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Lithium in vicuna bones by water ingestion in the andes

Lithium and boron in calcified tissues of vicuna and its relation to the 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 hyper arid Andean plateau. In the central part of the plateau, the Lithium Triangle defines a zone having lithium -rich salt pans. Brine pools naturally form within those 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, in order to assess its relation to the chronic exposure by water ingestion. We have 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 high lithium, boron, and arsenic grades. Lithium (13.50 to 40 mg kg⁻¹) and boron (40 to 46.80 mg kg⁻¹), but not arsenic, were found in the vicuna bones and teeth. Based on this and previously reported data on human tissues in the Andes, we have conducted statistical assessments on the relations between lithium and boron grades in body tissues and bearing waters, and discussed its environmental significance in the context of the lithium triangle.

Keywords: lithium; vicuna; bones; teeth; salt pan; Andes

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INTRODUCTION

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

As Li is a very reactive and geochemical incompatible element, it is often present in waters (i.e., in solution) in its ionic form Li^+ . The mean Li concentration in sea water is 0.18 mg L^{-1} (Riley and Tongudai 1964). Continental waters are characterized by containing large variable Li grades. In the Andean plateau, river waters involve relatively high Li contents that are typically up to 10 mg L^{-1} (López Steinmetz 2017). These rivers systems are frequently fed by hydrothermal sources that contain even higher Li grades, which is conducive to increasing Li concentrations in river waters often exceeding 15 mg L^{-1} (Giordano et al. 2013; Giordano et al. 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} (Fig. 2 A), and that can even exceed $1,000 \text{ mg L}^{-1}$ (Ericksen and Salas 1977; Risacher and Fritz 1991; Garret 2004; Kesler et al. 2012; Godfrey et al. 2013; López Steinmetz 2017; López Steinmetz et al. 2018).

Drinking water supply in Andean villages is obtained from such natural sources. The presence of Li in drinking waters can be traced back in time. Li was 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

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concentrations in the crown were high in enamel as well as in dentin, suggesting a prenatal exposure to Li-bearing drinking waters (Farell et al. 2013).

The passage of time has not changed these situations, and in modern times, the water potabilization process does not include the regulation of Li contents 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 Fig. 1), besides Li concentrations reaching 1 mg L⁻¹, 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 drinking water of such quality is observed to induce an increase of up to one order of magnitude in the concentration of these chemical elements in the urine, as well as in diverse body tissues including hair, blood and breast milk, relative to people unexposed (Concha et al. 2010; Figueroa et al. 2014; Harari et al. 2015).

Studies on the effect of ingesting Li have been, for a long period of time, mainly focused in its psychiatric use, which is the most common source of exposure to Li. Li salts, especially the carbonate (Li₂CO₃) and acetate (LiCH₃COO) have psychotropic properties, and are employed since the mid-1800s in the treatment of bipolar disorders (Cade 1949; Schou 1968; Aral and Vecchio-Sadus 2008). Treatment of these disorders require the surveillance of the Li concentration due to its toxicity (Price and Heninger 1964).

Some major differences exist between these two kinds of Li exposures. First, although psychiatric Li exposures are conducted under long-term durations, 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 grades are not

systematically subjected to serum surveys, contrarily to persons following a psychiatric treatment. For example, according to pharmacological Li carbonate leaflets, a standard posology during a psychiatric treatment for a young adult person weighing ~70 kg is from 500 to 1200 mg day⁻¹. This implies a daily absorption of 93 to 225 mg of Li. The resulting therapeutic serum concentrations are expected remain below 8.40 mg L⁻¹, as the toxic stage begins at about 10.50 - 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 about 30 min after oral ingestion. Li is distributed throughout body tissues, and is excreted primarily through the kidneys after approximately 24 h (Freeman and Freeman 2006). Additionally, a 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). 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 that are not exposed to Li (Alexander et al. 1951). Bone and tooth tissues are composed of water, inorganic, and organic constituents. The proportion of constituting components in bone and tooth are variable and depends on several factors such as the tissue type, the considered species, the age, etc. In human tooth, enamel is the most mineralized tissue

with 96%, and in dentin, the inorganic material, represents 70% (Gutiérrez-Salazar and Reyes-Gasga 2003). The mineral fraction in bone is also the most abundant constituent, where it is majorly composed of phosphate (PO_4^{3-}) and calcium (Ca^{2+}), and represents about 65% of normal human bone, and up to ~76% in bovines (Quelch et al. 1983).

Living beings in the Andean plateau are exposed to altitudes of more than 3,000 m above sea level (m asl), 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. 2012, and references therein; Fig. 2 B). During periods of enhanced aridity (from April to November), the adaptation strategy of vicuna consists of drinking from brine pools within the salt pans (Fig. 2 C). The salinity of these brines is $>100,000 \text{ mg L}^{-1}$, and dissolved elements include high concentrations of Li, As, and B, among other ions.

In this article we present results of chemical analysis on the bone and tooth composition of two 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.

THE STUDIED SPECIMEN AND ITS ENVIRONMENT

The 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 (*Vicugna vicugna*), an endangered species protected by laws, is found in the high plateau areas,

between 3,000 and 4,600 m asl (Franklin 2011; Fig. 2). Adult specimens of vicuna measure between 1.45 to 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. 2012).

The vicuna diet is based on grasses and shrubs (~80%), complemented by pseudograsses and herbs (Tirado et al. 2012). These proportions are however variable, and change following sedentary and migratory tendencies of different populations according to seasonal and environmental restrictions (Franklin 1982; Contreras et al. 2006). An interesting aspect of the 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 km² placed at 3,410 m asl within the largest (>17,000 km²) endorheic basin of the Argentinean plateau (Fig. 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 >18°C (Bianchi 1981; Buitrago and Larran 1994).

Water is scarce and contains high saline contents. 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 (Fig. 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, Fig. 2), which holds brines of the Na-K/Cl-SO₄ type, nearly neutral pH values (ranging from 7.10 to 8.00), and with 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 (Fig. 2 C). 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, while Li⁺ grades are largely variable ranging between 25 and 1,018 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.

METHODS

Water and brine sampling

We have collected water samples from the San Antonio de los Cobres (SAC1) and Cobres (CO) Rivers, and from a spring source (SAC2) in the headwaters of the SAC River. A brine sample (SG) 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 (LB; López Steinmetz 2017) and a brine sample (SG7; López Steinmetz et al. 2018). The locations of samples are summarized in Table 2, and shown in Fig. 1. All the water and brine sampling were conducted by employing the same sampling methodology: samples were collected from natural sources in 1.50 L

polyethylene bottles pre-cleaned with the water/brine being sampled, and were kept at ~0 °C until they were transported to the laboratory for analysis.

Electrical conductivity of samples were determined with a Hanna HI2314 multi-range conductivity meter. Total dissolved solids (TDS), representing the salinity of samples in mg L⁻¹, have been obtained by employing a conversion factor of 0.50 on the measured electrical conductivity. The concentrations of Li⁺, B, and As were conducted at the Agua de los Andes S.A. laboratory of water chemistry (Jujuy, Argentina) and were determined following standard recommendations (Clesceri et al. 1998). Li⁺ was determined by Atomic Emission Spectroscopy, colorimetry using carmine reactive was employed for B, and Arsenic was measured as total As by atomic absorption spectrometry. Analytical results are summarized in Table 3.

Bone and tooth sampling

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

Parts of the mandible with teeth of one specimen (HSG1) were sampled. Sample HSG1 is a composite sample that includes 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 (Fig. 3 B and C). Sample HSG1b only involves teeth (lower premolar, molars, and incisive). Sample HSG2 is a composite sample including partial femur, tibia, and metatarsus (hind leg) together that were selected from the second specimen (HSG2). For sample HSG2,

only the body of these bones were retained, and heads and bases were removed by cutting them (Fig. 3 D, E, 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 hours. Samples were then crushed with a special 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 drying the samples.

Sample processing included acid digestion and dilution by first treating the sampled bone and tooth with 9 mL of HNO₃ and 3 mL of HCl by microwave digestion using a Milestone Ethos Easy digester. Following which, 0.5 mg of each sample were considered and diluted to a final volume of 20 mL for HSG1 and HSG2, and to 25 mL for HSG1b.

The determinations of the total Li, B, As, calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K) were performed by inductively coupled plasma optical emission spectroscopy (ICP-OES) using an optic ICP (HORIBA JY 2000). Internal standards and standard solutions (HORIBA Advanced Techno Co., Ltd) were prepared in 20% HNO₃ 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 three-point curve method (0, 200 y 500 ppb), four-point curve fitting was employed for Li and As (0, 10, 100, 1000 ppb), and K (0, 0.5, 1, 10 mg L⁻¹), and five-point curves for Ca, Mg, and Na (0, 1, 10, 50, 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 employed 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 190-1100 nm (UV-visible) reference beam. Results are summarized in Table 4.

Statistical analysis

All analyses were conducted using the R Studio 3.1.1 software (2014-07-10) 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 (W), and the Bartlett tests (Bartlett's K-squared), respectively. Data are not normally distributed, consequently, further analyses were conducted using non-parametric tests by applying the Kruskal-Wallis test (K_{test}) and the Spearman (rho) correlation coefficient.

Assessment of differences and associations were analyzed first: i) between Li in water, brine, and vicuna calcified tissues (bones, bones plus teeth, and teeth) samples, and ii) between B in water, brine, and vicuna calcified tissues (bones, bones plus teeth, and teeth) samples, according to the data we have obtained in our study (Tables 3 and 4), which are summarized in Table A of the Electronic Supporting Information; and then between Li in water and body tissues (of vicuna and human) categorized in calcified, soft, keratinized and fluids, by considering the dataset presented in Table 5. The latter tested

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data include values reported in this study and in previous studies. Details concerning statistical computations are available in the Electronic Supporting Information.

RESULTS

Hydrochemistry

The salinity of samples ranged from ~1,000 to ~57,000 mg L⁻¹. According to the limnologic criteria (Cole 1983), water samples collected from rivers (CO and SAC1) are brackish, and the sample from the spring (SAC2) corresponds to brines (Fig. 4).

The Li concentrations in tributaries discharging into the Salinas Grandes are between 1.15 and 3.75 mg L⁻¹. Brines from pools in the salt pan are characterized by Li grades of 673 and 1,018 mg L⁻¹ (Table 3).

The B concentration in feeding rivers is measured between 18 and 44 mg L⁻¹, and reaches its maximum in brine pools with 406 mg L⁻¹. As was detected in stream channels, springs and brines, with concentrations of 0.06 to 1.32 mg L⁻¹.

Concentration ratios (in meq L⁻¹) between Li, B, and As are extremely variable. The B/Li ratio is comprised between 0.23 and 13.74, Li/As ranges between 177.33 and 5,503.34, and B/As grades from 1,807.77 up to 17,642.84 (Table 6). The concentration ratios with respect to the salinity (TDS normalized values) are of 58.72 to 371.65 for [(Li/As)/TDS], and 36.99 to 5,107.68 for [(B/As)/TDS] (meq L⁻¹ per mg L⁻¹; Table 6).

Vicuna bone and tooth chemistry

The measured concentrations of all analyzed constituents of sampled bones and teeth are higher in the HSG1 (mandible and teeth together as composite sample) than in HSG2 (femur, tibia, and metatarsus together), except for K (Table 4). PO₄ and Ca represent the majoritarian (98.54 - 98.53 %) constituents of the bone and/or tooth mineral

fraction, with 42.36 and 36.20 g% of PO₄ and 25.50 and 21.50 g% of Ca in HSG1 and HSG2, respectively. Na, Mg, and K are minor constituents, and their concentrations are in the range of 0.62 - 0.53 g% of Na, 0.32 - 0.24 g% of Mg, and 0.06 - 0.09 g% of K.

Traces of Li and B have been detected in bone composition. Li is 20.80 and 13.50 mg Kg⁻¹ in samples HSG1 and HSG2, respectively, and B is 46.80 and 40.00 mg Kg⁻¹. Teeth (HSG1b) contain 40 mg Kg⁻¹ of Li and 41.70 mg Kg⁻¹ of B. The concentrations of As were below the detection limit of the employed analytical method (0.50 mg Kg⁻¹). The B/Li concentration ratios (in meq L⁻¹) are comprised between 0.67 and 1.90 (Table 6).

Statistical analysis

Li and B.

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

Li in water and body tissues.

The assessment of the dataset shown in Table 5 reveal that there are no significant differences between the concentrations of Li in the four categories of human and vicuna tissues ($K_{\text{test}} = 3.75$, $df = 3$, $p\text{-value} = 0.29$). Significant differences are found between the concentrations of Li in water and those in body tissues ($K_{\text{test}} = 6.45$, $df = 2$, $p\text{-value} = 0.04$). The analysis of associations has shown that there is a significant, and positive

association between the concentrations of Li in water and body tissues ($\rho = 0.64$, p -value = 0.02, alternative hypothesis: two sided). For this association, with a significance level of 0.05 and $n = 13$, the power of the test is 70% (power = 0.70).

DISCUSSION

Available water resources in the Salinas Grandes area are characterized by containing high levels of salinity, Li, B, and As. In rivers and spring waters (samples SAC1, SAC2, CO, and LB), the concentrations of B are ~1 order of magnitude higher than those of Li, whereas concentrations are in the same order of magnitude in samples collected in brine pools (samples SG and SG7; Table 6). However, the analysis of differences between Li and B in the three groups of samples (water, brine, and calcified tissues) has revealed that these differences are not significant.

There is no clear proportionality between As and the concentrations of B and Li (Li/As and B/As ratios, Table 6). The lack of proportionality between As, Li and B even persists when the salinity of samples is taken into consideration (i.e., TDS normalized ratios, Table 6). On the other hand, the analysis of associations has revealed that the concentrations of Li and those of B are 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 grades 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 its relation with the Andean plateau environment. The analyzed calcified tissues contain up to 40 mg Kg^{-1} of Li and 46.80 mg Kg^{-1} of B. The B/Li ratios are between 0.67 and 1.90 (Table 6). No As has been found in vicuna bones

and teeth while it can accumulate in other animals (Rodríguez and Mandalunis 2018).

According to the results, either the amount of As is below the detection limit of the analytical technique used, or it is 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 to 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 to 4.55 Kg^{-1} ; Table 1) are consistent and within the range of the Li concentrations reported in Andean mummies (1.10 to 8.80 Kg^{-1} ; Table 1) (Concha et al. 2010; Farrell 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 that does not undergo the regulation of Li contents prior to human ingestion. This implies that the Li concentration 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 to 40 Kg^{-1} ; Table 4) are higher than those reported in human tissues (1.10 and 8.80 Kg^{-1} ; Table 1) (Concha et al. 2010; Farrell et al. 2013; Figueroa et al. 2014). The weight of a human being is comparable with that of an adult vicuna (measuring from 35 to 65 kg; Cajal 1989; Aguilar et al. 1995; Borgnia et al. 2008; Cassini et al. 2009; Franklin 2011; Tirado et al. 2012). The studied vicuna specimens are young and, despite this, the measured Li concentration in bones and teeth are higher than those reported from human in the Andes, especially concerning calcified tissues. Therefore, the comparatively higher

concentrations of Li in vicuna calcified tissues (20.80, 40.00, and 13.50 mg kg⁻¹) relative to human bones (8.80 mg kg⁻¹), and teeth (1.59 mg kg⁻¹), could be related to the peculiar adaptation of vicuna to the arid conditions of the plateau by ingesting saline waters rather than to the time of exposure (i.e., the age). Brackish waters and brines are richer in Li - from 1.15 to >1,000 mg L⁻¹ - than fresh drinkable water consumed by humans, i.e. 1 mg L⁻¹ at San Antonio de los Cobres (Concha et al. 2010), and 5.17 mg L⁻¹ in the Camarones River's waters (Table 1; Zaldívar 1980; Farrell et al. 2013). Indeed, we have found a positive association between the Li concentration in water with Li in all body tissues (i.e., soft, fluid, keratinized and calcified) in human and vicuna, which is consistent with the reported dependency of the Li concentration in serum and other tissues on the Li exposure in human and other species (Aral and Vecchio-Sadus 2008; Concha et al. 2010; Figueroa et al. 2012; Figueroa et al. 2014; Harari et al. 2015), and in human and rat bones and teeth (Birch 1974; Lee 1998; Pepersack et al. 1994; Zamani et al. 2009; Farrell et al. 2013).

The toxicology of Li is based on a serum-measuring scale, and the toxic stage in humans begins at about 10.50 - 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 is no data concerning toxic ranges in serum for vicunas, nor for humans in a bone nor tooth-Li concentration scale. The Li concentration measured in the vicuna calcified tissues is twice to triple higher than that was reported in humans, which begs the question: what would be the consequence of such levels of Li in bone tissues upon the health of vicunas?

In vicuna, teeth contain 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 to 96%; Gutiérrez-Salazar and Reyes-Gasga 2003) with respect to bones (about 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 Ca²⁺ by Mg²⁺ in the mineralized bone and tooth structure Ca₁₀(PO₄)₆(OH)₂ as Li⁺ and Mg²⁺ have similar ionic ratios (i.e., the size of these ions are quite similar, Li⁺ = 0.60 Å and Mg²⁺ = 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, involved osteological mechanisms are likely to be more complex than that and, for instance, the substitution of Ca²⁺ by Mg²⁺ (and/or Na⁺, K⁺, Li⁺?) results in the decrease of the crystal mesh size, and require the incorporation of HPO₄²⁻ in order 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 that increase consistently with the tissue's mineralized fraction was not found in Andean human tissues (i.e., there is 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 previously mentioned, the concentrations of Li in waters are positively associated with those in body tissues. In addition, we also found that Li concentrations correlate 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 would be reflecting the ingestion of saline, high Li and B-graded 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 if the presence of B and Li we have found might correspond to an anomaly of the studied specimens, or to a normal characteristic of the *Vicugna vicugna* species and the *camelidae* family, associated with the intrinsic characteristics of the Andean plateau environment.

CONCLUSION

Available water resources in the Salinas Grandes area are characterized by maximum concentrations reaching 1,019 mg L⁻¹ of Li; 406 mg L⁻¹ of B; and 1.32 mg L⁻¹ of As. This 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 has been found in vicuna calcified tissues, which would imply that, unlike Li and B, As does not seem to be assimilated from drinking water and incorporated to bones and teeth. The measured Li concentration in vicuna is higher than that was reported from calcified tissues of other species exposed to Li through drinkable water. The analysis of associations has shown that there are significant, and positive associations between: i) the concentrations of Li in water and body tissues, and ii) the concentrations of Li and B.

The toxicology of Li in vicuna should be assessed in order to address: i) the consequence of such levels of Li upon the health of vicunas, and ii) investigate 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 in order to set out whether these compositional values correspond to an anomaly of the studied specimens, or to a normal characteristic of the *Vicugna 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.xxxx.

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Data accessibility — Data, associated metadata, and calculation tools are available from the corresponding author (lucrecialopezsteinmetz@hotmail.com).

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Figure 1. Map showing the location of the study area and sampling sites. The water samples have been collected in San Antonio de los Cobres river (SAC1 on the map), Cobres river (CO) and from spring sources in the headwaters of the San Antonio de los Cobres river (SAC2) and the Las Burras river (LB). The brine samples have been collected from two 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)

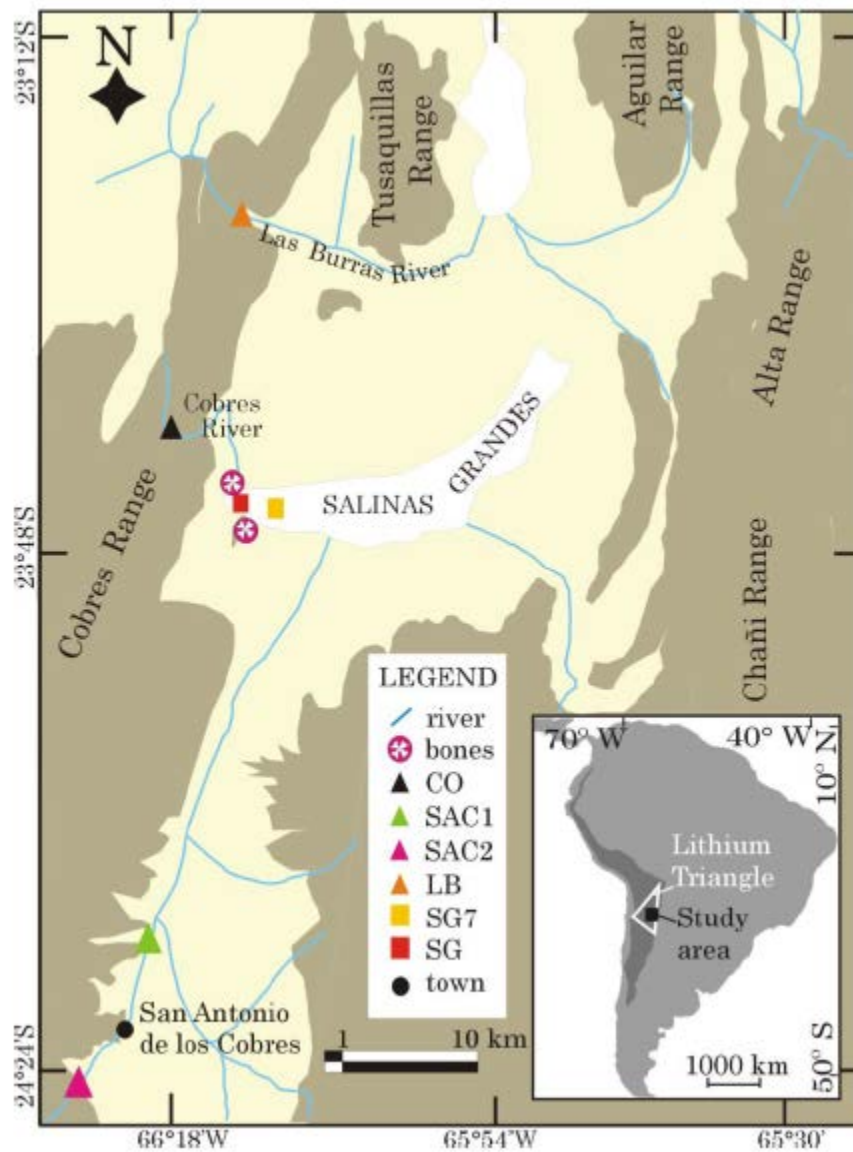


Figure 2. Pictures showing the salt crust (a), a caravan of vicunas (b), and a brine pool (c) at the Salinas Grandes salt pan



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 six lower incisive (c); the femur body (d), the tibia body (e), and the metatarsus body (f)

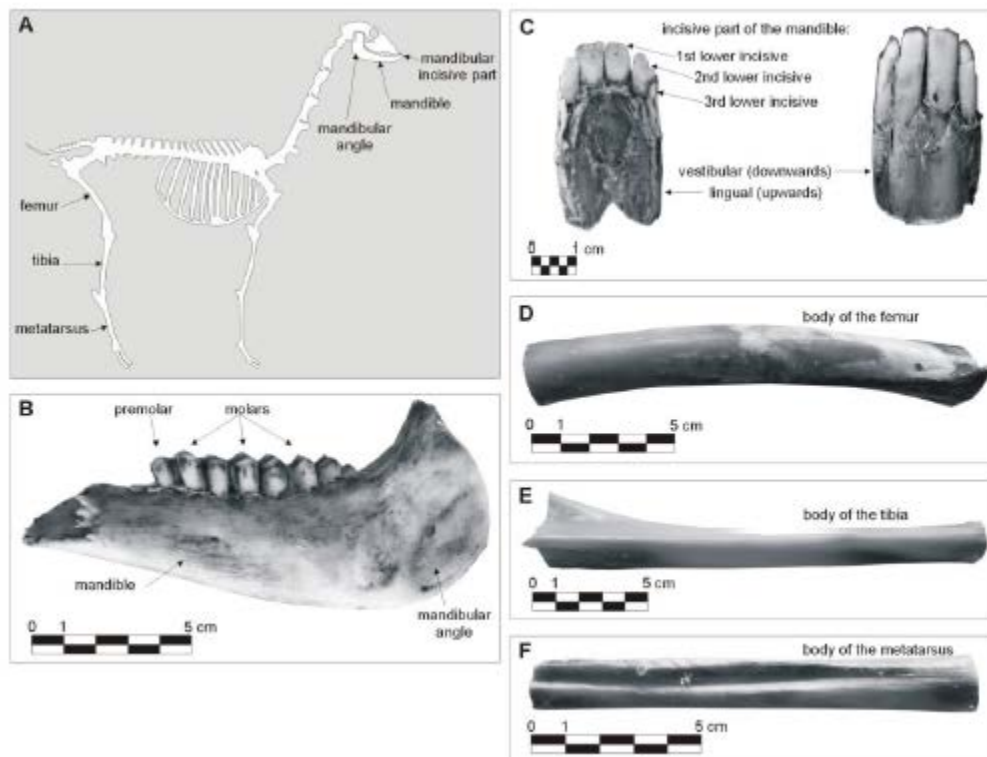


Figure 4. Water types according to the saline content

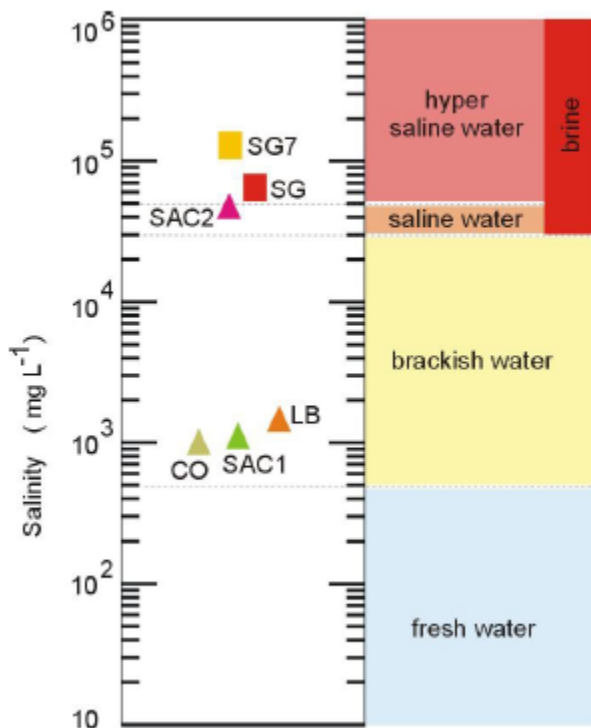


Table 1. Summary of lithium (Li) concentrations in urine, calcified, keratinized, and soft human tissues reported from the Andean plateau. Sample ID correspond to the source of data: C10 is from Concha et al. (2010); F13 is from Farell et al. (2013); F14, and F14a to F14g are from Figueroa et al. (2014).

Sample ID	Water (mg L ⁻¹)	Li concentration			Li water/Li tissue
		Human tissues (mg Kg ⁻¹)	Tissue type		
	1.00				
C10	San Antonio de los Cobres River	4.55	urine	modern	0.22
F14	5.17	2.82	urine		1.83
F13	Camarones	1.59	tooth	Mummies	3.25

F14a	River	8.80	rib	from the	0.59
F14b		8.00	hair (trunk)	Camarones	0.65
F14c		1.10	hair (ns)	Valley	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

Table 2. Detailed location of collected water, brine and vicuna samples considered in the present study.

sample	Latitude	Longitude
SAC1	24°08'58.23"	66°17'51.19"
SAC2	24°17'07.47"	66°22'25.86"
CO	23°39'01.55"	66°18'08.52"
LB	23°25'19.10"	6°13'36.80"
SG	23°43'18.73"	66°13'23.25"
SG7	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. TDS: total dissolved solids, Li: Lithium, B: Boron, As: Arsenic. All concentrations are expressed in mg L⁻¹. Sample LB correspond to data reported by López Steinmetz (2017), and SG7 is from López Steinmetz et al. (2018). Remaining samples are original data from the present study.

	Water samples				Brine samples	
	SAC1	SAC2	CO	LB	SG	SG7
TDS	1,054	45,370	1,002	1,240	57,640	116,136
Li	1.15	27.15	2.07	3.75	673.00	1,018.23
B	18.26	280.04	44.31	20.03	406.15	360.93
As	0.07	0.11	0.06	-	1.32	-

Table 4. Composition of vicuna bone and tooth samples.

	HSG1	HSG1b	HSG2	units
	bone+tooth	tooth	bone	
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%

Table 5. Summary of the dataset and the categories of body tissues considered during statistical analysis. ^a Mean value obtained from the lithium (Li) concentrations measured in water samples SAC1, SAC2, CO, LB, SG, and SG7. Sample ID correspond to the source of data: C10 is from Concha et al. (2010); F13 is from Farell et al. (2013); F14, and F14a to F14g are from Figueroa et al. (2014). Further details concerning these samples are given in Table 1. Remaining data is original from the present study.

Sample	Li in water	Li in body tissues	Categories of body tissues	Details
HSG1	287.56 ^a	20.80	calcified	bone+tooth
HSG2	287.56 ^a	13.50	calcified	bone
HSG1b	287.56 ^a	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 (not specified)
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)

Table 6. Ratios between lithium (Li), boron (B), arsenic (As), and total dissolved solids (TDS) normalized values. Samples of vicuna calcified tissues include bone plus tooth, bone and tooth samples. Ratios between Li, B, and As are expressed in meq L⁻¹, and TDS are in mg L⁻¹.

Ratio	Water samples				Brine samples		samples of vicuna calcified tissues		
	SAC1	SAC2	CO	LB	SG	SG 7	HSG 1	HSG1 b	HSG 2
B/Li	10.19	6.62	13.74	3.4 3	0.39	0.2 3	1.44	0.67	1.90
Li/As	177.33	2,664.17	372.40	-	5,503.3 4	-	-	-	-
B/As	1,807.7 7	17,642.8 4	5,117.9 0	-	2,132.3 3	-	-	-	-
(Li/As)/ TDS	168.25	58.72	371.65	-	95.48	-	-	-	-
(B/As) /TDS	1,715.1 5	388.87	5,107.6 8	-	36.99	-	-	-	-