Deciphering Exceptional Preservation of Fossils Through Trace Elemental Imaging

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Introduction

The fossil record consists essentially of biomineralized remains of shelly invertebrates and skeletons of vertebrates, but rare "soft-bodied" fossils have also been preserved over time. Soft-bodied organisms normally degrade too fast to become fossilized, but such fossils have been conserved in an exceptional preservation state over millions of years in specific deposits called *Konservat-Lagerstätten*. Exceptional preservation may include the preservation of complete organisms, hard-part mineralogy, detailed morphology of soft tissues at cellular or subcellular levels, and organic molecules or fragments. Decay-prone tissues can be preserved in the fossil record either in an altered organic form or as replicated authigenic minerals, commonly calcium phosphates through *phosphatization* [1]. For such soft tissue preservation to occur, processes normally involved during degradation had to be dramatically slowed or arrested soon after death [2–3]. Despite much effort in the past decades, processes governing exceptional preservation processes remain poorly understood.

Detailed knowledge of the chemical composition of fossils, including at trace levels, is likely to provide crucial information on the chemical and structural changes that may affect a specimen after burial. This has led to interdisciplinary efforts involving paleontologists, geochemists, and physicists who use and adapt cutting-edge analytical and microscopic techniques on fossils, for example: new sequencing approaches that triggered the hunt for ancient DNA in fossil humans or in a woolly mammoth found in the Scandinavian permafrost; computed X-ray tomography that is now commonly used to study the internal structure of fossils; and extraction of information about them from amber. In addition, recent synchrotron-based microscopic techniques have produced several unexpected discoveries regarding the preservation of ancient specimens (see [4, 5] for detailed reviews).

Among the new analytical tools available to paleontologists, synchrotron-based X-ray fluorescence (XRF) has proved a particularly valuable tool. While a century has passed since Henry Moseley invented the technique of X-ray spectrometry for element identification, the first setups allowing XRF mapping were developed in the 1960s [6]. Since then, XRF mapping has vastly benefited

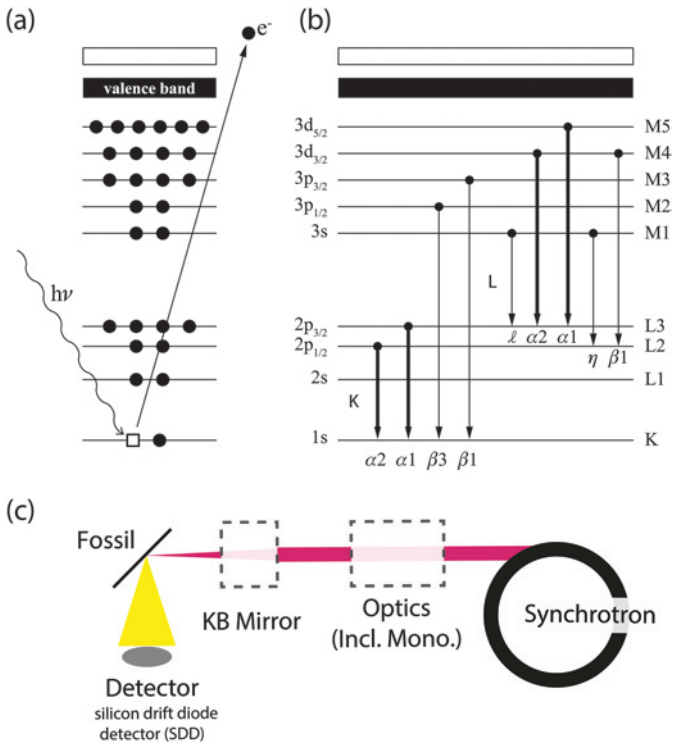


Figure 1: XRF principle. (a–b) Schematic representation of the photo-excitation process (a) and subsequent allowed XRF transitions (b), with the main emission lines in bold (after [4]). (c) Schematic experimental setup at the DIFFABS beamline, SOLEIL synchrotron, France.

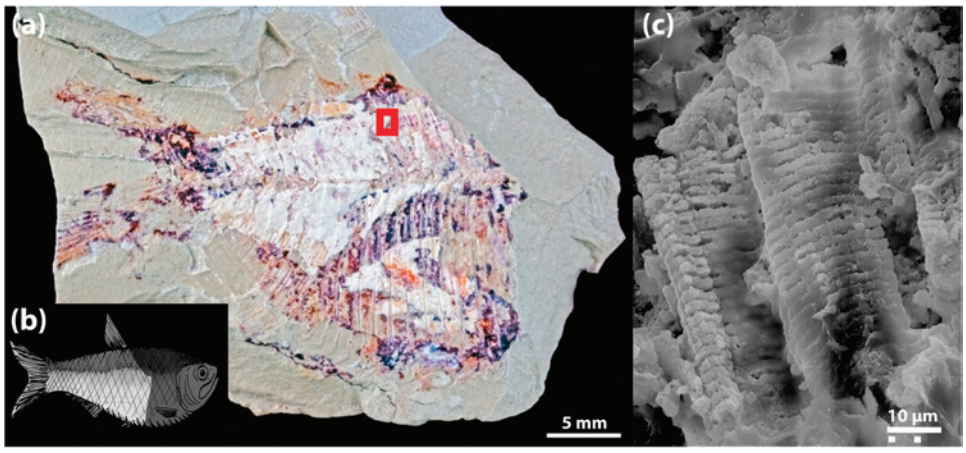
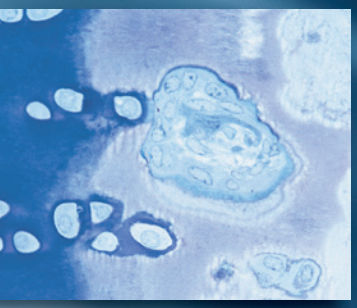


Figure 2: Optical photograph (a) and reconstruction (b) of the well-preserved clupeomorph fish *Diplomystus sp.* (Poi-SGM 10) from the Djebel Oum Tkout Lagerstätte (Upper Cretaceous, Kem Kem Beds, Morocco) exhibiting a high level of preservation, including finely mineralized muscles as shown by SEM imaging (c). The red box in (a) indicates where the SEM image in (c) was taken. Image courtesy of and modified with permission from DB Dutheil [16].



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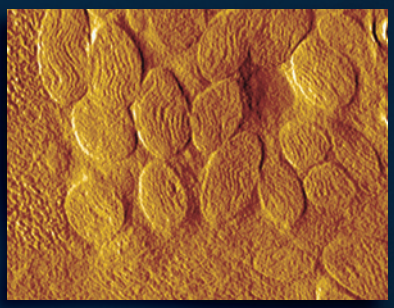

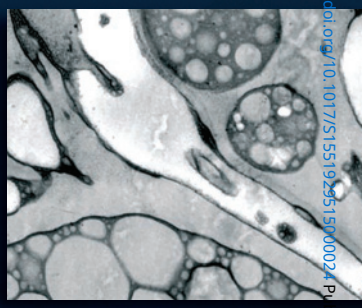

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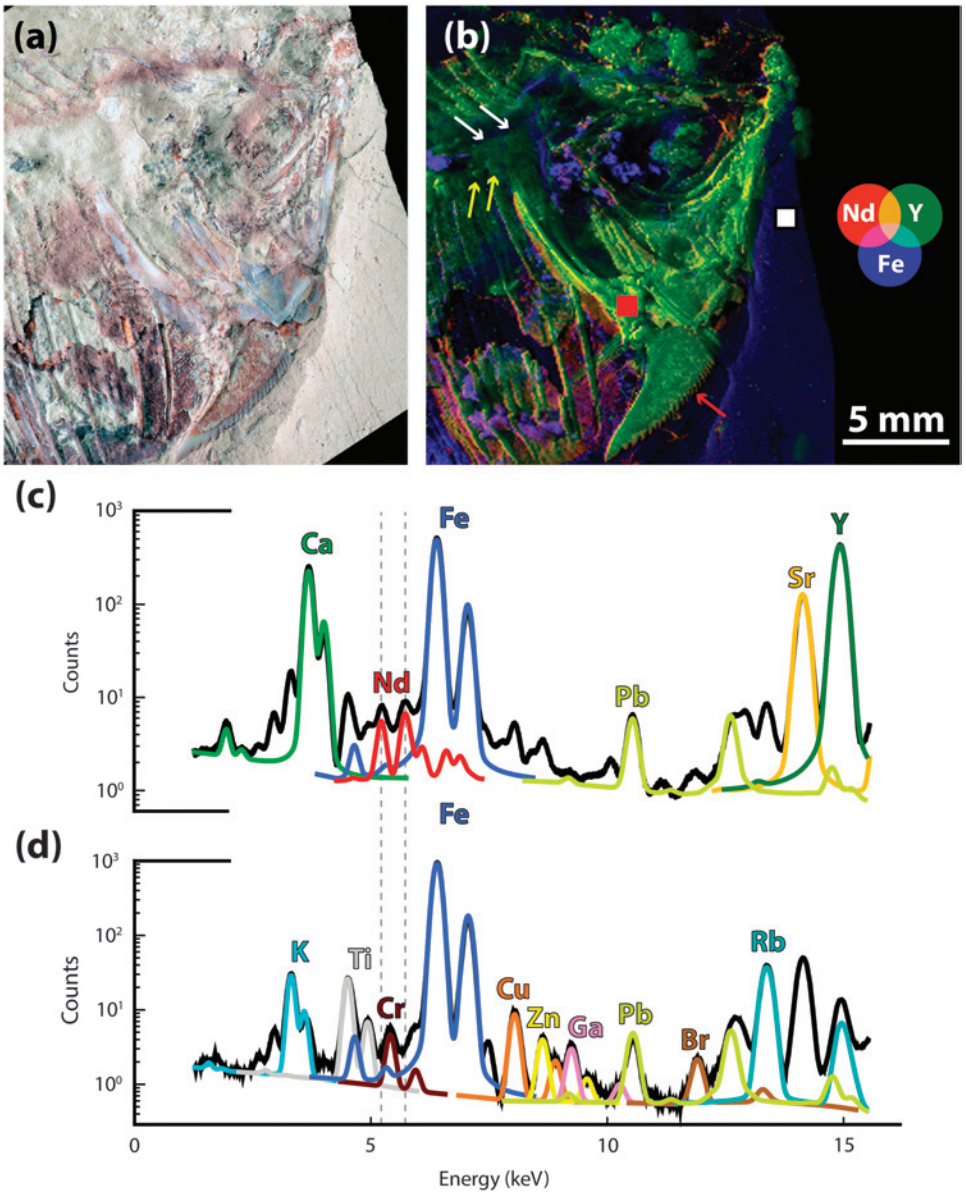

Figure 3: Synchrotron XRF mapping of major-to-trace elements of the anterior part (skull on the right) of a new fossil fish (MHNM-KK-OT 03a) from the Djebel Oum Tkout Lagerstätte (Upper Cretaceous, ~100 Myr, Morocco). Optical photograph (a) and false color overlays of iron (blue) and two REEs, neodymium (red) and yttrium (green), show distributions reconstructed from a full spectral decomposition of the data (b; modified from [13]). Acquisition parameters: 100 × 100 µm² scan step, 50,851 pixels. (c–d) Mean XRF spectra and main elemental contributions from the red (c) and white (d) box areas in (b) (100 pixels), respectively characteristic of fossil bone and the sedimentary matrix. Red, white, and yellow arrows in (b) respectively highlight the notched elongated bone, hidden vertebrae, and rib insertions.

from the increase in X-ray flux at synchrotron facilities and from the development of stable X-ray focusing optics, novel detectors, and new data collection strategies that minimize experimental dead times. Fast XRF mapping, based on the collection of integrated intensity in preselected spectral regions of interest, was used to map major and minor element concentrations, such as copper and zinc, in fossil bird feathers [7, 8]. The same group also studied lizard skins [9] and plants [10]. This article presents new methodological developments regarding trace elemental imaging in fossils, particularly focusing on strontium, yttrium, and the rare earth element (REE) series, known to be present in significant quantities in fossil bones and teeth, as well as sedimentary apatites [11]. REEs are also known to be critical proxies in paleoenvironment and taphonomic reconstructions because their fractionation depends on fossilization and diagenetic conditions [11–12].

Materials and Methods

Synchrotron XRF mapping. For the past twenty years, paleontologists have used point spectroscopy and mapping methods to analyze the elemental composition of flattened fossils. Scanning electron microscopy (SEM) can reveal surface anatomical details that are not evident under light microscopy. But while the spatial resolution of SEM X-ray emission spectroscopy is about 1 µm, this method is of limited interest for trace elemental imaging because the minimum elemental detection is in the range of 0.1 to 5 wt%, depending on the element analyzed. Laser ablation–inductively coupled plasma–mass spectrometry (LA-ICP-MS) allows microscale mapping of trace elements such as REEs in fossil bones, but local ablation of the area investigated precludes complementary characterization and use of this technique on rare specimens. Synchrotron-based XRF appears as a very promising tool to map trace elemental distributions in ancient fossils [7–10, 13]. XRF spectroscopy detects the secondary X-ray emission from atoms bombarded with X rays generated by an X-ray source, here a synchrotron (Figure 1a–1b). Radiative de-excitation results in lines in the emission spectrum that are characteristic of the chemical elements making up the sample. Used in raster-scanning mode, XRF allows microscale mapping of elements. In paleontology, synchrotron XRF mapping has previously been used in collecting integrated intensity in preselected spectral regions of interest, thereby allowing great analytical speed, but hampering trace REE mapping because most corresponding L-lines fall in an energy domain where K-lines from transition metals dominate the signal [13].

Data acquisition. Collection of full-range XRF spectra coupled with spectral decomposition for all constitutive elements, or a much faster statistical analysis based on the Kullback-Leibler divergence, which is a natural dissimilarities measure for probability distributions [14], was recently demonstrated to allow mapping a large set of trace elements, including REEs [13]. XRF maps were collected at the DIFFABS

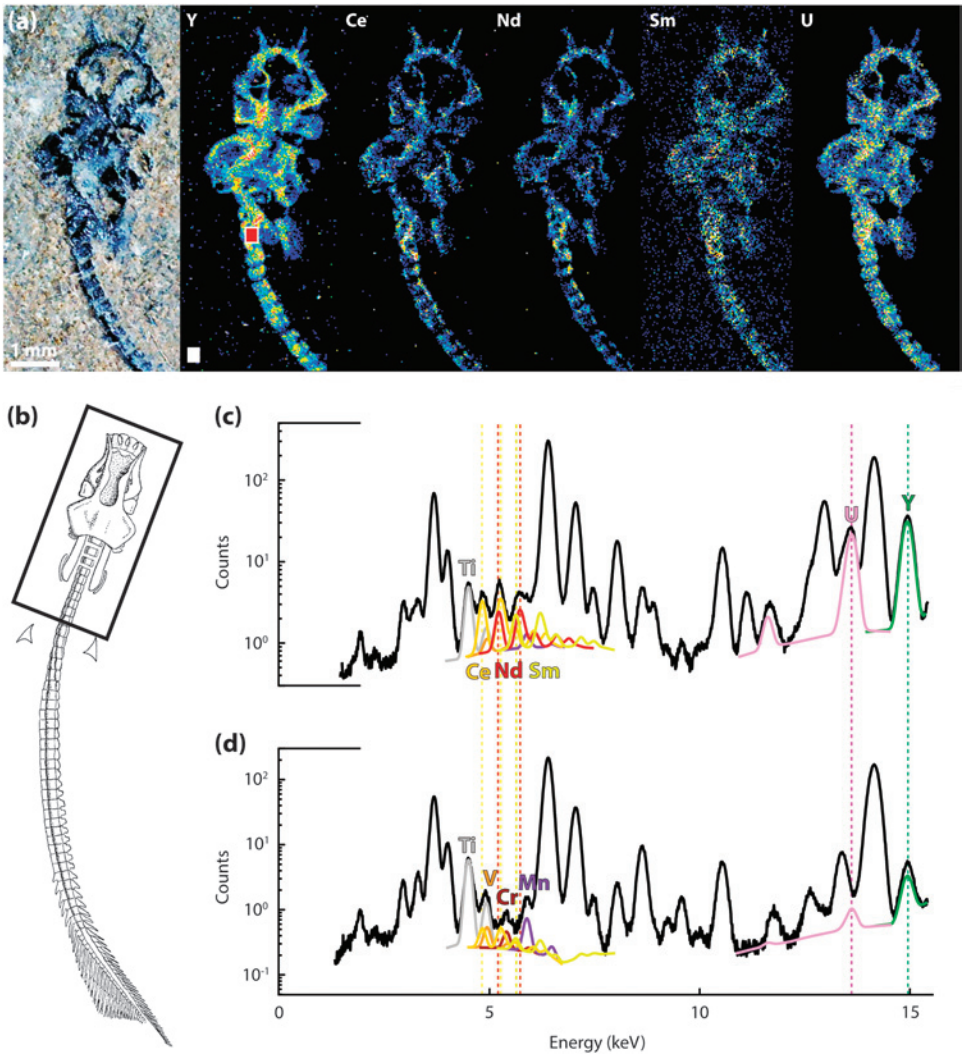

Figure 4: Synchrotron XRF mapping of trace elements in *Palaeospondylus gunni* (MNHN-GBP 92), an early vertebrate from the Middle Devonian (~390 Myr) of Scotland. (a) Light optical micrograph and distributions of yttrium; the REEs lanthanum, cerium, neodymium and samarium; and uranium that can be clearly mapped in this fossil (scan step: 30 × 30 µm², 28,231 pixels; distributions reconstructed from a full spectral decomposition of the data). (b) Reconstruction of the entire organism, with the box area corresponding to what is shown in (a). (c–d) Mean XRF spectra from the red (c) and white (d) box areas in the Y map (168 pixels), respectively characteristic of the fossil and the sedimentary matrix, showing contributions from transition metals and the elements mapped in (a).

beamline (SOLEIL synchrotron, France), at an excitation energy of 17.2 keV, selected for excitation of K-lines from phosphorous to yttrium and L-lines from cadmium to lead. The X-ray beam was collimated by 2 bendable mirrors, monochromatized using a Si(111) double-crystal monochromator, and focused using Kirkpatrick-Baez mirrors down to a spot size of 11 × 7 µm². The sample was mounted on a *xyz* scanner stage, allowing ±12 mm movements with better than 500 nm accuracy. The sample was oriented at 45° to the incident beam and at 45° to the XRF detector, a silicon drift detector (SDD), placed in the horizontal plane (Figure 1c). Counting time per pixel was set to 500 ms to attain good statistics on trace elements at this energy. All the elemental distributions presented herein have been reconstructed from a full spectral decomposition performed with the PyMCA data-analysis software [15] using batch-fitting procedure, Pseudo-Voigt peak shape, and polynomial baseline subtraction.

Results

This approach was applied to a series of flattened fossils as old as the middle Devonian (~390 Myr [million years ago]). In particular, the specimens studied included exquisitely preserved fish and shrimp from the late Cretaceous (~100 Myr) of Morocco, which display finely mineralized muscles observed by SEM imaging (Figure 2). The elemental distributions that were obtained, greatly improved the discrimination of hard tissues (bones, carapaces, or cuticles) from both the sedimentary matrix and the fossilized soft tissues (muscles) on the basis of variations in relative elemental concentrations (Figures 3 and 4). For instance, in a newly discovered teleost fish of unknown affinities bearing a large, notched elongated bone unique to the fossil record, the technique revealed certain bones, such as the entire skull together with vertebrae and rib insertions, concealed under a fine layer of unpreparable clay (Figure 3). Although this fossil is the only teleost ever reported to display such a peculiar bone, and therefore a yet undescribed taxon, most critical features of the skull, fins, vertebrae, and rib insertions remained hidden under the sediment, precluding an accurate description of this new genus and species. In *Palaeospondylus*, a mysterious, fish-like fossil vertebrate of highly debated affinities from the middle Devonian of Scotland (~390 Myr), the overall fossil morphology is easily distinguishable by REEs mapping, but also by mapping traces of actinides such as uranium (Figure 4).

Discussion

Visualization of as-yet unobserved anatomical details. Such an approach is particularly suited to flattened fossils given that X rays will penetrate the fossil to a depth of a several tenths of a millimeter (about 250 µm and 300 µm for strontium and yttrium, respectively). Comparing fluorescent lines from several elements allows tuning the probed depth and distinguishes, to some extent, surface from subsurface. Thus, synchrotron XRF mapping makes it possible to obtain a detailed, accurate view of the anatomy of a fossil without the need for prior delicate sample preparation and therefore appears to be a useful complement to light microscopy and SEM in visualizing anatomical features of fossils that are hidden under well-preserved decay-prone tissues or a sedimentary matrix that is not able to be prepared. The great advantages of the method are its non-destructiveness and its

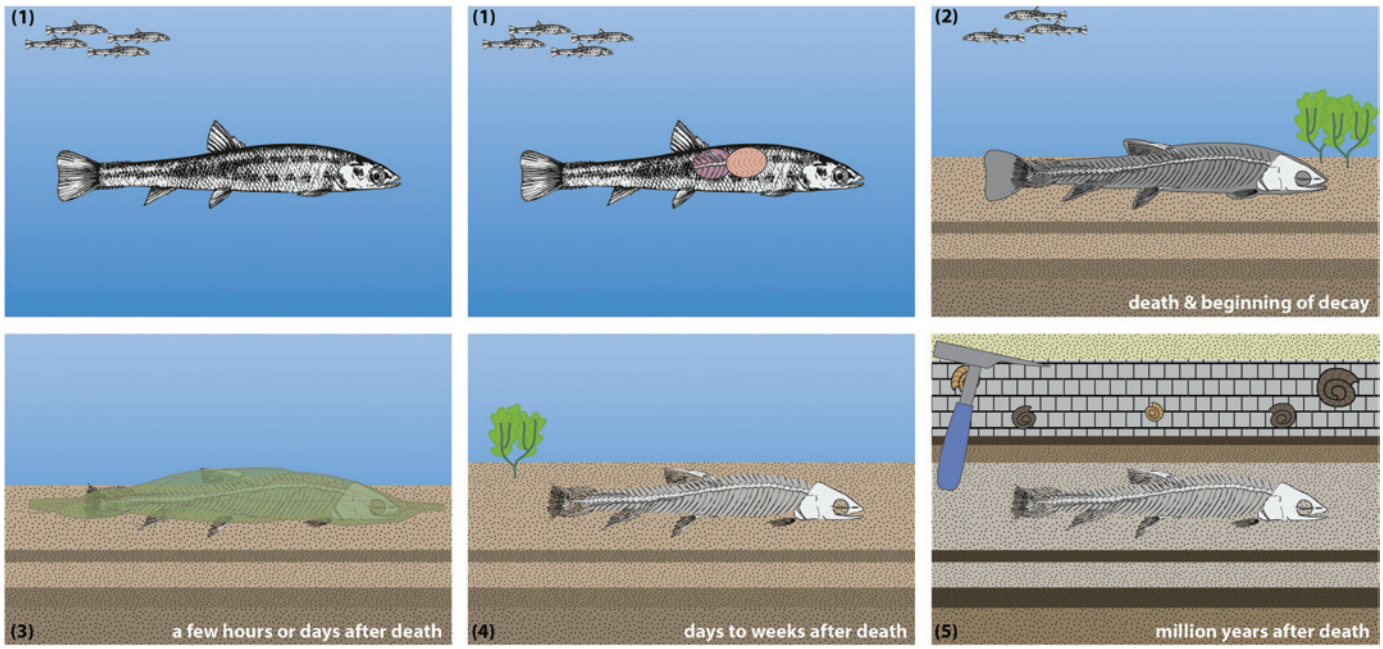

Figure 5: Schematic representation of the fossilization pathway in the Djebel Oum Tkout Lagerstätte, showing the acantomorph fish *Spinocaudichthys oumtkoutensis*. During fish life, bone apatite composition is controlled by biogenic uptake (1). After death, decay usually removes all soft parts (2), but in exceptional cases, soft tissues may be preserved in apatite minerals, here through microbially mediated phosphatization under a microbial mat that quickly forms around the carcass (3). Some days to weeks after death, the composition of bone apatite and soft tissue authigenic apatite is controlled by local taphonomic conditions (4). A million years after death, apatite appears further enriched or depleted in various elements (5).

non-invasiveness: no delicate sample preparation is needed beyond the preliminary usual cleavage of the sediment blocks on the field, as well as a better elemental detectability than SEM X-ray emission spectroscopy. It should therefore be highly beneficial for the study of those unique witnesses of ancient traces of life on Earth that are fossils from Lagerstätten, when compression during fossilization makes interpretation harder. The method can also be informative at the histological scale in higher resolution maps, that is, with a smaller step size between pixels and lower X-ray information depth, that is, in conditions where the XRF signal escapes from a limited depth (such as by collecting signal from lighter elements or lower energy lines).

Insights into local conditions of burial. Trace elements such as strontium, yttrium, and particularly REEs, have provided useful information on the provenance and environment for many geochemical samples such as rocks, waters, and minerals, including bone bioapatite and other apatite group minerals, as they simultaneously reflect the connectivity of the environmental water network, the local redox, the specific surface area of the bioapatite nanocrystals, the physico-chemical conditions, and the properties of substituted apatite [11, 17–19]. These elements were shown to be present in significant quantities in fossil bones and teeth (exceeding 100 ppm), a range straightforwardly detectable with synchrotron XRF techniques, whereas they are encountered *in vivo* in the lower ppt (parts per trillion) to ppb (parts per billion) range. Indeed, REEs concentration is known to increase by three to four orders of magnitude within thousands of years in fossil bones through intake from the fossilization context. REEs produce trivalent ions that can readily substitute for Ca^{2+} isomorphously in apatite minerals. Phosphatized soft tissues in well-preserved fossils are found in apatite minerals and also incorporate trace elements during diagenesis. However, unlike bone apatite, biogenic uptake in soft tissues is very limited during the life of the animal. In contrast, days to weeks after death, apatite in the bones and in the mineralized soft tissues incorporate trace elements depending on the local taphonomic conditions. Both will be further enriched or depleted in these elements during the millions of years up to present time (Figure 5). Contrasted elemental signatures therefore evidence differences in sorption and/or substitution rates, as well as initial composition, and can therefore provide relevant information on the fossilization and diagenesis processes at the sites.

Future directions. Besides morphological information, processed XRF spectra provide semi-quantitative elemental contents. Because it is possible to image numerous trace elements, including most of the lanthanides and some actinides (Figure 4), synchrotron-based XRF mapping can be used to draw local REE fractionation patterns, that is, the relative abundances of REEs normalized by proper reference materials. REE patterns are usually established from point quantification and profiling, whereas XRF techniques lead generally to limited radiation-induced side effects [20]. Such local quantification of REEs may therefore open new avenues for taphonomic and paleoenvironmental studies.

Conclusion

Synchrotron-based XRF mapping appears to be an efficient tool for obtaining critical morphological and chemical information at microscale on flat fossils for taxonomic, phylogenetic, paleoenvironmental, and taphonomic studies. These new developments are expected to provide significantly more accurate description of fossil characters, better understanding of fossilization processes, and gain of paleoenvironmental information from trace elemental fractionation patterns.

References

[1] DEG Briggs, *Annu Rev Earth Planet Sci* 31 (2003) 275–301.
[2] DM Martill, *J Open Univ Geol Soc* 10 (1989) 4–10.
[3] MH Schweitzer, *Annu Rev Earth Planet Sci* 39 (2011) 187–216.
[4] L Bertrand et al., *Phys Rep* 519(2) (2012) 51–96.
[5] DEG Briggs and RE Summons, *BioEssays* 36(5) (2014) 482–90.
[6] J Campbell et al., *Talanta* 15(11) (1968) 1205–16.
[7] U Bergmann et al., *PNAS* 107 (2010) 9060–65.
[8] R Wogelius et al., *Science* 333 (2011) 1622–26.
[9] NP Edwards et al., *Proc R Soc B* 278 (2011) 3209–18.
[10] NP Edwards et al., *Metallomics* 6 (2014) 774–82.
[11] D Herwartz et al., *Geochim Cosmochim Ac* 103 (2013) 161–83.
[12] CN Trueman and MJ Benton, *Geology* 25 (1997) 263–66.
[13] P Gueriau et al., *PLoS One* 9 (2014) e86946.
[14] S Kullback and RA Leibler, *Ann Math Stat* 22 (1951) 79–86.
[15] V Solé et al., Spectrochim. *Acta B* 62 (2007) 63–68.
[16] DB Dutheil, *Mesozoic fishes* 2 (1999) 553–63.
[17] L Kocsis et al., *Geochim Cosmochim Ac* 74 (2010) 6077–92.
[18] CN Trueman et al., *Palaeogeogr Palaeoclimatol Palaeoecol* 310 (2011) 124–32.
[19] CN Trueman, *Palaeontology* 56 (2013) 475–86.
[20] L Bertrand et al., *Trends Anal Chem* 66 (2015) 128–45.

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