Free webinar

September 28, 2021
10am EDT, 7am PDT, 2pm BST
1pm EDT, 10am PDT, 6pm BST

Evaluationg Stormwater to Identify & Quantify Causal Toxins from Tire Degradants in Coho Salmon Mortality

For decades, scientists had been concerned about water quality impacts on Pacific Northwest coho salmon that returned from the Pacific Ocean to spawn in local streams and rivers. After rain events in the area, acute and widespread mortality of adult coho salmon in the streams occurred; this was subsequently called urban runoff mortality syndrome (URMS). The cause of this phenomena was unknown for many years with many regulated chemicals and pathogens ruled out as culprits.

This webinar will take you through the journey of researchers at the University of Washington successfully identifying the primary chemical cause of this mortality – 6PPD-quinone. 6PPD-quinone is an oxidation product of 6PPD, an industrial antioxidant compound commonly used in tires. Ed Kolodziej will go through how his team were the first to identify the emerging contaminant using effect-directed analysis workflows paired with a high-resolution LC-Q/TOF and software tools. He will also demonstrate the steps that the led to linking coho mortality to 6PPD and its degradation product 6PPD-quinone.

Following this, researchers at Vogon Laboratories will discuss developing a routine quantitative method on a liquid chromatograph coupled to a triple quadrupole mass spectrometer (LC/TQ) for analysis of 6PPD-quinone. This presentation describes a fast, direct-inject analytical method for the quantitation of 6PPD-quinone in surface water.

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Environmental Toxicology

Lithium and Boron in Calcified Tissues of Vicuna and Their Relation to Chronic Exposure by Water Ingestion in The Andean Lithium Triangle

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Abstract: Vicuna is a wild, endangered species of Andean camelid living in the hyperarid Andean plateau. In the central part of the plateau, the Lithium Triangle defines a zone with lithium-rich salt pans. Brine pools naturally form within the salt pans, and the adaptation strategy of vicuna consists of drinking from brine pools. Together with reporting the first chemical data on vicuna bones and teeth, we analyzed lithium, boron, and arsenic in water and brines, with the aim of assessing their relation to chronic exposure by water ingestion. We collected and analyzed bones of vicuna specimens lying in an Andean salt pan, together with brine and water samples. Brine and water samples are highly saline and contain large amounts of lithium, boron, and arsenic. Lithium (13.50–40 mg kg^{-1}) and boron (40–46.80 mg kg^{-1}), but not arsenic, were found in the vicuna bones and teeth. Based on our results and on previously reported data on human tissues in the Andes, we conducted statistical assessments of the relationships between lithium and boron in body tissues and water samples, and discuss their environmental significance in the context of the Lithium Triangle. *Environ Toxicol Chem* 2020;39:200–209. © 2019 SETAC

Keywords: Lithium; Vicuna; Bones; Teeth; Salt pan; Andes

INTRODUCTION

The Lithium Triangle defines a zone within the Andean plateau that is characterized by the presence of lithium (Li)-rich salt pans (Figure 1). This region constitutes a major part of the known global Li resources (Kesler et al. 2012; Munk et al. 2016). Lithium is a relatively abundant element on Earth, and its concentration in the upper continental crust is approximately 35 ± 11 ppm (Teng et al. 2004).

Because Li is a very reactive and geochemically incompatible element, it is often present in waters (i.e., in solution) in its ionic form, Li^+. The mean Li concentration in seawater is 0.18 mg L^{-1} (Riley and Tongudai 1964). Continental waters are characterized by large, variable amounts of Li. In the Andean plateau, river waters have relatively high Li contents that are typically up to 10 mg L^{-1} (López Steinmetz 2017). These river systems are frequently fed by hydrothermal sources that contain even higher amounts of Li, which leads to increasing Li concentrations in river waters that often exceed 15 mg L^{-1} (Giordano et al. 2013, 2016; Peralta Arnold et al. 2017). Moreover, in the Lithium Triangle, brines within salt pans typically contain mean Li concentrations ranging between 200 and 400 mg L^{-1} (Figure 2A), and that can even exceed 1000 mg L^{-1} (Ericksen and Salas 1977; Risacher and Fritz 1991; Garrett 2004; Kesler et al. 2012; Godfrey et al. 2013; López Steinmetz 2017; López Steinmetz et al. 2018).

The drinking water in Andean villages is obtained from such natural sources. The presence of Li in drinking waters can be traced back in time, and it has been found in calcified, keratinized, and soft tissues of ancient mummies from the Puna de Atacama in northern Chile (Farell et al. 2013; Figueroa et al. 2014; Table 1). In sampled teeth, the concentrations in the crown were high in enamel as well as in dentin, suggesting prenatal exposure to Li-bearing drinking waters (Farell et al. 2013).

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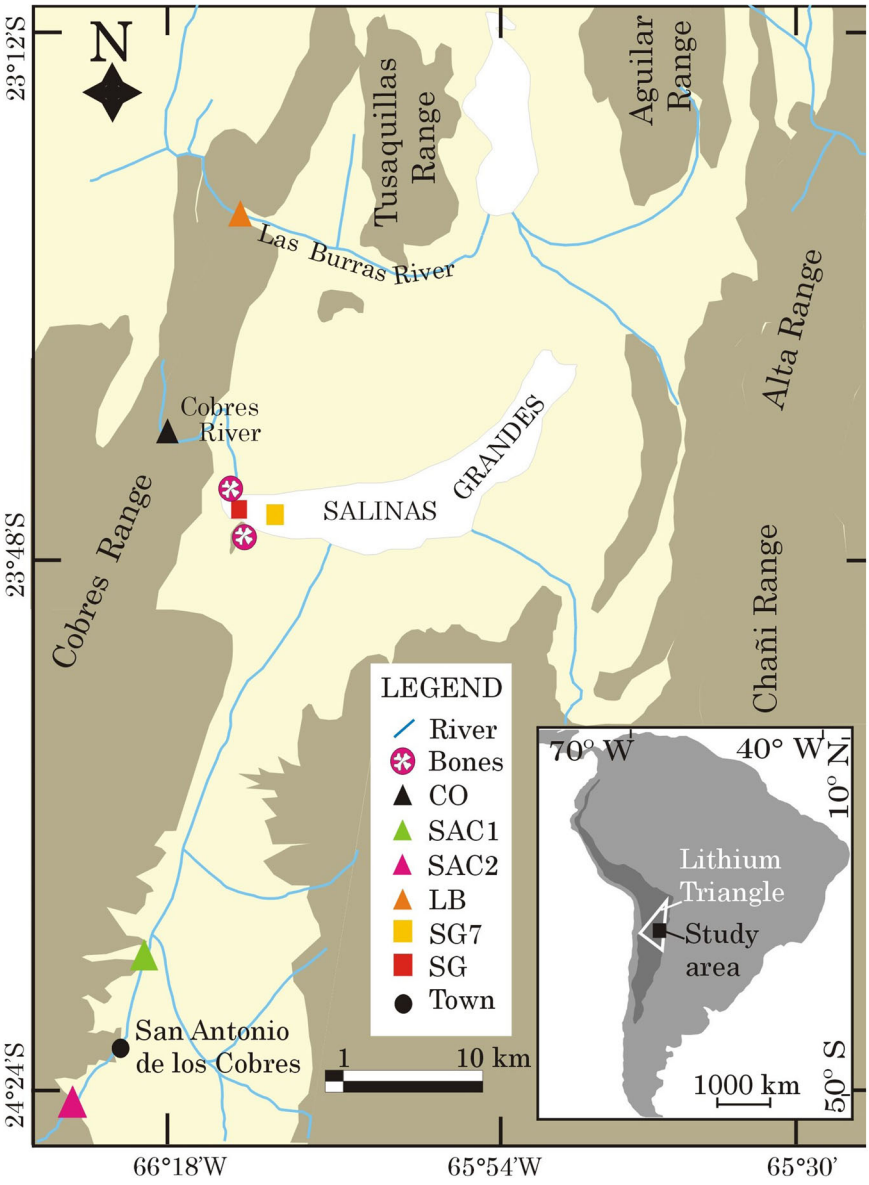

FIGURE 1: Map showing the location of the study area and sampling sites. The water samples were collected from the San Antonio de los Cobres River (SAC1 on the map), the Cobres River (CO), and spring sources in the headwaters of the San Antonio de los Cobres River (SAC2) and the Las Burras River (LB). The brine samples were collected from 2 brine pools within the salt pan in the western Salinas Grandes (SG and SG7). The samples LB and SG7 originally came from previous studies (López Steinmetz 2017; López Steinmetz et al. 2018).

Nothing has changed with the passage of time; in the modern era, the water potabilization process does not include the regulation of Li content prior to human ingestion (Figueroa et al. 2012). For example, in the town of San Antonio de los Cobres in northwest Argentina (for location, see Figure 1), Li concentrations have reached 1 mg L^{-1}, and in addition, up to 0.21 mg L^{-1} of arsenic (As), and 5.90 mg L^{-1} of boron (B) have been reported in drinking waters (Concha et al. 2010). The chronic ingestion of such drinking water has been shown to induce an increase of up to 1 order of magnitude in the concentration of these chemical elements in urine, as well as in diverse body tissues including hair, blood, and breast milk, relative to unexposed populations (Concha et al. 2010; Figueroa et al. 2014; Harari et al. 2015).

Studies on the effect of ingesting Li have long been mainly focused on its psychiatric use, which is the most common source of exposure to Li outside of the Andes. The Li salts, especially carbonate (Li_2CO_3) and acetate ($LiCH_3COO$), have psychotropic properties, and have been used since the mid-1800s in the treatment of bipolar disorders (Cade 1949; Schou 1968; Aral and Vecchio-Sadus 2008), with surveillance because Li is toxic at higher concentrations (Price and Heninger 1964).

Some major differences exist between these 2 kinds of Li exposure. First, although psychiatric patients are usually exposed to Li over a long term, the people living in Andean villages are exposed to Li during their entire life. Furthermore, inhabitants of the Andean plateau where drinkable water contains high Li concentrations are not systematically subjected

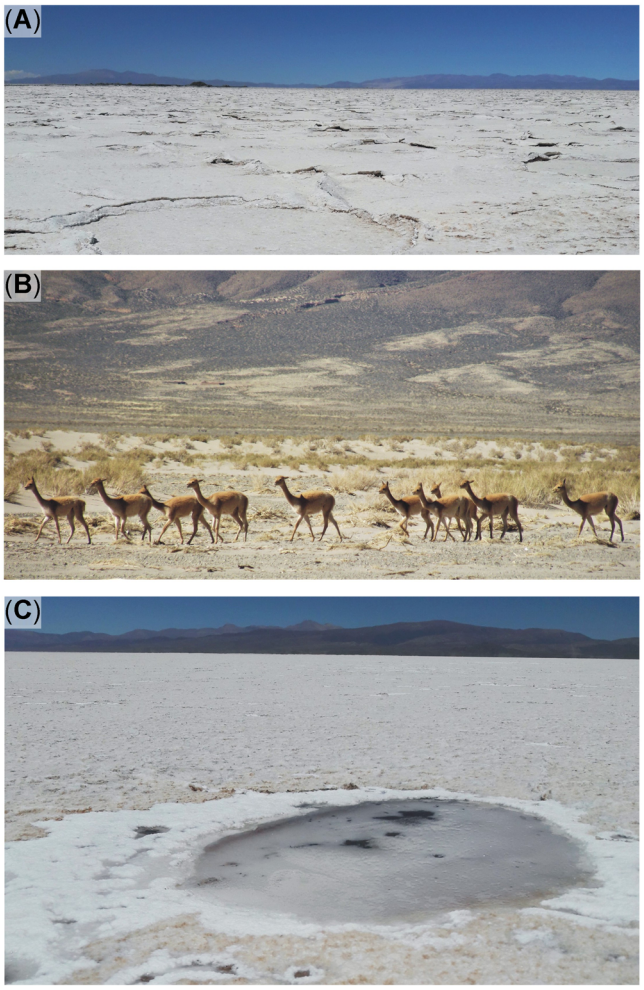

FIGURE 2: Photos showing the salt crust (**A**), a caravan of vicunas (**B**), and a brine pool (**C**) at the Salinas Grandes salt pan.

to serum testing, unlike psychiatric patients. For example, according to pharmacological Li carbonate leaflets, a standard posology during psychiatric treatment of a young adult weighing approximately 70 kg is from 500 to 1200 mg d^{-1}. This implies a daily absorption of 93 to 225 mg of Li. The resulting therapeutic serum concentrations are expected to remain below 8.40 mg L^{-1}, because the toxic stage begins at approximately 10.50 to 17.50 mg L^{-1} (Litovitz et al. 1994; Jaeger 2003). In populations residing in the Puna de Atacama, Figueroa et al. (2014) have reported average serum concentrations of 5.30 mg L^{-1} resulting from daily ingestions of high Li-graded drinkable waters.

Absorbed Li reaches its maximum concentration in serum approximately 30 min after oral ingestion. Lithium is distributed throughout body tissues, and is excreted primarily through the kidneys after approximately 24 h (Freeman and Freeman 2006). In addition, long-term metabolization of Li occurs through ossification; Li accumulates in mammal bones, and seems to be especially incorporated into the minerals of rapidly growing bones (Birch 1974, 1976, 1988). Naturally, this incorporation ends when the subject is no longer exposed to Li. However, only a small fraction is rapidly removed from the bone tissue. This slow decrease suggests a reduced bone turnover state (Peppersack et al. 1994; Mak et al. 1998; Zamani et al. 2009).

It is important to note that there is no Li in human bones of persons who are not exposed to Li (Alexander et al. 1951). Bone and tooth tissues are composed of water and inorganic and organic constituents. The proportions of these components in bone and tooth are variable and depend on several factors such as tissue type, species considered, age, and so forth. In the human tooth, enamel is the most mineralized tissue, at 96%, and dentin, the inorganic material, represents 70% (Gutiérrez-Salazar and Reyes-Gasga 2003). The mineral fraction is also the most abundant constituent in bone, where it is mostly composed of phosphate (PO_4^{3-}) and calcium (Ca^{2+}), and represents approximately 65% of normal human bone, and up to approximately 76% of bovine bone (Quelch et al. 1983).

Living beings in the Andean plateau are exposed to altitudes of more than 3000 m above sea level, high daily thermic amplitudes, and regional aridity. Vicuna (*Vicugna vicugna*) is a wild Andean camelid living under these extreme conditions in the high plateau areas (Tirado et al. 2016, and references therein; Figure 2B). During periods of enhanced aridity

TABLE 1: Summary of lithium (Li) concentrations in urine and calcified, keratinized, and soft human tissues reported from the Andean plateau[a]

	Li concentration				
Sample ID	Water (mg L^{-1})	Human tissues (mg kg^{-1})	Tissue type		Li water/Li tissue
C10	1.00 San Antonio de los Cobres River	4.55	Urine	Modern	0.22
F14	5.17 Camarones River	2.82	Urine		1.83
F13		1.59	Tooth	Mummies from the Camarones	3.25
F14a		8.80	Rib	Valley	0.59
F14b		8.00	Hair (trunk)		0.65
F14c		1.10	Hair (ns)		4.70
F14d		7.30	Nail		0.71
F14e		2.80	Liver		1.85
F14f		5.00	Kidney		1.03
F14g		3.40	Muscle (ns)		1.52

[a]Sample ID numbers correspond to the sources of the data: C10 is from Concha et al. (2010); F13 is from Farell et al. (2013); F14 and F14a–g are from Figueroa et al. (2014).
ns = not specified.

(April–November), the adaptation strategy of vicuna consists of drinking from brine pools within the salt pans (Figure 2C). The salinity of these brines is more than 100 000 mg L^{-1}, and dissolved elements include high concentrations of Li, As, and B, among other ions.

We present the results of a chemical analysis on the bone and tooth composition of 2 specimens of vicuna we have found at the salt pan of Salinas Grandes in the Andean plateau of northwestern Argentina. We analyzed the presence of Li, B, and As in bones, teeth, waters, and brines, and discuss its environmental significance in the context of the Lithium Triangle.

VICUNA AND ITS ENVIRONMENT

Vicuna sp.

Wild South American camelids represent one of the main groups of vertebrates in the Andean plateau (Franklin 1982). Andean camelids live in arid and semiarid environments, and are distributed according to the altitudinal range. Vicuna (*V. vicugna*), an endangered species protected by law, are found in the high plateau areas, between 3000 and 4600 m above sea level (Franklin 2011; Figure 2). Adult specimens of vicuna measure between 1.45 and 1.60 m, and usually weigh 35 to 65 kg (Cajal 1989; Aguilar et al. 1995; Borgnia et al. 2008; Cassini et al. 2009; Franklin 2011; Tirado et al. 2016).

The vicuna diet is based on grasses and shrubs (~80%), complemented by pseudograsses and herbs (Tirado et al. 2016). These proportions are variable, however, and change following the sedentary and migratory tendencies of different populations according to seasonal and environmental restrictions (Franklin 1982; Contreras et al. 2006). An interesting aspect of vicuna is that, together with guanaco (*Llama guanicoe*), they are one of the rare Andean mammals capable of drinking saline waters. This adaptation strategy is mainly employed during the driest periods, when caravans of vicunas can be observed traversing salt pans.

The Salinas Grandes salt pan

Salinas Grandes is a large salt pan of 280 km^2 located at 3410 m above sea level within the largest (more than 17 000 km^2) endorheic basin of the Argentinean plateau (Figure 1). The regional climate is arid and ruled by scarce rainfalls averaging 300 to 400 mm. Under these conditions, soils are skeletal, and vegetation is mostly represented by halophytes, grasses, and resinous shrubs. The relative humidity barely reaches 47%, and the annual average temperature is 8 °C, with day/night amplitudes of more than 18 °C (Bianchi 1981; Buitrago and Larran 1994).

Water is scarce and is highly saline. Fluvial systems mainly consist of ephemeral streams that infiltrate before reaching the salt pan. Some major fluvial collectors feeding the Salinas Grandes systems over the year are the San Antonio de los Cobres and Las Burras Rivers (Figure 1). Springs are abundant in the headwaters of the basin catchment, and supply large amounts of solubilized salts (López Steinmetz 2017).

The Salinas Grandes salt pan is composed of halite (NaCl; Figure 2), which holds brines of the Na-K/Cl-SO_4 type, with nearly neutral pH values (7.10–8.00) and salinities reaching 120 539 mg L^{-1} (López Steinmetz et al. 2018). These brines are stored beneath the salt crust, and often emerge through natural brine pools due to dissolution at certain sites of the salt pan (Figure 2C). Brines contain a large variety of dissolved elements, including B and Li. In the Salinas Grandes, the concentration of B is typically between 300 and 540 mg L^{-1}, whereas Li^+ concentrations are largely variable, ranging between 25 and 1018 mg L^{-1} (López Steinmetz et al. 2018). The highest saline concentrations in the Salinas Grandes, including B and Li, are found in the western margin of the salt pan.

MATERIALS AND METHODS

Water and brine sampling

We collected water samples from the San Antonio de los Cobres River and the Cobres River, and from a spring source (San Antonio de los Cobres 2) in the headwaters of the San Antonio de los Cobres River. A brine sample (Salinas Grandes) was collected from a brine pool within the salt pan, in the western Salinas Grandes. Two other samples from previous studies were included in the analysis: a river water sample (Las Burras River; López Steinmetz 2017) and a brine sample (Salinas Grandes 7; López Steinmetz et al. 2018). The locations of the samples are given in Table 2 and shown in Figure 1. All the water and brine samplings were conducted by employing the same methodology: samples were collected from natural sources in 1.50-L polyethylene bottles precleaned with the water/brine being sampled, and were kept at approximately 0 °C until they were transported to the laboratory for analysis.

Electrical conductivity of samples was determined with a Hanna HI2314 multirange conductivity meter. Total dissolved solids (TDS), representing the salinity of samples in mg L^{-1}, were obtained by employing a conversion factor of 0.50 on the measured electrical conductivity. The concentrations of Li^+, B, and As were determined at the Agua de los Andes Laboratory of Water Chemistry (Jujuy, Argentina) following standard recommendations (Clesceri et al. 1998). The Li^+ was determined by atomic emission spectroscopy, the B by colorimetry, carmine reactive, and the total As by atomic absorption spectrometry. The analytical results are summarized in Table 3.

TABLE 2: Locations of the water, brine, and vicuna samples collected

Location	Latitude	Longitude
San Antonio de los Cobres River 1	24°08′58.23″	66°17′51.19″
San Antonio de los Cobres River 2	24°17′07.47″	66°22′25.86″
Cobres River	23°39′01.55″	66°18′08.52″
Las Burras River (from López Steinmetz 2017)	23°25′19.10″	6°13′36.80″
Salinas Grandes	23°43′18.73″	66°13′23.25″
Salinas Grandes 7	23°43′45.23″	66°10′54.41″
HSG1	23°44′42.56″	66°13′18.32″
HSG2	23°42′30.58″	66°12′20.82″

TABLE 3: Chemical composition of water and brine samples[a]

	Water samples				Brine samples	
	SAC1	SAC2	CO	LB	SG	SG7
TDS	1054	45 370	1002	1240	57 640	116 136
Li	1.15	27.15	2.07	3.75	673.00	1018.23
B	18.26	280.04	44.31	20.03	406.15	360.93
As	0.07	0.11	0.06	—	1.32	—

[a]All concentrations are expressed in mg L^{-1}. Sample LB (Las Burras River) corresponds to data reported by López Steinmetz (2017), and SG7 is from López Steinmetz et al. (2018). The remaining samples are original data from the present study.
TDS = total dissolved solids; Li = lithium; B = boron; As = arsenic; SAC1 and 2 = San Antonio de los Cobres River 1 and 2; CO = Cobres River; SG = Salinas Grandes.

Bone and tooth sampling

We collected the remains of 2 young vicuna specimens lying in the western border of the Salinas Grandes (locations are shown in Figure 1). Partial skeletons were cleaned, and bones were classified following Sato Sato and Angulo Alvarez (2012) and Sierpe González (2015; Figure 3A).

Parts of the mandible with teeth of one specimen (HSG1) were sampled. Sample HSG1 is a composite sample that included the left side of the mandible with the mandibular angle, the lower premolar, and molars; the right mandible with the lower premolar and molars; and the mandibular incisive part with the lower incisive (Figure 3B and C). Sample HSG1b only included teeth (lower premolar, molars, and incisive). Sample HSG2 is a composite sample including a partial femur, tibia, and metatarsus (hind leg) together selected from the second specimen. For sample HSG2, only the bodies of these bones were retained, and the heads and bases were cut away (Figure 3D–F).

Tooth and bone samples HSG1, HSG1b, and HSG2 were prepared, processed, and analyzed at the IACA Laboratories (Bahia Blanca, Argentina). For sample preparation, the sampled bone and tooth were freed of soft tissue, and partially defatted with ether for 24 h. Samples were then crushed with a special

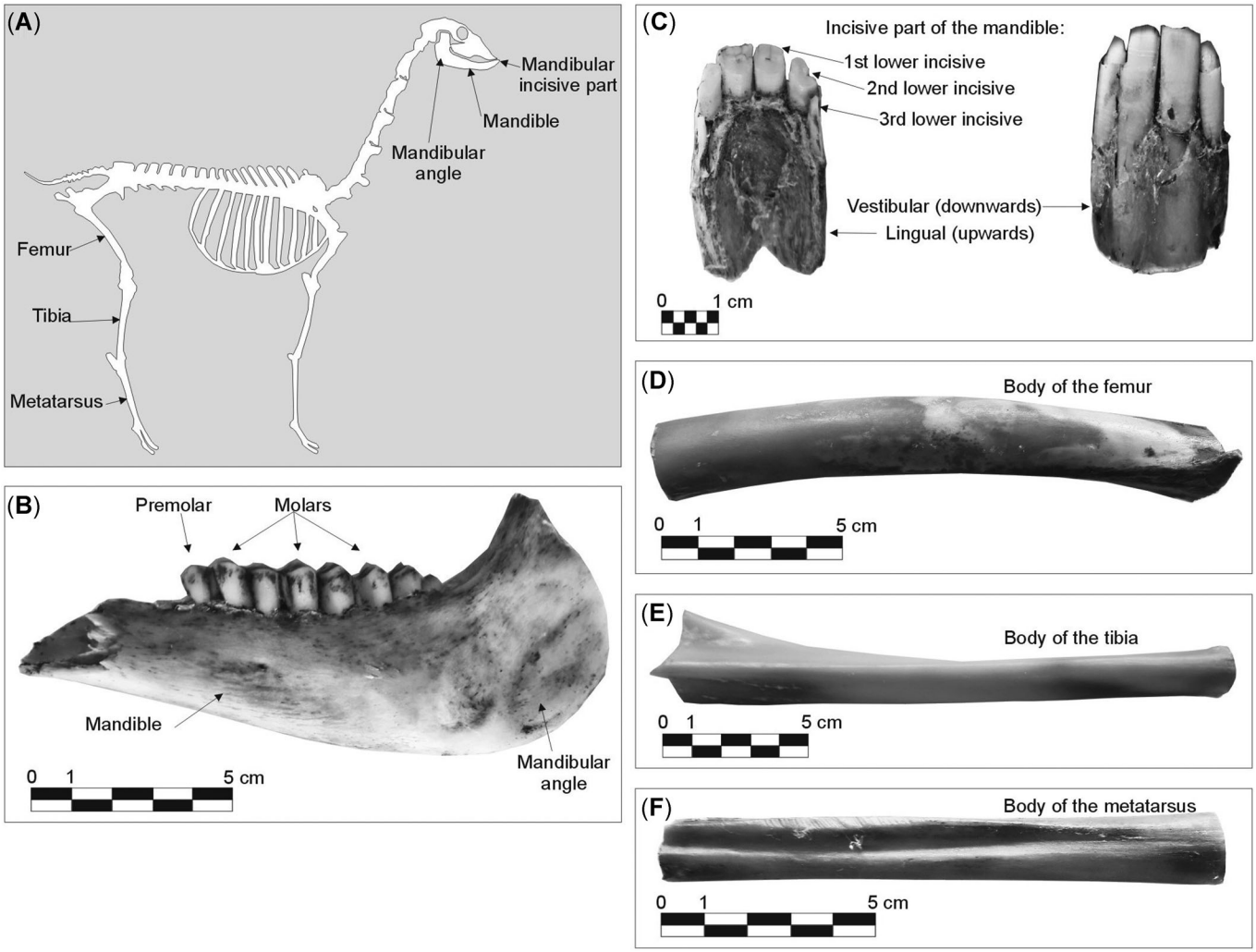

FIGURE 3: (**A**) Schematic and simplified osteology of the right side of camelidae, showing the parts of the skeleton and positioning the sampled bones and teeth. Pictures showing details of bones and teeth in samples HSG1 and HSG2: part of the right mandible with the mandibular angle, the lower premolar, and molars (**B**); the mandibular incisive part with the 6 lower incisive (**C**); the femur body (**D**); the tibia body (**E**); and the metatarsus body (**F**).

stainless steel cutting tool, and ground to obtain a fine powder using a mortar. Samples were homogenized to obtain representative fractions, and, finally, dry matter was determined gravimetrically as the residue remaining after the samples were dried.

Sample processing included acid digestion and dilution by first treating the sampled bone and tooth with 9 mL of HNO_3 and 3 mL of HCl by microwave digestion using a Milestone Ethos Easy digester. Then 0.5 mg of each sample was chosen and diluted to a final volume of 20 mL for HSG1 and HSG2, and to 25 mL for HSG1b.

Determination of the total Li, B, As, Ca, magnesium (Mg), sodium (Na), and potassium (K) was performed by inductively coupled plasma (ICP)–optical emission spectroscopy using an optic ICP device (HORIBA JY 2000). Internal standards and standard solutions (HORIBA Advanced Techno) were prepared in 20% HNO_3 and employed for calibrations. The limit of detection of measured elements was 3 times the standard deviation of the blank values. Different methods were used to make the determinations: B was determined using a 3-point curve method (0, 200, and 500 ppb), 4-point curve fitting was employed for Li and As (0, 10, 100, and 1000 ppb), and K (0, 0.5, 1, and 10 mg L^{-1}), and 5-point curves for Ca, Mg, and Na (0, 1, 10, 50, and 100 mg L^{-1}). Different dilutions were employed for each determination to adjust the concentrations found in samples with the different calibration standard solutions: Li, B, As, and K 1:2, Ca and Mg 1:100, and Na 1:10. The analytical wavelengths were: Li 670.784 nm, B 208.959 nm, As 189.042 nm, Ca 317.933 nm, Mg 279,806 nm, Na 558.995 nm, and K 766.490 nm. The determination of phosphates was performed using a spectral photometer by measuring the absorption of a 190- to 1100-nm (ultraviolet-visible) reference beam. Results are summarized in Table 4.

Statistical analysis

All analyses were conducted using RStudio 3.1.1 software, and an alpha value for rejection of null hypotheses was set at $p < 0.05$ for all analyses. The normality of distributions and homogeneity of variances were evaluated by applying the Shapiro–Wilk, and Bartlett tests (Bartlett's *K*-squared), respectively. The data were not normally distributed, and so further analyses were conducted using nonparametric tests by applying the Kruskal–Wallis test (K_{test}) and the Spearman (rho) correlation coefficient.

Assessment of differences and associations were analyzed first: 1) between Li in water, brine, and vicuna calcified tissues (bones, bones plus teeth, and teeth) samples, and 2) between B in water, brine, and vicuna calcified tissues (bones, bones plus teeth, and teeth) samples, according to the data we obtained (Tables 3 and 4), which are summarized in the Supplemental Data, Table A; then differences and associations were assessed between Li in water and body tissues (of vicuna and human), categorized as calcified, soft, keratinized, and fluids, by considering the dataset presented in Table 5. This dataset data includes values reported in the present and previous studies. Details on the statistical computations are available in the Supplemental Data.

TABLE 4: Composition of vicuna bone and tooth samples

	HSG1 Bone + tooth	HSG1b Tooth	HSG2 Bone	Unit
Li	20.80	40.00	13.50	mg kg^{-1}
B	46.80	41.70	40.00	mg kg^{-1}
As	<0.50	—	<0.50	mg kg^{-1}
Ca	25.50	—	21.50	g%
Mg	0.32	—	0.24	g%
Na	0.62	—	0.53	g%
K	0.06	—	0.09	g%
PO_4	42.00	—	36.20	g%

Li = lithium; B = boron; As = arsenic; Ca = calcium; Mg = magnesium; Na = sodium; K = potassium; PO_4 = phosphate.

TABLE 5: Summary of the dataset and the categories of body tissues considered during statistical analysis[a]

Sample	Li in water	Li in body tissues	Category of body tissue	Details
HSG1	287.56[b]	20.80	Calcified	Bone + tooth
HSG2	287.56[b]	13.50	Calcified	Bone
HSG1b	287.56[b]	40.00	Calcified	Tooth
C10	1.00	4.55	Fluid	Urine
F13	5.17	1.59	Calcified	Tooth
F14	5.17	2.82	Fluid	Urine
F14a	5.17	8.80	Calcified	Rib
F14b	5.17	8.00	Keratinized	Hair (trunk)
F14c	5.17	1.10	Keratinized	Hair (ns)
F14d	5.17	7.30	Keratinized	Nail
F14e	5.17	2.80	Soft	Liver
F14f	5.17	5.00	Soft	Kidney
F14g	5.17	3.40	Soft	Muscle (ns)

[a]Sample ID numbers correspond to the source of the data: C10 is from Concha et al. (2010); F13 is from Farell et al. (2013); F14 and F14a–g are from Figueroa et al. (2014). Further details concerning these samples are given in Table 1. The remaining data are original from the present study.
[b]Mean value obtained from the lithium (Li) concentrations measured in water samples San Antonio de los Cobres 1 and 2, Cobres River, Las Burras River (data from López Steinmetz 2017), Salinas Grandes, and Salinas Grandes 7.
ns = not specified.

RESULTS

Hydrochemistry

The salinity of samples ranged from approximately 1000 to approximately 57 000 mg L^{-1}. According to limnologic criteria (Cole 1983), water samples collected from rivers (Cobres and San Antonio de los Cobres 1) were brackish, and the sample from the spring (San Antonio de los Cobres 2) was briny (Figure 4).

The Li concentrations in tributaries discharging into the Salinas Grandes were between 1.15 and 3.75 mg L^{-1}. Concentrations of Li in brines from pools in the salt pan were 673 and 1018 mg L^{-1} (Table 3).

The B concentration in feeding rivers was 18 to 44 mg L^{-1} and reached its maximum in brine pools, at 406 mg L^{-1}. Arsenic was detected in stream channels, springs, and brines, at concentrations of 0.06 to 1.32 mg L^{-1}.

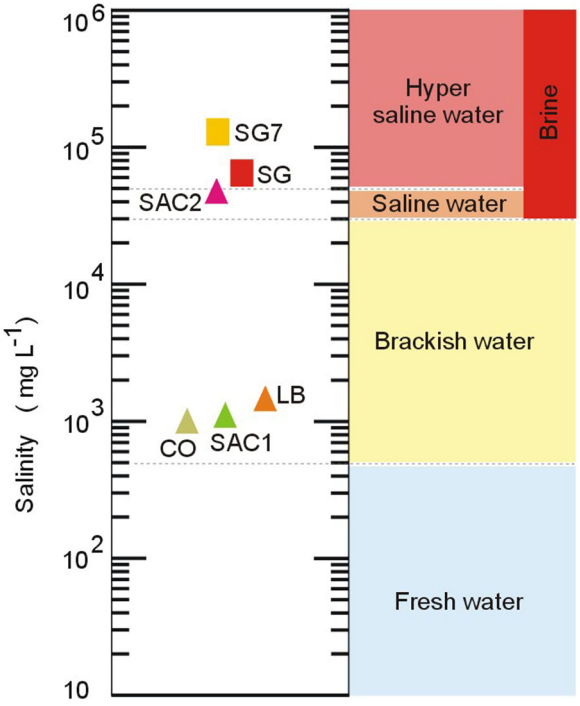

FIGURE 4: Water types according to saline content.

Concentration ratios (in meq L^{-1}) between Li, B, and As were extremely variable. The B to Li ratio was between 0.23 and 13.74, the Li to As ratio was between 177.33 and 5503.34, and the B to As ratio was between 1807.77 and 17 642.84 (Table 6). The concentration ratios with respect to salinity (TDS normalized values) were 58.72 to 371.65 for ([Li to As] to TDS) and 36.99 to 5107.68 for ([B to As] to TDS; meq L^{-1}/mg L^{-1}; Table 6).

Vicuna bone and tooth chemistry

The measured concentrations of all analyzed constituents of sampled bones and teeth were higher in sample HSG1 (mandible and teeth together as a composite) than in sample HSG2 (femur, tibia, and metatarsus together), except for K (Table 4). The major constituents of the bone and/or tooth mineral fractions were PO_4 and Ca (98.54–98.53%), with 42.36 and 36.20 g% of PO_4 and 25.50 and 21.50 g% of Ca in HSG1 and HSG2, respectively. The Na, Mg, and K fractions were minor constituents, with concentrations in the range of 0.62 to 0.53 g% of Na, 0.32 to 0.24 g% of Mg, and 0.06 to 0.09 g% of K.

Traces of Li and B were detected in bone composition, with Li being 20.80 and 13.50 mg kg^{-1} in samples HSG1 and HSG2, respectively, and B being 46.80 and 40.00 mg kg^{-1}. Teeth (HSG1b) contained 40 mg kg^{-1} of Li and 41.70 mg kg^{-1} of B. The concentrations of As were below the detection limit of our analytical method (0.50 mg kg^{-1}). The B to Li concentration ratios (in meq L^{-1}) were between 0.67 and 1.90 (Table 6).

Statistical analysis

Li and B. Analysis of the differences between the concentrations of Li and B in the 3 groups of samples (water, brine, and vicuna calcified tissues) revealed no significant differences (Li: $K_{test} = 5.44$, $df = 2$, $p = 0.07$; B: $K_{test} = 4.28$, $df = 2$, $p = 0.12$). The analysis of associations revealed a significant positive association between the concentrations of Li and B (rho = 0.80, $p = 0.01$, alternative hypothesis: 2-sided). For this association, with a significance level of 0.05 and $n = 9$, the power of the test was 80% (power = 0.80).

Li in water and body tissues. Assessment of the dataset shown in Table 5 revealed no significant differences between the concentrations of Li in the 4 categories of human and vicuna tissues ($K_{test} = 3.75$, $df = 3$, $p = 0.29$). Significant differences were found between the concentrations of Li in water and those in body tissues ($K_{test} = 6.45$, $df = 2$, $p = 0.04$). There was a significant positive association between the concentrations of Li in water and in body tissues (rho = 0.64, $p = 0.02$, alternative hypothesis: 2 sided). For this association, with $p = 0.05$ and $n = 13$, the power of the test was 70% (power = 0.70).

DISCUSSION

The available water resources in the Salinas Grandes area are characterized by high levels of salinity, Li, B, and As. In rivers and spring waters (samples San Antonio de los Cobres 1 and 2, Cobres River, and Las Burras River), the concentrations of B were approximately 1 order of magnitude higher than those of Li, whereas concentrations were in the same order of magnitude in samples collected in brine pools (Salinas Grandes and Salinas Grandes 7; Table 6). However, the analysis of differences between Li and B in the 3 groups of samples (water, brine, and calcified tissues) showed that these differences were not significant.

There was no clear proportionality between As and the concentrations of B and Li (Li to As and B to As ratios; Table 6).

TABLE 6: Ratios between lithium (Li), boron (B), and arsenic (As), and total dissolved solids (TDS) normalized values[a]

	Water sample				Brine sample		Sample of vicuna calcified tissues		
Ratio	SAC1	SAC2	CO	LB	SG	SG7	HSG1	HSG1b	HSG2
B/Li	10.19	6.62	13.74	3.43	0.39	0.23	1.44	0.67	1.90
Li/As	177.33	2664.17	372.40	—	5503.34	—	—	—	—
B/As	1807.77	17 642.84	5117.90	—	2132.33	—	—	—	—
(Li/As)/TDS	168.25	58.72	371.65	—	95.48	—	—	—	—
(B/As)/TDS	1715.15	388.87	5107.68	—	36.99	—	—	—	—

[a]Samples of vicuna calcified tissues include bone plus tooth, bone, and tooth. Ratios between Li, B, and As are expressed in meq L^{-1}, and TDS are in mg L^{-1}. SAC1 and 2 = San Antonio de los Cobres River 1 and 2; CO = Cobres River; LB = Las Burras River (from López Steinmetz 2017); SG = Salinas Grandes.

The lack of proportionality between As, Li, and B even persisted when the salinity of the samples was taken into consideration (i.e., TDS normalized ratios; Table 6). On the other hand, our analysis revealed that the concentrations of Li and B were positively associated. Based on the association between Li and B and the lack of proportionality with respect to the ionic ratios of As, it can be deduced that natural processes regulating the Li and B concentrations in waters are similar between them, but are different from those ruling As.

To the best of our knowledge, this is the first study to report chemical data on vicuna bones and teeth, and their relation with the Andean plateau environment. The calcified tissues analyzed contained up to 40 mg kg^{-1} of Li and 46.80 mg kg^{-1} of B. The B to Li ratios were between 0.67 and 1.90 (Table 6). No As was found in vicuna bones and teeth, whereas As can accumulate in other animals (Rodríguez and Mandalunis 2018). According to the results, either the amount of As was below the detection limit of the analytical technique used, or else As was simply absent in the vicuna calcified tissues. This would imply that, unlike Li and B, As does not seem to be assimilated from drinking water and then incorporated into bones and teeth in vicuna. In contrast, As ingestion has been reported to be related to glucose and lipid metabolism in mice (Zuo et al. 2019), and to accumulate in human soft and hard tissues (Rodríguez and Mandalunis 2018).

The measured concentrations of Li in persons living in the Andean region (2.82–4.55 kg^{-1}; Table 1) were consistent and within the range of the Li concentrations reported in Andean mummies (1.10–8.80 kg^{-1}; Table 1; Concha et al. 2010; Farell et al. 2013; Figueroa et al. 2014). It is important to note that, similar to ancient people living in the Andean plateau, modern settlements are supplied with drinkable water whose Li content is not regulated prior to human ingestion. This implies that the Li concentrations in mummy tissues can be considered as accurate and likely representative data of modern inhabitants.

The measured concentrations of Li in the vicuna calcified tissues (13.50–40 kg^{-1}; Table 4) were higher than those reported in human tissues (1.10 and 8.80 kg^{-1}; Table 1; Concha et al. 2010; Farell et al. 2013; Figueroa et al. 2014). The weight of a human being is comparable to that of an adult vicuna (weighing 35–65 kg; Cajal 1989; Aguilar et al. 1995; Borgnia et al. 2008; Cassini et al. 2009; Franklin 2011; Tirado et al. 2016). The vicuna specimens we studied were from young animals; even so, the measured Li concentrations in bones and teeth were higher than those reported from humans in the Andes, especially in calcified tissues. Therefore, the comparatively higher concentrations of Li in vicuna calcified tissues (20.80, 40.00, and 13.50 mg kg^{-1}) compared with human bones (8.80 mg kg^{-1}) and teeth (1.59 mg kg^{-1}) could be related to the particular adaptation of vicuna to the arid conditions of the plateau by ingesting saline waters rather than to the time of exposure (i.e., age). Brackish waters and brines are richer in Li (from 1.15 to more than 1000 mg L^{-1}) than fresh drinkable water consumed by humans, that is, 1 mg L^{-1} from the San Antonio de los Cobres River (Concha et al. 2010) and 5.17 mg L^{-1} from the Camarones River (Table 1; Zaldívar 1980; Farell et al. 2013). Indeed, we found a positive association between the Li concentration in water with Li in all body tissues (soft, fluid, keratinized, and calcified) in humans and vicuna, which is consistent with the reported dependency of Li concentrations in serum and other tissues on Li exposure in humans and other species (Aral and Vecchio-Sadus 2008; Concha et al. 2010; Figueroa et al. 2012, 2014; Harari et al. 2015), and in human and rat bones and teeth (Birch 1974; Peppersack et al. 1994; Zamani et al. 2009; Farell et al. 2013).

The toxicology of Li is based on a serum-measuring scale, and the toxic stage in humans begins at approximately 10.50 to 17.5 mg L^{-1} (Litovitz et al. 1994; Jaeger 2003). Andean populations exposed to Li through drinking waters have average serum concentrations of 5.30 mg L^{-1} (Figueroa et al. 2014). There are no data on toxic ranges in serum for vicunas, nor for humans in either a bone or tooth Li concentration scale. The Li concentration measured in the vicuna calcified tissues was 2 to 3 times higher than that reported in humans, which suggests the question: what would be the consequences for the health of vicunas of such levels of Li in bone tissues?

In vicuna, teeth contained more Li than bones (tooth sample HSG1b = 40 mg kg^{-1} > bone + tooth sample HSG1 = 20.80 mg kg^{-1} > bone sample HSG2 = 13.50 mg kg^{-1}; Table 4). Interestingly, this is consistent with the increasing proportion of the mineral fraction in human teeth (70–96%; Gutiérrez-Salazar and Reyes-Gasga 2003) compared with bones (~65%; Quelch et al. 1983). This Li concentration pattern is likely dependent on the mineralized fraction of the calcified tissue and might be related to mechanisms involved in the metabolization of Li. Such mechanisms might include, for instance, the incorporation of Li^+ during the substitution of Ca^{2+} by Mg^{2+} in the mineralized bone and tooth structure $Ca_{10}(PO_4)_6(OH)_2$, because Li^+ and Mg^{2+} have similar ionic ratios (i.e., the sizes of these ions are quite similar [Li^+ = 0.60 Å and Mg^{2+} = 0.65 Å], and thus they might occupy the same position in the crystalline structure of the mineral fraction in bone and/or tooth). It is also possible that isovalent replacements of Na^+ and K^+ sites by Li^+ might take place. However, the osteological mechanisms involved are likely to be more complex than that; for instance, the substitution of Ca^{2+} by Mg^{2+} (and/or Na^+, K^+, Li^+?) results in a decrease in the crystal mesh size, and requires the incorporation of HPO_4^{2-} for the charges to be compensated (Serre et al. 1998; Cabrejos-Azama et al. 2014, and references therein). Furthermore, the observed pattern of Li concentrations (a consistent increase with the tissue's mineralized fraction) was not found in Andean human tissues (i.e., there was no tooth > bone > soft and keratinized tissue Li concentration trend; Table 1), which highlights the complexity of processes ruling the metabolization of Li.

Alexander et al. (1951) have reported that human bones contain a normal average concentration of 63.70 mg kg^{-1} of B, which would not be dependent on the time of exposure. As mentioned just above, the concentrations of Li in waters are positively associated with those in body tissues. In addition, we also found that Li concentrations correlated positively with B in water, brine, and calcified tissues. According to these results, we consider that the concentrations of Li and B observed in vicuna reflect the ingestion of saline, high Li- and B-concentrated

waters. Despite the consistency of our reported data, due to the absence of other studies reporting the tissue compositions of vicuna for comparison, it is unclear whether the presence of B and Li we found might correspond to an anomaly of the specimens studied, or to a normal characteristic of the *V. vicugna* species and the Camelidae family, associated with the intrinsic characteristics of the Andean plateau environment.

CONCLUSIONS

Available water resources in the Salinas Grandes area are characterized by maximum concentrations reaching 1019 mg L^{-1} of Li; 406 mg L^{-1} of B; and 1.32 mg L^{-1} of As. The present study reports the first chemical data on vicuna bones and teeth with up to 40 mg kg^{-1} of Li and 46.80 mg kg^{-1} of B. No As was found in vicuna calcified tissues, which would imply that, unlike Li and B, As does not seems to be assimilated from drinking water and incorporated into bones and teeth. The measured Li concentrations in vicuna were higher than those reported from calcified tissues of other species exposed to Li through drinkable water. Our analysis of associations showed that there were significant positive associations between: 1) the concentrations of Li in water and in body tissues, and 2) the concentrations of Li and B.

The toxicology of Li in vicuna should be assessed, with the aim of addressing: 1) the health consequences for vicunas of such levels of Li, and 2) the potential existence of metabolic processes of adaptation in vicuna that would eventually reveal useful information for the treatment of human pathologies.

Further studies will be necessary to determine whether these compositional values correspond to an anomaly of the specimens studied, or to a normal characteristic of the *V. vicugna* species and the characteristics of the Andean plateau environment.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4608.

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