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Bioimaging of trace metals in ancient Chilean mummies and contemporary Egyptian teeth by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS)

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ABSTRACT

Distributional analysis of trace metals in biological tissue provides important archived toxicological and nutritional information. In this study, lead, strontium, zinc, and lithium concentrations were measured in dental tissue using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). Teeth from two populations were sampled: ancient Chilean mummies (n = 16) from Arica city as well as recently exfoliated deciduous teeth from contemporary Egyptian children (n = 20) living in the Nile Delta. In this study, LA-ICP-MS was used to obtain high-resolution (50 μm) elemental bioimages in dental tissue. The experiments consisted of fully quantitative Pb bioimages as well as semi-quantitative Zn and Li bioimages of one contemporary Nile Delta, Egypt tooth and one ancient tooth from the Andes. Lead levels in the Egyptian teeth averaged an order of magnitude greater than in the ancient Chilean teeth (14.93 ± 32.28 μg/g and 1.33 ± 0.84 μg/g, respectively), and had a much greater range of variation among teeth (1.02 μg/g–68.38 μg/g versus 0.36 μg/g–2.35 μg/g, respectively). Lithium, an element widely present in northern Chile, was found in higher concentrations in the Arica teeth than in the Kalama teeth, with the highest concentrations at two sites in Chilean burial grounds where water is tainted with natural lithium. Micrometer resolution images of quantified Pb, Zn, and Li concentrations from two teeth revealed higher concentrations of Pb and Zn in the pulp and surface enamel. Zn and Li are concentrated in the crown, potentially indicative of higher prenatal exposure.

1. Introduction

Calcified tissues such as bones and teeth are mainly composed of inorganic hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], and are therefore mostly chemically inert [1–4]. Teeth comprise three distinct tissues: the outer crown consists of enamel, the most highly mineralized and hardest dental tissue, followed by dentine, making up the bulk of the inner crown and root, and a thin layer of cementum, which is similar to bone and aids in the attachment of the root to the bony socket. In addition, a biologically active zone, the pulp canal, continues to provide blood and nerves with connective tissues from the end of the root into the crown dentine.

Trace metal bioaccumulation often occurs by isovalent replacement at the calcium sites. Teeth form chronologically, somewhat like tree rings. Enamel apposition is particularly under strong genetic control with a typical daily increment of 4 nm [5] and is then followed rapidly by almost complete calcification [6]. Thus, the location of a chemical change in enamel as well as the other dental tissues offers a permanent record of the time of deposition of particular chemical substitutions in apatite. This facilitates tracking of archived elemental exposure, giving information about chronology and dose of exposure to toxic trace metals (i.e. lead) [7] as well as dietary evidence concerning nutritionally important elements such as zinc and iron.

Teeth are well suited to bioimaging studies as well as environmental and anthropological studies. In deciduous teeth, calcification begins in utero around the end of the first trimester and continues into infancy, thus offers both pre- and post-natal trace metal exposure information. As a result, bioaccumulation readings allow identification of long-term trace metal exposure and may provide more archived information than traditional blood sampling [8,9]. In addition, teeth, particularly core dental enamel, are essentially inert once formed, making them ideal biomaterials for studying trace metal accumulation in contemporary, historic and prehistoric samples [10–14].

Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) has become an important elemental bioimaging technique [15] because of its high-sensitivity, temporal elemental analysis capability of dental tissue [16,17], minimal sample destruction, high resolution, and direct solid-state sampling. LA-ICP-MS can produce high-resolution images of trace metal distribution in biological tissue. Kang et al. [12] were first to demonstrate the applicability of LA-ICP-MS to bioimaging of micro-spatial distribution of trace metals in the different dental tissue zones of deciduous teeth using small-area raster sections. Later, Hare et al. [18] semi-quantitatively imaged the micro-distribution of Cd, Pb, Sr, and Zn in whole deciduous teeth using a single standard.

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In this study, we investigated LA-ICP-MS bioimaging capabilities and its applicability to fully quantitative bioimaging of Pb distribution in human dental tissue using a series of biologically incorporated lead solid standards; these quantifications were used to assess and compare Pb exposure in contemporary and ancient deciduous teeth. In addition, Zn and Li distributions of both cohorts were compared by semi-quantitative analysis using a single standard.

2. Experimental methods

2.1. Teeth and sample preparation

The teeth sampled were from two sources. The contemporary teeth were from infants that were involved in the Nutrition Collaborative Research Support Program (NCRSP) in the Nile Delta town of Kalama, Egypt in 1983–1985 (samples courtesy of Professor Alan H. Goodman, Hampshire College, USA and data collection and analysis was approved by the institutional review boards and human subjects committees at Hampshire College and Al Azhar University, Cairo, Egypt) and subsequently collected upon exfoliation (n = 20) (AH Goodman, *Developmental Stress and Enamel Hypoplastic Defects*. National Institutes of Health, no. R15 DE 11444). Kalama is a semi-agrarian village in the Nile Delta located 25 km north of Cairo where diet consists mainly of staples of rice and wheat supplemented with legumes, seasonal fruits, vegetables, and some animal products [19].

Chilean archeological samples (n = 16) come from the arid coast of northern Chile and ranges from the Archaic Chinchorro Period (circa 5000–2000 BCE) to the Inca Horizon (1470–1532 A.D.). All were coastal sites except the Lluta-54 sites (samples provided by Professor Bernardo Arriaza, Instituto de Alta Investigacion, Universidad de Tarapacá, Arica, Chile). Chinchorro samples (Camarones-17, Yungay-372, Morro-1) are fishing–hunter–gatherer populations of the Archaic Period, while Playa Miller-4, Camarones-8, and Camarones-9 are also coastal groups but of the Late Intermediate Period (1000–1470 A.D.). As such they had a more complex socio-political organization, and had fine woven polychromous textiles and ceramic. They complemented their coastal diet with terrestrial and domesticated food resources. Lluta-54 sample came from a nearby valley associated with the Inca Horizon [20–22]. All bodies were buried in the sands of the Atacama Desert, and depending on the period they were either wrapped in vegetal fiber mats or in camelid woven textiles. The dry desert conditions were especially suitable for natural mummification of these bodies.

Tooth samples were pre-cleaned following previously described methods [3,12]. Teeth mounted in epoxy resin were sliced longitudinally using a low speed steel-bladed saw (Buehler Low Speed Saw, Isomet 11-1180, Lake Bluff, IL) to expose all dental tissues. Approximately 2 mm longitudinal sections were removed from the center of the tooth, cut, polished, and etched with 1 M HCl, then washed with DI water and acetone.

2.2. Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS)

LA-ICP-MS (New Wave ESI NWR 213 Laser Ablation System, ESI, Portland OR and ICP-MS Perkin Elmer Elan 6000A, Shelton, CT) was used to determine ^{208}Pb, ^{64}Zn, ^{7}Li, and ^{88}Sr concentrations. ^{43}Ca was used as an internal standard. Calibration of LA-ICP-MS was achieved using NYS-RM (kindly provided by Prof. Patrick Parsons, Wadsworth Center, New York State Department of Health, Albany, NY) lead-dosed caprine bone pellet standards [(CRM-NYS RM05-01: 1.09 ± 0.03; RM05-02: 16.1 ± 0.3; NYS RM05-04: 31.5 ± 0.7 Pb µg/g)] with 1000 µm long linear scans (n = 3). Linear laser ablations began at the lingual (inner) side of the tooth crown and continued, through core dentine and pulp, to the labial (outer) enamel. Background measurements of analytes were collected 40 s prior to ablation and for at least 30 s post ablation using the argon blank. Samples included deciduous incisors, canines, and primary molars (n_{total} = 36).

Table 1
Laser ablation operating parameters.

Operating conditions	Spot ablations	Linear ablations
Sampling scheme	Spot ablations arranged in grids	Linear ablations across teeth
ICP radio frequency (W)	1000	1000
Nebulizer gas flow (L.min^{-1})	0.87	0.87–0.81
Carrier gas flow (L.min^{-1})	0.25	0.25
Average fluence (J.cm^{-2})	19.81–29.19	12.45–23.43
Average energy delivered (mJ)	0.388–0.573	0.245–0.460
Laser energy (%)	50	50
Spot size (µm)	50	50
Spot separation (µm)	50	n/a
Resolution (µm)	50	n/a
Repetition rate (Hz)	20	10
Speed (µm/s)	50	50
Ablation duration (s)	1	n/a
Inter-site dwell time (s)	5	n/a
Isotopes measured (m/z)	^{43}Ca, ^{208}Pb, ^{64}Zn, ^{7}Li, ^{88}Sr	^{43}Ca, ^{208}Pb, ^{64}Zn, ^{7}Li, ^{88}Sr

Spot ablation experiments consisted of spots arranged in grids covering whole teeth. One contemporary tooth from Kalama, Egypt (60015: female deciduous incisor) and one ancient Chilean tooth (PLM4 T14: 2 year child incisor; sex unknown) were sampled in this way; both teeth were deciduous incisors. Table 1 shows the linear and grid laser ablation parameters.

2.3. Data analysis

Raw elemental intensity LA-ICP-MS data were imported into Excel (Microsoft Office X 2004 for Mac, Microsoft, Redmond, WA). Lead concentrations were calculated from the calibration function generated from the NYS certified reference standards; Zn, and Li concentrations were calculated using a single-point NYS standard. Peak-concentration ablation sites were labeled and analyte concentrations were aggregated using R software (R Foundation for Statistical Computing, Vienna, Austria). These data were formatted into matrices and graphed as surface plots. Tooth images and corresponding elemental concentration data plots were imported into Flash (Adobe Flash Professional CS5, San Jose, CA), where surface elemental distributions, and their axes were overlaid on the ablated zones and scaled to fit.

3. Results and discussion

Laser ablation parameters were optimized to maximize signal-to-noise ratios, spatial resolution, and sample throughput (Table 1). Signal

Table 2
Elemental concentration in different dental tissues determined by LA-ICP-MS.

Sample/tissue region	n	Pb (µg/g)	Zn (µg/g)	Sr (µg/g)	Li (µg/g)	^{88}Sr/^{43}Ca
Ancient mummies Arica, Chile						
Enamel	15	1.00	40	35	1.41	
Dentine	16	1.13	26	42	1.64	
Pulp	9	2.25	107	45	1.77	
Total	16	1.33 ± 0.84	50 ± 57	40 ± 25	1.59 ± 2.06	0.86 ± 0.40
Contemporary Kalama, Egypt						
Enamel	16	3.38	119	87	0.12	
Dentine	20	5.70	92	87	0.10	
Pulp	13	43.35	109	65	0.13	
Total	20	14.93 ± 32.28	106 ± 115	81 ± 40	0.12 ± 0.07	1.02 ± 0.18

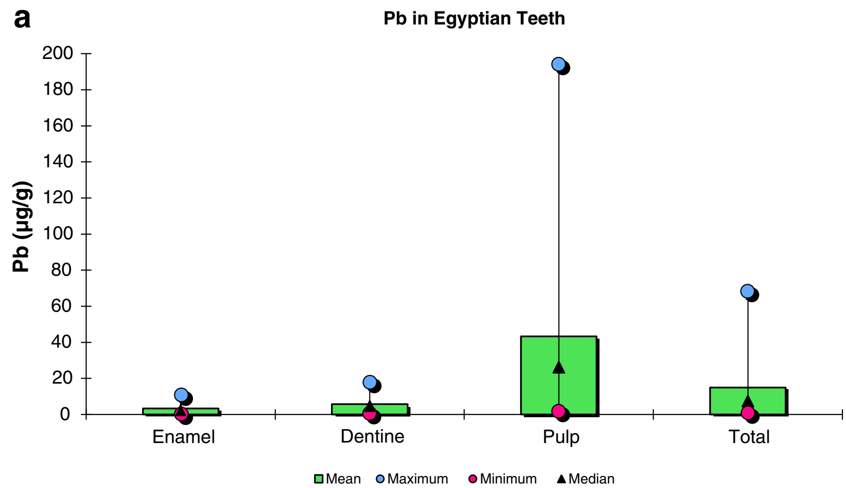

Fig. 1. Pb concentration distribution (µg/g) determined by LA-ICP-MS linear ablation scans across teeth. (a) Pb distribution in enamel, dentine, and pulp and total lead concentration in Kalama, Egypt teeth. (b) Pb distribution in enamel, dentine, and pulp and total lead concentration in Chilean mummy child teeth.

intensities were normalized by ^{43}Ca ($M^{+}/^{43}$Ca) to compensate for ablation variations. Pb concentrations in teeth tissue were quantified using NYS caprine bone standards and routine calibration functions had linear correlations (r^2) > 0.9964. Zn, Sr, and Li levels were semi-quantitatively determined by using a single standard [mean of all 4 NYS-RM informational values for Li (0.70 µg/g), Sr (160 µg/g) and Zn (82 µg/g) was

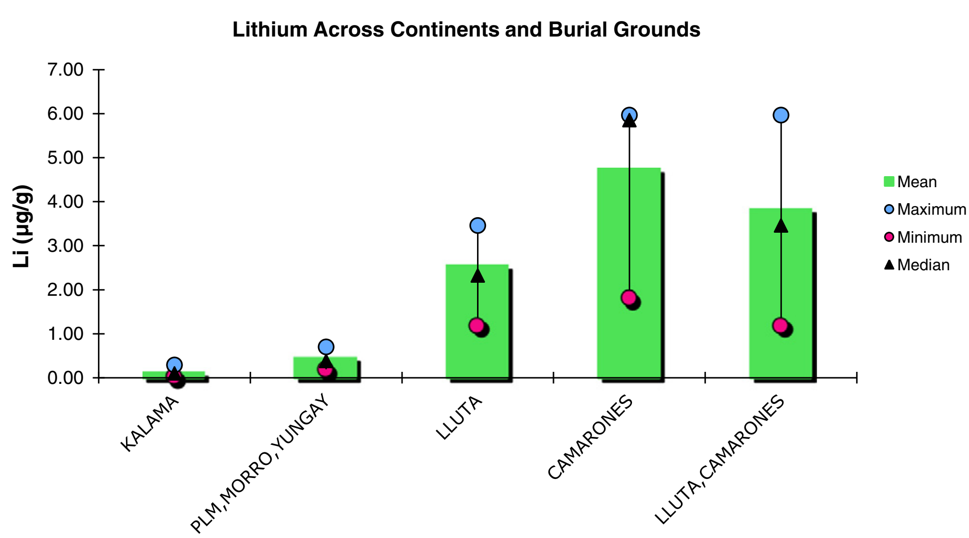

Fig. 2. Li concentration distribution (µg/g) determined by LA-ICP-MS linear ablation scans across teeth. Li concentration (µg/g) in teeth of contemporary deciduous teeth from Kalama, Egypt and ancient Chilean mummy teeth from several burial sites in Chile.

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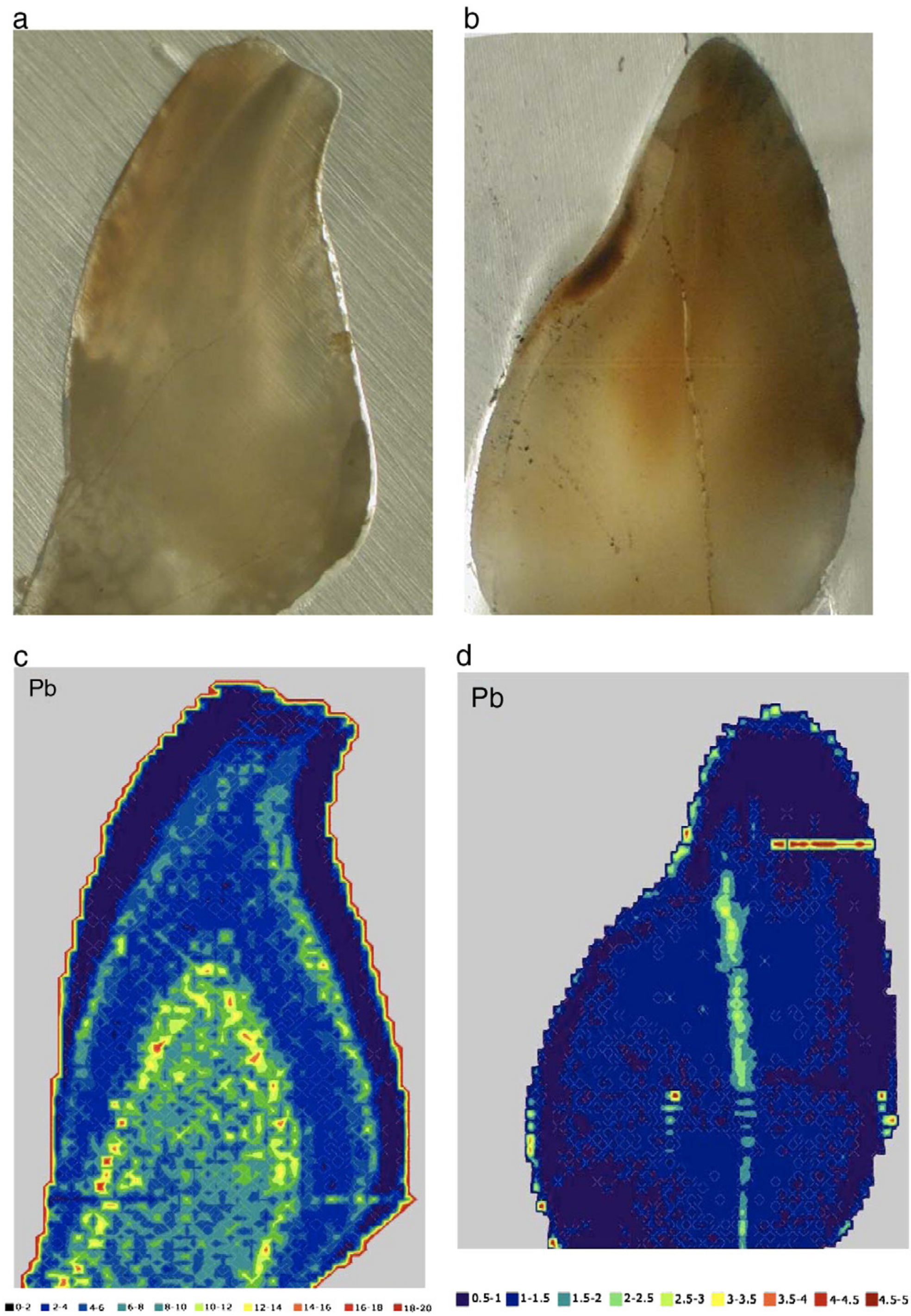

Fig. 3. Microphotographs of deciduous teeth: (a) Kalama, Egypt (60015) and (b) Chilean mummy deciduous incisor (PLM4 T14); magnification (× 12). Spatial Pb (μg/g) distribution: (c) a contemporary deciduous whole incisor from Kalama, Egypt (60015) [horizontal line on the bottom half of the image is an artifact due to a previous liner ablation line] and (d) an ancient Chilean mummy deciduous incisor (PLM4 T14). The legend for Pb concentrations of contours is shown below the elemental distribution bioimage.

used]. The detection limits for ^{208}Pb, ^{64}Zn, ^{88}Sr and ^{7}Li were 0.04, 0.0038, 0.0002 and 0.0002 μg/g ($n = 30$; $3 * \sigma_b$ where σ_b = standard deviation of the argon blank), respectively. Concentrations of these elements found in the sample teeth were consistently above detection limits.

3.1. Lead, zinc, and lithium concentrations, and Sr/Ca ratios

Table 2 shows the elemental concentrations of Pb, Zn, Sr and Li found in different dental tissues. In both cohorts of the samples, lead, and zinc distributions followed the same pattern: enamel < dentine ≪ pulp (see Fig. 1a and b). Elemental distribution generally follows this pattern with the exception of surface enamel, where a peak was often evident; this is from ion exchange between the calcium in hydroxyapatite and divalent cations, a phenomenon that does not generally penetrate further than 100–150 μm [23,24]. Enamel may be beneficial in assessing acute or chronic lead exposure; the core enamel Pb concentration in deciduous teeth formed in the mother's body is likely a reflection of prenatal Pb exposure whereas the dentine tissue of deciduous teeth offers an indication of early childhood lead exposure [25].

The distribution of lead in dental tissues has been observed before [12,17]. As expected, Pb concentrations were greater in the teeth from

Kalama, Egypt than in the teeth from Arica, Chile; the average concentrations differed by an order of magnitude ($[Pb]_{ancient}/[Pb]_{contemporary} = 0.0891$) and suggest anthropogenic lead contributions in the Egyptian contemporary teeth samples. The very low Pb also points to only natural Pb contribution from geological sources in pre-Columbian Chilean mummy teeth; there was no lead contribution from anthropogenic sources.

Although concentrations varied highly between individuals, the average zinc level in ancient Andean teeth was low (50 ± 57 μg/g; n = 16) and in modern-day Egyptian teeth (106 ± 115 μg/g; n = 20) was marginal, indicative of possible nutritional zinc deficiencies. Previously it has been reported the Zn concentrations < 90 μg/g in children's teeth could be due to borderline zinc bioavailability [26].

The observed Sr/Ca ratios between the two groups of teeth shown in Table 2 are not statistically significant ($p > 0.05$). Strontium decreases as trophic level increases due to biopurification or strontium discrimination [27,28], so it could be expected that Sr/Ca ratio would be lower in the Chilean teeth than in the Egyptian Kalama teeth due to dietary differences. But this was not confirmed in this study, even though the ancient Chilean hunter-gathered populations relied primarily on a marine based seafood diet [21,22] whereas the diet of the Kalama children was heavily plant-based and relied on bread and legumes [19].

Elevated lithium concentrations were found in all Chilean mummy teeth. Average lithium concentrations in the Chilean teeth (1.59 ± 2.06; μg/g; n = 16) were significantly higher ($p < 0.05$) than the lithium concentrations found in the Egyptian teeth (0.12 ± 0.07; μg/g; n = 20) (see Fig. 2). These elevated Andean lithium levels are consistent with lithogenic contributions; Li is relatively abundant around the Andes, including Bolivia and the Atacama region of Chile. Teeth from two of the Chilean burial sites, Lluta and Camarones (n = 5, combined) had significantly higher concentrations than the other burial sites (i.e. Playa Miller (PLM), Morro, and Yungay; located near the bluff of present day Arica, Chile, n = 11 combined). Through bioaccumulation in calcified tissues, environmental lithium (likely via drinking water from surface water sources) contributes to lithium present in teeth and bone. A study by Zaldívar [29] showed that Li concentrations in the contemporary Lluta River and the Camarones River were unusually high (1509 μg/L, 5171 μg/L, respectively) and elevated lithium levels were correlated with higher blood plasma lithium. The blood plasma therapeutic Li levels reported by the National Institutes of Health are 0.8 to 1.2 mEq/L and the "abnormal" values are set at anything in excess of 2.0 mEq/L [30]. Since trace metals generally bioaccumulate in calcified tissue at much lower levels than are measured in blood plasma values, it is likely that the ancient Arica people, particularly those who lived in the Lluta and the Camarones valleys, were exposed to potentially harmful lithium levels. Artificial mummification in this region might have arisen as reaction to widespread arseniasis due to drinking arsenic-laden river water [20,31]; yet lithium exposure could have played an equally integral role in beginning this ritual.

3.2. Bioimaging of lead, zinc, and lithium in deciduous teeth from contemporary Egypt and the ancient Andes

Spot ablations were arranged in grids with 50 μm resolution in orthogonal directions of Kalama, Egypt (60015, 1983–1985 A.D.) tooth (Fig. 3a) and ancient Chilean mummy (PLM4 T14, 1000–1470 A.D.) tooth (see Fig. 3b). Both teeth were deciduous incisors. Elemental distribution trends are consistent between the two teeth for all three elements measured: lead, zinc, and lithium. Micro-scale dental bioimaging using LA-ICP-MS has been successful before constructing qualitative [12] or semi-quantitative micro-scale dental bioimages using single standards [18]; this study presents quantitative lead distribution images of two

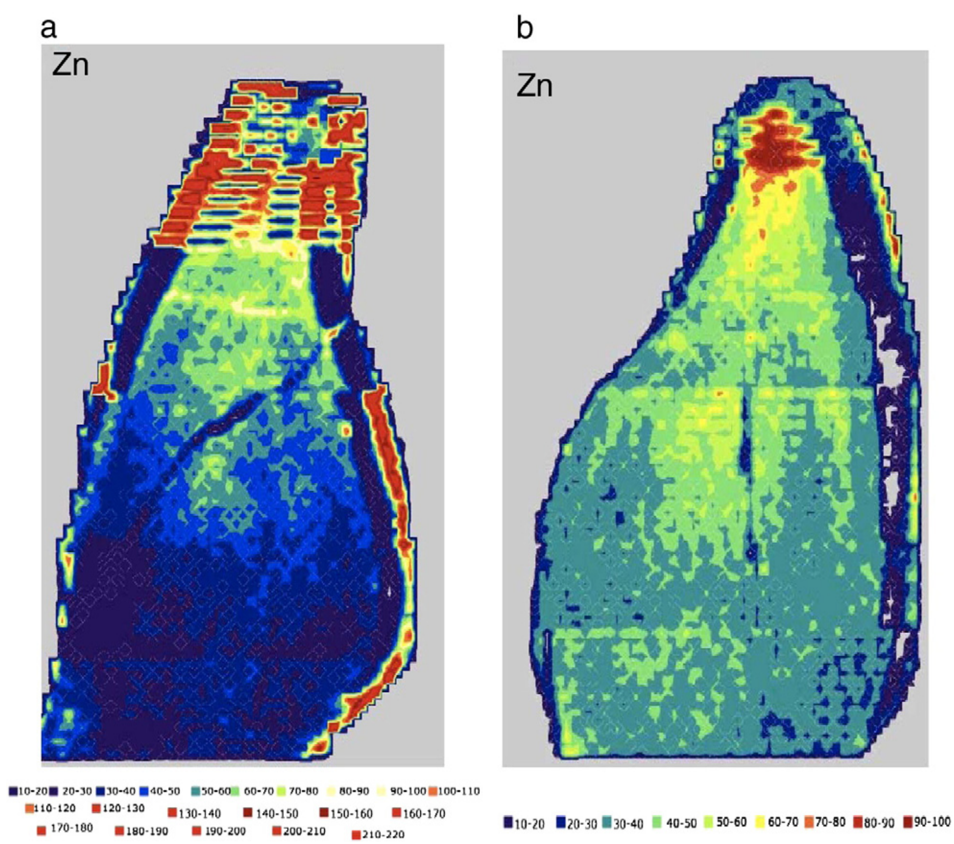

Fig. 4. Spatial Zn (μg/g) distribution: (a) a contemporary deciduous whole incisor from Kalama, Egypt (60015) and (b) an ancient Chilean mummy deciduous incisor (PLM4 T14). The legend for Zn concentrations of contours is shown below the elemental distribution bioimage.

deciduous teeth and semi-quantitative zinc and lithium elemental bioimages.

Fig. 3c shows the spatial distribution of lead in the contemporary Egyptian deciduous tooth (60015) from Kalama at 50 μm resolution. The lead concentration (μg/g) in this tooth shows elevated levels, indicative of exposure to anthropogenic lead. Lead distribution clearly followed the order previously observed with single linear scans across the tooth ($[Pb]_{enamel} < [Pb]_{dentine} \ll [Pb]_{pulp}$). Pb is lowest in the enamel except for a peak in the surface enamel, higher at the dentine enamel junction, greater in the dentine than in the enamel, and most concentrated in the pulp/secondary dentine. Elevated Pb zones are also distinguishable in surface enamel and at the dentine–enamel junction. The anomalous line in the bottom quarter of the Kalama tooth image (Fig. 3a) is an artifact left over from a previous linear ablation that preceded the spot ablations. It is likely that the tooth was not polished down past the previous ablation depth.

Lead concentrations measured in the ancient Chilean mummy tooth were much lower than lead measured in the contemporary tooth (Fig. 3d). Pb distribution does not vary greatly throughout this tooth and is relatively low; indeed, it does not exceed 1.5 μg/g in most of the tooth. It is likely that this sample reflects the "natural" background lead levels; the Late Intermediate Period Chilean mummies were part of a pre-industrial and pre-ceramic society [20]. In addition, there is no difference in Pb concentrations between early (i.e. Morro, Yungay), and late Chilean tribes (i.e. PLM4 T14) that have ceramic. Prehistoric lead exposure likely came from naturally occurring geological sources.

The distribution of zinc in both modern and ancient teeth and lithium in the ancient tooth (Fig. 4a and b) follows an interesting trend: these metals are concentrated in the crown of the tooth. In general, Zn is lowest in the enamel, higher at the dentine enamel junction, and greater in the dentine than in the enamel in both teeth. Zinc is also more concentrated at the tooth's crown, indicative of higher pre-natal zinc levels. It is possible that these levels reflect components of both maternal bone store levels and current maternal exposure levels [32].

Raw lithium data were gathered for the tooth from Kalama, Egypt; all data points were below detection limit (0.0002 μg/g), so this plot is not reported here. Lithium in the Andean tooth (see Fig. 5) from the laya Miller site (PLM4 T14; from the Late Intermediate Period (1000–1470 A.D.)) had a lower concentration than in those teeth from the Lluta and Camarones valleys. Still, this tooth had concentrations up to 2 μg/g in comparison to the lithium measured in the modern Egyptian teeth. As described previously the Li in the drinking water found in Lluta and Camarones rivers [29] in northern Chile is very high. Since trace elements in blood are at higher concentration than found in calcified dental tissue, it is likely that the individual was exposed to extreme high lithium concentrations from geological sources.

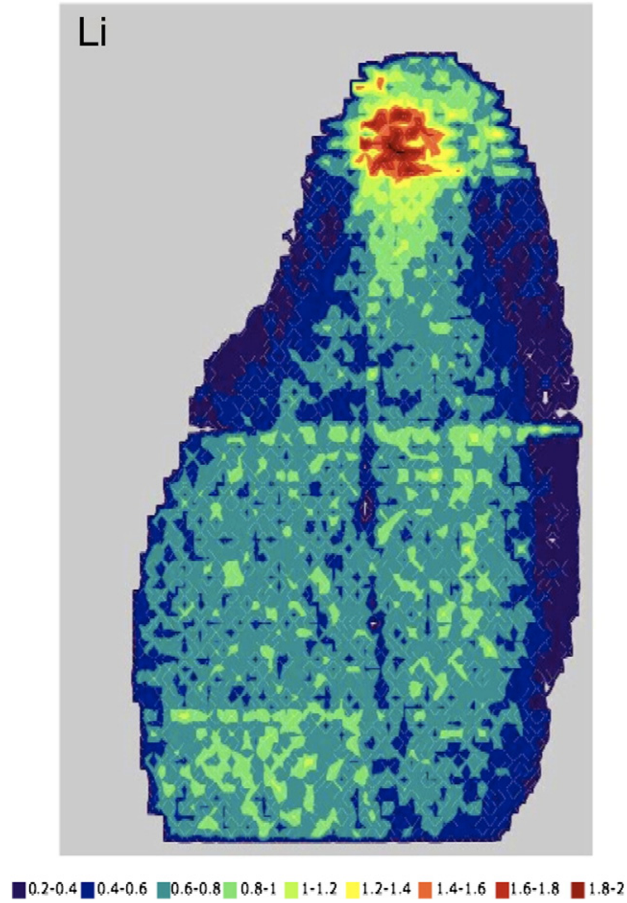

Fig. 5. Spatial Li (μg/g) distribution: in an ancient Chilean deciduous incisor (PLM4 T14). (Li levels were below the detection limit (0.2 ng/g) for the Kalama, Egypt tooth; the image is not shown here.) The legend for Li concentrations (μg/g) of contours is shown below the elemental distribution bioimage.

4. Conclusions

This study demonstrated the applicability of LA-ICP-MS to micro-scale imaging of trace metals in whole teeth. To our knowledge, this is the first study to accomplish fully quantitative lead bioimages of human dental tissue using biologically incorporated Pb standards. The images show heterogeneous distribution of trace elements across dental cross-sections. The pulp typically recorded the highest trace element concentrations whereas the dental calcified tissues recorded variations that may relate to the temporal dynamics of exposure.

Elevated lead levels were found in many of the teeth from Kalama, Egypt in comparison to ancient teeth from pre-Columbian Chilean mummies. The elevated lead levels in these contemporary teeth are likely due to anthropogenic lead sources, such as leaded gasoline. Zinc levels in both cohorts of deciduous teeth reflect their dietary contributions. Elevated lithium concentrations were present in Andean mummy teeth as compared to Egyptian teeth. Several mummy teeth from the Lluta and Camarones valleys showed elevated lithium levels consistent with a high lithium contribution from natural lithium sources in the surface waters and soils in the northern Atacama region of Chile. Comparison of linear and spatial (grid) ablation elemental images shows a heterogeneous distribution pattern of trace metal accumulation, with more accumulation in the pulp than in the other dental tissues.

The differences in elemental concentrations within and among teeth and groups may have significance for public health and suggest further study. It is important, for example, to further understand the sources of lead exposure in Kalama, Egypt. Further research would also be justified to better understand lead that is clearly accumulating before and after birth. Finally, it would be useful to know if lead in the calcified teeth is directly related to prenatal diets and breastfeeding or whether and to what degree it might reflect lead coming from bone decalcification and turn over during pregnancy and lactation.

Nonetheless, this study further demonstrates the use of multiple hard tissues to determine intake of pollutants and nutritionally important trace elements and furthermore, the use of these tissues to provide a retrospective, longitudinal picture of elemental incorporation into calcified tissues.

LA-ICP-MS is a versatile analytical approach for further studies in the bioimaging of archived elemental distribution in dental tissue. Environmental and nutritional stressors can be identified, and historical information can be accessed. With the availability of more solid elemental standards, other elemental distributions can be fully quantified using LA-ICP-MS.

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