Influence of depositional environment in fossil teeth: a micro-XRF and XAFS study.

I M Zougrou1, M Katsikini1*, F Pinakidou1, L Papadopoulou2, E Tsoukala2 and E C Paloura1

1 School of Physics, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece.
2 School of Geology, Aristotle University, 54124 Thessaloniki, Greece.

*E-mail: katsiki@auth.gr

Abstract. The formation of metal-rich phases during the fossilization of vertebrate fossil teeth, recovered from various deposition environments in northern Greece, is studied by means of synchrotron radiation X-ray fluorescence (SR-XRF) as well as Fe and Mn K edge X-ray absorption fine structure (XAFS) spectroscopy. XRF line-scans from the samples’ cross-sections revealed different contamination paths for Mn and Fe. The two-dimensional XRF maps illustrate the spatial distribution of P, Ca, Mn and Fe as well as the precipitation of Fe-rich phases in cementum, dentin and dentinal tubules. Goethite, lepidocrocite and ferrihydrite were detected in the samples’ cross-section by means of Fe K edge EXAFS spectroscopy. Moreover the Fe and Mn K edge EXAFS revealed the presence of vivianite and birnessite (MnO2) on the external surface of two samples.

1. Introduction
The study of fossil teeth composition and chemical alterations is of great importance not only for palaeoenvironment reconstruction and identification of the fossilization conditions but for conservation and restoration purposes as well. Mammal teeth consist typically of a crown composed of dentin covered by an enamel layer and a cementum layer which coats the tooth pulp. Although the inorganic component of all layers is carbonate hydroxylapatite (HAp), the microstructure and HAp percentage vary among the layers. More specifically, enamel consists of 97% wt. HAp crystals while in dentin and cement the corresponding component is 70% wt. Amelogenin proteins comprise the main organic component of enamel while dentin and cement comprise of type I collagen [1,2]. Contrary to other mammals, the structure of the occlusal surface in the cheek teeth of hypsodont animals such as the *Hipparion*, is more complex. Enamel in the *Hipparion* molar and pre-molar teeth forms several folds in the coronal dentin, named infundibula, which result in the formation of enamel ridges elevated from the occlusal surface. Cementum fills the infundibula and covers the enamel (coronal cementum). The *Hipparion* cheek teeth have five cusps: the protocone, the protoloph, the metaloph, the paracone and the metacone (figure 1a). The interior of the two complex enamel structures forming plications in the centre of the tooth are called prefossette and postfossette [3,4]. Although the inorganic phases of enamel, dentin and cementum are preserved during fossilization, porosity and degradation of the organic content contribute to the chemical alterations of the tooth induced by diagenetic processes. Dentin is more porous than enamel, containing dentinal tubules of 1-3 µm diameter which may be enlarged by dissolution or filled with diagenetic minerals. Moreover, the
collagen content of the dentin and cementum is more easily dissolved rendering these tissues more prone to diagenetic alterations than enamel [5].

In this study we investigate the diagenetic alterations of fossilized teeth of different animal taxa from three excavation sites in northern Greece. The samples were selected on the basis of the different dental tissue structure and different deposition environment. The contamination profiles of dental tissues were studied by micro-X-ray fluorescence (µ-XRF) line-scans across the cross-sections of the samples. Two-dimensional (2D) XRF maps were recorded to investigate the distribution of P, Ca, Mn and Fe in the dental tissue. The local bonding environment of Fe and Mn, which are the most common soil contaminants in fossil specimens, was determined by micro-X-ray absorption fine structure (µ-XAFS) spectroscopy. The bonding environment and oxidation state of Fe was assessed using the X-ray absorption near edge structure (XANES) portion of the Fe K edge XAFS spectra. The extended XAFS (EXAFS) part of the spectrum, in combination with XANES, provides information on the metal-rich phases. Color alterations at the external surface of two samples were also investigated by means of Mn and Fe K edge XAFS spectroscopy.

2. Materials and Methods

Fossil teeth of various geological ages recovered from three excavation sites in northern Greece were studied by means of µ-XRF and XAFS spectroscopies. The sample names and descriptions are listed in table 1 and corresponding photographs are shown in figure 1.

| Sample name | Animal taxum | Specimen | Excavation site | Age |
|-------------|--------------|----------|----------------|-----|
| MIL5 753    | Hipparion sp. | Upper molar tooth | Milia, Grevena (site 5) | Earlymost Villafranchian (MNQ 16) Plio-Pleistocene |
| MIL5 742    | Sus arvernensis | Canine tooth | Milia, Grevena (site 5) | MNQ 16 |
| XEK 13      | Elephas antiquus | Tusk fragment (Incisor tooth) | Xerias, Kavala | Middle Pleistocene |
| LAC 9141    | Ursus ingressus | Canine tooth | Almopia Speleopark, Aridaia | 42 ka BP (radiocarbon dating) Late Pleistocene |

The Milia fossils were found in Pliocene - Quaternary deposits of the Grevena basin. The deposits consist of Pliocene lacustrine sediments, followed by a Plio-Pleistocene sequence of clastic deposits, mainly sands, gravels and conglomerates, with sandstone intercalation and silty lenses [6]. The fossil teeth were found at a depth of approximately 15 m and thus their preservation state was not influenced by seasonal temperature and humidity changes occurring at the surface [7]. The Xerias fossils were found during the construction of the Egnatia Highway. The Middle Pleistocene fossil tusk was preserved in red clay deposits and had evident deformation marks in the form of circular and radial cracks propagating from the outer surface to the centre of the tusks’ cross-section, which were probably induced by the overlying sediment pressure [8]. Finally, the Almopia Speleopark is situated on the northern slope of the V-shaped Nikolau Rema gorge, on the slopes of Voras Mountain. The gorge consists of Maastrichtian limestone of the Almopia zone. The bulk of the cavities of the Almopia Speleopark developed under phreatic conditions while neotectonic activity lifted the caves to the vadose zone. The caves are located at 460 – 560 m altitude while hot springs of 30 – 37.5 °C water temperature lay under at altitudes of 360 – 390 m [9]. The fossil bear tooth was excavated from the V3 square in the LAC I b Chamber of the Bear Cave at approximately 1.1 m depth. The Chamber of the cave was periodically flooded and the fossils were sporadically submerged in water [10].

The SR-XRF and µ-XAFS measurements were conducted at the BESSY-II Synchrotron Radiation Storage Ring of the Helmholtz Zentrum Berlin. The measurements were performed at the KMC-II
beamline, at atmospheric pressure, using an energy dispersive fluorescence (XFlash-Rontec) silicon detector positioned on the horizontal plane at right angle to the incident beam. The total flux at the sample spot was in the range $10^9-10^{10}$ photons/sec [11]. The beam was focused using a circular capillary that reduced the beam size to 3.6 $\mu$m. The one dimensional (line-scans) and two-dimensional SR-XRF maps were recorded using a beam with excitation energy of 9.7 or 7.2 keV, impinging at 45° to the sample surface. For the construction of the XRF maps, the integrated count rate of the $K_\alpha$ emission peak of the corresponding element was taken into account [region-of-interest (ROI) integration]. The phosphor XRF maps were corrected for the Ca $K_\alpha$ escape peak. Macro-XAFS spectra were recorded at the same beamline after removing the capillary as well as at the C beamline of the DORIS storage ring at HASYLAB. In the latter case the beam size and photon flux on sample was $6 \times 1$ mm$^2$ and $10^9$ photons/sec, respectively, and the spectra were recorded using a 7-element fluorescence detector. XAFS spectra were recorded at the Fe and Mn $K$ edges provided that the metal concentration was sufficient. Porosity measurements are based on scanning electron microscopy - backscattering electrons (SEM-BSE) and optical microscopy images. For the porosity determination, the area of pores with diameter >7 $\mu$m existing in 500×500 $\mu$m$^2$ squares was taken into account.

For the XRF and XAFS characterization the samples were cut into transverse sections and then they were cleaned, air-dried and ground with 400-800 SiC grit papers. The measurements were conducted on the cross sections and on the external untreated surfaces of the bulk samples. For the analysis of the EXAFS spectra the FEFF8 package was used [12,13]. The XANES spectra were subjected to linear background subtraction and normalization to the edge jump while the fitting of the isolated pre-edge peak was performed with the PeakFit software. Characterization results on fossil bones excavated at the site of Milia were published elsewhere [14].

3. Results and discussion

3.1. Synchrotron radiation X-ray fluorescence mapping

The contamination profiles and the related alterations in different dental regions of the Hipparion molar and the Sus arvernensis canine teeth were assessed using $\mu$-XRF line-scans. A line-scan at the enamel – dentin region near the surface of the Hipparion molar and a second line-scan at the cementum region of the prefossette were recorded with excitation energy of 9.7 keV. The Sus arvernensis canine line-scan was recorded at a dentin region with excitation energy of 7.2 keV. The enamel surrounding the outer surface of the Hipparion tooth cusps is not well preserved and its thickness ranges from 0.5 to 1.5 mm. The line-scan of Ca, Mn and Fe $K_\alpha$ emission intensities are
shown in figure 2. In order to compensate for the different emission and absorption probabilities of each element, the profiles were normalized according to the fundamental parameter method by \( \varepsilon(E_{\text{fl}}) \omega_i p_{\text{ik}j} \tau_i(E_0) \) where \( \varepsilon \) is the detector’s efficiency at the fluorescence energy \( E_{\text{fl}} \), \( \omega_i \) is the fluorescence yield, \( p_{\text{ik}j} \) is the transition probability of the \( k^{\text{th}} \) line of the element \( i \) and \( \tau_i \) is the corresponding subshell photoelectric cross section at the energy of the impinging beam \( E_0 \) [14]. The profiles were not corrected for self-absorption effects due to the unknown matrix composition of the samples.

The distribution profile of Ca is related to the tooth bioapatite mineral, while Fe and Mn profiles might indicate incorporation in the bioapatite matrix and/or contamination from the burial environment. The profile of Ca is irregular in areas where the Fe concentration is high and fairly regular in areas where the Fe concentration is low. The decreasing Ca intensity in the Hipparion dentin region (figure 2a) extending to 1000-1200 \( \mu \)m length from the outer surface cannot be attributed to porosity. Based on SEM-BSE images the pore size diameter ranges from 7 to 70 ± 4 \( \mu \)m up to a distance of 1500 \( \mu \)m from the outer surface whereas in the same region the porosity ranges from 6.2 to 28.6 \( \times 10^{-4} \pm 0.2 \times 10^{-4} \). Thus, the variation in the Ca intensity is attributed to chemical changes in the matrix composition induced by dissolution and recrystallization processes rather than variations in the porosity values. In the Hipparion cementum region larger pores are observed (with size up to 98 \( \times 56 \) \( \mu \)m) and the corresponding porosity increases up to \( 6.9 \times 10^{-3} \pm 0.2 \times 10^{-4} \). The local intensity minimum observed between \( x=430 \) and 530 \( \mu \)m in figure 2b is attributed to the presence of such pores. The Fe profile is highly irregular in both near surface dentin regions (figure 2a and c) and in the cementum region in the centre of the Hipparion tooth (figure 2b) showing various local maxima. The observed contamination paths of Fe may have resulted from diffusion and/or groundwater infiltration processes. The presence of Mn is minimal near the surface however a Mn maximum is observed in the Hipparion cementum revealing that Mn contamination originated from infiltration processes through the empty Hipparion cusps. This observation is also confirmed by SEM-BSE images which reveal that the metal-rich phases mainly precipitate in the pre- and post- fossette regions.

Figure 2. \( \mu \)-XRF line-scans of Ca, Mn and Fe recorded at the cross sections of the (a) enamel-dentin region of the Hipparion molar tooth, (b) cementum region in the centre of the Hipparion molar tooth, (c) dentin region of the Sus arvernensis canine.

The Ca profile of the Sus arvernensis dentin region (figure 2c) is highly irregular revealing consecutive local variations in the apatite matrix. More specifically, local minima in the Ca concentration with a width of 25-85 \( \mu \)m, are observed. The Fe profile is also irregular but not directly correlated to the Ca profile. Local maxima in the Fe concentration, that denote Fe containing mineral precipitations, are observed both in spots of high and low Ca intensity. Reflected light optical microscopy images obtained in order to complement the \( \mu \)-XRF line-scans, revealed three characteristic alterations in the Sus arvernensis dentin: a) the presence of secondary minerals in the dentin region next to the Enamel-Dentin Junction (EDJ) due to diagenetic cracks traversing the
enamel, b) partially filled circular voids with diameter ranging from 1.6 to 12.5 \pm 0.8 \mu m in a dentin area up to a distance of 63\pm 4 \mu m from the EDJ, c) extensive microbial borings filled with secondary minerals extending up to 1000\pm 4 \mu m from the EDJ. The borings replace most of the apatite matrix up to approximately 450 \mu m from the EDJ while their density is significantly reduced at larger distances from the EDJ. This altered area is macroscopically distinguished by its yellow color. The size of the borings in the yellow area ranges from 9.4\times 10.9 \pm 0.8 \mu m^2 to 14\times 125 \pm 0.8 \mu m^2 whereas the size of the dentinal tubules ranges from 1.6 to 6.3 \pm 0.8 \mu m. Thus, the variation in the Ca intensity in the linear scan reflects the replacement of apatite by secondary minerals that fill the microbial borings.

The two-dimensional maps recorded at the dentin region of the *Hipparion* and the *Sus* teeth are shown in figures 3 and 4, respectively. The Ca and P maps reveal a similar spatial distribution of these elements indicating their co-participation in the formation of tooth apatite. A diagenetic crack observed in the *Sus* tooth is indicated by an arrow (figure 4). Metal containing, mainly Fe-rich, precipitates are observed in both cases whereas in the case of *Sus* tooth (figure 4) most of them appear to be of spherical shape filling dentinal tubules. The size of these precipitates is 5\pm 2.5 \mu m which is consistent with the size of the dentinal tubules, as they were determined from the optical microscopy images. The two larger precipitates with dimensions 10\times 10 \mu m^2 and 30\times 15 \mu m^2 located at the bottom left and top center areas of the Fe map respectively, are attributed due to their size, to precipitates filling microbial borings.

![Figure 3](image1.png)

**Figure 3.** XRF maps of the *Hipparion* molar tooth recorded at 650 \mu m from the outer surface showing dentin and a region of sedimentary metal-rich precipitates. The arrow in the Ca map indicates an area of metal containing precipitates in the dentin region.

![Figure 4](image2.png)

**Figure 4.** XRF maps of the *Sus arverensis* canine tooth recorded at a dentin region located at 2 mm from the outer surface. The arrow in the Ca map indicates a diagenetic crack.
3.2. X-ray Absorption Fine Structure

Fe is the most abundant contaminating element detected in the cross section of all samples except for the Ursus ingressus tooth where the metal concentration was very low. The Sus arvernensis tooth pulp is surrounded by a yellowish, approximately 1.5 mm thick, layer while the remaining part has a dark brown color as observed in the cross section. In order to investigate variations in the Fe speciation, Fe K edge µ-XAFS spectra were recorded from Fe-rich and Fe-poor spots properly selected from the XRF maps, whereas macro-XAFS spectra were recorded from regions of different color in the samples’ cross-sections. The Fe K edge XANES spectra shown in Figure 5 were recorded at a Fe-rich (a), Fe-poor (b), yellow-colored area (c) and brown-colored area (d) spots in the Sus arvernensis tooth cross-section. Fe K edge XANES spectra recorded at a Fe-rich spot located in the dentin region approximately 850 μm from the outer tooth surface (e), at a Fe-poor spot located in the cementum at the centre of the tooth cross-section (f) and a white region located at the lateral surface (g) of the Hipparion molar tooth as well as at the cross section of the Elephas antiquus tusk (h) are also shown.

The characteristic low intensity peak that appears before the absorption edge (hereafter it will be referred as pre-edge peak) is due to 1s→3d electronic transitions that become dipole allowed after mixing of the 3d with 4p orbitals induced by proper geometry or distortion of the Fe polyhedron [15,16]. The isolated pre-edge peaks fitted using lorentzian functions are shown in figure 5 and the fitting results are listed in table 2. During the fitting the widths of the L1 and L2 lorentzians were commonly iterated, while the width of L3 lorentzian was allowed to vary. The centroid position of the pre-edge peak, determined taking into account the positions of the L1 and L2 lorentzians that correspond to interactions of Fe with its 1st nearest neighbors, reveal that irrespectively of the local concentration of Fe or the dental region, Fe is trivalent and octahedrally coordinated [15,16]. The intensity of the L3 lorentzian corresponds to interactions of Fe involving 2nd nearest neighbors and was thus not included in the determination of the total area of the pre-edge peak and the centroid position. Slight variations in the intensity ratio of the L1-L3 components might be related to the formation of different Fe compounds and/or different degree of crystalline order. More specifically, in the Sus and Hipparion teeth we do not detect any variation in the pre-edge peak intensity between the Fe-rich and Fe-poor spots. However, the intensity of the pre-edge peak in the brown area of the Sus tooth and the white area of the Hipparion tooth is lower, revealing a higher centrosymmetry in the Fe site.

Figure 5. Fe K edge XANES spectra (left) and fitting of the corresponding isolated pre-edge peaks (right) of the: (a) Fe-rich spot, (b) Fe-poor spot, (c) yellow area and (d) brown area of the Sus arvernensis tooth; (e) Fe-rich spot, (f) Fe-poor spot and (g) white area at the lateral surface of the Hipparion molar tooth; (h) Elephas antiquus tusk. The spectra (a)–(f) and (h) were recorded at the sample cross sections and are shifted along the y-axis for clarity. The pre-edge peaks were fitted with three lorentzian components denoted as L1-L3.
Table 2. Fitting results of the pre-edge peak of the Fe K edge XANES spectra recorded at the cross sections of the *Sus arvernensis* canine tooth, the *Hipparion* molar tooth and the *Elephas antiquus* tusk. The Fe-rich and Fe-poor spots refer to sample positions determined by XRF mapping whereas the rest of the spectra are macro-XANES spectra recorded at characteristic sample regions. The three lorentzian functions are located at 7113.0 (L1), 7114.4 (L2) and 7116.8 (L3) eV.

| Spectrum                        | Component | Area (arb. units) | Total area (arb. units) | Centroid position (eV) |
|---------------------------------|-----------|-------------------|-------------------------|------------------------|
| *Sus* tooth (Fe-rich spot)      | L1, L2, L3 | 0.08, 0.08, 0.03  | 0.16                    | 7113.7                 |
| *Sus* tooth (Fe-poor spot)      | L1, L2, L3 | 0.07, 0.08, 0.03  | 0.15                    | 7113.8                 |
| *Sus* tooth (yellow area)       | L1, L2, L3 | 0.07, 0.08, 0.04  | 0.15                    | 7113.7                 |
| *Sus* tooth (brown area)        | L1, L2, L3 | 0.06, 0.06, 0.01  | 0.12                    | 7113.7                 |
| *Hipparion* molar tooth (Fe-rich spot) | L1, L2, L3 | 0.05, 0.10, 0.02 | 0.15                    | 7113.9                 |
| *Hipparion* molar tooth (Fe-poor spot) | L1, L2   | 0.09, 0.06        | 0.15                    | 7113.5                 |
| *Hipparion* molar tooth (White region at the lateral surface) | L1, L2, L3 | 0.04, 0.09, 0.03 | 0.13                    | 7114.0                 |
| *Elephas antiquus* tusk random spot | L1, L2, L3 | 0.04, 0.07, 0.05 | 0.11                    | 7113.9                 |

The bonding geometry of Fe is further investigated by means of Fe K edge EXAFS spectra. The spectra were subjected to subtraction of the atomic background and transformation from the energy- to the k-space. The resulting χ(k) spectra were fitted using photoelectron scattering paths constructed using the FEFF code [12]. The χ(k) spectra and the corresponding Fourier Transforms (FT) shown in figure 6 were recorded from the yellowish and brown area and from a larger region of the *Sus* tooth cross section, from a Fe-rich spot in the dentin region near the tooth surface and from a cementum region in the *Hipparion* tooth as well as from the *Elephas antiquus* tusk. The outer surface of the *Hipparion* tooth that exhibits a white Fe-rich area was also studied. The µ-EXAFS spectrum of *Sus* tooth brown area was fitted using the photoelectron scattering paths of ferrihydrite. The µ-EXAFS spectrum of *Sus* tooth yellow area as well as the macro-EXAFS from the *Sus* pulp cavity - yellow area were fitted with the goethite model. The same model was used for the fitting of the *Elephas antiquus* tusk macro-EXAFS spectrum. The µ-EXAFS spectrum of the Fe-rich spot at the *Hipparion* tooth was fitted with the lepidocrocite model whereas the macro-EXAFS spectrum of the *Hipparion* tooth was fitted with a mixed model of 54% goethite and 46% lepidocrocite contributions. Finally, the macro-EXAFS spectrum from the white region of the *Hipparion* tooth was fitted with the vivianite (FePO₄) model. During the iterations the coordination numbers (N) were kept fixed according to the respective reference models. The fitting results are listed in table 3.

Iron hydroxides are common diagenetic minerals in fossils revealing a fossilization process under oxidizing environment. Goethite is the most abundant contaminating mineral detected in the samples excavated from terrestrial sites. In goethite (α-FeOOH) the FeO₆ octahedra share edges and corners with Fe-Fe distances which are approximately 3.01 (3.28) and 3.46 Å, respectively. In lepidocrocite (γ-FeOOH) the octahedra are bonded only with edges with the Fe-Fe distance equal to 3.05 Å. Ferrihydrite is a low-symmetry Fe oxide polymer where the octahedra share edges but the presence of
Table 3. Fitting results of the Fe K edge μ- and macro-EXAFS spectra of the fossil teeth and tusk. N is
the coordination number, R in Å is the nearest neighbor distance, \( \sigma^2 \) in Å^2 is the Debye-Waller factor.
During the iterations the value of the coordination number N was kept fixed according to the respective reference model. The abbreviations Gt, Lp, Fh and Vn correspond to the goethite, lepidocrocite, ferrihydrite and vivianite (FePO_4) models.

| Specimen                      | Parameters | Sh1   | Sh2   | Sh3   | Sh4   |
|-------------------------------|------------|-------|-------|-------|-------|
| Sus tooth (brown area)        | Model: Fh  | N 6 O | 6 Fe  | -     | 8 O   |
|                               | R          | 1.99±0.01 | 3.00±0.02 | -      | 3.81±0.02 |
|                               | \( \sigma^2 \times 10^{-3} \) | 6.7 | 21.8 | - | 4.3 |
| Sus tooth (yellow area)       | Model: Gt  | N 6 O | 4 Fe  | 4 Fe  | 10 O  |
|                               | R          | 1.99±0.02 | 3.06±0.05 | 3.42±0.04 | 3.71±0.07 |
|                               | \( \sigma^2 \times 10^{-3} \) | 12.2 | 22.9 | 13.0 | 25.3 |
| Sus tooth (macro-EXAFS)       | Model: Gt  | N 6 O | 4 Fe  | 4 Fe  | 5 O   |
|                               | R          | 2.01±0.01 | 3.04±0.01 | 3.42±0.02 | 3.77±0.05 |
|                               | \( \sigma^2 \times 10^{-3} \) | 8.6 | 10.5 | 13.3 | 16.0 |
| Elephas antiquus tusk         | Model: Gt  | N 6 O | 4 Fe  | 4 Fe  | 6 O   |
|                               | R          | 2.01±0.01 | 3.04±0.01 | 3.41±0.01 | 3.80±0.04 |
|                               | \( \sigma^2 \times 10^{-3} \) | 7.83 | 9.41 | 11.07 | 17.5 |
| Hipparion tooth (Fe-rich spot)| Model: Lp  | N 6 O | 2.15 Fe | 2.15 Fe | 5.37 O |
|                               | R          | 2.02±0.01 | 3.28±0.03 | 3.45±0.06 | 3.77±0.06 |
|                               | \( \sigma^2 \times 10^{-3} \) | 9.9 | 6.4 | 12.2 | 24.7 |
| Hipparion tooth (white area at| Model: Vn  | N 6 O | 6 Fe  | -     | 6 O   |
| the outer surface)            | R          | 2.00±0.01 | 3.00±0.01 | -      | 3.74±0.06 |
|                               | \( \sigma^2 \times 10^{-3} \) | 9.5 | 9.1 | - | 8.1 |
| Hipparion tooth (white area at| Model: Vn  | N 6 O | 2 P   | 10 O  | 4 O   |
| the outer surface)            | R          | 1.99±0.01 | 3.18±0.04 | 3.78±0.05 | 4.33±0.05 |
|                               | \( \sigma^2 \times 10^{-3} \) | 4.7 | 8.4 | 18.8 | 2.3 |

Figure 6. Fe K edge \( \chi(k) \) (left) and corresponding amplitudes of the Fourier Transforms of the \( k^n \) weighted \( \chi(k) \) spectra (right) of: (a) brown region, (b) yellow region and (c) macro EXAFS spectra of the Sus tooth; (d) Elephas antiquus tusk; (e) macro EXAFS, (f) Fe-rich spot and (g) white region of the Hipparion molar tooth. The experimental spectra and the fitting curves are shown in thin and thick lines, respectively.
voids results in a less-compact structure [17]. SEM-EDX measurements from the cementum area of the *Hipparion* molar tooth and reflected light optical images from the pulp cavity of the *Sus arvernensis* tooth revealed the presence of both pyrite and goethite in the samples. The pyrite oxidation that results in the formation of metastable minerals ferrihydrite and schwertmannite, as well as jarosite, lepidocrocite, goethite and hematite has been reported in the literature [18,19]. The detection of various polytypes of Fe oxides even in teeth fragments found in the same depositional environment could be attributed to the locally different pH values that might result in structural transitions even at room temperature [17]. More specifically, the presence of pyrite and goethite near the pulp cavity of the Milia *Sus* tooth reveals pH conditions of 4-6 during this stage of fossilization [20,21].

Contrary to the Milia and Xerias terrestrial environments, the Aridaia caves of Speleopark seem to provide an ideal fossilization environment since no considerable amounts of Fe or Mn were detected in the *Ursus* tooth cross-section. However, a black region on the external surface of the *Ursus* tooth is found to contain Mn and thus it was studied by Mn $K$ edge EXAFS spectroscopy. Fitting of the $\chi(k)$ spectrum and the corresponding amplitude of the FT are shown in figure 7. The spectrum was fitted using the photoelectron scattering paths of birnessite ($\text{MnO}_6^2-$) [22]. Six oxygen and six Mn atoms were found at the distance of 1.90±0.01 and 2.87±0.01 Å, respectively. The corresponding Debye-Waller factors were found equal to 2.3 and 4.6×10^{-3} Å², respectively. The coordination number of the first neighboring shell (Mn-O) reveals that Mn forms octahedra, while the distance of the second neighboring shell (Mn-Mn) shows that the octahedra share mainly edges. It should be pointed out that Mn oxides and hydroxides exist in various polytypes that vary in the way the MnO$_6$ octahedra bind together by sharing mainly edges and thus forming chains. Phyllomorph phases are formed when corner linkages do not occur while tunnel structures in 3D networks are formed by corner-sharing cross-linking of chains. The similarity of Mn bonding environment in the mid-range order of the various polytypes renders their discrimination by EXAFS difficult [23].

**Figure 7.** Fitting of the Mn $K$ edge $\chi(k)$ (inset) and the corresponding amplitude of the Fourier Transform of the black region at the outer surface of the *Ursus* tooth. The experimental spectra and the fitting curves are shown in thin and thick lines, respectively.

4. Conclusions
The spatial distribution, contamination profiles and bonding environment of elements in fossil teeth and tusk excavated at sites in Northern Greece (Milia, Xerias, Almopia Speleopark) were studied by means of XAFS and XRF linear and 2D mapping. The dry sands where the Milia samples were recovered caused significant diagenetic alterations of the samples. Extensive microbial borings were detected by means of reflected light optical microscopy in the *Sus arvernensis* tooth while various Fe and Mn contaminant phases were identified in both Milia samples. The linear scans indicate that the Fe contamination paths are both through the sample surface via diffusion processes and through the
empty cusps via infiltration processes. The Mn precipitations were detected only in the cementum area in the centre of the *Hipparion* tooth. The high intensity of Mn indicates the exogenous origin of Mn that entered the sample via groundwater infiltration processes. SEM-BSE images of the *Hipparion* tooth confirm this conclusion and also reveal that the cementum area is mainly affected by metal-rich phases and to a lesser extent the dentin area around the pre- and post fossettes. The two-dimensional XRF mapping revealed the formation of Fe precipitates in the dentin region of the *Hipparion* tooth which was close to the outer surface as well as the precipitation of Fe-rich circular phases in the *Sus arvernensis* tooth dentin. Based on the size and distribution of the *Sus* Fe precipitates, as well as complementary reflected light optical microscopy images, Fe appears to fill various dentinal tubules and some microbial borings. Finally Fe K edge XAFS was used to assess the oxidation state as well as the coordination and bonding geometry of the metal-rich precipitates. In all the samples iron was trivalent and octahedrally coordinated. Iron hydroxides (ferrihydrite, goethite, lepidocrocite) were the only iron minerals detected in the cross-section of the samples revealing that the fossilization occurred under oxidizing conditions. Although the Xeria sample was heavily cracked along its entire length and filled with clay minerals, only iron hydroxides, and more specifically goethite, were detected. On the contrary in the Milia samples both goethite and pyrite were detected, revealing pH conditions during fossilization between 4 and 6. However the transformation was not complete. In the Almopia Speleopark sample, which was well preserved, we did not detect any considerable amounts of exogenous elements. The stable environment conditions of the cave, in addition to periodical flooding which wetted the samples and removed the organic components resulted in the complete fossilization of the sample. The burial environment affected only the external surface of the *Ursus* tooth. The structure of the black phase at the external surface of the *Ursus* tooth as well as a white phase at the external surface of the *Hipparion* tooth, were identified, without any destructive sample preparation, by means of Mn and Fe K edge EXAFS spectroscopy. The Pliocene lacustrine formation in which the *Hipparion* bone was found is correlated to the vivianite detected by EXAFS.

5. Acknowledgements
The measurements at the electron storage ring BESSY II of the Helmholtz Zentrum Berlin and HASYLAB-DESY were financially supported from the European Community’s Seventh Framework Program (FP7/2007-2013) under grant agreement n.º 226716.

6. References
[1] Ungar P S 2010 *Mammal Teeth: Origin, Evolution and Diversity* (Baltimore: The John Hopkins University Press) chapter 1 pp 9-19
[2] Lowenstam H A and Weiner S 1989 *On Biominalization* (New York: Oxford Univ. Press) p 324
[3] Budras K D, Sack W O and Röck S 2011 *Anatomy of the horse* (Hannover: Schlütersche Verlagsgesellschaft mbH & Co KG) chapter 4 pp 32-3
[4] Eisenmann V, Alberdi M T, De Guili C and Staesche U 1988 *Studying fossil horses: collected papers after the “New York Int. Hipparion Conf., 1981”* vol 1: Methodology ed M Woodburne and P. Sondaar, (Leiden: E J Brill) pp 1–71
[5] Dauphin Y and Williams C T 2004 *C. R. Palevol* 3 583-90
[6] Tsoukala E and Lister A 1998 *Boll. Soc. Paleontol. Ital.* 37 117-39
[7] Tsoukala E 2000 *Ann. Paleontol.* 86 165–91
[8] Tsoukala E, Mol D, Pappa S, Vlachos E, van Logchem W, Vaxevanopoulos M and Reumer J 2011 *Quatern. Int.* 245 339-49
[9] Lazaridis G 2006 *Scientific Annals, School of Geology, Aristotle University, Proc. of the 12th Int. Cave Bear Symp.* (Thessaloniki and Aridaia, GR, 2-5 November 2006) vol 98 ed E Tsoukala and G Rabeder (Thessaloniki: Aristotle University) pp 33-40
[10] Tsoukala E, Chatzopoulou K, Rabeder G, Pappa S, Nagel D and Withalm G 2006 *Scientific Annals, School of Geology, Aristotle University of Thessaloniki, Proc. of the 12th Int. Cave*
Bartoll J, Röhrs S, Erko A, Firsov A, Bjeoumikhov A and Langhoff N 2004 Spectrochim Acta B 
59 1587 -92
[12] Ankudinov A L, Ravel B, Rehr J J and Conradson S D 1998 Phys. Rev. B 58 7565–76
[13] Newville M, Ravel B, Haskel D, Rehr J J, Stern E A and Yacoby Y 1995 Physica B 208&209 
154–6
[14] Zougrou I M, Katsikini M, Pinakidou F, Paloura E, Papadopoulou L and Tsoukala E 2014 J 
Synchr. Rad 21 149-60
[15] Wilke M, Farges F, Petit P E, Brown Jr G E and Martin F 2001 Am. Mineral. 86 714–30.
[16] Westre T E, Kennepolh P, DeWitt J G, Hedman B, Hodgson K O and Solomon E I 1997 J. Am. 
Chem. Soc. 119 6297–314
[17] Manceau A and Drits V A 1993 Clay Miner. 28 165–84
[18] Nordstrom D K 1982 Aqueous Pyrite Oxidation and the Consequent Formation of Secondary 
Iron Minerals in Acid Sulfate Weathering ed J A Kittrick, D S Fanning et al. (Madison - Soil 
Science Society of Amerika) chapter 3 pp 37-56
[19] Huggins F E, Huffman G P, Kosmack D. A. and Lowenhaupt D. E. 1980 Internat. J. Coal Geol. 
1 75-81
[20] Andreeva D, Mitov I, Tabakova T and Andreev A 1994, J. Mater. Sci Mater.5 168-172
[21] Krishnamurti G S R and Huang P M, 1991 Clay Clay Miner. 39 28-34
[22] Korshin G V, Chang H S, Frenker A I and Ferguson J F 2007 Environ. Sci. Technol. 41 2560-5
[23] Manceau A, Gorshkov A I and Drits V A 1992 Am. Mineral. 77 1133–43