

High-resolution trace element distributions and models of trace element diffusion in enamel of Late Neolithic/Early Chalcolithic human molars from the Rioja Alavesa region (north-central Spain) help to separate biogenic from diagenetic trends

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ABSTRACT

High resolution in situ trace element μ XRF maps and profiles were measured on the enamel exposed in cross sections through archaeological human permanent molars from seven Late Neolithic/Early Chalcolithic funerary caves and megalithic graves of north-central Iberia. Changes in concentrations of Fe, Zn and Sr in inward direction into the enamel shed light on diagenetic and endogenous trace element concentrations in archaeological tooth enamel. Most of these profiles resemble sigmoid-shaped leaching profiles, suggesting that a combination of diffusion and advection processes govern the uptake of trace elements into the enamel from pore fluids on the outside of the tooth and in the more porous dentine. The present study shows how diffusion-advection (DA) models can be fitted to these trace element profiles to explain changes in trace element concentrations that happen during diagenesis. DA models explain a major part of the variation observed in leaching profiles into the enamel and can be used to reconstruct endogenous trace element concentrations, leaching times and leaching depth as well as trace element concentrations in ambient pore water during diagenesis. Models of trace element leaching together with trace element mapping reveal that Fe, Zn and Sr concentrations consistently increase during diagenesis, regardless of the type of burial site (i.e. funerary caves vs. megalithic graves). Profiles of Pb concentrations show much smaller concentration gradients, causing DA model fitting to be less accurate. Modelled leaching depths of 300–400 μ m warrant a careful approach when sampling for endogenous archaeological tooth enamel for trace element and stable isotope analysis. Results also show that it is possible to reconstruct endogenous trace element concentrations from these samples, even without applying pretreatment procedures, because leaching of trace elements into the enamel often remains limited to the outer 300–400 μ m of the enamel on archaeological timescales. Modelled leaching times are about ten times lower than the age of the samples, suggesting that the rate of trace element leaching into tooth enamel slows down or even halts during the burial period.

1. Introduction

The study of tooth and bone bioapatite chemistry has yielded much insight into the life history and (palaeo)environment of humans and animals (Lee-Thorp and van der Merwe, 1991; Balasse et al., 2003; Zazzo et al., 2006; Eagle et al., 2010; Pellegrini et al., 2011; de Winter et al., 2016). The abundance of bioapatites in the fossil record and the resistance of some types (e.g. tooth enamel) to diagenetic alteration make them an important record of geochemical proxy data (Zazzo et al., 2004; Sponheimer and Lee-Thorp, 2006; Kocsis et al., 2010). This interest in fossil and archaeological bone and teeth, as well as other bioapatite fossils (e.g. conodonts), has sparked research into the chemical structure of bioapatites (Legros et al., 1986; LeGeros, 1990; Skinner, 2005; Wopenka and Pasteris, 2005; Yoder et al., 2012). The

chemical composition of bioapatite can be summarized as $(\text{Ca}, \text{X}, \text{[]})_{10}(\text{PO}_4, \text{HPO}_4, \text{CO}_3)_6(\text{OH}, \text{F}, \text{CO}_3, \text{X}, \text{[]})_2$, in which X represents places where trace elements can be substituted and [] vacancies in the crystal structure. The formula shows that there are three phases in the bioapatite crystal: cations (mostly Ca), the phosphate group (PO_4 , partly substituted by CO_3) and the channel-filling group (e.g. OH and F). A wide range of compounds substitute into the bioapatite structure. Some of the most common are Mg, Sr, Ba, Fe, Zn and rare earth elements (REE; cation group; Trueman et al., 2011; Doat et al., 2004), carbonates (phosphate group; Chenery et al., 2012), anions (F, Cl, S) and structural water (channel-filling group; Legros et al., 1986; Yoder et al., 2012). Organic matter and non-structurally bound water are also present between bioapatite crystallites of endogenous bioapatites.

Due to the complexity of the bioapatite structure, not all bioapatites

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are equally suitable for palaeoenvironmental and archaeological reconstructions. Differences in crystallinity, organic matter content and mineral density between bioapatite in bone, tooth dentine and tooth enamel govern the resistance of these materials to diagenetic alteration (LeGeros, 1990; Lee-Thorp and Sponheimer, 2003). Tooth enamel is generally more crystalline, contains less organic material, and has larger apatite crystals, making it more resistant to post mortem alteration than bone and dentine (LeGeros, 1990). For this reason, tooth enamel has been a preferred substrate for stable isotope studies investigating palaeoenvironment and palaeodiet (Cerling et al., 1997; Zazzo et al., 2000; Sponheimer et al., 2003; Balasse et al., 2012). Similarly, trace element concentrations in bioapatites have been applied as proxies for palaeoenvironment and trophic level (e.g. Toots and Voorhies, 1965; Sillen, 1982; Balter et al., 2002; Trueman and Tuross, 2002).

However, the interpretation of trace element concentrations in archaeological tooth enamel is not straightforward. Previous studies show that fresh (unaltered) tooth enamel shows lateral variability in certain trace element concentrations. For example, geochemical profiles measured in tooth enamel show that concentrations of Zn and Pb increase towards the outer edge of the enamel and are elevated in the outer 100–300 µm. At the same time, Sr and Ba concentrations gradually increase towards the enamel-dentine junction (e.g. Humphrey et al., 2008; Müller et al., 2019). Furthermore, the preservation of original trace element concentrations in bioapatites over archaeological or palaeontological timescales is subject to debate (Sillen, 1982; Sponheimer et al., 2005; Sponheimer and Lee-Thorp, 2006; Kohn, 2008). While pretreatment techniques have been proposed to leach out elements that were incorporated post mortem, several studies show that the effects of diagenesis cannot be fully removed, even in enamel (Sillen, 1982; Hoppe et al., 2003; Lee-Thorp and Sponheimer, 2003). Still, careful application of pretreatment techniques can prevent sampling of diagenetically altered bioapatite, especially if diagenesis is still in an early stage (Budd et al., 2000; Sponheimer and Lee-Thorp, 2006; Snoeck and Pellegrini, 2015; Snoeck et al., 2015; McMillan et al., 2019).

In addition to applying pretreatments, attempts have been made to quantify the change in trace element concentrations that occurs during the process of diagenesis in bioapatites. Leaching experiments and diffusion and adsorption models can be used to understand trace element leaching profiles (Millard and Hedges, 1996; Kohn, 2008; Kohn and Moses, 2013). Leaching of trace elements into bone and dentine causes concentrations to approach homogeneous distribution after > 100 kyr (Williams and Marlow, 1987). On shorter, archaeological timescales, sigmoid (error function-shaped) concentration gradients are formed (Badone and Farquhar, 1982; Millard and Hedges, 1996). Since uptake into enamel is up to 50 times slower, concentration gradients may still be present in tooth enamel after millions of years of diffusive alteration (Kohn and Moses, 2013).

Migration of elements into or out of bioapatites can be understood in terms of two processes: intra-crystalline diffusion into the bioapatite and adsorption to the phosphate crystals (Millard and Hedges, 1996). Intra-crystalline diffusion coefficients (D) for uranyl in bone were determined at $6.8 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ by Haase and Kiem (1984), but can be modified for other particles and conditions by applying the Stokes-Einstein equation (Sutherland, 1905; Einstein, 1905; Von Smoluchowski, 1906; see also discussion in Millard and Hedges, 1996 and Kohn and Moses, 2013). Divalent ions (e.g. Zn^{2+} , Fe^{2+} , Sr^{2+} and Pb^{2+}) have higher D's (faster diffusion) than cations with a different charge, because their substitution does not require charge-coupled species (e.g. Na^+) to fit into the bioapatite mineral structure (Kohn and Moses, 2013). Similarly, larger ions with ionic radii farther removed from that of Ca^{2+} or P^{5+} (e.g. Sr^{2+} and Pb^{2+}) have lower D's (slower diffusion) than smaller ions (e.g. Fe^{2+} and Zn^{2+} ; Shannon, 1976; Kohn and Moses, 2013). Porosity ($p \approx 0.02$) and pore size distribution is different in enamel compared to bone, causing diffusion coefficients to be much lower (Van Dijk et al., 1983; Kohn and Moses, 2013). As a

consequence, migration of mono- and divalent ions in the inter-crystalline pore fluid in modern enamel typically happens with diffusion coefficients of $\sim 10^{-8} \text{ cm}^2 \text{ s}^{-1}$ (Van Dijk et al., 1983; Kohn, 2008). The crystal structure of enamel with nm-scale crystals in µm-scale bundles (with slow internal diffusion coefficients; Chermiak, 2000) separated by a small fraction ($\sim 2\%$) of larger (µm scale) pores and organic complexes (faster diffusion, especially near crystal surfaces; Stipp et al., 1992) may cause diffusion coefficients to vary internally (Kohn, 2008). Due to this possible internal variation of diffusion coefficients, it has been suggested that either a double-medium diffusion (DMD) or more simplified diffusion-advection (DA) model may approximate leaching of trace elements into bioapatite (Kohn, 2008). Partition coefficients (R) for the adsorption of elements from pore fluid onto apatite are listed in Rinklebe et al. (2016; see also Wright et al., 2004). Partition coefficients are generally larger than 10^5 and are stable over a wide range of pH and redox potential (Rinklebe et al., 2016).

A combination of diffusion and adsorption (DA) of trace elements in bioapatite can be modelled using Fick's second law modified by Crank (1975). This approach was applied by Millard and Hedges (1996) to model the uptake of uranyl in bone. Slow diffusion rates in enamel cause enamel thickness to be irrelevant, meaning that modelling can be simplified by assuming infinite enamel thickness. The solution to this modified version of Fick's second law (the diffusion-advection equation) for enamel is (Crank, 1975):

$$[M]_l(x) = [M]_0 + ([M]_1 - [M]_0) * \operatorname{erfc}\left(\frac{(x - x_0)}{2} * \sqrt{\frac{R + 1}{D * t}}\right).$$

Here, $[M]_l(x)$ is the concentration of the leaching element at time t and at distance x from the enamel surface (x_0 is the depth of the leaching front), $[M]_0$ is the original concentration in the enamel (assumed uniform) and $[M]_1$ is the concentration of bioapatite in equilibrium with ambient pore water (see Fig. 1). Parameters p , R and D respectively represent the enamel's porosity (0.02), volumetric equilibrium constant (related to the partition coefficient K_d , see Millard and Hedges, 1996) and diffusion coefficient ($7.7 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$, or $2.4 \times 10^{-6} \text{ µm}^2 \text{ yr}^{-1}$; Millard and Hedges, 1996). The term “ erfc ” represents the complementary Gauss error function that describes the sigmoid shape of modelled diffusion fronts. It is defined as (Glaisher, 1871):

$$\operatorname{erfc}(x) = 1 - \operatorname{erf}(x) = \frac{2}{\sqrt{\pi}} \int_x^\infty e^{-t^2} dt.$$

Note that ambient pore water concentrations ($[M]_w$) can be calculated from diagenetic bioapatite concentrations ($[M]_1$), which are in equilibrium with pore waters: $[M]_w = \frac{[M]_1}{p * R}$ (Millard and Hedges, 1996). The shape of the DA model has been shown to mimic leaching profiles of trace elements into enamel at the enamel-dentine junction (Kohn, 2008). Quantifying diffusion profiles in bioapatites yields information about diagenesis and the fossilization process on archaeological timescales (Millard and Hedges, 1996; Kohn, 2008). However, modelled durations of the formation of the trace element diffusion front may not be reliable predictors of the timing of fossilization (Kohn and Moses, 2013). Nevertheless, applying the DA model on trace element profiles in archaeological tooth enamel may yield important insights about the spatial distribution of diagenetic elements in enamel and whether original trace element concentrations can be reconstructed from these samples.

In this study, micro-X-Ray Fluorescence (µXRF) trace element mapping and profiling is applied on an assemblage of Late Neolithic/Early Chalcolithic (LN/EC) human permanent molars (M1 and M2) from six burial sites in the Rioja Alavesa region of north-central Iberia. The aim is to study the composition and distribution of trace elements in the teeth and to demonstrate the use of µXRF mapping and profiling in the study of trace element concentrations in tooth enamel. Furthermore, a method is presented to fit solutions of the DA model to

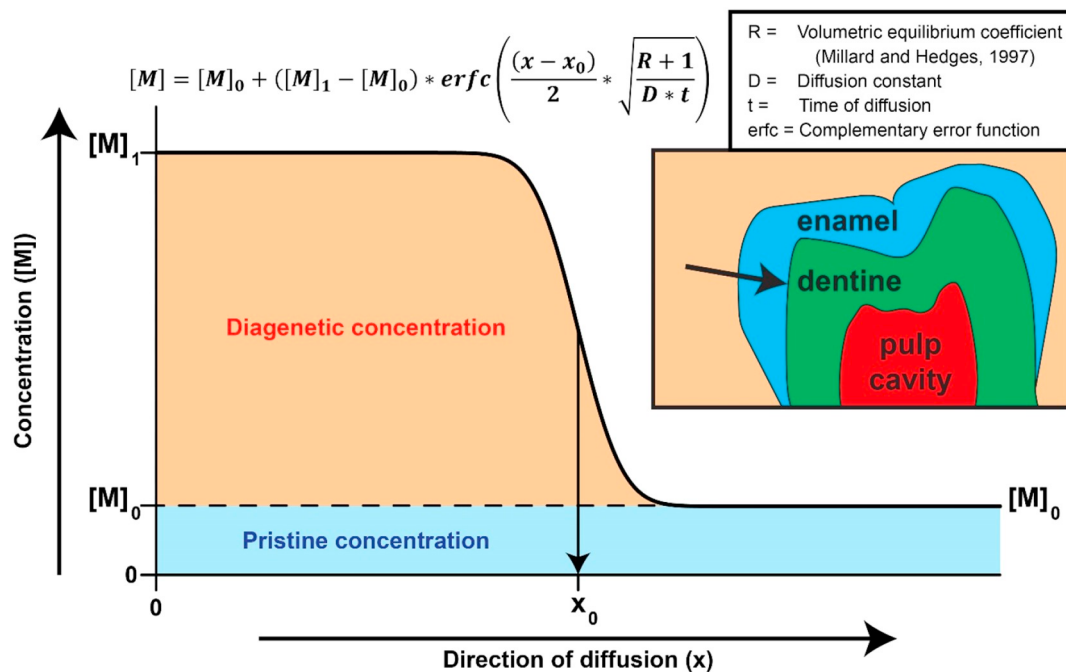


Fig. 1. Illustration of the typical shape of a leaching front going into the enamel. Concentrations of endogenous ($[M]_0$) and diagenetically altered ($[M]_1$) are indicated on the vertical axis. The depth of the leaching front (x_0) is defined as the location of the inflection point of the sigmoid-curve that describes the shape of the leaching front. The insert shows an example of the direction of diffusion relative to a cross section through the tooth.

trace element profiles and extract information about original and diagenetic trace element concentrations in archaeological tooth enamel.

2. Materials and methods

2.1. Site description

For this study, 63 human teeth (31 first and 32 second permanent molars) from six LN/EC burial sites in the Rioja Alavesa region of north-central Iberia were analyzed. Teeth were obtained with the help of several local institutes, and details of the excavation and availability of the specimens are found in [Fernández-Crespo and Schulting \(2017\)](#). Three megalithic grave sites, Chabola de la Hechicera (CH), Alto de la Huesera (LHUE) and Longar (LON) are located in the Ebro river plain (lowland sites). The substrate of these graves consists predominantly of Tertiary sandstone deposits, occasionally overlain by thin Quaternary deposits ([Arnedo and Urbina, 2000](#)). The remaining three sites, Los Husos I (LHI), Peña Larga (CPL) and Las Yurdinas II (LYII) are funerary cave sites located in the foothills of Sierra de Cantabria mountain range to the north of the river valley (mountain sites). The funerary caves are located in the Cretaceous limestones of Sierra de Cantabria. The contemporary use of these sites was dated between 3500 and 2900 cal. BC ([Fernández-Crespo and Schulting, 2017](#)). Skeletal remains were well-preserved in all sites, though most remains were found disarticulated ([Fernández-Crespo and Schulting, 2017](#)). An overview of the sites and the material that is used in this study is given in [Table 1](#). All molars were recorded using high resolution photogrammetry, shot-blasted with aluminum oxide pellets to remove surface debris and then embedded in Herculite II (a high-strength gypsum molding material) and sectioned longitudinally (in coronal-apical and distal-mesial direction) using a Buehler Isomet low-speed diamond saw with a micrometer gauge, an abrasive wafering blade and a cooling water bath ([Fernández-Crespo et al., 2018](#)). One complete tooth half was used for analyzing high resolution *in situ* trace element μ XRF maps and profiles, leaving the other for further micro-sequential stable isotope analysis.

2.2. XRF mapping

Tooth halves selected for analysis were mounted in hydrophobic sand to stabilize them and to level their polished surfaces for XRF analysis. Batches of 10–20 teeth and one pressed pellet of BAS-CCB01 bioapatite trace element standard reference material (Bureau of Analyzed Samples Ltd., UK) were loaded into a Bruker M4 Tornado μ XRF scanner (Bruker nano GmbH, Germany). The Bruker M4 is equipped with a 30 W Rh metal-ceramic X-ray tube operated at maximum energy settings (50 kV, 600 μ A) and two 10 mm² silicon drift detectors. X-rays are focused on a circular spot with a diameter of 25 μ m (calibrated for Mo- α radiation) using polycapillary focusing optics. Each daily batch subject to XRF mapping consisted of teeth from different sites to make sure that differences in measurement reproducibility between batches did not influence the comparison of XRF maps between sites. High-resolution XRF elemental maps were created of the entire cross section surface of teeth and BAS-CCB01 pellet using the M4 Tornado's mapping mode with an integration time of 1 ms per pixel and a pixel spacing of 25 μ m (25 mm/s scanning velocity). The integration time of 1 ms per pixel allowed semi-quantitative trace element mapping (see [de Winter and Claey, 2016](#)). Heatmaps of relative concentrations of Al, Fe, S, Sr and Zn in cross sections through the teeth were produced from the XRF map data to visualize the distribution of these elements throughout the teeth. An overview of XRF mapping results is given in [Fig. 2](#).

2.3. XRF profiles

XRF profiles were carried out on the enamel exposed in polished cross sections through the molars with a sample spacing of 100 μ m, a spot size of 25 μ m and an integration time of 60 s per measurement. The longer integration time used for profiles allowed the Time of Stable Reproducibility and Time of Stable Accuracy to be reached, which makes it possible to quantify trace element concentrations ([de Winter et al., 2017](#)). Trace element concentrations measured in individual point measurements in these profiles were calibrated using a set of 7 matrix-matched reference materials: NIST SRM1486, NIST SRM1400,

Table 1
Overview of the number of first permanent molars (M1) and second permanent molars (M2) from each of the sites used in this study. Note that megalithic graves were located in the Ebro river valley (lowland) while funerary caves were located in the foothills of Sierra de Cantabria mountain range to the north of the river valley (highland). Coordinates of site locations are given with reference to the European Terrestrial Reference System 1989 (ETRS89).

Locality	Type	Coordinates				# M1	# M2	Total
			X	Y	Z			
Chabola de la Hechicera (CH)	Megalithic grave	ETRS89 30N	536647	4712892	595	2	2	4
Alto de la Huesera (LHUE)	Megalithic grave	ETRS89 30N	535619	4712097	614	7	7	14
Longar (LON)	Megalithic grave	ETRS89 30N	549777	4714071	710	7	7	14
Los Husos I (LHI)	Funerary cave	ETRS89 30N	536786	4716755	840	3	3	6
Peña Larga (CPL)	Funerary cave	ETRS89 30N	539805	4717990	900	2	2	4
Las Yurdinas II (LYII)	Funerary cave	ETRS89 30N	524512	4719608	907	10	11	21
						31	32	63

NIST120c (National Institute of Standards and Technology, Gaithersburg, MD, USA), BAS-CCB01, MAPS-4 (U.S. Geological Survey, Denver, CO, USA), ENF and CBA (in-house, see [de Winter et al., 2016](#)). Linear calibration curves constructed through measurement results in these standards had R^2 coefficients better than 0.99 for all elements used in this study (see SI_cal_BioAp and SI_BioAp_cal_summary). In order to sample trace element variations in different directions through the enamel, three XRF profiles (AB, CD and DA) were measured per tooth, oriented in so-called “enamel triangles” (as suggested by [Bondioli et al., 2009](#); see [Fig. 3](#)). Profile AB was measured from the cervical margin (A) upwards along the enamel-dentine junction to the coronal surface of the enamel (B). Profile CD was measured from the enamel-dentine junction (C) to the distal or mesial edge of the tooth (D) at the location where enamel thickness in distal-mesial direction was largest. The final DA

profile was measured from the distal or mesial edge of the tooth (D) along the outside of the enamel to the cervical margin (A). These profiles allowed the study of changing trace element concentrations into the enamel in 6 directions (A → B, B → A, C → D, D → C, D → A and A → D). Enamel triangles were measured either on the distal or mesial side of the tooth in function of the amount of exposed enamel in cross section. A total of 9858 individual XRF point measurements contained in 366 line scans were carried out for this study. An example of the results of XRF profiles in a first molar from the Alto de la Huesera site (LHUE) is shown in [Fig. 3](#).

Reproducibility of trace element concentrations in XRF profiles was tested by repeating three profiles on the surface of the BAS-CCB01 pellet at the beginning, in the middle and at the end of every 24 h run used to measure a batch of teeth. Results of these measurements on the

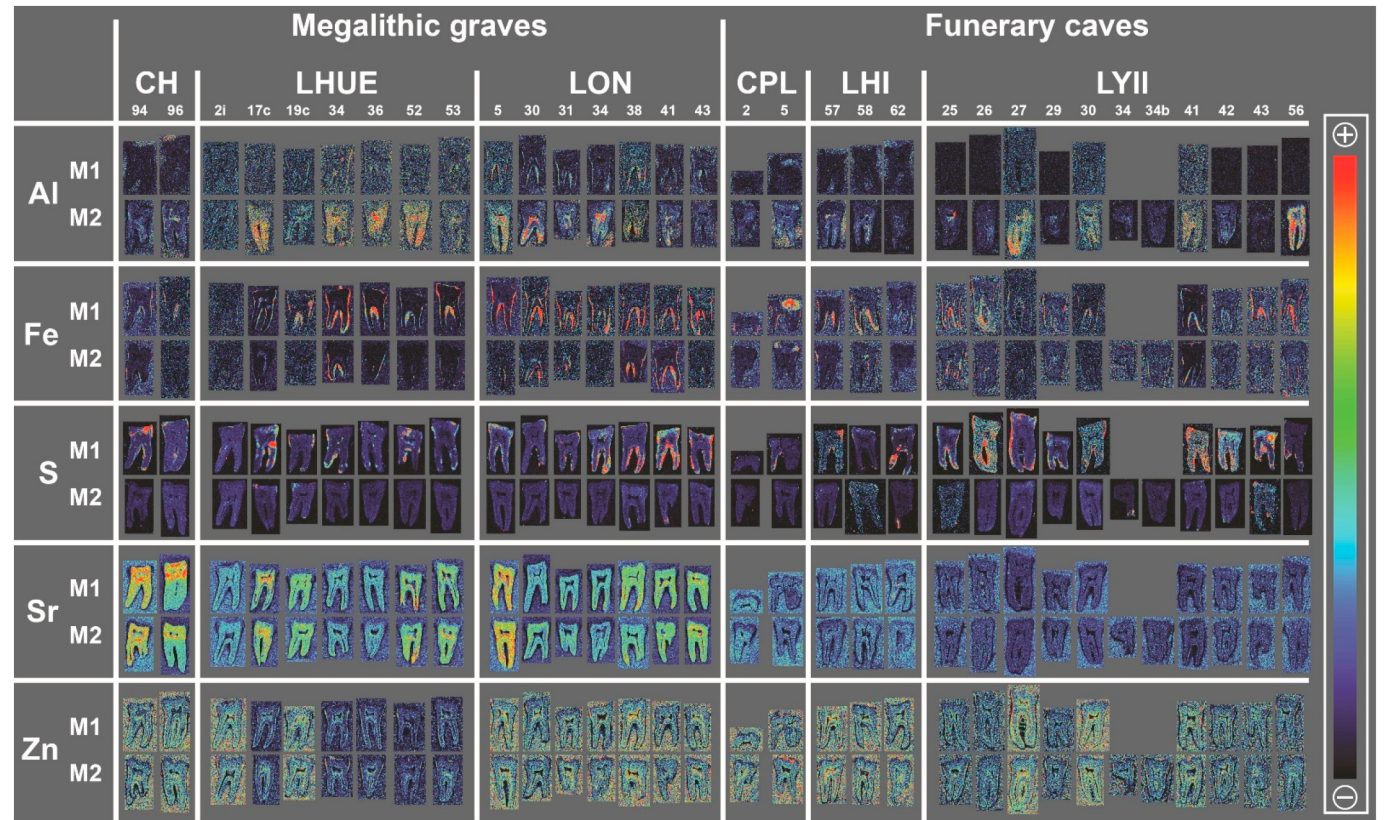


Fig. 2. Overview of μ XRF mapping results on all teeth used in this study. First and second permanent molars of the same individual are shown on top of each other. The elements Al, Fe, S, Sr and Zn were selected because they yielded visually interpretable results (concentrations of Pb were too low for mapping to be successful). Color scale of elemental maps is relative because dwell time during mapping is too short to allow pixel-by-pixel quantification of absolute concentrations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

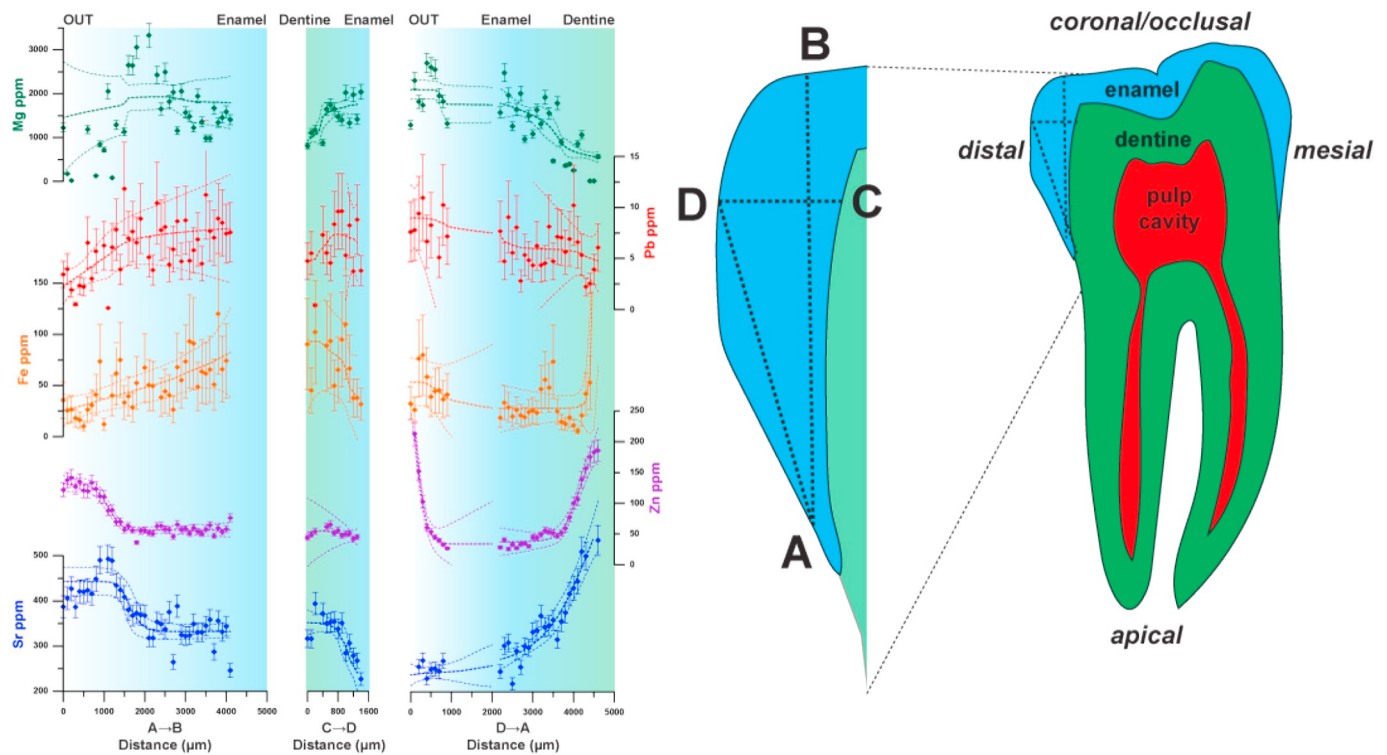


Fig. 3. Example of the result of XRF profiles measured in the first permanent molar of specimen 2i from the Alto de la Huesera site (LHUE). Plots on the left show results of XRF profiles along the three arrows indicated on the right hand illustration. All XRF profiles used in this study were measured according to the scheme shown on the right hand side of this figure, allowing 6 potential leaching profiles to be modelled.

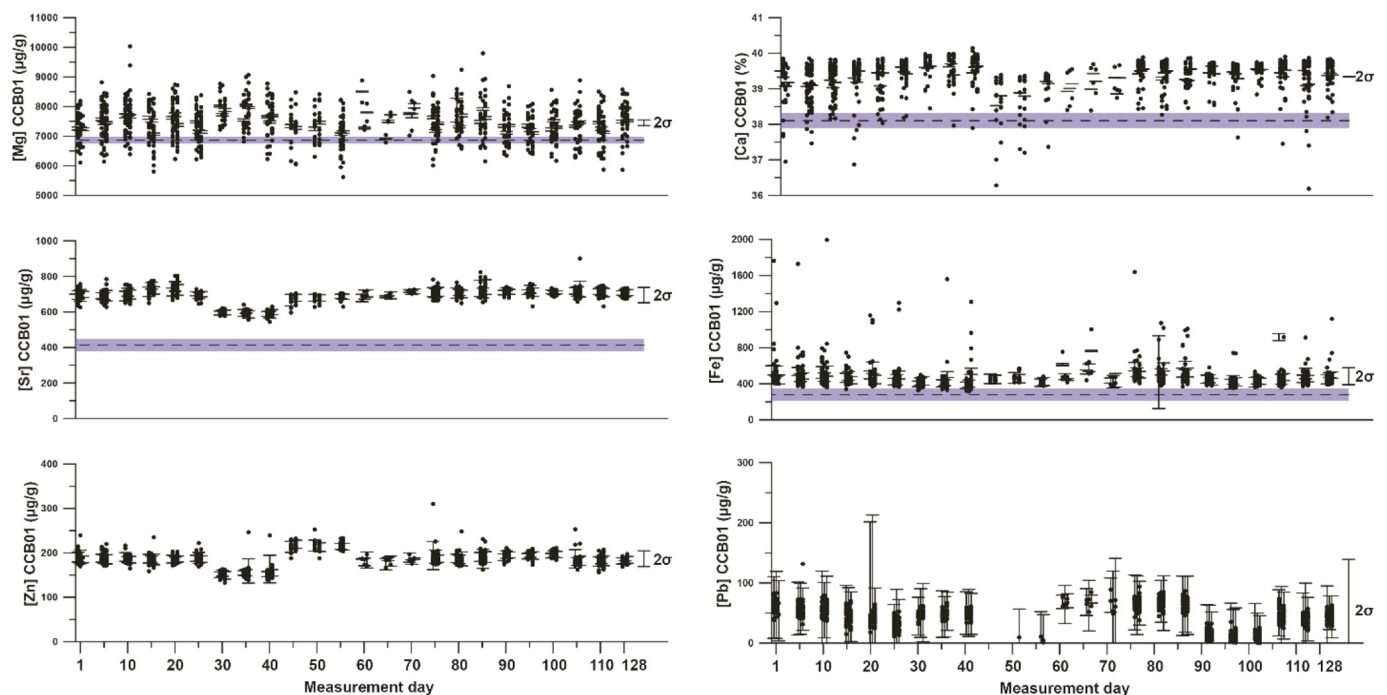


Fig. 4. Results of XRF point measurements on a pressed pellet of the BAS-CCB01 bioapatite standard showing long-term reproducibility of XRF profile measurements during the measurements carried out for this study. Individual clouds of points with error bars indicate the reproducibility (2σ) within one measurement run. Purple colored horizontal bars indicate the reference value of the standard. Error bars at the right end of the graphs indicate the long-term reproducibility (2σ) of all measurements on the Bas-CCB01 standard done for this study. Variability between runs can be corrected by routinely measuring this standard reference material. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

BAS-CCB01 standard are visualized in Fig. 4.

2.4. DA model

Diffusion-advection profiles were modelled by fitting Crank's solution to Fick's second law for infinite sample thickness (Crank, 1975; see above) to the obtained μ XRF trace element profiles. The sampling strategy of enamel triangles theoretically allows modelling of trace element diffusion from the outside of the tooth into the enamel in 5 directions (AB, BA, DC, AD and DA in Fig. 3, see methodology). In addition, diffusion from the dentine into the enamel could be modelled at location C in the direction of D (Fig. 3). Note that, while XRF profiles were placed carefully, it remains possible that leaching from the dentine side influenced the results in the AB-profile because the latter profile is located close to the enamel-dentine junction (see Fig. 3). Another caveat is that lateral variability in trace element concentrations that was present before burial is not taken into account in this model, as it cannot be reconstructed from archaeological teeth. The potential presence of in vivo variability in trace element concentrations should, however, not be ignored when discussing the model outcome. To model diffusion perpendicular to the surface of the enamel, the angle between the enamel surface and the XRF profile was used to calculate the shortest distance of each point on the XRF profiles to the enamel surface (x). The DA model was fit to the data by minimizing the residual sum of squares using the “optim” function in the open-source computational software R (R Core Team, 2013; SI_R_script). The “optim” function makes use of the Nelder and Mead algorithm (Nelder and Mead, 1965) and takes as input the above mentioned porosity (p), diffusion coefficient (D) and volumetric equilibrium constant (R), which are fixed for a certain element and material. The optimization algorithm is used to find values for original concentrations in the enamel ($[M]_0$), fully diagenetic concentrations in equilibrium with pore water ($[M]_1$), the location of the middle of the leaching front with respect to the enamel surface (x_0) and the modelled diffusion time it took for the leaching front to be established (t). Concentrations of the pore fluid ($[M]_w$) were calculated from diagenetic concentrations in the enamel and the volumetric equilibrium constant (R) and the porosity (p) following $[M]_w = \frac{[M]_1}{p \cdot R}$. The goodness of fit of the model with respect to the data was assessed by the standard error of the estimates on the parameters and by the coefficient of determination (R^2). To test the effect of measurement uncertainties on the model output, Monte Carlo simulations were carried out on the modelling routine of all trace element profiles measured in one of the teeth (first molar of LHUE 2i). Trace element concentrations in XRF profiles were randomly subsampled 1000 times, assuming normal distribution around the measured value and using the standard deviation of XRF measurements and 95% confidence levels were calculated from the result (see Fig. 3). All calculations used to fit DA models to XRF profiles and to carry out Monte Carlo simulations are reported in SI_R_script.

3. Results

3.1. Quality assessment of XRF measurements

Repeated measurements on the BAS-CCB01 reference material allowed the monitoring of long-term reproducibility of μ XRF trace element analysis on enamel over a period of 5 months (128 days). Standard deviations of reproducibility of a range of elements measured in XRF profiles are reported in Table 2. The results of these test show that standard deviations within a measurement run (24 h) are consistently lower than reproducibilities over the entire course of the measurement period (5 months). The scatter in XRF measurements is also illustrated for some elements in Figs. 4 and 5. While long term reproducibility is lower than the reproducibility within a measurement run, the results of this experiment show that correcting individual runs

relative to a certified reference material allows μ XRF measurements on bioapatite to stay reproducible on the long term. Such μ XRF profile measurements should therefore always include regular measurements of a reference material like BAS-CCB01 to correct for instrumental drift. Table 2 also shows that μ XRF measurements on enamel yield reproducible results for Ca, P, Si, Mg, Cl, Sr, Ba, Fe, Zn, Cr, Pb, Br and Cu present at concentrations as low as tens of parts per million. This means that μ XRF profiling, when the right measurement conditions are used, is a reliable method for the measurement and quantification of trace elements in tooth enamel.

3.2. XRF mapping results

Fig. 2 shows an overview of XRF mapping results of all teeth used in this study. Heatmaps of Al, Fe, S, Sr and Zn illustrate relative distributions of these elements through the teeth. While XRF mapping yields semi-quantitative results, comparing heatmaps from different sites can still reveal general patterns about the distribution of elements in the samples. The fact that tooth cross sections were measured in random order means that patterns in the overview cannot be ascribed to instrumental drift and show real variability between teeth, individuals and sites. A comparison of XRF maps shows that Al, Fe and S are typically present in elevated concentrations at the edges of teeth, while Sr and Zn are more abundant in the dentine and pulp cavity. Concentrations of Al, Fe and S are highly localized in the maps in contrast to Sr and Zn, which are more homogeneously distributed through the teeth. Interestingly, Al concentrations seem to be higher in second molars, while Fe and S concentrations are elevated in first molars. Teeth from megalithic graves in the Ebro river valley seem to contain much higher Sr concentrations than those found in funerary caves in the foothills of the mountain range. This difference associated with burial types is only observed in Sr maps. There is distinct variability between individuals from the same site, regardless of the type of burial site (megalithic grave or funerary cave), especially in Zn and Sr results. This variation between individuals is clearly observed when both the first and second molar from the same individual show the same trace element pattern (see Fig. 2).

3.3. XRF profile results

Examples of μ XRF trace element profiles on tooth cross sections are illustrated in Fig. 3. All data collected in μ XRF profiles through all teeth are reported in SI_XRF_data. While a range of trace elements could be quantified using μ XRF profiling (see Fig. 5), display of results in Fig. 3 is limited to 5 common trace elements: Mg, Pb, Fe, Zn and Sr. Profiles through the enamel of the first molar of LHUE 2i (individual 2i from the Alto de la Huesera site) show how the concentrations of these elements vary through the enamel. The “enamel triangles” measurement strategy allows the assessment of changes in trace element concentrations in inward profiles both from the outside of the tooth (e.g. profile B $\rightarrow$ A and D $\rightarrow$ C) and from the enamel-dentine junction (e.g. profile C $\rightarrow$ D). Note that profiles from A to B should record leaching from the outside of the tooth, but their proximity to the enamel-dentine junction may cause them to be influenced by leaching from the dentine. The results show clear sigmoid leaching profiles into the enamel for both Zn and Sr. Profiles for Pb and Fe are less clear due to the comparatively low concentrations (10–150 μ g/g) of these elements and the associated higher measurement uncertainties (see also Fig. 5). Profiles of Mg in this tooth are hard to interpret, even though Mg concentrations are quite high (1000–3000 μ g/g) and measurement uncertainty low. Sigmoid-shaped profiles reflect trace element leaching into the enamel both from the outside of the tooth and from the dentine. Contrary to Sr, Zn, Fe and Pb profiles, Mg profiles seem to lack the typical shape of leaching profiles with a sigmoid shape with increasing (or decreasing) concentrations towards the edge of the enamel. Clear leaching profiles, such as those observed in Zn and Sr measurements in Fig. 3 allow the

Table 2

Overview of internal (“within batch”) and external (“all data”) reproducibility standard deviations of the full range of elements quantified from repeated XRF measurements on the BAS-CCB01 standard reference material.

#	Concentration	SD all data	SD within batch	RSD all data	RSD within batch
O	39.42%	0.19%	0.16%	0.49%	0.40%
Ca	39.34%	0.51%	0.45%	1.30%	1.14%
P	18.24%	0.24%	0.21%	1.30%	1.14%
Na	1.09%	0.89%	0.84%	81.15%	76.73%
Si	8392 ppm	2984 ppm	2566 ppm	35.56%	30.58%
Mg	7450 ppm	616 ppm	550 ppm	8.26%	7.38%
Cl	6958 ppm	2023 ppm	605 ppm	29.08%	8.70%
Al	1008 ppm	535 ppm	372 ppm	53.10%	36.90%
S	695 ppm	578 ppm	376 ppm	83.14%	54.05%
Sr	695 ppm	43 ppm	20 ppm	6.21%	2.85%
Ba	505 ppm	94 ppm	49 ppm	18.58%	9.61%
Fe	484 ppm	238 ppm	129 ppm	49.21%	26.60%
K	421 ppm	447 ppm	297 ppm	106.27%	70.67%
Zn	187 ppm	18 ppm	10 ppm	9.46%	5.29%
Cr	48 ppm	18 ppm	8 ppm	38.62%	17.70%
Pb	45 ppm	19 ppm	9 ppm	41.99%	19.71%
Br	28 ppm	6 ppm	4 ppm	21.98%	14.51%
Cu	20 ppm	7 ppm	5 ppm	36.09%	26.64%
Mn	16 ppm	21 ppm	10 ppm	132.22%	62.42%
Ti	14 ppm	27 ppm	10 ppm	199.50%	72.59%
Ni	6 ppm	7 ppm	5 ppm	107.95%	89.13%

Red shading indicates reproducibility standard deviations exceeding 50% of the measured value.

separation of concentrations associated with the edge of the enamel from those measured at the center. The latter are thought to represent parts of the tooth less effected by diagenetic leaching. In the example in Fig. 3, high Sr (700–800 µg/g) and Zn (250–300 µg/g) concentrations at the edge of the enamel (e.g. left-hand side of the AB profile) are distinguished from lower concentrations of Sr (600–650 µg/g) and Zn (30–70 µg/g) in the middle of the enamel. The depth of the shift in

concentration observed in these profiles (~1500 µm from the enamel edge), as well as their typical sigmoid shape, makes a strong case for the hypothesis that these profiles indeed show diagenetic leaching rather than in vivo trace element variability. Previous work shows that these in vivo changes in, for example, Zn are only present in the outer 200 µm of enamel and lack the “plateau” of elevated trace element concentrations that are typical of the sigmoid-shaped leaching profiles observed

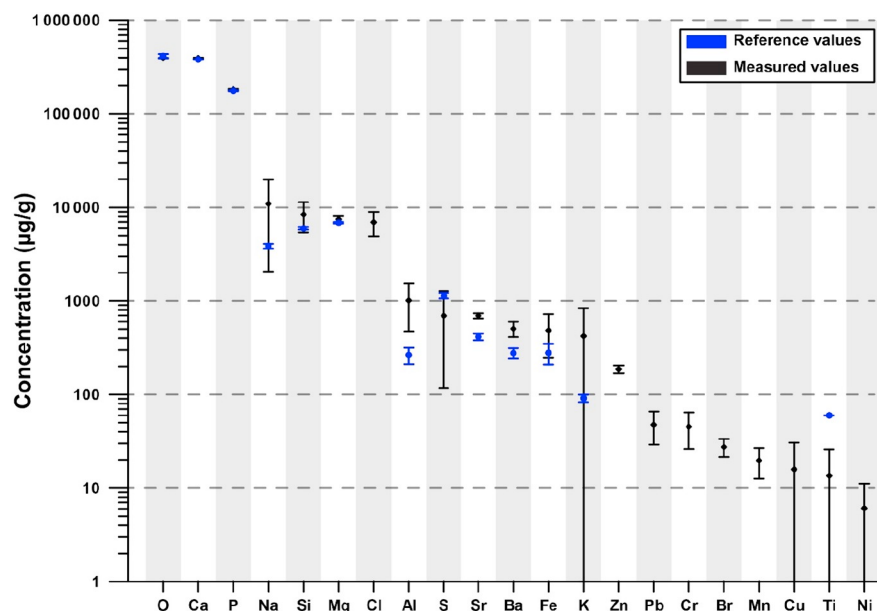


Fig. 5. Plot showing the uncalibrated measurement results and external reproducibility (2σ) of XRF measurements on the BAS-CCB01 standard reference material in black. Blue dots and error bars indicate the reference value and error on the standard. Note that the vertical scale is logarithmic. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

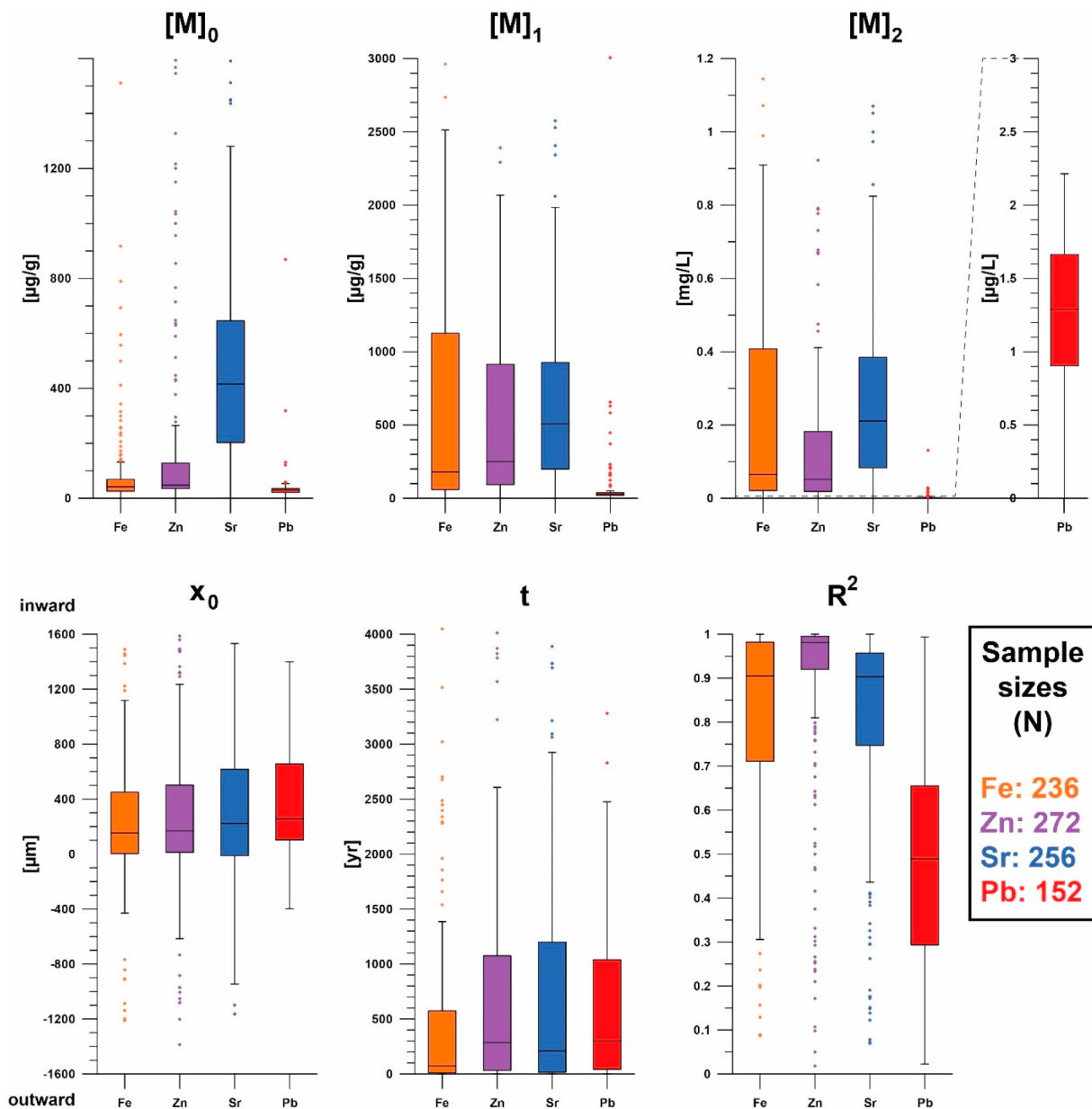


Fig. 6. Box-whisker plots giving an overview of the results of fitting the DA model to XRF profiles of Fe (orange), Zn (purple), Sr (blue) and Pb (red). Different subplots represent data of different parameters (e.g. $[M]_1$ and x_0) obtained by fitting the model to the XRF data as well as the R^2 value of the model fit. Colored bars of boxplots represent interquartile range (IQR, containing 50% of the data) while vertical lines in the bars show the median value. Outliers are plotted as colored dots above and below the box-whisker plots. Results were considered outliers if they plot > 1.5 times the IQR away from the top or bottom of the IQR (colored box). The number of successful DA model fits (N) is given in the bottom right part of the figure and differ per element due to variations in the degree of success of model fitting. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in Fig. 3 (Humphrey et al., 2008; Müller et al., 2019). However, incomplete or less well-developed leaching profiles, such as those in Fe and Pb profiles (Fig. 3) are less straightforward to interpret. The DA model fitted through trace element data in Fig. 3 reflects these observations and shows that the model fits profiles of Sr and Zn much better than those of other elements. Confidence levels on the model fits calculated by Monte Carlo simulation show that the DA model explains a large portion of the variation in these trace element profiles and that the remaining variability falls within the measurement error. Even in Fe and Pb profiles, fitting of the DA model yields information about differences in concentrations between areas of the enamel that are affected and those that are less affected by trace element leaching.

3.4. Model results

Attempts were made to fit the DA model to all trace element data collected in μ XRF profiles measured in “enamel triangles” through all 63 molars (SI_R_script). In cases where leaching profiles are not recognizable in the trace element profiles, the fitting algorithm of the DA model will not converge towards a stable result and no data is produced, meaning that not all quantified elements in all profiles on all teeth yielded estimates of fitted model parameters. A total of 4539 leaching fronts were successfully modelled this way, and statistics and resulting parameters of DA models fitting all trace element profiles are reported in SI_model_data. However, for sake of brevity, the discussion of DA models will be limited to those successfully applied to profiles of Fe, Pb, Sr and Zn. Fig. 6 gives an overview of the modelled leaching

Table 3

Overview of the results from DA model fitting on records of Fe, Zn, Sr and Pb from all teeth sorted by site. Median values of modelled endogenous concentrations (C_0), diagenetic concentrations (C_1), pore water concentrations (C_2), depth of leaching front (x_0), time required for leaching (t) and the goodness-of-fit of the model (R^2) are listed. Errors (1σ) given are those resulting from model fitting uncertainty.

element name	Type of site	Site	N	C_0 ppm	$\pm 1\sigma$ ppm	C_1 ppm	$\pm 1\sigma$ ppm	C_2 ppb	$\pm 1\sigma$ ppb	x_0 μ m	$\pm 1\sigma$ μ m	t yr	$\pm 1\sigma$ yr	$C_1 - C_0$ ppm	R^2
Fe	Caves/ Rock shelters	CPL	4	1006	± 149	1170	± 101	423	37	309	± 3	1062	± 56031	365	0.44
		LHI	20	42	± 3	252	± 83	92	35	22	± 177	158	± 556	227	0.94
		LYII	75	42	± 3	165	± 28	71	13	152	± 163	74	± 213	130	0.84
	Megalithic graves	CH	13	59	± 7	112	± 18	40	6	200	± 101	44	± 238	61	0.85
		LHUE	57	42	± 5	200	± 27	72	14	161	± 70	56	± 102	172	0.94
		LON	67	46	± 4	180	± 26	65	9	154	± 58	53	± 107	128	0.91
	TOTAL		236	44	± 4	185	± 40	71	14	154	± 91	72	± 176	129	0.90
	Zn	CPL	8	69	± 13	403	± 17	80	3	99	± 3	1059	± 47509	326	0.93
		LHI	21	50	± 3	492	± 16	98	3	189	± 40	944	± 521	328	0.98
		LYII	90	55	± 5	433	± 25	87	5	182	± 21	328	± 187	329	0.99
		CH	13	41	± 3	191	± 162	378	60	28	± 34	592	± 666	353	0.96
		LHUE	61	51	± 2	139	± 13	28	3	186	± 31	298	± 205	87	0.98
		LON	79	44	± 3	161	± 20	33	4	166	± 81	207	± 395	123	0.97
	TOTAL		272	48	± 4	260	± 21	62	4	169	± 35	356	± 246	200	0.98
Sr	Caves/ Rock shelters	LHI	20	481	± 12	548	± 12	228	5	409	± 126	228	± 488	90	0.85
		LYII	81	164	± 5	213	± 10	101	4	367	± 77	273	± 359	51	0.88
	Megalithic graves	CH	11	646	± 15	876	± 508	375	351	299	± 63	252	± 292	397	0.91
		LHUE	66	408	± 12	520	± 26	216	11	209	± 90	171	± 270	134	0.91
		LON	78	616	± 13	808	± 59	362	379	185	± 286	466	± 631	464	0.92
	TOTAL		256	422	± 11	526	± 34	224	14	223	± 126	269	± 365	138	0.91
Pb	Caves/ Rock shelters	LHI	17	33	± 2	36	± 2	1.60	0.10	545	± 93	935	± 2632	9	0.54
		LYII	49	34	± 2	32	± 3	1.41	0.12	238	± 171	144	± 1723	8	0.39
	Megalithic graves	CH	6	34	± 3	32	± 2	1.39	0.07	442	± 102	164	± 2318	5	0.44
		LHUE	36	26	± 2	29	± 3	1.25	0.15	258	± 111	267	± 2399	10	0.61
		LON	44	29	± 2	29	± 3	1.26	0.11	196	± 149	277	± 1759	6	0.42
	TOTAL		152	32	± 2	32	± 3	1.38	0.12	257	± 136	300	± 1894	8	0.49

Green colors indicate high coefficients of determination (R^2) of the DA model fits, while red colors mark fits with a lower correlation.

parameters and a quantitative assessment of the result of model fitting for these elements. Large variations in data quality, tooth preservation and measurement uncertainty cause the results of model fitting to be highly variable, as is illustrated by the boxplots in Fig. 6. Nevertheless, patterns in the model results could still be discussed when discarding outliers caused by less successful model fitting attempts.

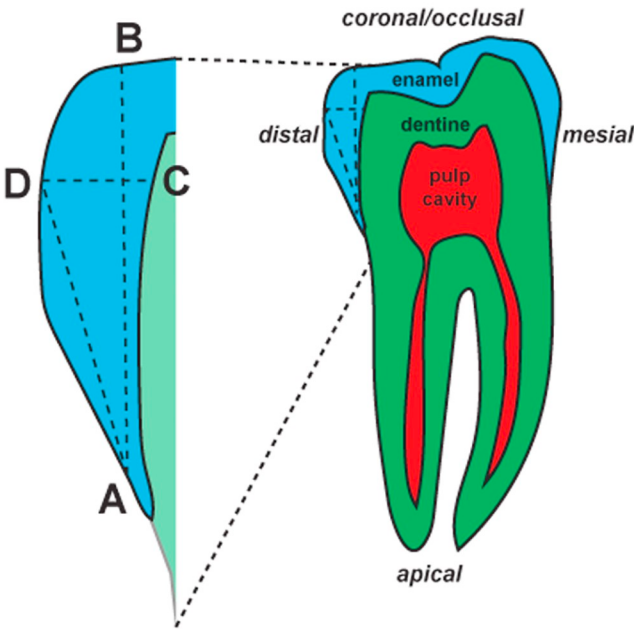
The DA model fits the XRF data well in general, with goodness-of-fit (R^2) values exceeding 0.7 in > 75% of the fitting attempts on Fe, Zn and Sr data. The best results are obtained for Zn profiles, with a median R^2 value of 0.98. Median R^2 values of Sr and Fe are 0.91 and 0.90 respectively, while Pb profiles achieved the worst results with > 75% of fitting attempts failing to reach R^2 values above 0.7 (median $R^2 = 0.49$). The same results broken down per site in Table 3 show that R^2 values are not very variable between sites except for bad model fits (median $R^2 = 0.44$) for Fe results in the Peña Large site (CPL). Most of the variation in goodness-of-fit of the model is explained by differences between modelled elements. A comparison with other parameters shows that the difference in Pb concentration between modelled endogenous concentrations (C_0) and diagenetic concentrations (C_1) is very low (8 μ g/g) compared to the measurement error (9–19 μ g/g; Table 2; Fig. 3). Table 4 shows a comparison of the result of modelling leaching fronts in different profiles constituting the “enamel triangles”. These

results show that, while comparing profiles of the same element, the best model fits are generally obtained in records on the CD profile (both from the dentine side, C, as from the outside, D). In addition, records along the AB profile also yield good model fits when leaching from the outside on the bottom side of the tooth (from point A) is considered (see Fig. 3).

While there is some spread in modelled endogenous (C_0) and diagenetic (C_1) concentrations, the majority of modelling attempts show similar results between teeth from the same site. The effect of diagenesis (e.g. the difference between C_0 and C_1) on Fe and Zn is easily observed, while the effects on Sr and Pb are more ambiguous. Breaking down the results per site shows more distinct patterns in modelled concentrations (Table 3). The effect of diagenesis on Fe and Zn is larger in funerary cave sites while megalithic grave sites show a larger diagenetic increase in Sr. As mentioned above, the difference between endogenous and diagenetic Pb concentrations is very small and falls within the measurement error. This effect of diagenesis is illustrated in Fig. 7, which shows the direction of diagenetic alteration in the trace element concentrations per site. Consequently, modelled pore fluid concentrations (C_2) yield relatively high (± 300 μ g/L) Sr concentrations in megalithic grave sites compared to lower concentrations (± 150 μ g/L) in funerary cave sites, mirroring the more extensive Sr

Table 4
Goodness-of-fit of the model (R^2) listed by element and by transect. In general, transects from C to D, from D to C and from A to B show the best fit with the DA model, illustrating that these transects have better developed sigmoid-shaped leaching fronts.

element	profile	leaching side	N	R2
Fe	AB	A	45	0.92
		B	33	0.83
	CD	C	27	0.87
		D	38	0.95
	DA	D	43	0.91
		A	47	0.83
Zn	AB	A	57	0.99
		B	37	0.86
	CD	C	36	0.99
		D	37	0.99
	DA	D	47	0.97
		A	55	0.98
Sr	AB	A	52	0.90
		B	42	0.87
	CD	C	38	0.95
		D	35	0.90
	DA	D	41	0.82
		A	47	0.93
Pb	AB	A	31	0.51
		B	22	0.42
	CD	C	18	0.58
		D	16	0.65
	DA	D	32	0.36
		A	27	0.48



diagenesis in megalithic grave sites. Modelled pore fluid Fe and Zn concentrations are higher in funerary cave sites ($\pm 80 \mu\text{g/L}$ and $\pm 90 \mu\text{g/L}$ respectively) than in megalithic grave sites ($\pm 60 \mu\text{g/L}$ and $\pm 30 \mu\text{g/L}$ respectively). The difference in Sr concentrations between funerary caves and megalithic graves is also visible in the μXRF maps (Fig. 2), while the observed variation in Fe and Zn is not clearly observed in μXRF maps.

Interestingly, modelled endogenous concentrations (C_0) show that not all difference in trace element composition between sites is a result of diagenesis. Endogenous Zn concentrations in tooth enamel are higher in funerary caves ($\pm 55 \mu\text{g/g}$) than in megalithic graves ($\pm 45 \mu\text{g/g}$). The opposite is true for endogenous Sr concentrations, which are generally higher in megalithic graves ($\pm 600 \mu\text{g/g}$) than in funerary caves ($\pm 450 \mu\text{g/g}$). No significant difference is observed in endogenous Fe and Pb concentrations. The latter is likely a result of higher relative measurement errors, lower concentrations of Pb and less successful model fitting (lower R^2).

In general, modelled leaching fronts of Pb and Sr seem to penetrate deeper into the enamel than those of Zn and Fe, as is evident from the modelled leaching depths (x_0 ; Fig. 6). However, care must be taken while interpreting these results, as the errors of the model prediction (Table 3) increase quickly when model fit (R^2) decreases. This results in high uncertainties on x_0 , especially in Pb records. Uncertainties are lower for Fe, Sr and especially Zn leaching models. No difference is observed in the depth of Zn and Fe leaching between funerary caves and megalithic grave sites, but a Sr leaching fronts penetrated deeper in funerary cave sites ($\pm 400 \mu\text{m}$) than in megalithic grave sites ($\pm 300 \mu\text{m}$). This result seems counterintuitive, as diagenetic enamel and pore fluid Sr concentrations were higher in megalithic grave sites.

The time it took to establish leaching fronts in the enamel (t) could also be modelled using the DA model proposed in this study. However, even more so than the depth of the leaching front, the reliability of this result is strongly dependent on the quality of the model fit. As a result, leaching times of all elements show some variability (Fig. 6). Errors on individual leaching times can be extensive, but the amount of leaching

profiles analyzed in this study allows discussion of differences in average leaching time per element and between different burial types. Modelled times needed to establish Fe leaching fronts (± 100 yrs) are almost always shorter than for Zn, Sr and Pb (± 300 yr). Like leaching depth, modelled leaching times are also generally larger in funerary cave sites than in megalithic grave sites.

4. Discussion

4.1. X-Ray Fluorescence analysis on tooth enamel

Results presented in this study highlight the use of lab-based μXRF scanning for the determination of lateral variations in trace element composition in tooth enamel. When correcting results using a matrix-matched certified reference material, reproducibility relative standard deviations better than 10% can be reached when integration time is long enough to achieve time of stable reproducibility (TSR, de Winter et al., 2017) of 60 s per analysis (Table 2). This allows up to 1300 data points to be collected in a 24 h timespan without damage to the sample, giving μXRF advantages over other methods for trace element analysis such as ICP-OES, ICP-MS and LA-ICP-MS. Trace element concentrations down to tens of $\mu\text{g/g}$ could be determined by μXRF measurements, allowing several elements of interest in bioapatite studies (e.g. Ca, Mg, Sr and Ba; see Table 2) to be quantified. The addition of long-term reproducibility monitoring and calibration with multiple reference materials ensures quality control on the accuracy and reproducibility of measurements (Fig. 4). We therefore strongly suggest that μXRF laboratories adopt this strategy to facilitate intra-lab comparison of trace element data.

The combination of XRF mapping and profiling along “enamel triangles” allows for detailed analysis of the lateral variability of trace elements in tooth enamel. XRF mapping using commercial lab-based μXRF devices presently only allows the semi-quantitative visualization of trace element concentrations. However, the small ($25 \mu\text{m}$) spot size and comparatively short measurement time (up to 5 tooth surfaces can

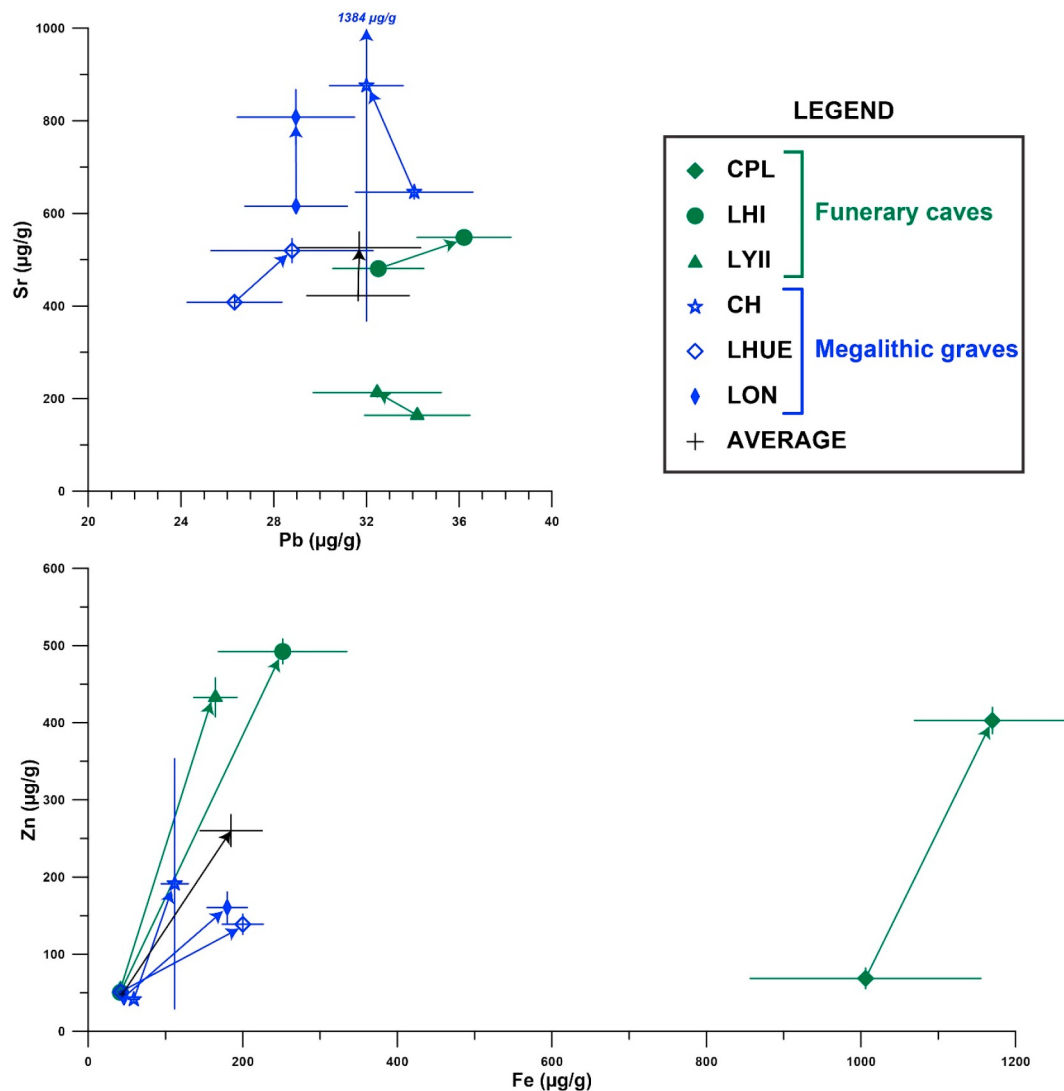


Fig. 7. Cross plots of modelled endogenous and diagenetic concentrations of Fe vs. Zn and Sr vs. Pb for different sites. The arrow points towards diagenetic concentrations and illustrates the pathway of diagenesis (assuming flat trace element profiles before burial). Note that diagenetic alteration always results in increased values of Fe, Zn and Sr. Megalithic grave sites show less increase in Zn and more increase in Sr during diagenesis. Error bars on Pb results are too large to discern the direction of diagenesis. Medians and error bars are the same as those reported in Table 3.

be mapped per hour) makes lab-based XRF mapping a very useful non-destructive tool for screening samples for diagenesis. Furthermore, combining μ XRF maps of several teeth into an image, as was done in this study, creates a very quick overview of the concentrations of common trace elements in the teeth which can help guide further sampling (Fig. 2). In this study, μ XRF mapping revealed that dentine, the pulp cavity of the teeth and the outer edges of enamel are more susceptible to diagenetic alteration over archaeological timescales. This is a result of the higher porosity of these materials and/or their closer contact with pore fluids outside the tooth (LeGeros, 1990). XRF mapping also reveals a difference in susceptibility to diagenesis between first and second molars. Diagenetically altered parts of first molars were distinctly more enriched in Fe and S than second molars of the same individuals, while second molars were more enriched in Al. Perhaps higher susceptibility to diagenesis in first permanent molars may be linked to dental wear, as they erupt earlier in life and are usually more worn than second permanent molars (Gleiser and Hunt, 1955). Wear causes enamel to become thinner or to wear away locally, causing pore fluids to penetrate the tooth more easily. However, the fact that differences in Fe and S concentrations between first and second molars are also observed in specimens which showed no dental wear (LYII29,

LHUE34, LHUE52, LHUE53, CH94, CH96, see Fig. 2) shows that reduced enamel thickness due to dental wear cannot fully explain these differences. The fact that unworn enamel thickness in first molars is lower than in second molars (Macho and Berner, 1993; Fornai et al., 2014), and enamel density varies between tooth position (Lynch et al., 2010) may also explain the increased susceptibility of first molars to diagenesis. Elevated Al concentrations in second molars are hard to explain in terms of diagenetic processes. Al fluoresces with comparatively low energy X-rays at the edge of the detectable spectrum, making it hard to measure using the applied μ XRF setup (e.g. de Winter and Claeys, 2016). Therefore, XRF maps of Al should not be over-interpreted. It is possible that differences between the compositions of first and second permanent molars arise from original differences in in vivo tooth composition. These may reflect differences between breastfeeding (infant) and fully weaned (childhood) diet, since the first molar mineralizes during the first 0–3 years of life while the second molar mineralizes during year 2.5–8 (AlQahtani et al., 2010). However, since XRF maps are semi-quantitative and because of the aforementioned difficulties in detecting Al, the distinction between differences in vivo Al concentrations and concentration differences due to diagenesis cannot easily be made based on mapping results. Comparison between

stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of sequentially sampled tooth dentine (formed during infancy) and bone collagen (formed later in life) shows that dietary differences between these life stages are observed (Fernández-Crespo et al., 2018). While these differences could potentially explain the observed variations in trace element composition, to the extent of our knowledge no earlier studies have observed a link between concentrations Al, Fe and S in tooth enamel and diet.

Typical sigmoid-shaped trace element profiles measured using μXRF profiling suggest that leaching of trace elements from surrounding pore fluids in the soil and the dentine play a dominant role in the alteration of trace element composition of buried teeth on archaeological timescales (see Fig. 3). Shapes of these leaching profiles resemble the error function-shaped profiles that result from DA models adapted from Fick's second law (Millard and Hedges, 1996; Kohn and Moses, 2013; Fig. 1). Characteristic flat concentration profiles on the outside (C_i) and inside (C_0) of the tooth divided by sharp concentration slopes, such as those found in Zn and Sr concentrations in specimen LHUE 2i (Fig. 3) indicate that leaching is better approximated by a DA model (diffusion-absorption) than by a double-medium diffusion (DMD) model, which is characterized by a relatively high concentration tail (Kohn, 2008). The suitability of the DA model is further demonstrated by the high goodness-of-fit (R^2) parameters obtained from fitting the model to Zn, Sr and Fe profiles.

4.2. Modelling of leaching profiles

Model fitting statistics show indeed that a large part of the variability in Zn, Sr and Fe concentrations in the enamel profiles can be explained by leaching of these elements into the tooth by means of diffusion and adsorption. R^2 values of model fitting are higher than 0.7 in > 75% of the analyzed profiles and often exceed 0.90, meaning that this leaching explains 70 to 90% of the variability in trace element concentrations along the profile. The majority of the residual variation can be explained by measurement uncertainty (Table 2) and natural heterogeneity in tooth composition. The latter includes in vivo trace element variability as described by Humphrey et al. (2008) and Müller et al. (2019). Fig. 3 shows that the 95% confidence levels determined by Monte Carlo simulation of the measurement and model fitting uncertainty encompass almost the entire variability in trace element profiles. This also explains the poor goodness-of-fit of DA models on Pb data, which has a higher relative standard deviation of measurement error. The measurement error on Fe is also relatively large, but large differences between endogenous and diagenetic concentrations still allow DA models to fit the data relatively well. This shows that the success rate of the DA model, and therefore the accuracy of the predicted model parameters, mostly depends on the ratio between the concentration gradient present in the tooth and the measurement uncertainty. As a consequence, the data on successful model fitting presented in this study is slightly biased against teeth that are either completely endogenous or fully diagenetic. In both cases, little or no difference in concentration between the outside and the inside of the enamel will be present, causing the model to fail to fit the data.

When successful, modelling of leaching fronts in archaeological tooth enamel can yield additional information about the diagenetic process, such as the depth of leaching and the time it took to establish leaching fronts. While diagenetic and endogenous concentrations could be estimated from trace element profiles without modelling, model fitting provides an automated way to estimate these parameters without user bias as well as an uncertainty on the estimate. Modelling also allows more accurate estimation of diagenetic and endogenous concentrations when the full sigmoid shape of the leaching front is not present in the tooth and manual estimation of these concentrations is difficult. An example of this is visible in the AD-profile of LHUE specimen 2i (Fig. 3). This latter application comes with a caveat however, since trace element profiles that lack the full sigmoid shape are hard to distinguish from in vivo trace element profiles, which also show a steep

drop in concentration away from the outer edge of the enamel. This is especially true in the case of Zn and Pb, whose enrichment on the outer edge was demonstrated by Humphrey et al. (2008) and Müller et al. (2019). Therefore, care must be taken in interpreting trace element profiles without a fully developed leaching front in terms of diagenetic leaching, especially when the thickness of the enriched layer at the edge of the enamel is very similar to that found in in vivo trace element profiles ($\sim 100\text{--}300\ \mu\text{m}$). An example of such an ambiguous trace element profile is the left hand side of the Zn-profile in AD direction (Fig. 3), while the right hand side of this profile shows a wider, more fully developed sigmoid-shaped leaching profile. In the following discussion, ambiguous profiles without a clearly developed sigmoid shape are excluded from the interpretation and only successful model fits were selected based on goodness of fit (R^2) and visual observation of the sigmoid leaching front.

According to DA model fitting, leaching of Fe into tooth enamel happens during a shorter time period ($< 100\ \text{yr}$) than Zn, Sr and Pb ($\pm 300\ \text{yr}$). Pore fluid concentrations and the type of burial site do not seem to affect this leaching time. The time over which leaching occurs is about an order of magnitude shorter than the age of the samples (ca. 5500–5500 yr). This result seems to suggest that leaching of trace elements slows down or halts at some point during fossilization. The applied DA model assumes a constant diffusion coefficient, while diagenetic processes such as secondary mineral deposition and reduction of permeability of the bioapatite may slow down diffusion rates over time (Kohn, 2008). Since uptake of trace elements into bioapatite has been demonstrated to continue millions of years after deposition, it is unlikely that trace element leaching into enamel stopped after several hundreds of years (Peppe and Reinert, 2007). Instead, diffusion rates probably slowed down over time, causing modelled diffusion times to underestimate the actual time it took to establish diffusion fronts.

Leaching fronts of Sr and Pb penetrate marginally deeper into the enamel than those of Fe and Zn. This is counterintuitive, because diffusion of Sr and Pb should be slower due to their larger ionic radii. This shows that other processes counteract this effect and promote deeper penetration of Sr and Pb into the enamel. No clear influence of ambient pore fluids or burial type is observed, except for Sr leaching, which seems to be deeper in funerary cave sites where pore fluid concentrations are lower. Modelling attempts seem to indicate that leaching of Pb into the teeth is very limited, as is evident from shallow concentration gradients and poor success in fitting a DA model to the Pb data ($R^2 < 0.5$). However, since the error on Pb μXRF measurements is of the same order of magnitude as the observed concentration gradient, modelling Pb leaching fronts did not yield good quantitative estimates of leaching parameters in this study. Nevertheless, the fact that the concentration gradient of Pb in the enamel is so shallow indicates that environmental Pb concentrations were most likely low and diagenetic leaching of Pb was limited to concentrations which were barely detectable by μXRF (tens of $\mu\text{g/g}$). The presence of leaching fronts of Fe, Sr and Zn after thousands of years of inhumation means that endogenous concentrations of these elements can be retrieved from archaeological samples. These concentrations may serve as tools for palaeodiet and palaeoenvironmental reconstructions, if a link between them and diet or environment can be demonstrated (as was done for Sr, but not for Fe and Zn). The discrepancy between times of leaching and the actual age of the samples shows that trace element leaching profiles (in this case of Fe, Sr, Zn and Pb) are not an accurate tool for dating archaeological samples. This same conclusion was drawn by Kohn and Moses (2013) based on investigation of leaching profiles in archaeological bones.

Model fitting trace element profiles in archaeological tooth enamel seems to be a successful tool for the detection and quantification of the effect of diagenesis on trace element compositions (Fig. 7). Comparison of endogenous and diagenetic trace element concentrations reveals statistically significant differences in the extent of diagenetic alteration between different burial types. Fig. 7 shows that teeth from funerary

caves are characterized by higher diagenetic Zn concentrations while Zn concentrations in megalithic graves are less altered. There seems to be less difference in alteration of Fe concentrations. The exception are teeth from the Peña Larga site (CPL), which exhibit very high ($\geq 1000 \mu\text{g/g}$) Fe concentrations in both altered and endogenous values, though only 4 teeth were available from this site and R^2 values for fitting of CPL Fe profiles are low (0.44). The latter leads to the assumption that DA model fitting on Fe profiles of CPL was not successful and the modelled parameters are unreliable. Interestingly, most of the osteological assemblage from the Peña Larga site was found charred, suggesting that samples from this site were exposed to high temperatures. It is known from studies of cremated remains that enamel tends to develop pores and fissures when exposed to increased temperatures (Shipman et al., 1984). This process may explain why Fe concentrations are high ($> 900 \mu\text{g/g}$; see Fig. 7) throughout the enamel in specimens from the CPL site, as fractures may have facilitated increased migration of Fe into the enamel. However, it remains unclear why such a process would only affect concentrations of Fe while leaving concentrations of other trace elements (e.g. Zn; see Fig. 7) unaffected. Perhaps the migration of iron oxides into the enamel facilitated by this increased porosity might explain the elevated Fe concentrations. This process of post-burial iron oxide precipitation in bioapatites has indeed been described in other studies (Jacques et al., 2008; Kuczumow et al., 2010). Results for Sr show an opposite trend, with lowland sites showing much stronger diagenetic increases than mountain sites. Despite differences between sites, diagenesis causes Sr, Zn and Fe concentrations to increase in all sites, meaning teeth absorb these trace elements from their surroundings. The trend in both Sr and Zn diagenesis is most likely a result of differences in soil composition which would have affected concentrations of these elements in the pore fluid, which is indeed reflected in modelled pore fluid concentrations (C_2 ; Table 3). The calcareous sandstones that make up the substrate of lowland sites may have facilitated more transfer of pore fluids rich in Sr, Zn and Fe to the interred remains than the more massive limestone deposits that contain the mountain sites, leading to increased rates of leaching of these elements into the enamel of individuals interred in the lowland sites. No direct measurements of soil composition are available to test this hypothesis, but the effect of varying trace element concentrations in the soil on diagenetic trace element uptake in bioapatite has been demonstrated (Millard and Hedges, 1996; Kohn and Moses, 2013).

4.3. Burial-type differentiation between trace element patterns

The difference between endogenous concentrations of Sr and Zn in teeth of funerary graves and megalithic sites may hold clues to differences in life history and environment between the peoples living in these different sites. The differences in endogenous Sr concentrations between megalithic graves and funerary caves found by DA model fitting are in agreement with those found by bulk Sr concentration measurements on tooth enamel from the same sites reported in Fernández-Crespo, personal communication. The latter study ascribes elevated Sr concentrations to a difference in diet and to people interred in megalithic graves having more privileged access to valued resources (e.g. salt) than people interred in funerary caves. This result, together with meaningful differences in stable isotope ratios suggests socioeconomic inequalities between those interred in distinct burial locations (Fernández-Crespo, personal communication).

It is possible that a difference in diet explains the opposite trend observed in endogenous Zn-concentrations. Some previous studies have postulated that higher Zn concentrations may point towards a higher contribution of meat in the diet, while higher Sr concentrations may indicate a higher contribution of leafy greens and legumes (Burton and Wright, 1995; Subar et al., 1998). This interpretation would be in agreement with palaeodietary reconstructions, suggesting a more agriculture-based lifestyle with a more plant-based diet in lowland communities and potentially a pastoral lifestyle with a more meat-

based diet in mountain communities in the Rioja Alavesa region (Fernández-Crespo, personal communication). However, it must be stressed that an unambiguous link between Zn concentrations in tooth enamel and diet has not been demonstrated. The fact that Zn concentrations (as an essential metal) are strongly bioregulated in human tissue renders such an interpretation highly tentative (Ezzo, 1994; Humphrey et al., 2008). Endogenous Zn concentrations found by DA model fitting ($40\text{--}60 \mu\text{g/g}$) are in good agreement with typical Zn concentrations found in modern human tissues (Underwood, 2012), showing that the presented technique for separating diagenetic from endogenous trace element concentrations yields reliable results.

4.4. Implications for sampling strategies

Results presented in this study show that trace element leaching during diagenesis has a strong effect on the distribution of trace elements in archaeological tooth enamel. If DA model fitting is reliable, the comparatively short times ($< 500 \text{ yr}$) required to establish leaching fronts means that Zn, Sr and Pb concentrations in almost all archaeological sites will be affected by trace element leaching. Leaching fronts in Fe concentrations seem to form even faster, with typical leaching times < 100 years. Diagenesis of trace element concentrations happens fast regardless of the type of burial site examined in this study. These results should, however, be interpreted with care, as it is well possible that modelled leaching times underestimate the actual time needed for the formation of leaching profiles because diffusion coefficients are not constant through time. Depth of leaching ($300\text{--}400 \mu\text{m}$) is substantial compared to the typical thickness of enamel in cross section ($1\text{--}3 \text{ mm}$), and leaching occurs both from the outside of the tooth and from the more porous dentine into the enamel (see Fig. 3). If these results are representative for archaeological sites in general, they indicate that only a small part of the enamel in archaeological teeth may be used for the determination of endogenous (in vivo) trace element concentrations. Considering a leaching penetration of $300 \mu\text{m}$ from the outside of the tooth and the enamel-dentine junction, only the central part of the enamel where enamel thickness is greatest (at the location of CD-profiles, see Fig. 3) may be used for the determination of endogenous trace element concentrations. In this case, both the outer edge and the region directly bordering the enamel-dentine junction should be avoided in these measurements as they stand a higher chance of being contaminated by trace element leaching. The results in this study show that the thickness of the leaching front to be avoided strongly depends on the elements of interest and their concentration in the burial environment. It must be noted that the teeth used for this investigation were not subject to any pretreatment methods. Such pretreatment procedures may successfully remove (part of) trace elements leached into bioapatites during diagenesis, especially when diagenesis is in an early stage (McMillan et al., 2019). However, pretreatment procedures have been shown not to be fully efficient (Snoeck et al., 2015). The best way to avoid contamination due to leaching is to carefully remove the outer $300\text{--}400 \mu\text{m}$ of enamel and to sample core enamel from the resulting surface of the tooth, avoiding enamel close to the enamel-dentine junction. Another way to obtain an unaltered sample from the enamel core would be to section the teeth as done in this study such that the core enamel is exposed. This procedure also allows (semi-)non-destructive in situ measurements to be carried out previous to destructive sampling, preserving more information about the composition of the tooth in multiple dimensions. Differences in DA model fitting success show that the best results are obtained in CD profiles, which cross the part of the enamel most likely to be unaffected by diagenesis (Table 4). Sampling trajectories along the enamel-dentine junction (profile AB) and along the edge of the tooth (profile DA) often fail to capture the full transition from diagenetic concentrations to endogenous concentrations, explaining reduced success in DA model fitting and higher uncertainties on the estimates of associated parameters.

The construction of “enamel triangles” on tooth cross sections and

subsequent fitting of DA models to extract endogenous trace element concentrations ensures the separation of diagenetic from endogenous concentrations and should be favored over bulk measurements. This illustrates that in situ measurement techniques like μ XRF and LA-ICP-MS offer more control on sampling for trace element concentrations than dissolution-based techniques such as ICP-MS and ICP-OES, if the detection limit of in situ measurements allows these concentrations to be determined reliably. The large difference in trace element concentrations between altered and endogenous enamel (up to hundreds of $\mu\text{g/g}$; Table 3) demonstrates the need for careful sampling using such in situ techniques if trace element concentrations are to be used as proxies for diet and life history in archaeological studies. The same holds true for isotopic studies, since a large diagenetic input of Sr and Zn from the direct surrounding may skew results of isotopic analysis. Even a 10% mixing of diagenetic enamel ($\pm 525 \mu\text{g/g}$ Sr; $\pm 260 \mu\text{g/g}$ Zn; $\pm 180 \mu\text{g/g}$ Fe) with 90% endogenous enamel ($\pm 425 \mu\text{g/g}$ Sr; $\pm 50 \mu\text{g/g}$ Zn; $\pm 50 \mu\text{g/g}$ Fe) would cause 12% of Sr, 37% of Zn and 25% of Fe in the sample to originate from diagenetically altered enamel. This would significantly affect the result of isotope analysis on these elements and would, for example, bias provenance studies towards values found locally in the burial locality. Smaller diagenetic leaching gradients in Pb profiles are most likely a result of comparatively low concentrations of Pb in the samples close to the detection limit of μ XRF analysis (Fig. 3). Comparison with previous studies shows that error function-shaped leaching gradients can also be observed in Pb profiles, and that other measurement techniques (e.g. LA-ICP-MS) should be used to quantify Pb concentrations in archaeological tooth enamel to aid sampling for Pb concentration and isotope ratio measurements for archaeological reconstructions (Kohn and Moses, 2013; Müller and Fietzke, 2016).

4.5. Perspective

The results of measurements and DA model fitting presented in this study reveal that leaching of trace elements into tooth enamel is an important process controlling trace element concentrations on archaeological timescales. The presented dataset shows that leaching depths for Fe, Sr and Zn are generally in the order of hundreds of micrometers. This implicates that this outer part of the enamel should be avoided when sampling preserved enamel for trace element and stable isotope analysis. Further research is needed to investigate whether pretreatment procedures are successful in removing leached trace elements from the outer part of the enamel. Beyond this outer rim of affected enamel, it has been demonstrated that original trace element concentrations are preserved and modelling diffusion-adsorption fronts allows these concentrations to be reconstructed with confidence. Modelled leaching of Sr, Fe and Zn also shows that concentrations of these elements almost always increase in enamel during diagenesis. This means that decreasing trace element profiles likely indicate preserved trends in life history or environment rather than diagenesis. Leaching times modelled in the specimens in this study are an order of magnitude lower than the age of the specimens. This shows that either diagenetic leaching only takes place over a few hundreds of years after burial and then stops, or that diffusion coefficients decrease during the fossilization process due to secondary mineralization, decreasing porosity and changing pore size distribution. Analyzing trace element concentrations in teeth of various ages and environments is required to confirm this observation and to understand why the process of trace element leaching does not continue at the same rate during the entire burial period.

5. Conclusions

Results in this study demonstrate the versatility of micro X-Ray Fluorescence scanning and its application in the study of variability of trace element compositions in tooth enamel. The combination of semi-quantitative XRF mapping and quantitative XRF profiling according to

the “enamel triangles” sampling strategy on cross sections through archaeological molars allows a detailed investigation of the variability of trace element concentrations throughout the enamel. The results of these analyses shed light on the post mortem leaching of trace elements into the enamel from both the outside of the tooth and from the more porous dentine, and aid in developing sampling strategies to determine in vivo trace element concentrations. Diffusion-advection models explain a large part (70–90%) of the spatial variability observed in the enamel profiles and fitting DA models to trace element profiles allows accurate estimation of in vivo and diagenetically altered concentrations. Furthermore, model fitting also sheds light on the timing and depth of diagenetic trace element leaching and can be used to reconstruct pore water compositions in the burial environment. Leaching of Fe, Sr and Zn into enamel is likely being slowed down by fossilization processes which cause modelled leaching times to underestimate fossilization times and sample age. However, leaching fronts penetrate to a depth of about 300–400 μm in archaeological teeth with an age of ± 3000 years, meaning endogenous concentrations can still be reconstructed from these samples. The extent to which enamel seems to be affected by these leaching processes shows that great care should be taken in sampling archaeological enamel for trace element concentration and isotope analysis. Since Fe, Zn and Sr are all demonstrated to leach into the tooth enamel from the surrounding sediment and/or the enamel-dentine junction, provenance studies using (isotope ratios of) these elements may be biased towards values found in the soil of the burial location when sampling diagenetically altered enamel. Studies aiming to determine endogenous enamel trace element and stable isotope compositions should therefore either include in situ measurement techniques such as μ XRF and LA-ICP-MS scanning, or apply careful sample preparation and avoid the outer 300–400 μm of the enamel and the enamel-dentine junction while sampling for bulk analyses.

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