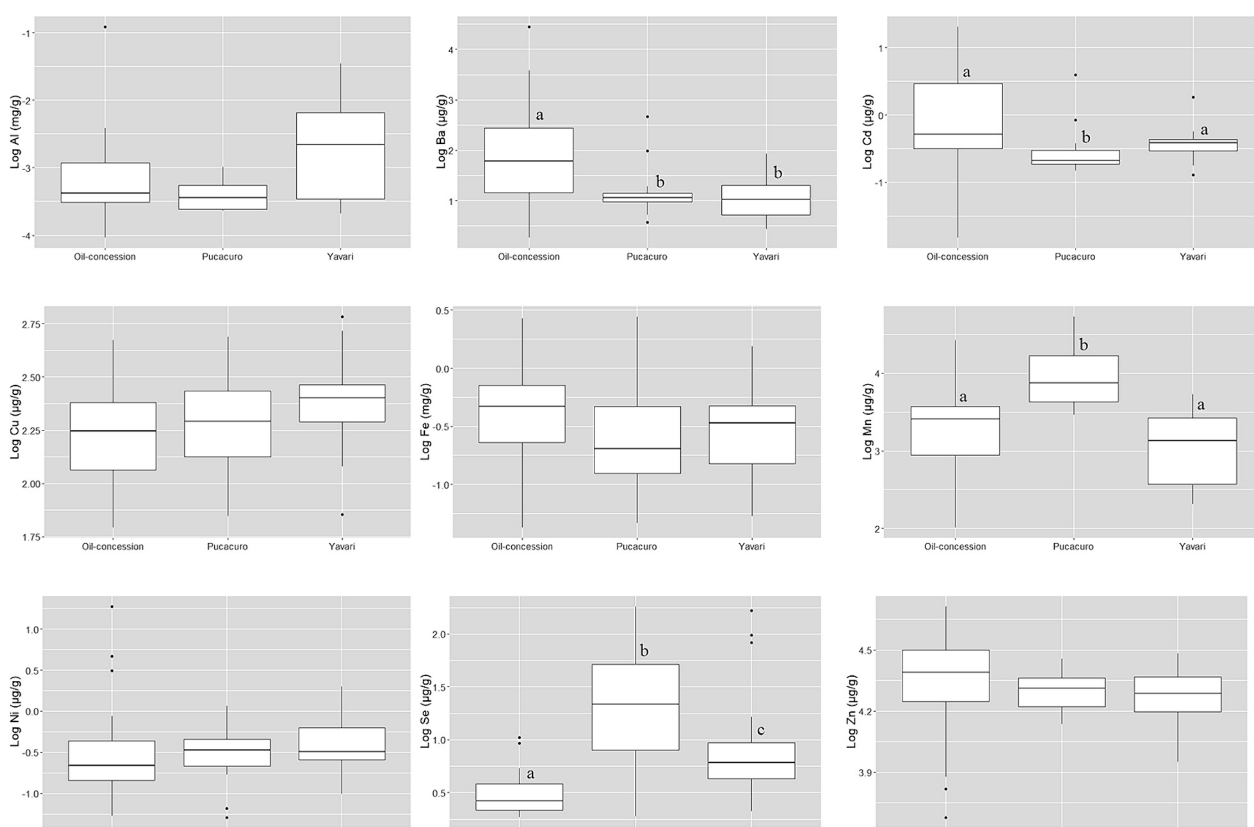

Fig. 3. Heavy metal concentrations (in log) in pacas (n = 78) from the three studied areas in the Northern Peruvian Amazon. a,b,c Values that appear in parentheses with different letters are significantly different ($P < 0.05$).

from other countries, the low toxicity of these elements makes them a minor concern for public health (Jorhem et al., 1989; European Food Safety Authority - EFSA, 2006, 2008; European Food Safety Authority - EFSA, 2015; Kovarich et al., 2020). Despite Fe, Zn and Cu are generally present in produced water (Fakhru'l-Razi et al., 2009), these elements are widely and irregularly distributed along the terrestrial crust, and their geographical variability is highly influenced by local geological features (Okoye et al., 2022). Therefore, it is difficult to argue the anthropogenic sources and the cause-effect relationship that explains the strong trends observed in these heavy metals between the different study areas.

5. Conclusion

Our results reveal the assimilation and bioaccumulation of petrogenic heavy metals (notably, Cd and Ba) by Amazonian wildlife, and most importantly, the introduction of hazardous heavy metals to the trophic chain. They also suggest the potential concomitant introduction to the trophic change of other petrogenic compounds non covered by this study, such us polycyclic aromatic hydrocarbons, with detrimental mutagenic and carcinogenic effects. Elevated Cd levels are widely found in livers of paca hunted for food in oil extraction areas of the Peruvian Amazon posing a health risk to the Amazonian wildlife, and local rural and Indigenous Peoples that rely on subsistence hunting for their daily protein intake. In the oil extraction area, 7.6–79.5 % of liver samples had Cd concentrations that exceed international standards for safe human consumption. Cd levels in livers of lowland paca from oil extraction areas points at oil activities as the source of the high and unsafe blood Cd levels reported for the indigenous population of this oil extraction area in the Peruvian Amazon. Given that the occurrence of redirected geophagy is particularly frequent in oil-polluted sites by oil spills and the discharge of produced water (Orta-Martínez et al., 2018b) -and that these sites are often hunting hotspots-, the environmental and health risks may be widespread in all of the oil and gas extraction areas in the world's rainforests, even in remote areas.

The oil-concession block 1AB/192, which include the Pastaza and Corrientes River basins, was leased in the late 1960s and have become the longest-running and the most productive oil project in the Peruvian rainforest (Orta-Martínez et al., 2007), with an accumulated production of 1038 million barrels of oil (Orta-Martínez et al., 2018a). Inadequate operational practices have resulted in the regular dumping of produced water into the local streams and rivers, becoming an important source of heavy metals in the northern Peruvian Amazon (Yusta-García et al., 2017). However, in 2009, produced water started to be reinjected back into the oil reservoirs (Orta-Martínez et al., 2018a) and the impact of oil-extraction activities has decreased from 2009 onwards (Moquet et al., 2014). Our results attest the bioaccumulation of heavy metals in wildlife caused by the oil industry after the application of this corrective measure, but a considerably worse scenario should be expected before 2009.

Analogous operational practices are common in wide spans (7.33×10^5 km^2) of Amazon rainforests, and even other tropical forests (Finer et al., 2015). In the Amazon, hydrocarbon extraction activities started in the 1920s (Finer and Orta-Martínez, 2010) and, in 2019, oil concessions covered 59 %, 34 %, 36 % and 19 % of the Ecuadorian, Bolivian, Colombian, and Peruvian Amazon (Codato et al., 2019). Oil reserves overlap with the 39.4 % of the Amazon tropical forests, including indigenous territories and of Indigenous Peoples in voluntary isolation (Orta-Martínez et al., 2018a). Therefore, it is urgent to better understand the bioaccumulation and biomagnification of oil-related pollutants in the tropical ecosystems of the world and to implement preventive measures to avoid and mitigate the potential health risks for the local indigenous population that rely on subsistence hunting.

Article impact statement

Hazardous petrogenic heavy metals are assimilated and bioaccumulated by Amazonian wildlife, and introduced to the trophic chain posing a health risk to wildlife and local Indigenous Peoples that rely on subsistence hunting.

Ethical standards

This research abided by the STOTEN guidelines on ethical standards. This study was performed in accordance with the Dirección General de Flora y Fauna Silvestre from Peru (0350-2012-AG-DGFFS-DGEFFS) and the Head of the National Reserve of Pucacuro (03-2012-SERNANPRN Pucacuro). Export permits were as follows: 000605-CITES-Perú and 003106-CITES-Perú, 001309-MINAGRI-DGFFS, and 003005-SERFOR.

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CRediT authorship contribution statement

Pedro Mayor: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lucía Soliño:** Writing – review & editing, Writing – original draft, Software, Investigation, Formal analysis. **Mar Cartró-Sabaté:** Methodology, Investigation. **Martí Orta-Martínez:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

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