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Catherine Kooyomjian
Nova Southeastern University

Dimitrios G. Giarikos
Nova Southeastern University, Halmos College of Arts and Sciences

Michael J Adkesson
Chicago Zoological Society

Amy Hirons
Nova Southeastern University

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ARTICLE

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Maternal offloading of arsenic and other trace elements in Peruvian fur seals

Catherine Kooyomjian[1] | Dimitrios G. Giarikos[1] | Michael J. Adkesson[2] | Amy C. Hirons[1]

[1]Halmos College of Arts and Sciences, Nova Southeastern University, Dania Beach, Florida

[2]Chicago Zoological Society, Brookfield Zoo, Brookfield, Illinois

Correspondence
Amy C. Hirons, Halmos College of Arts and Sciences, Nova Southeastern University, 8000 N Ocean Drive, Dania Beach, FL 33004.
Email: hirons@nova.edu

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Abstract

The maternal transfer of 15 elements was examined in Peruvian fur seal (*Arctocephalus australis*) dam and pup paired vibrissae (whiskers), serum, and milk samples collected from 2009 to 2019. Pup vibrissae, grown in utero, represented gestational transfer, while milk represented lactational transfer. Element concentrations, except arsenic, were highest in vibrissae compared to serum and milk for both dams and pups. Mean arsenic concentrations in pup vibrissae (0.44 µg/g) and milk (0.41 µg/g) were twice as high as dam vibrissae concentrations (0.19 µg/g) and nearly ten times higher than dam (0.06 µg/g) and pup serum (0.04 µg/g) concentrations. Mean arsenic concentrations from 2011 to 2019 increased in dam vibrissae (0.026 µg/g to 0.262 µg/g) and milk (0.361 µg/g to 0.484 µg/g). Pup vibrissae had significantly higher concentrations for 11 of the 15 elements analyzed compared to dam vibrissae, suggesting that element transfer is occurring through recent exposure and remobilization of elements from dam body stores. Potentially high concentrations of aluminum, arsenic, copper, and lead in pup tissues may impact their survival and population health. The impact of regional mining activities can contribute to elevated trace elements through runoff and pose a possible threat to local marine environments.

KEYWORDS

arsenic, maternal transfer, milk, pinniped, SECLER[1], serum, trace elements, vibrissae

1 | INTRODUCTION

[1]Peruvian fur seals (*Arctocephalus australis*; PFS), a subpopulation of South American fur seals, are found along the coast of Peru and northern Chile (Cárdenas-Alayza & Oliveira, 2016). Peruvian fur seals are categorized as "vulnerable" by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species and protected under Peruvian law (Decreto Supremo No. 013-99-AG; Cárdenas-Alayza & Oliveira, 2016). The Punta San Juan marine protected area (PSJ; 15°22′S, 75°11′W; Figure 1) in southern Peru is a key rookery for PFS and the site of the most intense upwelling core in the Humboldt current system (Bakun & Weeks, 2008; Majluf & Trillmich, 1981). The upwelling of cold, nutrient-rich water correlates to the highest primary productivity in the region, supporting numerous economically and ecologically important species (Bakun & Weeks, 2008). Under normal conditions, PFS prey heavily on abundant Peruvian anchoveta (*Engraulis ringens*), but dramatic food web changes, including the collapse of the anchoveta fishery, can occur during periodic El Niño-Southern Oscillation (ENSO) events (Arias-Schreiber, 2000; Taylor et al., 2008). The combined effects of commercial fishing and ENSO events have adversely impacted PFS populations in Peru.

Due to their long life-span and high trophic position, pinnipeds can accumulate elements at high concentrations, making them ideal bioindicators for the marine ecosystem (Das et al., 2003). Trace elements naturally occur in the environment, but they can also be released through anthropogenic activities. Terrestrial and deep-sea mining and their related activities can lead to enrichment of bioavailable elements in seawater and sediments causing negative impacts on coastal marine community structure, including high trophic level consumers (Ramirez et al., 2005; Stauber

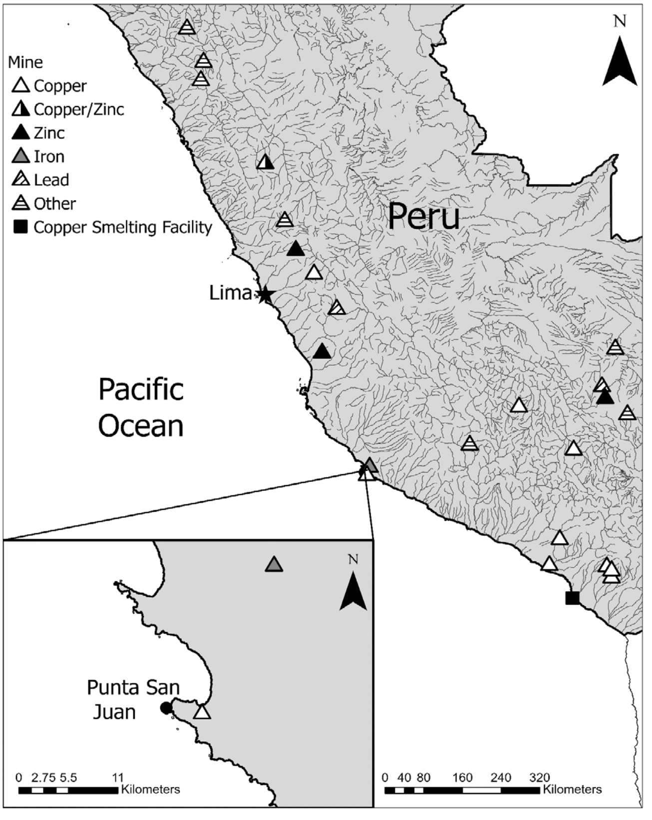

FIGURE 1 Map of Peru including major mine locations, copper smelting facility, and Punta San Juan reserve (study site).

et al., 2005; Wagemann, 1989). Deep-sea mining has the potential to release toxic elements that may impact exposure and uptake of elements in marine mammals (Miller et al. 2018).

Punta San Juan, the study site, is located 20 km from the largest open-pit iron ore mine in Peru and 6 km from an expanding copper mine (Figure 1). Several mine waste and sewage dumping sites can be found along the Peruvian coast with one site only 4 km from PSJ (Adkesson et al., 2018). Multiple elements, including arsenic, lead, and zinc can be remobilized during copper mining and smelting and may have negative impacts on the surrounding environment (Ayres et al., 2003; Ramirez et al., 2005).

Trace elements, present in minute amounts in a sample or environment, are classified as either essential or nonessential. Essential elements have biochemical and physiological functions, while nonessential elements do not have a biological function in the body (Tchounwou et al., 2012). In mammals, essential elements include, among others, chromium, cobalt, copper, iron, manganese, nickel, selenium, vanadium, and zinc. Elements such as aluminum, arsenic, cadmium, lead, mercury, and tin traditionally have been defined as nonessential (Das et al., 2003; Tchounwou et al., 2012), although some debate exists on whether trace levels of arsenic should be considered essential (Hunter, 2008; Mayer et al., 1993; Nielsen, 2000) Trace elements are considered toxic when they exceed a certain threshold and have negative impacts on the organism. Essential elements can be harmful at high levels, but nonessential elements may be toxic even at low concentrations.

The uptake of elements by marine organisms occurs mainly through ingestion of food; however, trace elements can also be maternally transferred via the placenta and lactation (Das et al., 2003). Adult PFS females give birth to one pup a year, which they nurse for 6 months to 3 years, depending on prey availability and pup growth rate. The weaning process is flexible, and dams can nurse a newborn, a yearling, and a juvenile at the same time (Majluf, 1987). The maternal transfer of trace elements during gestation and lactation are of particular concern since these are crucial developmental periods for offspring. In humans, early exposure to high concentrations of certain elements, including arsenic, cadmium, and mercury, can lead to impaired growth and development in newborns (Espart et al., 2018; Yu et al., 2011).

Continuously growing, keratinous tissues, such as fur (hair) and vibrissae (whiskers), incorporate elements as they grow, making them valuable minimally invasive samples for element analysis (Ferdinando, 2019; Habran et al., 2013; Ikemoto et al., 2004; Kooyomjian, 2021). Otariid adult vibrissae grow continuously for up to 7 years until they are shed (Hirons et al., 2001). Pup vibrissae begin to grow in utero and can be utilized to examine placental transfer of nutrients and trace elements (Rea et al., 2013). The gestation period for South American fur seals last 11 months, consisting of a 2–3-month embryonic diapause followed by embryonic growth (Boshier,1981). Rea et al. (2015) examined stable isotopes ($\delta^{13}C$) in Steller sea lion (*Eumetopias jubatus*) dam and pup paired vibrissae. By aligning the stable isotope profiles, they found that pup vibrissae grew throughout mid to late gestation, showing approximately 5–6 months of growth. Serum reflects elements currently circulating through the body, either from recent exposure or remobilization from tissues such as blubber (Yordy et al., 2010). Milk is a nutrient-rich solution produced as needed by the dam to nourish her pup, reflecting hours to days of exposure or remobilization. Milk content in otariid species contains about 42% lipid, 12% protein, and 42% water (Arnould & Hindell, 1999; Georges et al., 2001). South American fur seal milk is composed of 28.3%–57.1% lipid (Ponce de León & Pin, 2006).

Maternal offloading of elements has significant potential impacts for both the dam and pups, sparing the dam potentially toxic elemental concentrations while subjecting the pup to levels that may incur developmental challenges. This study examined vibrissae, serum, and milk from paired PFS dams and pups for select years from 2009 to 2019. Analysis of lipid-rich milk provided information about which elements were transferred during lactation, while pup vibrissae showed elemental transfer during gestation. Elemental concentrations in the serum of both dams and pups show the current circulating values based on diet and remobilization. The objective of this study was to examine the maternal transfer of 15 elements in PFS. This information can be used to determine how Peru's coastal mining, agriculture, and industry may affect this important apex predator.

TABLE 1 Tissue types collected for all individual dams and pups. X = sample collected.

	Dams			Pups	
Year	Vibrissae	Serum	Milk	Vibrissae	Serum
2009 ($n = 11$)		X			X
2010 ($n = 15$)		X			X
2011 ($n = 1$)			X		
2011 ($n = 2$)	X		X		
2011 ($n = 3$)		X	X		
2011 ($n = 10$)	X	X	X		
2015 ($n = 3$)	X	X		X	
2015 ($n = 3$)	X	X	X	X	
2019 ($n = 2$)	X	X	X	X	
2019 ($n = 5$)	X	X		X	X
2019 ($n = 9$)	X	X	X	X	X

2 | MATERIALS AND METHODS

2.1 | Sample collection

Archival vibrissae, serum, and milk samples were used from 48 PFS dam/pup pairs. In addition to the 48 PFS dam/pup pairs, 16 PFS dams in 2011 were also analyzed. All three sample types were not available for every individual (Table 1). Of the 64 total number of dams, there are the following pairings: 22 paired dam and pup vibrissae, 40 paired dam and pup serum, 27 paired milk and dam serum, and 9 paired milk and pup serum for a total of 112 individuals and a total of 187 samples. The archived samples were collected in 2009, 2010, 2011, 2015, and 2019 from fur seals that were anesthetized as part of an ongoing population health monitoring program at PSJ. All sample collection was authorized by the Peruvian government and performed under the direct veterinary supervision of one of the authors (MJA) during the breeding season (November). Vibrissae, including the follicle, were pulled, and stored in plastic bags until analysis. Blood was collected from the jugular vein and placed in royal blue trace element tubes (no additive; Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ). Blood samples were centrifuged (1,132 $\times$ g for 10 min) and resultant serum was separated and then frozen. Milk samples were collected from the teat through manual expression or use of gentle suction from a syringe. Serum and milk samples were kept on ice packs for up to 8 hr, frozen at -20°C for up to a week, and then stored at -80°C until analysis.

2.2 | Sample preparation and analysis

Vibrissae, serum, and milk were digested and analyzed as described in Kooyomjian (2021). Vibrissae (dry weight) were cleaned and dried before being digested in 5:1 trace metal basis nitric acid and 30% hydrogen peroxide on a ModBlock at 60°C for at least 24 hr. Serum and milk samples were digested using a microwave digestion system by combining approximately 0.5 g (wet weight) of sample with 4 ml of trace metal basis nitric acid, 1 ml of 30% hydrogen peroxide, and 1 ml of ultrapure water (Rey-Crespo et al., 2013). Analysis was performed via sector-field inductively coupled plasma mass spectrometer for fifteen elements: aluminum (Al), arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), selenium (Se), tin (Sn), vanadium (V), and zinc (Zn). Prior to analysis, digested samples were diluted 5-fold in 0.64 M ultrapure nitric acid (Seastar Baseline) containing 2 ppb indium as an internal standard. External standards were used, and a blank rerun every eight samples. Two United States Geological Survey (USGS) reference water concentrations were assessed as part of each analytical run to verify the standardization. No certified reference materials were used for this study since high lipid milk and vibrissae were not available. Detection limits (μg/g) were calculated as three times the standard deviation of the blank as follows: Al (0.1), As (0.00003), Cd (0.00001), Cr (0.0001), Co (0.00002), Cu (0.005), Fe (0.004), Pb (0.0004), Mn (0.00008), Hg (<0.00001), Ni (0.0005), Se (0.00003), Sn (0.001), V (0.00004), Zn (0.02).

2.3 | Statistical analysis

All analyses were conducted using the statistical program R version 3.6.0 (R Core Team, 2019). The Shapiro–Wilk test was used to test for normality of data and Bartlett's test was used to verify homogeneity of variances. Data were log10-transformed to meet normality and homogeneity of variances as needed. Normally distributed data are reported as mean ± standard deviation, while log10-transformed and non-normal data are reported as median and 95% confidence interval throughout. Statistical significance was determined as $p < .05$. Differences in elemental concentrations in paired dam and pup tissues were tested using paired *t*-tests and Mann–Whitney Wilcoxon tests. For paired *t*-test, the test statistic and degrees of freedom (*df*) are reported as *t*(*df*), while the Mann–Whitney Wilcoxon reports the test statistic as *V*. Correlations among sample types and elements were tested using Spearman rank correlation ($n \leq 30$) or Kendall's tau correlation ($n > 30$).

Due to lack of concentrations above the detection limit, Sn was not included in any serum analyses. Paired *t*-test could not be performed for Hg in serum since there were no dam-pup pairs with serum Hg concentrations above the detection limit. Cadmium, Hg, and Sn were not analyzed for milk versus pup serum due to lack of paired data above the detection limits.

3 | RESULTS

3.1 | Element concentrations in vibrissae, serum, and milk

Data summary for vibrissae, serum, and milk samples from PFS dams and pups are presented in Table 2. Elemental concentrations were higher in dam and pup vibrissae compared to both serum and milk, except for As. For example, mean Cd concentration was 300 times higher in pup vibrissae compared to pup serum and Hg was over 700 times higher in dam vibrissae compared to dam serum. Mean As concentrations in pup vibrissae and milk (0.44 and 0.41 μg/g, respectively) were two times higher than dam vibrissae (0.19 μg/g) and nearly ten times higher than dam and pup serum (0.06 and 0.04 μg/g, respectively). Elemental concentrations were higher in dam milk compared to dam serum for all elemental concentrations except for Fe (dam serum: 5.75 μg/g vs dam milk: 3.63 μg/g) and Pb (dam serum: 0.07 μg/g vs. dam milk: 0.05 μg/g).

Peruvian fur seal dam vibrissae and milk were both collected in 2011, 2015, and 2019 (Table S1). Arsenic concentrations in dam vibrissae and milk increased throughout this period. The mean dam vibrissae As concentration in 2011 was 0.026 μg/g, in 2015 was 0.127 μg/g, and in 2019 was 0.262 μg/g. From 2011 to 2015 the As concentration increased five-fold and then increased two-fold again from 2015 to 2019. While the vibrissae As concentration increased significantly, the mean milk As concentrations increased only slightly. In 2011 it was 0.361 μg/g, in 2015 was 0.404 μg/g, and in 2019 was 0.484 μg/g.

3.2 | Element concentrations in dam/pup paired vibrissae and serum

Paired samples of dam and pup vibrissae and dam serum were collected in 2015 ($n = 6$) and 2019 ($n = 16$). Dam serum concentrations were significantly lower than pup vibrissae concentrations for all elements ($p < .01$). Vanadium was significantly negatively correlated between dam serum and pup vibrissae (Table S2).

TABLE 2 Trace element concentrations for Peruvian fur seal dam and pup vibrissae, serum, and milk. Normally distributed data are presented as mean ± standard deviation while log10-transformed and nonnormal data are presented as median (95% confidence interval) in μg/g. N/A = not available. Vibrissae samples (dry weight); serum and milk (wet weight).

Element	Dam vibrissae	Pup vibrissae	Dam serum	Pup serum	Milk
n	34	22	61	40	30
Al	19.82 (17.73–22.85)	145.5 (129.4–174.5)	3.78 (3.41–4.83)	3.40 (3.02–4.08)	3.95 (3.65–4.32)
As	0.19 ± 0.11	0.44 ± 0.18	0.06 ± 0.02	0.040 (0.037–0.045)	0.41 ± 0.15
Cd	0.19 (0.17–0.25)	0.49 (0.18–0.79)	0.0022 (0.0017–0.0025)	0.0012 (0.0010–0.0015)	0.005 (0.003–0.008)
Cr	0.24 (0.16–0.33)	0.26 (0.24–0.40)	0.029 (0.022–0.033)	0.026 (0.021–0.031)	0.04 (0.03–0.06)
Co	0.014 (0.013–0.019)	0.035 (0.031–0.039)	0.0011 (0.0010–0.0013)	0.0011 (0.0009–0.0014)	0.0026 (0.0018–0.0030)
Cu	20.88 ± 1.93	19.50 ± 4.01	0.83 (0.79–0.88)	0.56 (0.51–0.65)	2.23 (1.67–2.63)
Fe	8.76 (7.58–9.96)	21.85 (18.85–26.27)	4.99 (3.74–6.14)	6.27 (5.04–7.39)	2.70 (1.95–3.88)
Pb	0.10 (0.08–0.13)	0.21 (0.17–0.27)	0.025 (0.019–0.040)	0.04 (0.03–0.05)	0.032 (0.027–0.047)
Mn	0.26 (0.24–0.30)	0.64 (0.60–0.72)	0.014 (0.010–0.016)	0.016 (0.013–0.017)	0.04 (0.03–0.05)
Hg	2.11 (1.97–2.20)	1.46 ± 0.40	0.0009 (0.0001–0.0036)	0.018 ± 0.007	0.004 (0.002–0.025)
Ni	0.17 (0.15–0.20)	0.37 (0.32–0.42)	0.007 (0.006–0.009)	0.009 (0.007–0.010)	0.02 (0.01–0.04)
Se	1.07 (0.95–1.11)	3.97 ± 1.03	0.31 ± 0.05	0.14 ± 0.02	0.35 ± 0.13
Sn	2.88 (2.44–3.27)	0.022 (0.011–0.069)	N/A	N/A	0.05 ± 0.03
V	0.034 (0.030–0.040)	0.062 (0.052–0.075)	0.0023 (0.0019–0.0026)	0.0025 (0.0019–0.0031)	0.003 (0.002–0.004)
Zn	192.2 ± 26.54	162.9 ± 23.60	1.28 (1.21–1.41)	1.30 (1.23–1.40)	8.10 (7.39–9.69)

Dam vibrissae concentrations were significantly higher than pup vibrissae for Cu ($t[21] = 2.01$, $p = .02$), Hg ($t[21] = 5.51$, $p < .001$), Sn ($V = 49$, $p = .01$), and Zn ($t[21] = 3.50$, $p = .001$). Dam vibrissae had significantly lower concentrations than pup vibrissae for Al ($t[21] = -24.08$, $p < .001$), As ($t[21] = -6.10$, $p < .001$), Cd ($V = 40$, $p = .008$), Cr ($t[21] = -2.19$, $p = .02$), Co ($V = 22$, $p < .001$), Fe ($t[21] = -6.71$, $p < .001$), Pb ($V = 20$, $p < .001$), Mn ($V = 28$, $p < .001$), Ni ($t[21] = -6.18$, $p < .001$), Se ($t[21] = -13.45$, $p < .001$), and V ($t[21] = -5.91$, $p < .001$; Figure 2).

Dam and pup paired serum were collected in 2009 ($n = 11$ pairs), 2010 ($n = 15$ pairs), and 2019 ($n = 14$ pairs). Dam serum had significantly higher concentrations than pup serum for As ($t[39] = 5.67$, $p < .001$), Cd ($V = 501$, $p < .001$), Cu ($t[39] = 7.07$, $p < .001$), and Se ($t[39] = 20.76$, $p < .001$; Figure 3). Dam and pup serum were significantly positively correlated for As ($T = 533$, $\tau = 0.37$, $p < .001$), Pb ($T = 499$, $\tau = 0.28$, $p = .01$), and Ni ($T = 294$, $\tau = 0.26$, $p = .04$) while Al showed a significant negative correlation ($T = 292$, $\tau = -0.25$, $p = .02$).

Paired pup vibrissae and serum were available for 2019 samples ($n = 14$ pairs). Pup vibrissae concentrations were significantly higher than pup serum concentrations for all elements ($p < .001$). Lead concentrations were significantly positively correlated between pup vibrissae and serum ($S = 168$, $\rho = 0.63$, $p = .02$; Table S2).

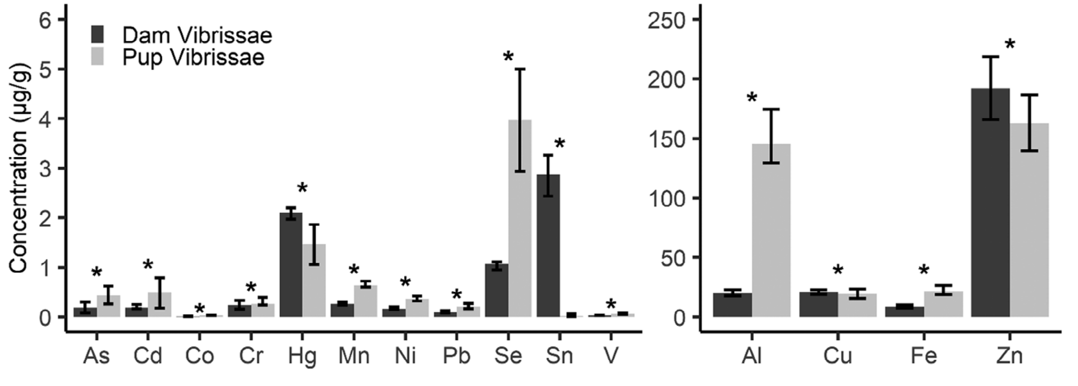

FIGURE 2 Element concentrations in the vibrissae from 22 Peruvian fur seal (*Arctocephalus australis*) dam (black) and pup (gray) pairs from Punta San Juan, Peru. Normally distributed data are presented as mean ± standard deviation while log10-transformed and nonnormal data are presented as median (95% confidence interval) in μg/g. Asterisks indicate significant difference between dams and pups.

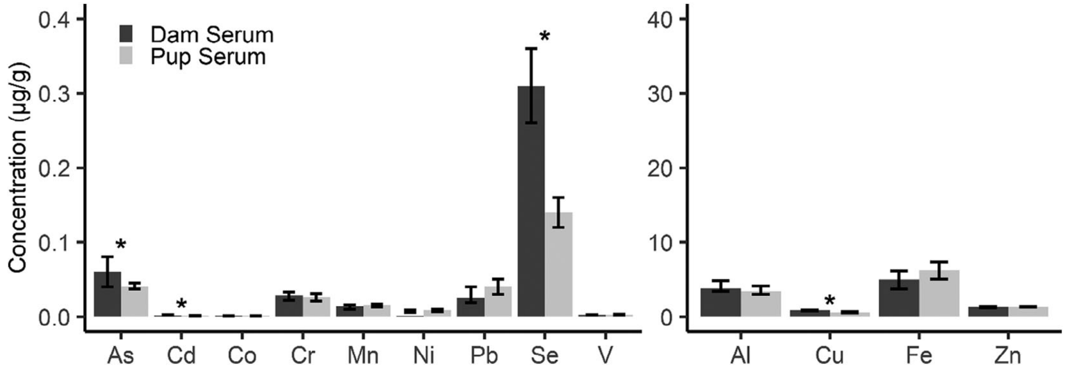

FIGURE 3 Element concentrations in the serum from 40 Peruvian fur seal (*Arctocephalus australis*) dam (black) and pup (gray) pairs from Punta San Juan, Peru. Normally distributed data are presented as mean ± standard deviation while log10-transformed and nonnormal data are presented as median (95% confidence interval) in μg/g. Asterisks indicate significant difference between dams and pups.

3.3 | Element concentrations in milk compared to vibrissae and serum

Peruvian fur seal milk samples were collected in 2011 ($n = 16$), 2015 ($n = 3$), and 2019 ($n = 11$). A clear increase in As, Cu, Se, and Zn milk concentrations occurred across years sampled. All element concentrations in milk were significantly lower than pup vibrissae concentrations except for As. Mean As concentrations were similar for pup vibrissae and milk (0.44 and 0.41 μg/g, respectively) and significantly higher than dam vibrissae (0.19 μg/g) and dam serum (0.06 μg/g), Table S3.

Milk concentrations were significantly higher than dam serum concentrations for As, Cd, Co, Cu, Mn, Ni, Se, and Zn, but significantly lower than dam serum for Fe. Selenium and V concentrations were significantly positively correlated between dam serum and milk (Table S2).

Paired pup serum and milk samples were collected in 2019 ($n = 9$). Milk concentrations were significantly higher than pup serum concentrations for As, Cu, Se, and Zn, but significantly lower than pup serum for Fe (Table S4). Pup serum and vibrissae Pb concentrations were both significantly negatively correlated with milk Pb concentrations (Table S2).

4 | DISCUSSION

4.1 | Element concentrations in vibrissae, serum, and milk

Vibrissae, serum, and milk accumulate elements over various temporal scales. Peruvian fur seal adult female vibrissae are retained for an average of 3.5 years and consist mainly of proteinaceous keratin (Edwards, 2018; Hirons et al., 2001; Ling, 1977). Serum represents a much shorter time frame of hours to weeks and contains mostly water and proteins (Laker, 1982). Fur seal milk is rich in lipid and protein and represents a time frame of hours to days as it is produced and secreted as needed (Arnould & Hindell, 1999; Georges et al., 2001).

Vibrissae concentrations for all elements in this study were always greater than dam and pup serum concentrations. For example, the smaller differences in concentrations between vibrissae and serum were for Fe and Pb, which were two to five times higher. Conversely, Hg was over 700 times higher in vibrissae concentrations than serum (Table 2). Gray et al. (2008) found similar results with hair concentrations consistently higher than serum concentrations for 14 elements analyzed in leopard seals (*Hydrurga leptonyx*) and Weddell seals (*Leptonychotes weddellii*) from Antarctica (Tables 2 and S5). Dam vibrissae concentrations for all elements in this study, except As, were always greater than milk concentrations (Table S6). The mean milk As concentration was two times higher than the mean dam vibrissae concentration showing that As is likely offloaded through lactation. Vibrissae provide an ideal offloading matrix as they are not metabolically active and can sequester elements until they are shed.

Arsenic concentrations in dam vibrissae increased from 2011 (0.026 μg/g) to 2019 (0.262 μg/g) and in milk samples from 2011 (0.361 μg/g) to 2019 (0.484 μg/g) (Table S1). This may indicate an increase in bioavailable As due to expanding Cu mining and agricultural runoff. Peru's annual Cu production doubled during the study period from 1.2 million tonnes in 2011 to 2.5 million tonnes in 2019, and production is expected to increase to 3.5 million tonnes annually by 2025 (Ministry of Energy and Mines 2019, 2021). Copper smelting is the largest anthropogenic source of As into the environment (Matschullat, 2000). The Humboldt current may carry waste from the Cu smelting facility in Ilo, Peru north, to areas near PSJ. Coastal agriculture and associated runoff may also increase bioavailable As in the marine environment (Bedoya-Perales et al., 2018; Tchounwou et al., 2012). Once released into the environment, As is taken up in the food web and bioaccumulates in marine mammals. The findings of increasing As concentrations are consistent with the findings of Loaiza et al. (2021) indicating that As is a concern in the Peruvian marine environment.

Iron concentrations in adult PFS vibrissae (8.76 μg/g) and milk (2.70 μg/g) were 8–10 times lower than Fe concentrations in hair (73.1–87 μg/g) and milk (24.7 μg/g) reported for pinnipeds in Antarctica and Scotland (Gray et al., 2008; Habran et al., 2013; Table S5). Low Fe may be due to the extremely low Fe concentrations found in the upwelling water of southern Peru compared to northern and central Peru (Bruland et al., 2005). Phytoplankton from the Humboldt Current and Peru upwelling was found to be limited in Fe (Hutchins et al., 2002). Low Fe concentrations in Peruvian pinnipeds may be worth investigating as Fe deficiency can lead to improper growth and reduce immune response (Valko et al., 2005). Dam (192.2 μg/g) and pup vibrissae (162.9 μg/g) and dam (1.28 μg/g) and pup serum (1.30 μg/g) Zn concentrations were slightly higher than hair (101–137 μg/g) and three times higher than serum (0.36–0.48 μg/g) Zn concentrations found in pinnipeds from Antarctica and Scotland (Gray et al., 2008; Habran et al., 2013; Table S5). Zinc is often found in Cu ores, and the growing Cu mine near the study site may be the source of increased bioavailable Zn (Ayres et al., 2003).

4.2 | Dam and pup vibrissae concentrations

Samples were collected from pups within 1–2 weeks of their birth; therefore, the pup vibrissae reflect mostly gestational transfer from dams. Since vibrissae were pulled and not cut, the follicle containing the most recent growth was included in the analysis. Although pup vibrissae only reflect 5–6 months of gestational growth and dam vibrissae contain approximately 42 months of growth, pup vibrissae had significantly higher concentrations of 11 (Al, As, Cd, Cr, Co, Fe, Pb, Mn, Ni, Se, and V) of the 15 elements analyzed (Figure 2). This suggests elements are maternally offloaded to pups during gestation. Pup vibrissae concentrations of Al (145.5 μg/g), Pb (0.21 μg/g), and Se (3.97 μg/g) were nearly two to eight times higher than dam vibrissae concentrations (Al: 19.82 μg/g; Pb: 0.10 μg/g; Se: 1.07 μg/g), suggesting these elements are released from maternal tissue stores and may indicate high concentrations in maternal tissues. Mercury and Cd are mainly transferred from dam to pup during gestation with little transfer during lactation (Grajewska et al., 2019; Habran et al., 2013). This is confirmed for PFS through the undetected and low concentrations of Hg and Cd found in milk and higher Hg and Cd concentrations in pup vibrissae.

Because little information exists on element concentrations in pinniped pup vibrissae, results were compared to keratinous hair and lanugo concentrations. Habran et al. (2013) determined element concentrations in dam hair and pup lanugo for gray seals (*Halichoerus grypus*) from Scotland. Their findings were similar to the current study with Cu, Hg, and Zn concentrations higher in dams, but they also showed dam hair had higher Cd, Cr, Fe, Pb, Ni, Se, and V concentrations than pup lanugo. Mean Cu concentrations were approximately five times higher in PFS dam and pup vibrissae (20.9 and 19.3 μg/g, respectively) compared to dam hair and lanugo in gray seals (4.2 and 2.8 μg/g, respectively; Habran et al., 2013). However, Hg concentrations were lower in PFS dam and pup vibrissae (2.11 and 1.46 μg/g, respectively) than in gray seal hair and lanugo (7.7 and 4.6 μg/g, respectively; Habran et al., 2013) and northern fur seal (*Callorhinus ursinus*) dam and pup fur from Alaska (4.87 and 7.84 μg/g, respectively; Beckmen et al., 2002).

Dam vibrissae Al concentrations (19.82 μg/g) were more than twice as high as hair Al concentrations found in adult leopard and Weddell seals from Antarctica (7.62 and 9.13 μg/g, respectively; Gray et al., 2008). High concentrations of Al were also reported in adult Humboldt penguin (*Spheniscus humboldti*) feathers collected from PSJ (67 μg/g; Adkesson et al., 2019; Table S6), suggesting elevated bioavailable Al near PSJ. Since Al is the most abundant metal in the earth's crust, local mining activities may increase the release of Al into the environment (Agency for Toxic Substances and Disease Registry, 2008). Pup vibrissae had the highest Al concentrations with a median concentration of 145.5 μg/g. Aluminum toxicity can affect bone growth and development in mammals, and in children, hair Al concentrations over 8 μg/g were linked to developmental disorders (Blaurock-Busch et al., 2012; Jaishankar et al., 2014).

In human hair, As concentrations between 0.1 and 0.5 μg/g are indicative of concerning, chronic As exposure (Ratnaike, 2003). Approximately 53% of dam vibrissae contained As concentrations over 0.1 μg/g, and all 2019 dam vibrissae contained concentrations above 0.1 μg/g. All pup vibrissae As concentrations exceeded 0.1 μg/g with 23% greater than 0.5 μg/g. Arsenic is a known carcinogen and exposure to high levels of As in humans is associated with increased risk of stillbirths (Ehrenstein et al., 2006). With PFS populations at PSJ declining, additional research is needed to evaluate As speciation and the potential deleterious effects of As toxicity on reproductive health in pinnipeds.

4.3 | Dam and pup serum concentrations

Dam and pup serum concentrations only differed significantly for four (As, Cd, Se, and Cu) of the 13 elements (Hg and Sn were not analyzed). Serum samples were collected within two weeks of birth, mainly reflecting concentrations during gestation, where dam and pup serum would be the most similar. Habran et al. (2013) examined whole blood in dam and pup paired gray seals and, similar to the present study, found higher Cu and Se concentrations in dam serum compared to pup serum, during early lactation.

In dam serum, As was highest during the extreme El Niño of 2015. This is consistent with the finding in Kooyomjian (2021) that there is more bioavailable As during El Niño years compared to La Niña years. Increased smelting and agricultural runoff due to heavy rainfall during El Niño may lead to increased As in the marine environment, where is it taken up into the food web and bioaccumulates in Peruvian pinnipeds (Holmgren et al., 2001). Lead was positively correlated between dam and pup serum ($\tau = 0.28$) and pup vibrissae and pup serum ($\rho = 0.63$). Similar correlations for Pb in dam and pup whole blood ($\rho = 0.63$) and pup lanugo and blood ($\rho = 0.5$) were found in gray seals (Habran et al., 2013).

Dam and pup serum Al concentrations (5.64 and 4.47 μg/g, respectively) were more than an order of magnitude higher than serum concentrations reported for adult leopard and Weddell seals (0.25, 0.08 μg/g, respectively; Gray et al., 2008). However, serum Al concentrations were on the same order of magnitude as concentrations reported for adult Humboldt penguins from PSJ (2.14 μg/g) further suggesting higher bioavailable Al near PSJ compared to Antarctica (Adkesson et al., 2019; Table S6).

Dam and pup Pb serum concentrations (0.07 and 0.05 μg/g, respectively) were at least an order of magnitude higher than serum concentrations for adult leopard and Weddell seals (<0.001 and < 0.005 μg/g, respectively; Gray et al., 2008; Table S6). Peruvian fur seal Pb serum concentrations were also an order of magnitude higher than whole blood concentrations reported for bottlenose dolphins in Florida (0.003 μg/g; Bryan et al., 2007). Chronic Pb exposure can cause brain and kidney damage in humans and PFS should be further assessed for signs of toxicity (Jaishankar et al., 2014).

4.4 | Milk concentrations

Concentrations of As, Cu, Se, and Zn increased in milk from 2011 to 2019. Increasing As concentrations were also found in dam vibrissae and are likely linked to both Cu mine waste and agricultural runoff. Elevated Cu and Zn concentrations may be due to local Cu mining dust and waste or leeching from naturally rich Cu deposits and should be monitored. The median milk Zn concentration (8.10 μg/g) was only slightly higher than concentrations found in gray seals from Scotland (7.3 μg/g; Habran et al., 2013) and harp seals (*Pagophilus groenlandicus*) from the Gulf of Saint Lawrence (6 μg/g; Wagemann et al.,1988), but the median Cu concentration (2.23 μg/g) was four times higher than concentrations reported for gray seals (0.45 μg/g; Habran et al., 2013) and harp seals (0.54 μg/g; Wagemann et al., 1988; Table S5).

Milk concentrations were higher than both dam and pup serum for As, Cu, Se, and Zn. In gray seals, milk had higher concentrations than dam and pup blood for Zn, but not for Cu or Se (Habran et al., 2013). Lead was positively correlated between pup serum and vibrissae, but negative correlations were seen for Pb between pup serum and milk ($\rho = -0.73$) and pup vibrissae and milk ($\rho = -0.55$) (Table S2). This may indicate that Pb is mainly transferred to the pup during gestation with little transfer through lactation. Habran et al. (2013) found a positive relationship between Pb, V, Ni, and Cr in gray seal milk. Similar relationships were seen in this study between Ni and V ($\rho = 0.464$), Cr and V ($\rho = 0.642$), Cr and Ni ($\rho = 0.546$), and Cr and Pb ($\rho = 0.669$) (Table S7). This indicates that these elements follow similar pathways in milk production.

Although there are no reported values in the literature for Al in pinniped milk, the median concentration in PFS milk, 3.95 μg/g, is quite high compared to Al concentrations in human milk. Aluminum concentrations in human milk range from approximately 0.015 to 0.030 μg/g, although much higher concentrations of up to 0.7 μg/g have been detected in infant formulas (Fanni et al., 2014). The Food and Drug Association (FDA) recommend concentrations of Al in drinking water do not exceed 0.2 μg/g, which is an order of magnitude lower than the PFS milk concentrations and therefore may warrant further investigation (FDA, 2020a).

Arsenic concentrations in dam milk were high (0.41 μg/g). Otariid milk contains more than 40% of fat and 12% of protein, much higher than any other mammal. It is known that arsenite (AsO_3^{3-}, As^{3+}) is more lipid soluble than arsenate (AsO_4^{3-}, As^{5+}) and arsenite has the highest acute toxicity among the different arsenic forms (Saha et al., 1999). In marine organisms, arsenic is mainly present in a less toxic, organic form (Kunito et al., 2008). Arsenic speciation conducted on marine mammal livers found that As was primarily present in the form of arsenobetaine (Kubota et al., 2002). However, in the lipid-rich blubber of ringed seal (*Pusa hispida*) the most prevalent form of As was dimethylarsinic acid (Ebisuda et al., 2003). More studies should be conducted on lipid-rich fur seal milk to determine the species of arsenic present since it can also significantly affect the rate at which it is excreted (Saha et al., 1999). No evidence exists in the literature that As concentrations have been assessed for pinniped milk elsewhere. However, As has been detected in other mammalian milk, including cows and humans (Bansa et al., 2017; Chandra Sekhar et al., 2003; Hameed et al., 2019). Chandra Sekhar et al. (2003) found elevated mean As concentrations (up to 0.33 μg/g) in cattle fed contaminated grass compared to the control (0.02 μg/g). The authors did not indicate any signs of toxicity or disease in the cattle with high milk As concentrations. Bansa et al. (2017) examined As in breast milk of women from mining communities in Ghana and found a geometric mean As concentration of 0.027 μg/g, which is more than 2.5 times higher than the World Health Organization (WHO) limit for drinking water (0.01 μg/g; WHO, 2011). The FDA recommends that As concentrations do not exceed 100 ppb (0.1 μg/g) in infant rice cereals (FDA, 2020b). All milk samples in the present study contained As concentrations above the WHO limit for drinking water and the FDA suggested limit for infant rice cereals indicating potential As contamination in Peruvian pinnipeds. At PSJ, South American sea lion adult female vibrissae As concentrations were more than two times higher than adult PFS vibrissae As concentrations (Kooyomjian, 2021). South American sea lion milk from PSJ should also be analyzed for As concentrations.

4.5 | Conclusion

Evidence of both gestational and lactational transfer was found for all 15 elements analyzed. Pup vibrissae appear to be viable indicators of placental transfer and accumulation of elements; pup vibrissae had significantly higher concentrations for 11 of the 15 elements compared to dam vibrissae. Elemental concentrations were higher in dam and pup vibrissae compared to both serum and milk, except for As. The highest concentrations for As were found in pup vibrissae and milk, nearly an order of magnitude higher than dam and pup serum. It was determined that the As concentrations increased throughout the study period in dam vibrissae and milk which places As as an element of concern in the Peruvian marine environment. Arsenic speciation must be conducted to determine if As is present in the more toxic inorganic form. High concentrations of Al, As, Cu, and Pb also suggest anthropogenic contamination from regional mining and agriculture and should be examined as potential sources of harmful pollutants in the marine environment.

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AUTHOR CONTRIBUTIONS

Catherine Kooyomjian: Investigation; methodology; writing – original draft. **Dimitrios Giarikos:** Data curation; methodology; supervision; validation; writing – review and editing. **Michael J. Adkesson:** Conceptualization; data curation; resources; writing – original draft; writing – review and editing. **Amy C. Hirons:** Conceptualization; methodology; project administration; supervision; writing – review and editing.

ORCID

Dimitrios G. Giarikos https://orcid.org/0000-0003-3853-6356
Amy C. Hirons https://orcid.org/0000-0003-3464-3541

ENDNOTE

[1] SECLER: Study of Environmental Conservation through Leading-Edge Research

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SUPPORTING INFORMATION

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