APPLICATIONS OF SYNCHROTRON RADIATION TECHNIQUES TO THE STUDY OF TAPHONOMIC ALTERATIONS AND PRESERVATION IN FOSSILS

A Thesis

Submitted to the Faculty of Graduate Studies and Research

In Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy

in

Physics

University of Regina

by

Anezka Popovski Kolaceke

Regina, Saskatchewan

February, 2019

Copyright 2019: A. P. Kolaceke
Anezka Popovski Kolaceke, candidate for the degree of Doctor of Philosophy in Physics, has presented a thesis titled, *Applications of Synchrotron Radiation Techniques to the Study of Taphonomic Alterations and Preservations in Fossils*, in an oral examination held on December 21, 2018. The following committee members have found the thesis acceptable in form and content, and that the candidate demonstrated satisfactory knowledge of the subject material.

External Examiner: *Dr. Phil Bell, University of New England*

Co-Supervisor: Dr. Mauricio Barbi, Department of Physics

Co-Supervisor: Dr. Ryan McKellar, Department of Physics

Committee Member: Dr. Josef Buttigieg, Department of Biology

Committee Member: **Dr. Maria Velez Caicedo, Department of Geology**

Committee Member: Dr. Garth Huber, Department of Physics

Chair of Defense: Dr. Fanhua Zeng, Faculty of Graduate Studies & Research

*via ZOOM Conferencing
**Not present at defense*
Abstract

Fossils have traditionally been seen as sedimentary rocks that preserve little of the original composition of animals, except for their shapes, and perhaps some original material from recalcitrant mineralized structures, such as bones, and teeth. However, recent studies have shown that not the case. Researchers have identified preserved organic molecules, such as collagen and melanosomes, as well as mineralized soft tissues, including feathers, muscle tissue and skin, tens of millions of years after the animal’s death. These results have improved our understanding of extinct species, and have been obtained using a variety of characterization techniques, including the synchrotron-based approaches that are the focus of the research presented in this thesis. The main goal of the research discussed in this thesis was the application of synchrotron radiation techniques (X-ray fluorescence and X-ray absorption spectroscopy, in particular) in order to determine the taphonomic alterations that fossils experience, and examine how different materials are preserved.

In this thesis, I discuss the results of the chemical characterization on the remains of the *Tyrannosaurus rex* known as “Scotty”, turtle shells, and a rare specimen of fossilized hadrosaur skin. I also examine the applicability of X-ray fluorescence to determine the composition and elemental distribution of insect inclusions in amber. The results presented herein offer possible explanations on how some of these specimens were preserved and the extent of the chemical alterations they underwent during their taphonomic history.

Beyond the specific results for each specimen, the overall research presented in this thesis shows that synchrotron radiation techniques have great potential to advance palaeontological research, as it becomes necessary to evaluate the chemistry of specimens in high resolution. These characterization techniques were able to confirm that more original material is preserved after fossilization than would have been believed possibly even a decade ago.
Acknowledgements

I would like to thank my supervisors, Mauricio Barbi and Ryan McKellar, for all the help and advice during the development of this project, for the suggestions and corrections on this thesis, and for all other learning opportunities I was offered. I would also like to thank the members of my Ph. D. committee for all the suggestions and comments throughout the last years. All the help and discussions were fundamental for the development of this thesis.

I am grateful for the support from the Physics Department at the University of Regina and all faculty members, students and staff, and the Faculty of Graduate Studies and Research for the funding through a Graduate Research Fellowship, teaching assistantships and other scholarships and awards I received during my studies. I would also like to thank all the Royal Saskatchewan Museum staff who helped in the selection and preparation of samples and received me so well every time I visited, specially Wes Long.

The research described in this thesis was performed at the Canadian Light Source. I would like to thank all the beamline scientists and CLS staff that were always so helpful and friendly. I also acknowledge the receipt of support from the CLS Graduate Student Travel Support Program.

I thank all the researchers that co-authored manuscripts with me for this research, including Maria Velez, Ian Coulson, Tim Tokaryk, and my supervisors. I am also grateful to all the comments by reviewers and editors from the submitted papers.

Finally, I would like to express my gratitude to my family and friends for their patience and emotional support. You are part of the reason I was able to finish this thesis.
Contents

List of Tables viii

List of Figures ix

1 Introduction and thesis structure 1

2 Taphonomy 4

   2.1 Abstract ................................................. 4
   2.2 Introduction ........................................... 4
   2.3 Taphonomy of vertebrates ................................. 5
       2.3.1 Decay ............................................. 5
       2.3.2 Physical processes .................................. 6
           Weathering ......................................... 6
           Transport ........................................... 8
           Time-Averaging ..................................... 12
           Bioturbation ....................................... 13
       2.3.3 Chemical processes .................................. 14
           Silicification ...................................... 14
           Pyritization ....................................... 15
           Phosphatization .................................... 17
   2.4 Preservation in vertebrates ............................... 18
       2.4.1 Bones ............................................. 18
       2.4.2 Soft-tissues ...................................... 19
       2.4.3 Integument ....................................... 20
   2.5 Special preservation ...................................... 22
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5.1 Konzentrat-Lagerstätten</td>
<td>23</td>
</tr>
<tr>
<td>2.5.2 Konservat-Lagerstätten</td>
<td>23</td>
</tr>
<tr>
<td>Preservation in amber</td>
<td>25</td>
</tr>
<tr>
<td>2.6 Conclusions</td>
<td>29</td>
</tr>
<tr>
<td>3 Synchrotron light sources and techniques</td>
<td>31</td>
</tr>
<tr>
<td>3.1 Abstract</td>
<td>31</td>
</tr>
<tr>
<td>3.2 Introduction</td>
<td>31</td>
</tr>
<tr>
<td>3.3 Synchrotron light sources</td>
<td>33</td>
</tr>
<tr>
<td>3.3.1 Acceleration</td>
<td>33</td>
</tr>
<tr>
<td>3.3.2 Beam focusing</td>
<td>36</td>
</tr>
<tr>
<td>3.3.3 Generating radiation</td>
<td>37</td>
</tr>
<tr>
<td>3.4 X-ray fluorescence (XRF)</td>
<td>40</td>
</tr>
<tr>
<td>3.4.1 Theory and techniques</td>
<td>40</td>
</tr>
<tr>
<td>Spatially resolved XRF</td>
<td>41</td>
</tr>
<tr>
<td>XRF mapping</td>
<td>42</td>
</tr>
<tr>
<td>3.4.2 Quantitative analysis</td>
<td>44</td>
</tr>
<tr>
<td>3.5 X-ray absorption fine structure (XAFS)</td>
<td>46</td>
</tr>
<tr>
<td>3.5.1 The XANES spectra</td>
<td>48</td>
</tr>
<tr>
<td>Edge</td>
<td>49</td>
</tr>
<tr>
<td>Pre-edge</td>
<td>49</td>
</tr>
<tr>
<td>Post-edge</td>
<td>50</td>
</tr>
<tr>
<td>3.5.2 Qualitative and semi-quantitative analysis</td>
<td>50</td>
</tr>
<tr>
<td>Edge shift</td>
<td>50</td>
</tr>
<tr>
<td>Linear combination analysis (LCA)</td>
<td>50</td>
</tr>
<tr>
<td>Principal component analysis (PCA)</td>
<td>51</td>
</tr>
<tr>
<td>Deconvolution of XANES features</td>
<td>51</td>
</tr>
<tr>
<td>3.5.3 Quantitative analysis</td>
<td>51</td>
</tr>
</tbody>
</table>
4 Chemical diagenesis of *Tyrannosaurus rex* bones from the Frenchman Formation

4.1 Abstract

4.2 Introduction

4.2.1 Scotty, the Saskatchewan *T. rex*

4.3 Material and methods

4.4 Results

4.5 Discussion

4.6 Conclusions

5 Non-destructive chemical analysis of insect inclusions in amber

5.1 Abstract

5.2 Introduction

5.3 Material and methods

5.3.1 Specimens and preparation

5.3.2 Measurements and analyses

5.4 Results

5.4.1 XRF applied to insect inclusions

5.4.2 Preservation of ant inclusions in Baltic amber

5.5 Discussion

5.5.1 XRF applied to insect inclusions

Modern ants

Baltic amber

North Carolina amber
5.5.2 Preservation of ant inclusions in Baltic amber . . . . . . . . . 106
5.6 Conclusions . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 109

6 Chemical diagenesis of turtle shells 112
6.1 Abstract . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 112
6.2 Introduction . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 113
6.3 Material and methods . . . . . . . . . . . . . . . . . . . . . . . . . . 115
6.4 Results . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 117
6.5 Discussion . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 127
6.6 Conclusions . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 132

7 Chemical diagenesis of individual structures in hadrosaurid skin
layer 134
7.1 Abstract . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 134
7.2 Introduction . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 135
7.3 Material and methods . . . . . . . . . . . . . . . . . . . . . . . . . . 138
    7.3.1 Specimen and geological settings . . . . . . . . . . . . . . . . 138
    7.3.2 Methodology . . . . . . . . . . . . . . . . . . . . . . . . . . . . 139
7.4 Results . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 142
7.5 Discussion . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 150
7.6 Conclusions . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 154

8 Synchrotron applied to taphonomic studies 155
8.1 Abstract . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 155
8.2 Introduction . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 155
8.3 Results . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 155
8.4 Discussion . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 163
    8.4.1 Relative concentrations . . . . . . . . . . . . . . . . . . . . . . 163
    8.4.2 Correlation plots . . . . . . . . . . . . . . . . . . . . . . . . . . 165
List of Tables

1. List of specimens analyzed. 87
2. Data acquisition parameters for insect inclusion samples used in XRF measurements. 88
3. Elemental maps data acquisition parameters for turtle samples at the VESPERS beamline. 117
4. Energy range and dwell time for the STXM measurements of different elements. 141
5. Calcium peaks for each spectrum in fig. 56. 146
6. Carbon peaks for each spectrum in fig. 58. 148
7. Relative areas of Fe, Mn, Sr and Y relative to Ca for dinosaur bones and turtle shells used in the research presented in previous chapters. 156
## List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Principle of focusing.</td>
</tr>
<tr>
<td>2</td>
<td>Energy levels in an atom.</td>
</tr>
<tr>
<td>3</td>
<td>Strontium XAFS spectrum with XANES region selected. Also represented the different regions in XANES spectrum: pre-edge, edge and post-edge (multiple scattering region). (Original in colour)</td>
</tr>
<tr>
<td>4</td>
<td>“Bone anatomy”. Modified from [139]. Licensed under the terms and conditions of the Creative Commons Attribution (CC BY). (Original in colour).</td>
</tr>
<tr>
<td>5</td>
<td>Microscope images of a) <em>T. rex</em> rib (RSKM_P2523.8), b) <em>T. rex</em> rib (RSKM_P2523.8), c) <em>T. rex</em> vertebra (RSKM_P2523.8), d) hadrosaur tendon (RSKM_P2610.1), and e) swan femur (RSKM_A-8637). Braces show the general region mapped in each sample, which corresponds in all fossils to the region in the interface between bone and sedimentary matrix. Blue arrows show the region with bone, while red arrows shows regions with sedimentary matrix in the samples. The scale bars correspond to 3 mm. (Original in colour).</td>
</tr>
<tr>
<td>6</td>
<td>XRF elemental maps of <em>T. rex</em> rib (RSKM_P2523.8), showing transition region from outer cortical bone (left side) to sediment (right side), separated by Mn layer. These maps were measured in steps of 5.0 x 5.0 µm (total area of 880.0 x 219.8 µm) and 2.0 s per point. The colours representing each element are assigned to the right of the respective maps. (Original in colour).</td>
</tr>
</tbody>
</table>
XRF elemental maps of *T. rex* rib (RSKM_P2523.8), showing transition region from sediment (left side) to outer cortical bone (right side), separated by Mn layer. These maps were measured in steps of 10.0 x 10.0 µm (total area of 500 x 400 µm) and 2.0 s per point. The colours representing each element are assigned below the respective maps. (Original in colour) ........................................ 69

XRF elemental maps of *T. rex* vertebra (RSKM_P2523.8), showing transition region from sediment (bottom) to outer cortical bone (top). These maps were measured in steps of 20.0 x 20.0 µm (total area of 900 x 2100 µm) and 5.0 s per point. The colours representing each element are assigned below the respective maps. (Original in colour) ........... 70

XRF elemental maps of hadrosaur tendon (RSKM_P2610.1), showing transition from bone (top) to sediment (bottom). These maps were measured in steps of 5.0 x 5.0 µm (total area of 700 x 750 µm) and 2.0 s per point. The colours representing each element are assigned below their respective maps. (Original in colour) ...................... 71

XRF elemental maps of recent swan femur cortical bone (RSKM_A-8637). These maps were measured in steps of 5.0 µm (total area of 400 x 300 µm) and 2.0 s per point. The colours representing each element are assigned below their respective maps. (Original in colour) ....... 71

Average normalized spectra of the bone region in *T. rex*, hadrosaur and swan bones with selected peaks labeled. *T. rex* rib from fig. 6 is represented in black, rib from fig. 7 in red, hadrosaur in blue, and swan in green. (Original in colour) ...................... 72
12 XANES measurements for a *T. rex* bone (RSKM_P2523.8) still attached to its surrounding sediment for a) calcium, b) sulfur, c) iron and d) strontium. Arrows show some of the regions of the spectra where differences between measurements can be seen. (Original in colour).

13 Pre-edge fitting for the Fe XANES of *T. rex* bone (RSKM_P2523.8), using an ERF function to fit the edge and a pseudo-voigt function to fit the pre-edge peak. a) shows the data (blue), fitting (red) and residual (x10, in green), b) shows the data (blue), and fitting components (red; the pseudo-voigt is shown with a solid line and ERF function with a dashed line). (Original in colour).

14 Pre-edge fitting for the Fe XANES of the transition between *T. rex* bone and sediment (RSKM_P2523.8), using an ERF function to fit the edge and a pseudo-voigt function to fit the pre-edge peak. a) shows the data (blue), fitting (red) and residual (x10, in green), b) shows the data (blue), and fitting components (red; the pseudo-voigt is shown with a solid line and ERF function with a dashed line). (Original in colour).

15 Pre-edge fitting for the Fe XANES of sediment surrounding the *T. rex* bone (RSKM_P2523.8), using an ERF function to fit the edge and a pseudo-voigt function to fit the pre-edge peak. a) shows the data (blue), fitting (red) and residual (x10, in green), b) shows the data (blue), and fitting components (red; the pseudo-voigt is shown with a solid line and ERF function with a dashed line). (Original in colour).

16 Elemental maps of Ca, Cl, Fe and K for RSKM P3314.1 compared to its microscope image. (Original in colour).
<table>
<thead>
<tr>
<th>Page</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Elemental maps of Ca, Cl, Fe and K for RSKM P3314.2. (Original in colour)</td>
</tr>
<tr>
<td>18</td>
<td>Iron elemental distribution (top, split into two scans that correspond to the head and thorax, and the abdomen) for Baltic amber ant RSKM P3000.15 compared to its microscope image (bottom). Arrows show examples of features that can be matched between elemental map and microscope image. (Original in colour)</td>
</tr>
<tr>
<td>19</td>
<td>Iron elemental distribution (left) for Baltic amber ant RSKM P3000.16 compared to its microscope image (right). White arrows show lines with high amount of iron due to the infiltration of minerals in cracks in the amber. (Original in colour)</td>
</tr>
<tr>
<td>20</td>
<td>Iron elemental distribution (left) for Baltic amber ant RSKM P3000.17 compared to its microscope image (right). (Original in colour)</td>
</tr>
<tr>
<td>21</td>
<td>Iron elemental map (left) for North Carolina amber SEMC NC 272-276 compared to its microscope image (right). In the elemental map the scale goes from blue to red, representing lower to higher concentrations of iron, respectively. White areas were not mapped. (Original in colour)</td>
</tr>
</tbody>
</table>
a) CT rendering (ventral view) and b) XRF results for Baltic ant P3000.15. The elemental maps show iron in red and calcium in green. The overlay of the images can be seen in c). In a), the arrows show the two surfaces visible in the image. The inner and outer surfaces are represented by the blue and red arrows, respectively. In c), the white arrow shows the regions where iron and calcium are present in large quantity in the insect’s head. The orange arrows show areas with higher iron concentrations in the insect’s abdomen. The blue arrow shows the region with large quantity of calcium in the insect’s abdomen. (Original in colour) .................................. 96

a) CT rendering (lateral view) and b) XRF results for Baltic ant P3000.16. The elemental map shows iron in red and calcium in green. The overlay of the images can be seen in c). (Original in colour) ... 97

a) CT rendering (ventral view) and b) XRF results for Baltic ant P3000.17. The elemental map shows iron in red and calcium in green. The overlay of the images can be seen in c). (Original in colour) ... 97

a) CT rendering of the head of Baltic amber ant RSKM P3000.15 (dorsal view) and b) the diagram of the preserved tissues observed in the rendering. In b), the cuticle reinforcements of the tentorium are represented in blue, mandibular muscles in pink, and traces of either brain or digestive glands are in white. ................................. 98
Iron K-edge XANES results for measurements taken from the head capsules of Baltic amber ants compared to a modern ant (RSKM P3314.1). The spectra were normalized and are presented a) together and b) stacked with 0.2 interval between different spectra. RSKM P3000.15 is shown in blue, RSKM P3000.16 in purple, RSKM P3000.17 in green, and RSKM P3314.1 in red. The arrows mark the position of the edge. (Original in colour) .......................... 100

Iron pre-edge fit for Baltic ant specimen RSKM P3000.15, showing a) fitting line and residual and b) a magnified image of the pre-edge region and the individual functions used in the fitting. (Original in colour) 100

Iron pre-edge fit for Baltic ant specimen RSKM P3000.16, showing a) fitting line and residual and b) a magnified image of the pre-edge region and the individual functions used in the fitting. (Original in colour) 101

Iron pre-edge fit for Baltic ant specimen RSKM P3000.17, showing a) fitting line and residual and b) a magnified image of the pre-edge region and the individual functions used in the fitting. (Original in colour) 101

Iron pre-edge fit for Baltic ant RSKM P3314.1, showing a) fitting line and residual and b) a magnified image of the pre-edge region and the individual functions used in the fitting. (Original in colour) 102

Elemental maps for Ravenscrag Fm. turtle (RSKM_P3314.1), which includes part of the shell and of a rib bone. Orange arrows show presence of strontium within marrow spaces in the shell. (Original in colour) ......................................................... 118

Elemental maps for Ravenscrag Fm. turtle (RSKM_P3314.2), which includes part of the shell and of a rib bone. (Original in colour) 118

Elemental maps for Ravenscrag Fm. turtle (RSKM_P3314.3), which includes part of the shell and of a vertebra. (Original in colour) 119
34 Elemental maps for Ravenscrag Fm. turtle (RSKM_P3314.4), which includes part of the shell. (Original in colour) 119

35 Elemental maps for Ravenscrag Fm. turtle (RSKM_P3314.5), which includes part of the shell. (Original in colour) 120

36 Elemental maps for Ravenscrag Fm. turtle (RSKM_P3314.6), which includes part of a pectoral girdle. (Original in colour) 120

37 Elemental maps for Ravenscrag Fm. turtle (RSKM_P3314.7), which includes part of a limb bone. (Original in colour) 121

38 Elemental maps for Frenchman Formation turtle (FF, RSKM_P3314.8), which includes part of the shell. Orange arrows indicate examples of regions within the marrow spaces with high amounts of iron. (Original in colour) 121

39 Elemental maps for Dinosaur Park Formation turtle (DPF, uncatalogued shell fragment), which only includes part of the cancellous bone region of the shell. (Original in colour) 122

40 Elemental maps for the modern turtle (MT, RSKM comparative collection), which only includes part of the cancellous bone region of the shell. (Original in colour) 122

41 Average spectra for 125 points in the external cortex, cancellous bone and rib of the Ravenscrag Fm. RSKM_P3314.1 turtle specimen in linear (left) and logarithmic (right) scale. Peaks for the elements discussed in this chapter are marked. The red line corresponds to external cortex (EC), black corresponds to cancellous bone (CB) and green corresponds to the section of rib bone (RB). (Original in colour) 123

xv
42 Average spectra for 125 points in the cancellous bone and vertebra (Vert) of the Ravenscrag Fm. RSKM_P3314.3 turtle specimen in linear (left) and logarithmic (right) scale. Peaks for the elements discussed in this chapter are marked. The black line corresponds to cancellous bone (CB) and green corresponds to the section of vertebra bone (Vert). (Original in colour) . . . . . . . . . . . . . . . . . . . . . . . . . . . . 124

43 Average spectra for 125 points in the external cortex, cancellous bone and internal cortex of the Ravenscrag Fm. RSKM_P3314.4 turtle specimen in linear (left) and logarithmic (right) scale. Peaks for the elements discussed in this chapter are marked. The red line corresponds to external cortex (EC), black corresponds to cancellous bone (CB) and blue corresponds to the internal cortex (IC). (Original in colour) . . . . . . . . . . . . . . . . . . . . . . . . . . . . 124

44 Average spectra for 125 points in the external cortex and cancellous bone of the RSKM_P3314.5 Ravenscrag Fm. turtle specimen in linear (left) and logarithmic (right) scale. Peaks for the elements being discussed in this chapter are marked. The red line corresponds to external cortex (EC) and black corresponds to cancellous bone (CB). (Original in colour) . . . . . . . . . . . . . . . . . . . . . . . . . . . . 125

45 Average spectra for 125 points in the external cortex and cancellous bone of the FF turtle specimen (RSKM_P3314.8) in linear (left) and logarithmic (right) scale. Peaks for the elements being discussed in this chapter are marked. The red line corresponds to external cortex (EC) and black corresponds to cancellous bone (CB). (Original in colour) . 125

xvi
Average spectra for 125 points in the cancellous bone for a specimen from each deposit and the modern turtle in linear (left) and logarithmic (right) scale. Peaks for the elements being discussed in this chapter are marked. The green line corresponds to the spectra of the modern turtle (MT; RSKM comparative collection), cyan corresponds to DPF (uncatalogued), orange corresponds to RF (RSKM_P3314.5), and purple corresponds to FF (RSKM_P3314.8). (Original in colour) ......................................................... 126

Average spectra for 125 points in the external cortex for a specimen from Ravenscrag Formation (RSKM_P3314.5) and the Frenchman Formation (RSKM_P3314.8) in linear (left) and logarithmic (right) scale. Peaks for the elements being discussed in this chapter are marked. The orange line corresponds to the spectra of RSKM_P3314.5 and purple corresponds to RSKM_P3314.8. (Original in colour) ......................................................... 126

Elemental map for calcium and manganese for the Ravenscrag Formation turtle RSKM_P3314.5 with a) the same saturation for Mn used in fig. 35 and b) the saturation changed so that the portion of the distribution with lower intensity is shown in more detail. (Original in colour) ......................................................... 128

Region (20 x 20 µm) of the skin sample associated with UALVP 53290 containing a layer of approximately with substructures (left) and the measured region (5 x 5 µm) containing one of these substructures (inset and to right). The images were obtained in transmission mode and, thus, dark areas correspond to detected matter. The scale bar corresponds to 0.5 µm in the images. (Original in colour) ......................................................... 140
50 Mapped region (5 x 5 μm) of hadrosaurid skin associated with UALVP 53290 in a) transmission mode and b) absorption (O. D.) mode. . . . 142

51 Calcium XAS spectra for different regions of the substructures found in the UALVP 53290 skin layer. The colour of each spectrum (right) corresponds to the colour of the region in the image (left), where white areas represent a strong signal and black represents no signal. The vertical line represents the energy in the plot for which the image in the left was taken. (Original in colour) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 142

52 Carbon and potassium XAS spectra for different regions of the substructures found in the UALVP 53290 skin layer. The colour of each spectrum (right) corresponds to the colour of the region in the image (left), where white areas represent a strong signal and black represents no signal. The vertical line represents the energy in the plot for which the image in the left was taken. None of the spectra found in this image show the presence of potassium. (Original in colour) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 143

53 Iron XAS spectra for different regions of the substructures found in the UALVP 53290 skin layer. The colour of each spectrum (right) corresponds to the colour of the region in the image (left), where white areas represent a strong signal and black represents no signal. The vertical line represents the energy in the plot for which the image in the left was taken. Only the dark green spectrum had any identifiable iron. (Original in colour) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 143
Manganese XAS spectra for different regions of the substructures found in the UALVP 53290 skin layer. The colour of each spectrum (right) corresponds to the colour of the region in the image (left), where white areas represent a strong signal and black represents no signal. The vertical line represents the energy in the plot for which the image in the left was taken. Manganese was not found in any region of the measured area. (Original in colour) . . . . . . . . . . . . . . . . . . . 144

Map showing the distribution of the two different states of calcium in fossil skin associated with UALVP 53290, where a) shows the distribution of the spectrum found in red in fig. 51 (calcium 1), b) shows the distribution of the other spectrum (calcium 2) and c) shows a combination of both distributions. The circle in a) shows the approximated region where the substructure is believed to be. The arrow shows the region of strong calcium 1. (Original in colour) . . . . 145

Average spectra of calcium 1 (blue) and calcium 2 (red) in fossil skin associated with UALVP 53290, calculated based on the regions where the spectra are found. Blue arrows point at the main peaks of the spectra, while black arrows point at the secondary peaks. The red arrow points at detail at the second main peak, where two “bumps” can be observed. (Original in colour) . . . . . . . . . . . . . . . . . . . 145

Map showing the distribution of the three different states of carbon in fossil skin associated with UALVP 53290, where a) shows the distribution of carbon-1, b) shows the distribution of carbon-2, c) shows the distribution of carbon-3 and d) shows a combination of all distributions. (Original in colour) . . . . . . . . . . . . . . . . . . . 147
58  Average spectra of carbon 1, 2 and 3 in fossil skin associated with UALVP 53290, calculated based on the regions where the spectra are found. (Original in colour) .............................................. 147

59  Map showing the distribution of the only measured state of iron in fossil skin associated with UALVP 53290 as seen in the green spectrum of fig. 53. (Original in colour) .............................................. 148

60  Average spectrum of iron in fossil skin associated with UALVP 53290, calculated based on the regions where the spectrum are found. (Original in colour) .............................................. 149

61  Maps comparing the distributions of carbon-1, iron and a) calcium-1 and b) calcium-2 in fossil skin associated with UALVP 53290. (Original in colour) .............................................. 149

62  Maps comparing the distributions of carbon-2, iron and a) calcium-1 and b) calcium-2 in fossil skin associated with UALVP 53290. (Original in colour) .............................................. 150

63  Distribution of the ratio iron/calcium for a) *T. rex* rib RSKM_P2523.8 (fig. 6), b) *T. rex* rib RSKM_P2523.8 (fig. 7), c) DPP turtle (uncatalogued) and d) RF turtle RSKM_P3314.5. The numbers on the axes correspond to the number of steps in each direction. (Original in colour) .............................................. 157

64  Distribution of the ratio manganese/calcium for a) *T. rex* rib RSKM_P2523.8 (fig. 6), b) *T. rex* rib RSKM_P2523.8 (fig. 7), c) DPP turtle (uncatalogued) and d) RF turtle RSKM_P3314.5. The numbers on the axes correspond to the number of steps in each direction. (Original in colour) .............................................. 158

xx
Distribution of the ratio strontium/calcium for a) *T. rex* rib RSKM_P2523.8 (fig. 6), b) *T. rex* rib RSKM_P2523.8 (fig. 7), c) DPP turtle (uncatalogued) and d) RF turtle RSKM_P3314.5. The numbers on the axes correspond to the number of steps in each direction. (Original in colour) ................................ 159

Distribution of the ratio yttrium/calcium for a) *T. rex* rib RSKM_P2523.8 (fig. 6), b) *T. rex* rib RSKM_P2523.8 (fig. 7), c) DPP turtle (uncatalogued) and d) RF turtle RSKM_P3314.5. The numbers on the axes correspond to the number of steps in each direction. (Original in colour) .................................. 160

Scatter plots relating calcium and iron for dinosaur bones. The quantities are represented in arbitrary units, corresponding to the number of counts under the peak associated to a given element, corrected for variations in the electron beam current which affects the X-ray beam intensity at the synchrotron facility. .......................... 161

Scatter plots relating calcium and iron for turtle bones. The quantities are represented in arbitrary units, corresponding to the number of counts under the peak associated to a given element, corrected for variations in the electron beam current which affects the X-ray beam intensity at the synchrotron facility. .......................... 161

Scatter plots relating calcium and manganese for dinosaur bones. The quantities are represented in arbitrary units, corresponding to the number of counts under the peak associated to a given element, corrected for variations in the electron beam current which affects the X-ray beam intensity at the synchrotron facility. .......................... 162
70 Scatter plots relating calcium and iron for dinosaur bones. The quantities are represented in arbitrary units, corresponding to the number of counts under the peak associated to a given element, corrected for variations in the electron beam current which affects the X-ray beam intensity at the synchrotron facility.

71 Schematics of the magnetic field in a quadrupole magnet.

72 Simplified representation of the electron oscillations within a wiggler, as well as the radiation produced by their acceleration. (Original in colour)

73 Schematic representation of incident light on a finite slab of material and some of its possible reflections [113].
List of Abbreviations

BMIT  Bio-Medical Imaging and Therapy Facility
CB  cancellous bone
CC  Creative Commons
CLS  Canadian Light Source
CLSM  Confocal Laser Scanning Microscopy
CT  Computed Tomography
DPF  Dinosaur Park Formation
EC  external cortex
EDS  Energy Dispersive Spectrometer
EDX  Energy Dispersive X-ray Spectroscopy
ERF  error function
ESRF  European Synchrotron Research Facility
eV  electronvolt
EXAFS  Extended X-ray Absorption Fine Structure
FEG-SEM  Field Emission Gun Scanning Electron Microscopy
FF  Frenchman Formation
FMS  Full Multiple Scattering
FTIR  Fourier-Transform Infrared Spectroscopy
IC  internal cortex
IR  Infrared
K  Cretaceous
KB  Kirkpatrick-Baez
MT  modern turtle
NDF  number of degrees of freedom
NEXAFS  Near Edge X-ray Absorption Fine Structure
OD  optical density
Pg Paleogene
rf radio frequency
RGB red-green-blue
RF Ravenscrag Formation
ROI region of interest
RSM Royal Saskatchewan Museum
SEM Scanning Electron Microscopy
SM Soft X-ray Spectromicroscopy Beamline
SRS-XRF Synchrotron Rapid Scanning X-ray Fluorescence
STXM Scanning Transmission X-ray Microscopy
SXRMB Soft X-ray Microcharacterization Beamline
TEM Transmission Electron Microscopy
ToF-SIMS Time-of-Flight Secondary Ion Mass Spectroscopy
VERSPERS Very Sensitive Elemental and Structural Probe Employing Radiation from a Synchrotron
XAFS X-ray Absorption Fine Structure
XANES X-ray Absorption Near Edge Structure
XAS X-ray Absorption Spectroscopy
XPS X-ray Photoelectron Spectroscopy
XRF X-ray Fluorescence
1 Introduction and thesis structure

For many years, fossils were seen as lithified remains that mostly preserved the shape of the original animal, and, in some cases, materials that were originally mineralized, such as bones and teeth. That conception has changed in the past decades due to several published results suggesting the presence of preserved molecules and structures in fossils (e.g., [1, 2, 3, 4, 5]). These findings changed the research goals and the types of analyses being performed in fossils. But, with the changes in the type of studies developed in the field of palaeontology, the techniques used for the analysis of specimens also have to change. Such changes include the opportunity for multidisciplinary research with the application of techniques from other disciplines to study preservation and taphonomic alterations in fossils.

Taphonomy is the study of the processes that remains undergo between the animal’s death and discovery [6]. This includes physical effects, such as transport and weathering, and chemical effects that alter how the remains are positioned, where they are located, the way they look, and their composition [6]. The quality of the preservation in fossils is determined by how the remains are affected by these processes and when in their taphonomic history they occur. As an example, the fossilization of soft tissues depends on the early mineralization of the remains, such that they are preserved before significant decay occurs [6, 7].

The main goal of this thesis is to use synchrotron-based techniques (X-ray fluorescence and X-ray absorption fine structure, in particular) to study the taphonomy of different fossil specimens, including dinosaur bones and skin, turtle bones and insect inclusions in amber. The objective is to study the extent to which these techniques can be applied to different specimens to obtain information on their preservation and taphonomic alterations. With the use of synchrotron techniques, I want to be able to identify some of the possible mechanisms responsible for the exceptional preservation of fossils (especially soft tissues), and to
understand some of the diagenetic changes the specimens undergo. This thesis is primarily an application of high-energy physics to palaeontological samples. It extends a new suite of techniques to a wide range of palaeontological samples to test the limits of these techniques.

This thesis is divided into nine chapters, including this introduction. Chapters 2 and 3 provide the theoretical background for the discussions presented in the later chapters. Chapter 2 introduces the main concept of taphonomy in general, and the special conditions required to preserve vertebrates and invertebrates (specifically those preserved in amber). It also includes the explanation of the main physical and chemical alterations experienced by fossils. Chapter 3 discusses synchrotron light, how it is produced, its properties, and the main techniques used in the analyses for this thesis, X-ray fluorescence (XRF) and X-ray absorption fine structure (XAFS). Chapters 4, 5, 6 and 7 each discuss the research developed based upon different sets of specimens. The chapter structure contains a brief introduction and the theory specific to the research being developed (if the topic has not been covered in chapter 2 or 3), as well as the methods employed, the results, discussions, and conclusions relative to that particular topic. This is followed by chapter 8, which presents a discussion combining data from the previous four chapters, and chapter 9 with the conclusions for the thesis.

Chapter 4 presents results from the research developed on the *Tyrannosaurus rex* (*T. rex*) informally known as “Scotty”. In this study, I compared chemical maps of the *T. rex* bones with those from a hadrosaur found in the same formation, and from an extant swan, in order to identify diagenetic alterations and the specific characteristics responsible for “Scotty’s” preservation. In chapter 5, I discuss the application of synchrotron techniques to non-destructively characterize the chemical composition of insect inclusions in amber. The goals of this research were to understand the strengths and pitfalls of using XRF to chemically map inclusions
non-destructively, and to apply this technique to study ants in Baltic amber and their preservation. Chapter 6 discusses the research I developed on turtle shells with the intention of understanding how different bone tissue types, age, and depositional environment are affected by taphonomic modifications. The research I developed to characterize a very rare specimen of hadrosaur skin is discussed in chapter 7 (as part of a larger effort involving other researchers) to characterize a rare specimen of hadrosaur skin. Previous results have shown that the specimen preserved skin-like (i.e., epidermal) layers composed of approximately round substructures. My objective was to chemically characterize one of these single substructures in order to find possible mechanisms responsible for the preservation of this specimen. Chapter 8 synthesizes some of the data from chapters 4 and 6. It also compares the results of measurements in terms of the relative concentration between certain chemical elements, and how they correlate to one another.

All the measurements, analyses and discussions described in this thesis were developed by me, unless it is stated otherwise.
2 Taphonomy

2.1 Abstract

Taphonomy is the name given to the study of the processes remains go through from death until their discovery. Taphonomic studies have been developed by researchers for centuries. They are essential for the understanding of how fossils are changed and preserved, and how they relate to each other and their palaeoenvironment. Taphonomic effects can be divided into physical and chemical processes. Each of these processes will produce distinct types of modifications within the fossil remains. The objective of this review is to provide an overall introduction to the taphonomic process in general, and the details of these processes in the context of vertebrate and insect remains. I will also comment on the conditions that result on exceptional preservation scenarios, also known as Lagerstätten. This review will be used as the background for all discussions in subsequent chapters of this thesis. They will include studies on the taphonomic alterations encountered by different specimens of dinosaurs, turtles, and insects (as amber inclusions), some of which are exceptionally preserved.

2.2 Introduction

The term taphonomy was first proposed by Efremov in 1940 [8] as the science that uses concepts from both geology and biology to study the history of specimens, from death to diagenesis. This, however, was not the first time that taphonomy was studied. In fact, taphonomic researches had been developed since Leonardo da Vinci in the 15th century, who used the observation of the remains of bivalves and their extant counterparts to conclude that the remains found in Monferrato (Italy) mountains had not been transported by the biblical deluge, but were originally from that location [6].
By the end of the 19th and beginning of the 20th century, the study of taphonomy was mostly centered around paleoenvironmental arguments. Efremov, in particular, was a vertebrate paleontologist who focused on the loss of information due to taphonomic processes, which associated the science of taphonomy with the study of biases in the fossil record produced by these processes [6, 8]. Behrensmeyer and Kidwell [9] were critics of this limited interpretation of taphonomy, as fossil assemblages can also be changed over time due to the addition and alteration of information. They saw the addition of certain chemical elements that were not originally present in the remains, or changes in the location where the remains are found as more than just a loss of information. Thus, they proposed a new definition for this science: “the study of processes of preservation and how they affect information in the fossil record”. In the most recent applications, taphonomy sets methods to study palaeobiology [10]. These allow for better answers for questions regarding trends in biodiversity, characteristics of major extinctions, and rates of evolution, among others [10].

The modern study of taphonomy can be divided into two branches; 1) biostratinomy, including events that occur following the death of the animal, and extending until its burial, and 2) diagenesis, encompassing events that occur after burial, extending until the remains are found [6]. Since some of the processes in these two stages are continuous and the limits between the two are somewhat blurred, the following discussion will treat them together.

2.3 Taphonomy of vertebrates

2.3.1 Decay

According to Weigelt [11], when an animal dies, the lower temperature of the body causes the muscles to contract, limiting joint mobility. This process is called rigor mortis and it sets, usually, around ten hours after death and lasts between ten and
eighteen hours. Once rigor mortis is over, the decay and putrefaction processes begin. The decomposition of the remains is caused both by aerobic (external to the body) and anaerobic (internal to the body) bacteria, with contributions from other organisms, such as insects, which will lay their eggs in carcasses.

Weigelt [11] also proposed a very well-defined sequence for the disarticulation of mammals. His observations suggested which parts of the body are more likely to disarticulate first, such as the lower jaws (due to the way they are attached to the body and the preference of predators and scavengers for attacking animals around the head and neck). This hypothesis was challenged by Toots [12], who concluded that, although rules can be established for the normal sequence of disarticulation of carcasses, they depend on the taxonomic group being observed. For Toots, the sequence of disarticulation depends on the type of joint and the interlocking mechanisms keeping it together, and the amount of easily decomposable material around the articulation. Therefore, aside from the activity of scavengers, bones that are connected very strongly, such as the animal’s vertebral column, will be the last to decay and disassociate. Meanwhile, body regions surrounded by a large amount of easily decomposable material, such as the abdomen, will decompose very fast. This is also true for tissues holding joints in vertebrates. The work of Hill and Behrensmeyer [13], however, studying the disarticulation of African mammals, showed that the process is very consistent among animals of different taxa. Hill and Behrensmeyer [13] believe the differences observed by Toots [12] could be specific to the animals he was studying, and that Weigelt’s [11] hypothesis was probably more correct.

2.3.2 Physical processes

Weathering  Weathering refers to the effects on remains caused by exposure to environmental factors. The weathering of mammal bones subject to the natural
environment has been studied by Behrensmeyer [14] based on the observation of bones in Amboseli Park, Kenya. She observed that the exposed side of bones exhibited more weathering effects than the side touching the soil. These differences can be attributed to variations in environmental temperature, and moisture (the bone parts in contact with the soil would be less affected by these conditions). This idea was further supported by the fact that buried bones, which are not affected by the variations in the soil surface, did not show significant weathering. Moreover, parts of bones extending more than 10 cm above the soil showed less weathering than lower bones.

In special cases, weathering may act differently. For example, when the soil is alkaline and salt crystals form on the surface of bones, the part of a bone in contact with the soil weathers faster than that of the exposed side [14]. The action of insects, roots and bacteria can also cause different patterns of weathering [14, 10]. Weathering can also be caused by physical oxidation, hydrolysis, UV light, and dissolution, among other factors [10].

Behrensmeyer [14] suggested six weathering stages to be used in comparisons between fossils and modern remains, and the corresponding exposure time that would result in these stages, based on carcasses observations:

- **Stage 0**: Bones do not present cracking or flaking caused by weathering. They can also present other preserved structures, such as skin, muscle, ligaments or marrow tissue. This corresponds to 0–1 years exposed.

- **Stage 1**: Bones present cracks, usually, in the direction of the fiber structure. Cracking can also be present in the articulation surface of bones. Sometimes, other tissues are also present. This corresponds to 0–3 years exposed.

- **Stage 2**: Initially, flaking occurs in the outermost bone layers, usually associated with cracks. Eventually, the inner layers of the bone also show flaking until the outer layers of bone are completely gone. Portions of ligament, cartilage and
skin tissues can also be present. This corresponds to 2–6 years exposed.

- Stage 3: Patches to whole bones are uniformly covered by a rough and homogeneously weathered layer of compact bone, which does not penetrate more than 1.0–1.5 mm deep. Other tissue preservation at this stage is rare. This corresponds to 4–15+ years exposed.

- Stage 4: Overall, the bones present a rough and fibrous surface. Weathering can penetrate to the bone’s inner cortical or medullary layers; splinters are formed and can separate from the bone if it is moved. This corresponds to 6–15+ years exposed.

- Stage 5: Bones are falling apart with splinters around them. The bones’ original shape can be difficult to determine and they become very fragile and easily breakable. This corresponds to 6–15+ years exposed.

In case of heterogeneity, the patch with the most advanced stage is to be considered, and, when possible, edges and damaged surfaces of bones should not be used in this analysis.

These stages have been used with success over the years to characterize remains. However, identifying weathering patterns from other features acquired after burial or during diagenesis can sometimes be complicated [10].

Transport  Transport is the effect of moving remains from one location to another. The degree of transport will determine if fossils are autochthonous (found in the same area where the animals died), paraautochthonous (moved but not transported to a different community\(^1\), which usually includes disarticulation or reorientation), or allochthonous (found far from where the animal died, sometimes

\(^1\)Community here refers to the set of populations sharing the same geographical area at the same time.
mixed with other assemblages) [6, 15]. Both fresh remains and fossilized bones can be transported, the latter is usually referred to as reworking.

Apart from predation and scavenging, a common transport means for animal remains and sediments is water. The motion of the fluid may cause the transport or reorientation of bones and the transport of sediments related to their burial. The capacity of transport by a fluid depends on its properties of viscosity and density [6]. The density of the fluid $\rho$ can be calculated by:

$$\rho = \frac{m}{V} \quad (1)$$

where $m$ is the mass of the fluid dislocated by a body of volume $V$ measured at a certain temperature. As the temperature of the fluid increases, its density decreases [16].

The dynamic viscosity of a fluid, according to Isaac Newton’s formulation in his Philosophiae Naturalis Principia Mathematica [17], is given by:

$$\tau = \mu \frac{du}{dy} \quad (2)$$

where $\tau$ represents a force per unit area (shear stress), which acts parallel to the surface of the fluid; $u$ is the velocity of the fluid; $y$ is the distance between layers in the fluid; $\frac{du}{dy}$ is the rate of deformation of the fluid in the direction perpendicular to the fluid’s surface; and $\mu$ is the dynamic viscosity, a measure of how easy it is for the fluid to flow [6].

Fluid can move in two regimes: laminar and turbulent flow. In laminar flow, parallel fluid layers move in relation to each other without much interference, while turbulent flows have motion of eddies perpendicular to the fluid layers. Laminar flow can by disturbed by the geometry of the fluid’s bed, and, therefore, turbulence depends on the roughness of the sediments present in the environment [6].
According to Reynolds [18], eddies are produced when the quantity, known as Reynolds number, is above a certain threshold:

\[ Re = \frac{cpU}{\mu} \]  

(3)

where \( c \) is a linear parameter, such as the radius of a tube in which the fluid is moving; \( \rho \) is the density of the fluid; \( U \) is the mean velocity of the fluid and \( \mu \) is the dynamic viscosity of the fluid.

A body will be moved by a fluid if the force acting on it is larger than the force holding the body in place (usually friction). The former depends on the fluid velocity, shear stress, fluid viscosity and body size, shape and density [6].

Many experiments have been performed to determine the behaviour of bones and other remains when they are subjected to water currents. In his pioneering work, Voorhies [19] used a 45-feet-long by 4-feet-wide stream table with a sand bed (particle size between 0.25 and 0.50 mm) in which the velocity of the water could be controlled to study how mammal bones behave under different conditions. The results showed that long bones, such as the femur or tibia, tend to align parallel or perpendicular to the current. The latter orientation was more common when the water levels were shallow and part of the bone was exposed. The parallel alignment was more common when the bone was completely submerged. They also tend to have their larger ends oriented downstream.

Voorhies [19] also tested other types of bones with complex shapes, such as jaws, which tend to align convex-up when higher fluid velocities are reached. Small bones were more affected by the roughness of the bed than larger bones, and, in some occasions, became trapped and were buried as the materials moved. Although the velocities achieved in this experiment were not enough to move most heavier skulls, when they moved, their long axes remained perpendicular to the current while they
rolled. The pelvis tended to align upside-down, with the ilia oriented downstream. He did not notice patterns when looking at ribs and vertebrae.

In a second series of experiments, Voorhies [19] used the same setup on disarticulated remains of coyote and sheep in order to evaluate the sequence in which they are transported. He noted three groups of bones: in the first, the bones moved immediately when subjected to a current, by saltation or flotation (e.g., ribs, vertebrae); in the second, the bones tended to move some time after the bones in group one, by traction (e.g., femur, tibia, pelvis); in the third, the bones tend to resist the current and to stay in their original positions within the velocities tested (e.g., skull, mandible). These groups are now referred to as “Voorhies’ groups”.

Later research by Behrensmeyer [20] and Hanson [21] showed that most transport of bones in groups two and three occurs during floods, when the body of water moves faster and has the force necessary to move these bones. More recently, Coard [22] experimentally determined that articulated remains have a higher transport potential than their disarticulated counterparts and the same can be said, to a lesser extent, about dry bones in comparison to wet ones.

Dodson [23] was one of the first researchers to extend this type of analysis to fossil reptile bones. When studying the Oldman Formation (Alberta, Canada), he placed bones in eleven classes, according to how complete and articulated the remains were when found. The specimens varied from complete and articulated skeletons, some with fossilized integument, to isolated bones, reflecting different conditions of burial and transport.

Experimental studies of the behaviour of dinosaur bones in fluvial environments have been conducted by Peterson and Bigalke [24], using casts of pachycephalosaurid domes with mass and density similar to non fossilized bones. Their research suggested that, although the mammal models discussed before provide good approximations to the behaviour of their specimens, the shape and size of the dome result in differences
in transport distances and orientations. Ultimately, their study indicated that more research is necessary to better understand the transport of dinosaur remains and their peculiarities.

**Time-Averaging**  The time scales involved in biology (e.g., organisms’ lifespans) are very short when compared to the geological timescale. Therefore, before large geological changes take place, many organisms will usually live and die in a region. The result is that many animals may die and have their remains accumulated over the span of years before they are buried by sediments. The fossils later found will thus be an average of the life in that space over many years (up to hundreds of thousands of years, depending on the depositional environment). This is known as time-averaging and must be considered when interpreting a fossil assemblage [6, 25]. Time-averaging would not occur if, for example, a large catastrophic event occurred, immediately burying the animals.

The effects of time-averaging in fossil assemblages are discussed in [6], and for terrestrial settings more specifically in works such as [25]. One of these effects is related to the abundance of fossils of a single species. For a long accumulation of fossils in an area, more members from a single species will die in the region. This means that species that are preserved easily, or that live in the same region for long time intervals will have a disproportionate number of preserved individuals, even if they were not dominant at the time of their deaths.

Time-averaging can also interfere in the estimated diversity of a paleoenvironment. Depending on the duration of the averaging process, many communities can have their members included in an assemblage, and these could be difficult to differentiate from a community with a larger diversity living for a longer amount of time in the region. Time-averaging also smooths out short-term variations in the local diversity, causing problems in the interpretation of the local paleoecology, since species that
never coexisted would seem like they did [15].

Stratigraphic disorder can also be caused by time-averaging, where the chronological order of the deposition of remains in relation to the stratigraphic layers is not maintained due to reworking, or due to condensing of fossil horizons through winnowing of sediments [15].

**Bioturbation**  Bioturbation refers to the movement of sediments caused by the action of living organisms, which can alter the chemistry and preservation of remains. It will also modify the stratigraphic record by eliminating the short-term, high-frequency events (e.g., occasional floods) while maintaining the long-term, lower-frequency ones (e.g., formation of a lake in the region) [6]. While terrestrial bioturbation has not been studied in depth (most research focuses on feeding traces and trackways), many models have been developed to explain bioturbation in aquatic systems. The diffusion models for bioturbation are the most famous, and they are based on chemical and thermal mathematical diffusion models [6, 26].

As an example, a prominent diffusion model for deep-sea water was proposed by Guinasso and Schink [27]. In this model, the concentration distribution of a tracer material is studied both in terms of depth and time, according to the differential equation:

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} - v \frac{\partial c}{\partial x}$$

where $c$ is the concentration (measured in cm$^{-3}$), $t$ is the time (measured in kiloyears), $D$ is the eddy diffusion coefficient (measured in cm$^2$ kyr$^{-1}$), $v$ is the sedimentation rate (measured in cm kyr$^{-1}$) and $x$ is the depth from the surface (measured in cm). This model successfully describes the ranges known for some systems even though it has simplifications (such as the fact that $D$, in this model, does not vary with depth).

The diffusion models have been successful in modeling in these conditions;
however, the assumption that thermochemical diffusion and bioturbation work in the same way is not necessarily correct [6]. Diffusion models should only be applied in cases in which the particles in the sediment move randomly across space and time. The distance travelled by the particles are much smaller, on average, than the scale of the process being considered, and the time considered for mixing events is much smaller than the time necessary for reaction or for the introduction of the tracer in the sediments [6].

2.3.3 Chemical processes

Apart from the physical processes discussed in section 2.3.2, animal remains also go through chemical alterations over time. Some processes occur fairly early in the diagenetic history of the fossil, but the interactions between animal tissue and sediment persist over the years. Mineralization in fossils can produce detailed structural preservation by 1) permineralization, when fluids with minerals infiltrate the remains in sediments rich in organic matter, creating molds of the structures; 2) petrification, when minerals are added to hardparts, usually when they are porous enough to facilitate the process; 3) replacement, when minerals substitute other materials [6, 28]. How quickly tissue mineralization occurs in relation to its decay is a factor in its preservation. Early mineralization plays an important part in the preservation of fossils [6, 29]. Therefore, this section will discuss the alterations caused by chemical processes throughout the remains’ history, followed, in section 2.4, by a discussion on how different vertebrate structures are preserved in this context, and the conditions necessary for preservation to occur.

Silicification Silicification is the process of permineralization, petrification or replacement by silicon-based minerals, such as quartz and opal. These minerals can form crystals of various sizes, influencing which features are preserved in this
process [6]. The source of silicon is found in saturated or near-saturated solutions, in the form of silic acid ($H_4SiO_4$). This acid can eliminate water to form silica ($SiO_2$) and is usually deposited in animal remains via water with neutral or slightly acidic pH. Several mechanisms can give origin to the diluted silicon, such as volcanic and hydrothermal activities or biogenic activity [6, 30]. Some studies (e.g., [30, 31]) suggested that biogenic silicon might enhance, or even control the chances for silicification to occur, introducing an important bias in the fossil record. According to [30], organisms living in close proximity to siliceous sponges had a higher chance of preservation through silicification. The number of silicified fossils in marine environments may dramatically fall in geological periods when these sponges were not as common [30].

In terrestrial settings, this method of preservation is more common for wood and invertebrates. However, examples have been found of vertebrate preservation through silicification. One of such discovery was reported by Channing et al [32], where a bird from a Quaternary hot spring was found silicified with a remarkable preservation of details, which was probably facilitated by the action of microbial organisms. Another example is the Australian Cretaceous Griman Creek Formation, where terrestrial faunas and floras were preserved in opal [33]. Among the animals found are dinosaurs [34], molluscs [35], and mammals [36].

**Pyritization** Pyritization is the mineralization process by pyrite. In general, pyritization occurs when sulfides (usually produced by the reduction of sulfates) interact with dissolved iron. The iron is usually produced by one of the three following processes:

$$H_2S + 2FeOOH + 4H^+ \rightarrow S^0 + 2Fe^{2+} + 4H_2O \quad (5)$$

$$CH_2O + 4FeOOH + 8H^+ \rightarrow CO_2 + 4Fe^{2+} + 7H_2O \quad (6)$$
\[ 7O_2 + 2FeS_2 + 2H_2O \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \]  

These processes represent the reduction of iron oxides by sulfide, the reduction of iron oxides by organic compounds, and the oxidation of iron sulfides, respectively [6, 29, 28]. The resulting iron sulfides can take different shapes, but, framboidal pyrite, for example, is obtained as follows:

\[ 18CH_2O + 9SO_4^{2-} \rightarrow 18HCO_3^- + 9H_2S \]  
\[ 6FeOOH + 9H_2S \rightarrow 6FeS + 3S^0 + 12H_2O \]  
\[ 3FeS + S^0 \rightarrow Fe_3S_4 \]  
\[ Fe_3S_4 + 2S^0 \rightarrow 3FeS_2 \]

These equations show the formation of pyrite as a process, where pyrite is just the end product of the reaction [6, 28]. Also, since six FeS are formed in equation 9, and only three can be converted into pyrite with elemental sulfur produced in this reaction (see equations 10 and 11), another source of sulfur is necessary so that all the FeS can be converted into pyrite. This source is still unknown [6, 28].

In practice, porewaters cannot contain a large concentration of both dissolved sulfide and iron: when the concentration of one increases, the other decreases rapidly. As a result, the mechanism that allows pyritization of fossil vertebrates is the decay of organic matter [37]. In this case, the decay of the organics produce sulfur locally, which can be combined with iron present in the water for pyrite formation. The preservation of soft-tissue through pyritization will occur when decaying carcasses are placed in organic-poor sediments and the concentration of dissolved Fe is much higher than the concentration of sulfides being produced by decay [29]. That could occur for example with the presence of another factor (such as low organic carbon content) that limits sulfate reduction [29]. These sulfides can also be produced by
bacterial sulfate reduction when microbial mats are present in the specimen [38].

**Phosphatization** Phosphatization occurs when a large concentration of phosphorus is available. This element usually belongs to the apatite group (i.e., with general formula $Ca_{10}(PO_4,CO_3)_6(F,OH,Cl)_{≥2}$, where $PO_4^{3−}$ and $CO_3^{2−}$ can substitute one another) [6]. According to Briggs [29], the decay of organic matter and the dissolution of bioapatite, the fixation in microbial mats, and adsorption of Fe(II) ions produced by Fe(III) reduction are three agents that are the most important when increasing the concentration of phosphorus in the environment. Although the conditions allowing for the preservation of soft parts are localized, when it occurs, the highest levels of preservation are possible in phosphatization, including the preservation of cellular details [6].

Dissolved phosphorus usually combines with iron, but when in an anoxic environment, the iron is reduced and the phosphate concentration increases. Therefore, phosphatization occurs when the carcass remains at the oxic/anoxic interface long enough for phosphorus to be liberated after the reduction of iron [39]. However, in some cases, the conditions local to a specimen can result in preferential phosphatization of a single species. A good example of this phenomenon is when the animal itself provides enough phosphorus and microbial activity responsible for its decay provides the reducing environment [40]. In this case, the microbial activity will determine the level of preservation: if the decay has been generally extensive, most original features will be replaced by phosphatising microbes; while, if only some parts of the animal have decayed, other parts can be preserved in great detail.

The precipitation of calcium carbonate or calcium phosphate (from apatite) is controlled by local conditions (mainly the pH of pore waters) and can vary within the same individual. Consequently, different parts of the animal can contain either carbonates or phosphates [29]. The presence of microbial mats may contribute to
trapping phosphate and creating the reduced pH environment, which can promote phosphatization.

2.4 Preservation in vertebrates

The physical and chemical taphonomic agents previously mentioned act differently depending on the tissue being analyzed. In the next section, the most important of these agents and how they affect each of the commonly preserved vertebrate tissues will be discussed, with special attention to dinosaur remains.

2.4.1 Bones

Bones are mostly made of hydroxyapatite, water and organic compounds, of which the most common is collagen. Skeletal remains can experience a series of processes that will lead to their destruction or preservation during fossilization and diagenesis [41]. Before burial, the most common source of damage to bones is predation or scavenging. They can leave marks on the bones, such as scratches, punctures, and fractures. Chemical alterations can also be caused by digestion, which can create changes in the Ca/P ratios, or the concentration of elements such as Sr, Mn, Fe and Zn, among others [42]. Additional pre-burial processes include weathering, which can cause bone fragmentation, an increase in strontium levels, crystallographic alterations, and protein degradation. Trampling, which can also cause bone fragmentation; and transport, which can lead to the abrasion, dispersal, or artificial accumulation of bones can also occur [43].

After burial, the bones may be subject to bioturbation, damages caused by plant roots and lichens, or dissolution and corrosion of bone material by the surrounding soil. The bones can also go through diagenetic chemical changes, which can cause an increase in the amounts of Ca, Mg, S, Fe, Sr [41] and Y [44]. The types and degrees of changes experienced are correlated more strongly with the nature of the sediments
around the bone than to the duration of the diagenetic process [41].

Apart from the mineral portion of the bones, many recent studies have focused on the preservation of collagen, a protein that gives bones elasticity and resistance to tensional forces [1, 41, 45]. Collagen belongs to a group of proteins that also includes keratin and fibrinogen, which are more resistant to biodegradation due to their repetitive structure (high crystallinity and chemical bonds between strands) [46]. Although proteins are usually rapidly decomposed after death, they can be preserved if they experience mineral replacement early in their diagenetic history [46]. In fact, Avci et al. [45] have found great similarity in measurements comparing the collagen from extant bones with their fossilized counterparts. In a study involving exceptionally preserved embryonic bird bones from the Upper Cretaceous Rio Colorado Formation, they found similar results for extant and fossil bones when analyzing their immunological response, enzymatic degradation, and force-extension distribution measured using Atomic Force Microscopy (AFM). This suggested that the collagen found in the extinct birds may have preserved its functionality and integrity. Furthermore, studies (e.g., [1, 2]) have claimed the measurement of peptide sequences in preserved dinosaur collagen. This would suggest a very high level of preservation at the molecular level and provide extensive information on dinosaur biology. However, questions have been raised about these measurements, suggesting that the possibility of laboratory contamination cannot be dismissed [47].

2.4.2 Soft-tissues

Soft tissues have been found in several dinosaur fossils, such as vessels and collagen fibrils in a phalanx [48], and vessels and erythrocytes in a femur and phalanx [3] from the Upper Cretaceous of the Gobi Desert, Mongolia. The preservation of tissues that were not originally mineralized is rare and cannot be completely explained by the current understanding of fossilization processes. Many theories have been proposed
to explain it, such as the presence of microbial stabilization, and early replacement or mineralization of structures, among others [7, 29, 39, 49]. Experimental results, however, have suggested that iron (goethite) particles may play an important role in the preservation of soft-tissues, since the association of such particles and tissues has been observed. Although the mechanism by which iron acts on the preservation of remains is not understood, hypotheses have been made suggesting fixation mediated by free-radicals and by anti-microbial activity [7]. Iron could be acting to direct protect proteins by blocking sites where degradation enzymes act, or by indirectly protecting them from oxidative damage by binding with oxygen, for example [7].

2.4.3 Integument

Although more rarely preserved than bones, many specimens of dinosaur integument have been found over the years, including fossilized skin, external impressions of skin, and integumentary structures such as feathers. The first impressions of dinosaur skin were found in 1884 by J. L. Wortman and belonged to a hadrosaurid (*Edmontosaurus annectens*) [50]. Since then, many other skin fossils have been found, with reports for hadrosaurids being the most common [51]. Some early work includes that of Osborn [52], who reported finding impressions that almost provide a complete picture of the outer covering of the same hadrosaurid species. He compared the patterns of dermal tubercles in different areas of the animal’s body and found that there were a greater number of larger tubercles in the exposed parts of the dinosaur (back, limbs, and, to some extent, sides of the body). Based on an analogy with modern lizards and snakes, this skin patterning suggested a darker appearance in these areas when compared to the rest of the body. Also, Lambe [53] described skin impressions from a Cretaceous ceratopsian, which showed these animals were covered by small polygonal plates, similar to those found in hadrosaurids. A review of hadrosaurid skin impressions can be found in the work of Bell [50], with descriptions of the integument for different
species and their comparison. Although they are referred to as impressions in early works, detailed study of specimens such as the one presented in the chapter 7 of this thesis have shown that, at least in some cases they are actual fossilized skin. Not only can these fossils preserve the three-dimensional structure of dinosaur skin, but also chemical information, and biogenic molecules (e.g., [51, 54]). Specimens such as *Borealopelta* have been analyzed for traces of the original integument, including melanosomes (resistant pigment bodies). These integumentary components and the outlines of soft tissues preserved around skeletons, have improved our understanding of the appearance of dinosaurs, providing an indication of display structures (e.g., [55]) and integumentary colouration (e.g., [56]).

The majority of integumentary fossils suggest dinosaurs (including most ornithischians, all sauropodomorphs and some theropods) had scaly skin [57]. However, some ornithischians presented quills, and some theropods had feathers or “protofeathers” (although there is some uncertainty whether all known forms of protofeathers are homologous to those of modern birds) [57, 58]. The first report of a feathered dinosaur in [59]. Since then the preservation of dinosaur feathers in the fossil record have been documented in several instances. For example, in the work of Chen et al. [60], two almost complete skeletons belonging to the theropod *Sinosauropteryx* were identified with the preservation of integumentary structures like simple feathers surrounding them. These feathers are not believed to have aerodynamic characteristics, but suggest a step in the evolution of birds. In fact, the remarkable preservation of some specimens, with detailed structures such as feathers, has helped reinforce the hypothesis that birds descended from theropod dinosaurs [61]. On the other hand, the feathers in dinosaurs were not always complex with multiple tiers of branching, as is typically seen in extant avians. Instead, dinosaur feathers originated with a more simple form, comparable to mammalian hairs [62]. These filament-like feathers were found in the
heterodontosaurid *Tianyulong* [63] and the ceratopsian *Psittacosaurus* [64]. Early feathers with more complex branching patterns were present in other taxa, such as 1) *Sinornithosaurus* [65] and *Anchiornis* [66] (multiple filaments joined basally); 2) *Sinornithosaurus* [65] (multiple filaments branching from a single filament); 3) *Sinornithosaurus* [65] and *Anchiornis* [66] (filaments branching laterally from a central shaft); 4) *Epidexipteryx* [67] (multiple parallel filaments originating from a membrane). Pennaceous feathers comparable to modern birds were present in some non-avian theropods, such as *Caudipteryx* [68], *Protarchaeopteryx* [68], and *Microraptor* [69]. A full review of feathers in dinosaurs can be found in [70].

Beyond examining structural evolution, preserved feathers can also be used to study animal colouration if pigment structures (melanosomes) are preserved. The shape of these intracellular structures can be associated with certain colours, as has been done in studies such as by Clarke et al. [4] for an Eocene penguin, and by Carney et al. [5] for the primitive Jurassic bird *Archaeopteryx*. Originally classified as microorganisms [71], these round structures were later reinterpreted as melanosomes [72, 73]. However, there are questions if this interpretation is correct. Some researchers believe the hypothesis that the structures are in fact microorganisms have not been satisfactorily disproved [74, 75, 76], while others question the distortion of the structures during fossilization and how this may affect the colour interpretation based on these structures [77]. A review of the debate can be found in [78].

### 2.5 Special preservation

In general, the preservation of remains as fossils is a rare event. Most organisms die and their constituents are recycled by the environment. This fact brings extreme importance to the rare cases where exceptional preservation is found, as in the specimens studied in this thesis. Seilacher [110] named these special cases
Fossil-Lagerstätten, a term with origin in German mining tradition, used to refer to rock bodies of economic interest [111]. In the same way, a Fossil-Lagerstätte is a rock that has valuable fossils, justifying its exploration – either in terms of quantity (Konzentrat-Lagerstätten), or in terms of the quality of the preservation (Konservat-Lagerstätten). Among all the fossil deposits, the Lagerstätten would represent the best case scenarios for concentration or preservation of fossils [6] (to be discussed in the next sections).

2.5.1 Konzentrat-Lagerstätten

Konzentrat-Lagerstätten refer to sites with a large concentration of not necessarily well-preserved remains. This type of assemblage can be caused by many factors, including catastrophic events, resulting in mass mortality in a region; reworking of sediments containing fossils, such that they end up in the same area; or in concentration traps (e.g., cavities) that help protect remains and fossils from mechanical damage or diagenetic effects [111]. The processes that lead to preservation in Konzentrat-Lagerstätten are special cases of the physical processes discussed in section 2.3.2, so they will not be discussed again here.

2.5.2 Konservat-Lagerstätten

Konservat-Lagerstätten are special because of the quality of fossil preservation, even if fossils are not abundant. Exceptional preservation of animal remains is achieved when the decay and necrolysis processes are stopped early on and the organic components are not consumed by microbial activity [111]. Many sets of characteristics can be in play to generate the circumstances necessary for this to occur. This can include conservation traps, which involve localized preservational conditions, such as the action of bacteria causing early phosphatization or pyritization, permafrost, or the entrapment in resin, which matures into amber. It
can also include stratiform conservation deposits, which consist of larger areas in which exceptional preservation is a more general trend, caused by larger-scale factors, such as sediment smothering and anoxic conditions [111]. The discussion of preservation in amber can be found in the following section, so here I will focus on stratiform conservation deposits.

Sediment smothering (obrution) deposits are caused by rapid burial by sediments, which can be important in preventing disarticulation and bioturbation effects, depending on the thickness of the sediment layer [6]. Preservation related to obrution is an episodic event, which is taxon dependent – animals belonging to different taxa are more or less vulnerable to burial in these conditions [111]. An example of a very vulnerable group is the echinoderms, with an ambulacral system that is easily clogged by sediments, causing their death (and preservation). This explains why this group is often overrepresented in obrution deposits [111]. This mechanism usually results in the preservation of articulated hard parts, but, in most cases, it is not responsible for the preservation of soft parts by itself [112].

Anoxic conditions (stagnation deposits) are related to areas with a low concentration of oxygen, which helps retard decay, as well as disarticulation due to scavenging and bioturbation. Although decay can still occur in anoxic environments, the mineralization of soft parts also benefits from the lower oxygen conditions [6]. Therefore, stagnation, much like obrution, favours the preservation of articulated remains, but not, necessarily, of soft parts by itself [112].

Other factors can also contribute to the detailed preservation of animal remains, such as the presence of microbial mats, which prevent organic decay, erosion, bioturbation, and scavenging [6]. The presence of microbial mats can also be linked to authigenic mineralization of soft parts by concentrating elements such as phosphorus around the animal and favouring the phosphatization process [6].

In most cases, the preservation of soft parts is due to early mineralization of the
remains, which is controlled by environmental settings and conditions, such as the levels of pH, Eh, organic content, salinity, rate of burial and oxygenation [112]. In general, the characteristics of exceptional deposits include rapid mineralization, and limited pre-burial decay. The latter process is influenced by the nature of the decomposing carbon, the supply of oxidants to the bacteria responsible for decomposition, and the sediment type [112]. Anoxic conditions, for example, increase the precipitation of diagenetic minerals, by reducing the production of reactive ionic species. These minerals can promote the preservation of soft parts by three different modes: 1) permineralization, which is usually restricted to more resistant tissues, such as cellulose and chitin, but can rarely occur in more labile tissues, such as muscles, if the process starts early enough during diagenesis (in this case, preservation is usually by phosphatization); 2) mineral coats, which use the soft parts as a template for mineral formation, preserving tissue outlines; and 3) tissue casts and molds, which involves coarser replacement and results in a two- or three-dimensional mold of the soft tissue, involving a comparatively larger amount of information loss [112].

**Preservation in amber** The oldest record of an insect is from the Early Devonian, found at the Gaspé Peninsula (Quebec, Canada). This was a bristletail (Archaeognatha) with bulging compound eyes, monocondylic mandibles and many sensory setae [79]. The presence of this fossil at this time suggests that insect diversification was nearly contemporary with the emergence of vascular plants in the Silurian [79]. Since then, insects have grown to become the most diverse and successful group among multicellular animals, being of the utmost importance to many ecosystems [80]. The preservation potential of these organisms has strongly influenced our understanding of their evolutionary history and distribution, because they require exceptional preservation for detailed study [81].
The cuticle is one of the tissues in insects with the highest probability of preservation, but preservation still depends on factors such as the composition of the cuticle, the environment where it is deposited, and its diagenetic history [80]. Insects are usually considered along with soft-body organisms, because they require exceptional conditions to be preserved in the fossil record. The best preservation in sedimentary rocks occurs in fine-grained, laminated carbonates that are produced in lacustrine and shallow marine settings [80]. These settings have been reviewed in [80]. Entrapment in amber is another common condition for the preservation of insects and is the focus of the current work. The taphonomy of amber is different than in carbonates, starting with the types of insects preserved. Amber will preserve any kind of insect, but is biased toward smaller insects, while preservation in carbonates is correlated to the susceptibility to decay of the insects present [80]. Section 2.5.2 describes preservation in amber in greater detail.

The preservation of insects in amber has been recently reviewed by [80]. Amber is fossilized resin, produced and secreted by multiple types of trees throughout the fossil record [82]. The resin is made of terpenoid and phenolic compounds, which means that it is soluble in alcohol, but not in water [83]. The detailed composition of most ambers is not very well known, mostly due to its insolubility [80]. However, comparative studies between amber and resins can be used to establish its tree producers (e.g., [84, 85]).

Many trees produce resin, but only some are responsible for the amber found in the fossil record: the conifers Pinaceae, Araucariaceae and Taxodiaceae; and the angiosperms Leguminosae, Bursaraceae, Dipterocarpaceae, Hamamelidaceae and Combretaceae [80]. The oldest record of fossilized resin is from the Upper Carboniferous in England, and is thought to have been produced by a pteridosperm [86]. Amber only became more common after Araucariaceae became more abundant in the Early Cretaceous, with the first insect inclusions dating back to the Lower
Cretaceous, in Jezzine and Hammana (Lebanon) [80].

The accumulation of resin can occur in different parts of the tree, including internal channels and cracks inside the wood, within the bark, or on the outside of tree. These locations may influence resin polymerization, due to different access to polymerizing agents [80]. Also, resins can have different functions for the trees (e.g., antimicrobial [87]) and its secretion can vary daily and seasonally [80]. The explanation for resin production is not completely known and many theories have been proposed [80], including: serving as a defense mechanism against insect and fungal attacks [88, 89], reaction to damage [90], storage of unnecessary compounds produced by cellular metabolism and growth [91], protection against high temperatures and dehydration [92], and to attract pollinators [93].

The chance of entrapment for insects in amber depends on a series of factors, one of which is the viscosity of the resin [80]. The viscosity determines the amount of time the resin will be able to function effectively as a trap, as well as the surface tension, which, in turn, determines the force necessary for an insect to penetrate the resin and get trapped [80]. The more viscous the resin, the larger the insect needs to be in order to be able to penetrate it. However, the larger the insect, the more chances it has of escaping the resin if other agents (such as predation, dessication, or chemical interactions while trapped) are not considered [91]. Therefore, the fossil record in amber is biased toward smaller taxa [80].

Another factor that contributes to an insect’s chance of being trapped in resin is its behaviour [80]. Insects that depend on trees for living, such as for food or habitat, are more likely to be trapped [94], so are insects that forage for resin [90]. Insects that exhibit swarming behaviour, for example, have more chances of being trapped in large numbers when compared to others [95]. The resin produced by a tree can also attract or deter insects, which would increase or decrease, respectively, their chances of being trapped [80].
Finally, environmental factors can also contribute to the insects’ chance of being trapped [80]. These factors include the temperature (and season), which, as mentioned before, changes the amount of secretion; as well as soil type, water availability and amount of solar radiation, which are correlated with the amount of resin produced by a tree. Catastrophic events, such as fires, and insect infestations can also increase resin production [80, 96, 97, 98].

The insects usually die of asphyxia when they are trapped by amber, with the exception of larger individuals, which need more than one layer of resin to be completely covered. In this case, the insect may die of fatigue, chemical interactions, or predation [80]. The amount of time necessary for the insect to be completely covered by resin depends on the resin viscosity, quantity and how it is deposited [99]. If more than one layer of resin is necessary, disarticulation may occur, while if there was resin flow at the time of entrapment, the insect appendages may be oriented accordingly [80].

Amber deposits are usually allochthonous and, therefore, have been transported to some extent [80, 100, 101]. However, in some deposits, very fragile structures have been preserved alongside the amber produced by ancient forests, suggesting that transport distance was not long [102]. The fact that amber is only very rarely found in association with fossil trees suggest that amber and fossil wood are not transported together or that the resin within the trees is often destroyed during diagenesis [80].

Because it is not clear in insect inclusions which taphonomic processes occur before or after burial, the diagenesis of insects is usually considered to start after entrapment, while the diagenesis of the resin itself starts after burial [80]. Dehydration and carbonization are examples of processes that start before burial [80, 103]. Also, since many kinds of resin possess antimicrobial and antifungal properties, they protect the insect carcass from decay, providing the unique opportunity to study the effects of diagenesis without considering the effects of
external agents [80, 104]. In this case, decay is usually the result of the action of endogenous bacteria, which may be halted if early dehydration occurs [105]. While in most cases the preserved remains of insect inclusions are limited to their cuticle, sometimes other tissues, such as internal organs, can be preserved when early dehydration occurs [104, 106].

Insects preserved in amber usually retain their three-dimensional appearance, due to the action of the resin and its polymerization [80]. Interactions with the resin are probably also responsible for the preservation of tissues that would commonly decay quickly [103]. Although the exact mechanism that results in this preservation is not known, some theories have been proposed (see e.g., [91]; [107]).

The reworking of amber deposits is possible and the ease with which amber specimens can be transported depends on the amber characteristics, such as density, and the transport medium characteristics, such as salinity [80]. Other factors can also influence the diagenetic history of the specimen, such as the levels of oxygen (amber easily oxidizes, which suggests preservation is better in anoxic sediments); overburden pressure, which may result in distortion or fracturing of the amber, especially when it is more brittle (more polymerized); and high temperature, which can cause darkening of the amber and thermal cracking [80, 108, 109]. The mineralization of insect inclusions is also possible: when conditions are favourable, minerals can penetrate through cracks in the amber and reach the insect tissues [80].

2.6 Conclusions

Taphonomic process can significantly alter the characteristics of animal remains between their death and their discovery. Physical changes can influence our interpretation of the local palaeoenvironment and palaeoecology if not well understood. Chemical alterations can provide information on the local environmental conditions during fossilization and diagenesis, and on the factors that
favoured the preservation of fossils. It is for assessing the latter features that synchrotron radiation techniques can be used more effectively. They provide means to analyze in micro or macro scales the chemical composition and the spatial distribution of different chemical species in the specimens. This could be used, for example, to detect the presence of unexpected compounds (or the absence of expected ones) that would suggest processes the fossil underwent. They could also be used to identify compounds with organic origin in fossilized specimens and offer further insight on the characteristics of extinct species.
3 Synchrotron light sources and techniques

3.1 Abstract

The characterization of taphonomic effects in fossils in this thesis was performed using several techniques, most of which based on synchrotron light. Synchrotron facilities provide radiation with high brightness, meaning that samples can be analyzed with great resolution in a relatively short amount of time. This radiation is the result of the changes in acceleration of charged particles (electrons) travelling in a circular trajectory with the help of magnets. Synchrotron radiation can be used in many characterization techniques, among them X-ray fluorescence (XRF) and X-ray absorption fine structure (XAFS). XRF can be used to detect the presence, quantity and distribution of different chemical elements in the sample. XAFS is used to provide information on the chemical state of the elements present on the sample. This chapter provides an introduction on synchrotron light sources and the techniques used throughout this thesis.

3.2 Introduction

The theory of a synchrotron accelerator was first proposed by V. I. Veksler [113] in 1944 and, independently, by E. M. McMillan [114] in 1945, with the intention of its application to the study of particle physics. Using these new ideas, the first large synchrotron accelerator, which could reach energies of 300 MeV, was built and started operating in 1949 at the University of California [115] to develop studies involving the interactions between matter and X-rays or relativistic electrons.

Synchrotrons are circular particle accelerators, which usually operate using electrons. The electrons’ circular path is created with the use of bending magnets, which change the direction of motion for the charged particles, producing radiation as a byproduct [116].
In the first years of research using synchrotron facilities, the radiation emitted was considered an unavoidable problem, since it resulted in major energy losses for the electron beam. After the 1960s, researchers realized that this radiation could be used in condensed matter analyses and beamlines started being built, but still as a secondary project on high energy physics facilities. These laboratories are called “first generation” synchrotron radiation facilities [116].

In the mid-70s, facilities designed specifically for research using synchrotron light were built, starting the second generation sources. In these laboratories, the radiation was mostly produced using bending magnets, with some use of wigglers (see Appendix B for more on these devices) [116].

Most recently, third and fourth generation light sources are considered most desirable for research. The third generation sources differ from the second generation in the more common use of insertion devices (wigglers and undulators) to produce synchrotron radiation (see Appendix B) [116].

A synchrotron source is considered to be “fourth generation” if any important property surpasses the third generation sources by one order of magnitude or more [117]. The most common innovation to reach such status is the use of free electron lasers (FELs), which offer short light pulses with high peak intensity and brightness [116, 117].

For studies using synchrotron radiation, researchers would be interested in using the facility with the highest brightness possible, which would allow for faster and better results. The advances in technology between different generations of light sources mean later generations see an increase of multiple orders of magnitude in the brightness when compared to each previous generation.
3.3 Synchrotron light sources

3.3.1 Acceleration

Synchrotron light sources are typically composed of an electron gun, a linear accelerator (linac), a booster ring, a storage ring, and the beamlines and work stations used in research. The electron gun creates electrons that make up the beam. These electrons are ejected from a heated metal (usually tungsten) when it is under high voltage [118]. The free electrons then enter the linear accelerator.

Linear accelerators are used in synchrotron facilities to give the initial boost to electrons, reaching energies on the order of millions of electron volts (MeV). The ones in synchrotron facilities usually use RF-fields\(^2\) (radiofrequency fields) to accelerate the particles, by making their phase velocity equal to the velocity of the particles, with the former given by

\[
v_{ph} = \frac{c}{\sqrt{\epsilon \mu}} \leq c
\]

(12)

where \(c\) is the speed of light, \(\epsilon\) is the dielectric constant and \(\mu\) is the magnetic permeability [119].

The most common approach to adjust the phase velocity such that it is equal to the velocity of the particles is through the insertion of metallic plates in the waveguide that generates the RF-field. These metallic plates are positioned at periodic intervals and allow the passage of the particle beam through apertures. By choosing the right geometry, the plates will create magnetically coupled cavities, which filter out the undesired frequencies and the phase velocity is adjusted to the necessary values. In the case of most synchrotron light sources, the cavities are designed to produce a phase velocity equal to the speed of light, because this is approximately the velocity of the electrons [119].

\(^2\)RF-fields are electromagnetic fields created by alternate currents.
Both the booster ring and the storage ring are synchrotron devices. In the former, the electrons are accelerated to energies on the order of giga electron volts (GeV). In storage rings, the electrons can also be accelerated, if necessary, but their main function is to keep the energy of the electron beam constant in spite of the losses due to the radiation emitted. In both cases, however, RF cavities are used to boost the electrons [116].

Electrons receive energy from longitudinal electric fields present in the RF cavities. Assuming a situation in which there are no losses of energy, the frequency of the field would be such that an electron traveling at the exactly desired orbit would cross the cavity when the electric field is zero [120]. If a particle crosses the cavity earlier than the optimal electron (electron travelling at the desired orbit), the electric field will have a non-zero value, which will accelerate it, giving it energy. The added energy increases the relativistic mass of the particle and, therefore, its angular velocity decreases. After each revolution, the particle’s phase will become closer to the desired, until it crosses the cavity at zero electric field. However, this particle will still have energy higher than the necessary to cross the cavity at the zero field and its tendency in later revolution is to arrive at a time after the optimal electron. The electric field, in this case, will act to decrease the particle’s energy towards the synchronous value. Thus, the particles oscillate in phase and energy around the constant synchronous values generating the so-called synchrotron oscillations. If the goal is to increase the energy of the particles at each turn, one must increase the synchronous value, which, in synchrotron facilities, is done by increasing the magnetic field of the magnets, while the frequency of the electric field remains constant [120].

Mathematically, following the derivation presented in [119], one can assume two accelerating sections (which can be the same, if the particles are passing through it in periodic intervals) separated by a distance L. To accelerate the particles, the field is designed so that particles arriving in each accelerating section will be submitted
to the same phase, resulting in a total acceleration that is equal to the number of sections times their individual acceleration. This is known as synchronous phase and it is defined as:

$$\psi_s = \omega t - kz = \text{const.} \quad (13)$$

where $\omega$ is the frequency of oscillation of the electromagnetic field. The synchronicity condition is given when the time derivative of $\psi_s$ vanishes:

$$\dot{\psi}_s = \dot{\omega} - k\beta c = 0 \quad (14)$$

using $dz/dt = \beta c$. Setting

$$k = \frac{2\pi}{L} \quad (15)$$

the condition is met and the frequency of the electromagnetic field is given by:

$$\omega_1 = k_1 \beta c = \frac{2\pi}{L} \beta c = \frac{2\pi}{\Delta T} \quad (16)$$

where $\omega_1$ is the lowest frequency that satisfies the synchronous condition (it can also be satisfied by integer multiples of $\omega_1$) and $\Delta T$ is the time particles traveling at speed $\beta c$ take to travel between two accelerating sections. The last equation is known as synchronicity condition [119].

When particles are accelerating one has the option to increase $L$ or the frequency of the electromagnetic field as the particle velocity $\beta$ (or energy, in the case of electrons) increases. In the case of synchrotrons, only the second option is feasible, since the accelerating sections are fixed [119].

In the case of a storage ring, RF cavities are used to maintain the energy of the beam, by compensating for losses due to the emission of synchrotron radiation.
However, only half of the field’s period causes the acceleration effect (the other half would deaccelerate the particles) and, in order to maintain the stability of the orbit, only a quarter of the period can be employed. This comes from the fact that not all electrons will arrive at the cavity at the same time. Supposing that an electron arrives at the moment when the electric field provides exactly the amount of energy to compensate for the losses in the last turn, this electron will always reach the cavity at the right moment (i.e., a synchronous electron). If one electron arrives before the synchronous electron, it has to be met with a higher intensity field, in order to approximate the orbits of the two electrons, otherwise it would just increase their distance, which is the same mechanism presented before when the electrons are being actively accelerated. The opposite would occur for an electron arriving after the synchronous electron. Therefore only a quarter of the field’s period can be used [116].

More conditions must be applied to maintain the stability of the beam — as a result only five to ten percent of the RF period can be used, and any electron that does not arrive in this interval will be lost because it does not belong to the same stable orbit as the others. For this reason, the electron beam in synchrotron facilities travel in bunches with time length of 5–10% of the RF period. The minimum number of bunches allowed in the ring is one (single bunch mode), in which case its time interval is the revolution period for the electrons. More bunches can be placed in the ring, as long as the time interval between them is an integer multiple of the RF period. Since the time length of the bunches is limited, so is the number of electrons in each bunch, which means that the more bunches injected into the ring, the higher the beam current [116].

3.3.2 Beam focusing

The principle of focusing is that the angle through which the particles are deflected by the focusing device increases as the distance to its axis increases (see fig. 1). The
same principle can be applied to focusing beam; however, instead of lenses, magnetic devices must be used [119].

\[ B = [\mathbf{n} \times \mathbf{E}]_{ret} \] (17)  

\[ \mathbf{E}(x,t) = e \left[ \frac{\mathbf{n} - \beta}{\gamma^2 (1 - \beta \cdot \mathbf{n})^3 R^2} \right]_{ret} + \frac{e}{c} \left[ \frac{\mathbf{n} \times \{(\mathbf{n} - \beta) \times \dot{\beta}\}}{(1 - \beta \cdot \mathbf{n})^3 R} \right]_{ret} \] (18)

Figure 1: Principle of focusing.

The properties of the magnetic devices used to focus the electron beam in a synchrotron light source are derived and discussed in Appendix A.

3.3.3 Generating radiation

The following discussion on the radiation emitted by accelerated charged particles is based on [121], unless otherwise stated.

The relation between the electric and magnetic fields caused by a moving charged particle is given by:

\[ B = [\mathbf{n} \times \mathbf{E}]_{ret} \] (17)  

\[ \mathbf{E}(x,t) = e \left[ \frac{\mathbf{n} - \beta}{\gamma^2 (1 - \beta \cdot \mathbf{n})^3 R^2} \right]_{ret} + \frac{e}{c} \left[ \frac{\mathbf{n} \times \{(\mathbf{n} - \beta) \times \dot{\beta}\}}{(1 - \beta \cdot \mathbf{n})^3 R} \right]_{ret} \] (18)
where \( \mathbf{n} \) is a unit vector in the direction pointing from the particle to the point where
the measurement is being taken, \( \beta = v/c \), \( v \) is the velocity of the particle, \( \gamma = 1/\sqrt{1-\beta^2} \),
and \( R \) is the distance between particle and measurement. In equation 18, the first
term refers to the field generated by the particle’s velocity (it is independent of the
acceleration), while the second term is the field generated by its acceleration.

If the particle’s velocity is small when compared to the speed of light, then
equation 18 can be reduced to:

\[
E_a = \frac{e}{c} \left[ \mathbf{n} \times \left( \mathbf{n} \times \dot{\beta} \right) \right] \quad (19)
\]

In this case, the Poynting vector \( \mathbf{S} \) gives the instantaneous flux of energy:

\[
\mathbf{S} = \frac{c}{4\pi} \mathbf{E} \times \mathbf{B} = \frac{c}{4\pi} |E_a|^2 \mathbf{n} \quad (20)
\]

and the power radiated per unit solid angle is given by

\[
\frac{dP}{d\Omega} = \frac{e^2}{4\pi c^3} |\dot{\mathbf{v}}|^2 \sin^2 \Theta \quad (21)
\]

This result implies that the distribution of radiation for a nonrelativistic charged
particle has a \( \sin^2 \Theta \) dependence, where \( \Theta \) is the angle measured relative to the
direction of the acceleration.

When the particle is moving at relativistic speeds, the field will also depend on
terms related to the velocity of the particle. In this case, the radial component of the
Poynting vector can be obtained from equation 18

\[
[S \cdot \mathbf{n}]_{\text{ret}} = \frac{e^2}{4\pi c} \left\{ \frac{1}{R^2} \left| \mathbf{n} \times \left[ \mathbf{n} - \beta \times \dot{\beta} \right] \right|^2 \right\}_{\text{ret}} \quad (22)
\]

\(^3\)The Poynting vector represents the energy flux (magnitude and direction) for an electromagnetic
field.
while the power radiated per unit solid angle is defined as

$$\frac{dP(t')}{d\Omega} = R^2 S \cdot n (1 - \beta \cdot n) \quad (23)$$

Assuming that the particle is accelerated for only a small amount of time, such that $\beta$ and $\dot{\beta}$ can be considered constant in magnitude and direction, and that the radiation is observed from far away, making $n$ and $R$ constant during the particle acceleration interval, then equation 23 can be rewritten as

$$\frac{dP(t')}{d\Omega} = \frac{e^2}{4\pi c} \left| n \times \left\{ (n - \beta) \times \dot{\beta} \right\} \right|^2 \left(1 - n \cdot \beta\right)^5 \quad (24)$$

where $S \cdot n$ was substituted from equation 22.

Taking the linear motion as an example (i.e., $\beta$ and $\dot{\beta}$ are parallel), then

$$\frac{dP(t')}{d\Omega} = \frac{e^2 v^2}{4\pi c^3} \frac{\sin^2 \theta}{(1 - \beta \cos \theta)^5} \quad (25)$$

where $\theta$ is the angle measured from the direction of $\beta$ and $\dot{\beta}$. This result is the same as the one in equation 21 for $\beta \ll 1$ (i.e., for a nonrelativistic particle). If $\beta \rightarrow 1$, however, the angular distribution of the emitted radiation is tipped forward. The radiation intensity is maximum at $\theta_{max}$, which is given by:

$$\theta_{max} = \cos^{-1} \left[ \frac{1}{3\beta} \left( \sqrt{1 - 15\beta^2} - 1 \right) \right] \quad (26)$$

This result shows that the angles at which most radiation is emitted by relativistic particles are very small, and that the radiation is, thus, mostly emitted in a very narrow cone around the direction of motion of the particles. It can be shown [121] that this result is equivalent to the one for particles traveling in circular motion with instantaneously constant speed, as is in the case of synchrotron sources.

In the case where $\beta$ and $\dot{\beta}$ are perpendicular, such as in a synchrotron facility,
considering a coordinate system such that $\beta$ is in the $z$ direction and $\dot{\beta}$ is in the $x$ direction:

$$
\frac{dP(t')}{d\Omega} = \frac{e^2 |\dot{v}|^2}{4\pi c^3 (1 - \beta \cos \theta)} \left[ 1 - \frac{\sin^2 \theta \cos^2 \phi}{\gamma^2 (1 - \beta \cos \theta)^2} \right]
$$

(27)

where $\theta$ and $\phi$ are the polar angles defining the direction of observation. In this case, although the angular distribution is different than in the parallel case, the radiation is also emitted in a narrow cone in the particle motion direction.

Appendix B explores the different devices used in synchrotron facilities to change the direction of charged particles, causing their acceleration and the production of synchrotron radiation. Appendix C discusses X-ray optics and interactions between X-rays and matter, which are necessary to fully understand the techniques discussed in sections 3.4 and 3.5.

### 3.4 X-ray fluorescence (XRF)

#### 3.4.1 Theory and techniques

X-ray fluorescence measurements take into account the emission effects from the X-ray interactions with matter to produce spectra (as discussed in Appendix C). The energy of the peaks in the resulting spectrum can be associated with a chemical element, according to their characteristic energy levels, and the peak intensity is related to the quantity of the element in the sample [122].

In XRF analysis, the peaks are named according to the electron transition from which they originate, as shown in fig. 2. Transitions to the K shell of the atom are called K$\alpha$ if the electron comes from the L shell, K$\beta$ if the electron comes from the M shell, and so on. Transitions to the L shell are called L$\alpha$ (from M shell), L$\beta$ (from the N shell) and so on for other energy levels. In most XRF analysis, K and L transitions are the most commonly visible [122].
The next sections will discuss different ways of applying XRF in materials research, such as spatially resolved XRF, XRF mapping and quantitative measurements.

**Spatially resolved XRF** Spatially resolved XRF refers to the control over the area in the sample being measured in order to obtain the best conditions for the experiment. Large excitation areas are usually used in homogeneous samples, when there is no change in the element composition and concentration throughout the sample. In this case, a large area in the sample is probed with limited need of beam collimation, resulting in larger intensities and good statistics even for short measurement times [123].

When using a smaller collimator, the probed area in the sample is smaller, providing information on how the composition and concentration of elements change spatially. The intensity of the beam is, however, lower in this case. Since the intensity drops with the square of the spot diameter in collimators, there is a limitation on how small the beam spot can be in order for enough statistics to be
obtained in a feasible amount of time [123].

If an even smaller beam spot is necessary, a different set of optics must be used. An example is the polycapillary optics, which can provide a very small spot with high flux. Very small spots are necessary to study very complex materials, such as archaeological or geological samples [123].

In all the examples discussed so far, the size of the area studied depends on the size of the beam spot on the sample. However, this is not the only way to control how much of the sample is analyzed. A different approach is to shine radiation in a large area of the sample and use a piece of X-ray optics (such as slits) between the sample and the detector to select the fluorescence radiation produced in the desired part of the sample. This approach requires that the incident radiation of very high intensity, i.e., much higher than when using a collimated beam [123].

Another possible geometric arrangement for spatially resolved XRF involves the use of the focusing optics. This can be used not only to collimate the beam, but also to produce a confocal geometry. In this case, only the volume within the intersection between both beam paths will be probed. Notice that, in the previous examples, the collimation selects the area to be probed, but the depth is defined by the characteristics of the radiation and sample material. In the confocal approach, the method provides depth resolution as well, since it chooses a volume to be probed [123].

A very common application of spatially resolved XRF is μ-XRF, where the beam spot in the sample is on the order of micrometers. This technique can be used for either single point measurements or to map larger areas in the sample [123].

**XRF mapping**  XRF maps are single point measurements made in a grid, such that the space between points either in the horizontal (x) or vertical (y) directions are always the same (the distances do not need to be equal between the two directions). 42
In this case horizontal and vertical directions are perpendicular to each other and to the direction of the beam propagation (z). The number of points in each direction, and the space in between them (which will ultimately define the size of the map), are decided according to the structure that is being measured [123].

A typical XRF map contains thousands of points. Even with very short measurement times at each point, the time necessary to measure a complete map is in the range of hours and depends on the experimental setup of the beamline: in “stop-measure-go” setups, where the sample has to completely stop before data are collected, the time consumed is much larger than in “on-the-fly” setups, where the data are collected while the sample moves (and, therefore, the sample never stops) [113]. The very short time spent in each point means that the intensity of the data collected is very low, which necessitates very high beam intensity in experiments of this kind [123].

The resulting data set provides information on the position of the measurement (x and y in case a bidimensional map), on the energy E of the fluorescence photons, and on the intensity I of fluorescence photons at a given energy by means of recording a complete spectrum for each point (x,y) [123].

The data collected in a map is represented by a series of spectra, one for each scanned point with fixed x and y coordinates. This allows to chemically characterize spots and regions in the map. For example, specific regions of each spectrum, representing a given chemical element, can be selected. The map can then be showed applying this selection so that the distribution of this particular element can be observed in the sample. Bidimensional distributions (x,y) of single or multiple elements, or simply the selection of any given energy range, can be displayed this way. In these distributions, a single chemical element map can be represented using a colour scheme, where each colour corresponds to an intensity (obtained from the integration under the area in the spectrum corresponding to this particular
element). If multiple elements are shown, they are usually each represented by a
different colour, while the intensity is represented by the colour’s brightness [123].

In order to analyze the data obtained in XRF maps, one must take into account the
experimental setup used and the possible detector artifacts that would influence the
element distribution in the map. The detectors normally used in XRF experiments,
as well as the possible detector artifacts present in the data collected, are discussed
in Appendix D.

3.4.2 Quantitative analysis

Qualitative analysis of XRF measurements can provide information on which elements
are present in the sample through simple visual analysis of the spectra. Another
qualitative method is the positive material identification (PMI), which compares the
measured spectrum with the spectra of known materials measured under the same
conditions for a fast identification [123].

Even though the qualitative analysis can provide valuable information about the
sample analyzed, quantitative analysis is also possible and often very accurate.
X-ray fluorescence has a very strong dependence on the sample’s matrix (i.e.,
measured intensities depend not only on the element concentration and
measurement conditions, but also on the other elements and geometry in the
sample). Therefore, calibration properties have to be changed for each sample, but
all the parameters are very well described by physical models [123]. The basic
physical model to describe how measured intensity is related to the weight fraction
is given by the Sherman equation:

$$I_i = G \int_E \frac{w_i \tau_i(E) S_{-1}}{\sin \psi_{\text{ein}}} \frac{p_i \omega_i}{\sin \psi_{\text{aus}}} I_0(E) dE$$  \hspace{1cm} (28)

where $I_i$ is the intensity of element $i$, $G$ is a factor related to the geometry, $w$ is
the weight fraction, $\tau$ is the linear absorption coefficient, $\frac{S-1}{S}$ is the jump ratio, $p$ is the transition probability, $\omega$ is the fluorescence yield, $\mu$ is the linear mass absorption coefficient, $\psi$ is the incident and take off angle and $I_0(E)$ is the excitation spectrum [123].

However, the issue with this equation is that it calculates the intensity using the weight fraction, which is the opposite of the problem that usually needs to be solved. In XRF measurements, one wants to obtain the weight fraction of an element based on its measured intensity. Since equation 28 cannot be solved analytically, models have been developed to find approximated solutions for it [123].

Concentration correction models can be used, where

$$w_i = f(I_i, w_j)$$  \hspace{1cm} (29)

with $i$ representing the element of interest and $j$ representing all the other elements. However, since the weight fraction for the other elements is not usually known, this model has to be solved using iterative calculations [123].

Intensity correction models try to solve the previous problem by assuming that the matrix elements’ influence is linear:

$$w_j = a \times I_j$$  \hspace{1cm} (30)

where $a$ is the sensitivity [123]. Then,

$$w_i = f(I_i, I_j)$$  \hspace{1cm} (31)

and the calculation does not need to be performed iteratively, since the intensities can be obtained from the measured spectrum [123].

A common requirement for all these models is the necessity of a calibration
procedure, which is used to find specific parameters of the measurement, such as characteristics of the detector and beamline. The calibration is usually performed using a sample with known weight fraction [123].

### 3.5 X-ray absorption fine structure (XAFS)

The X-ray Absorption Fine Structure (XAFS) spectrum provides information of the local properties of the material being analyzed [116]. These spectra show a sharp increase in the absorption when the energy of the incident X-rays correspond to specific values characteristic for each element [124]. The energy values are determined by the energy necessary for an electron in the atomic core to be ejected. The increases in absorption are called edges and are named according to the original electronic level of the ejected electron. In this way, the K-edge corresponds to electrons originating from a 1s level and a L-edge corresponds to electrons originating from a 2s or 2p level [124].

An incident X-ray beam is attenuated as it goes through a sample, which is given by an exponential equation:

$$\Phi = \Phi_0 e^{-\mu(\omega)x}$$  \hspace{1cm} (32)

where $\Phi$ and $\Phi_0$ are the beam flux (number of photons per unit time per unit cross-section) after and before going through the sample, respectively, $\mu(\omega)$ is the attenuation coefficient as a function of the energy of the photons $h\omega$, and $x$ is the sample thickness [116]. As the energy of the incident photons increases, the attenuation coefficient decreases and, thus, the resulting flux increases smoothly. This behaviour is only different when the energy of the X-rays becomes high enough that a core electron can be ejected and the absorption edge appears. Since the energy necessary to eject a photoelectron from a determined shell increases with the atom’s atomic number, its value is characteristic for each atomic species [116].
The edges present in the X-ray absorption fine structure provide information on the local structure of the atom. In the case of isolated atoms, such as noble gases, the fine structure is contained within a few eV around the edge and is the result of the multiple allowed transitions to other bound levels in the atom, each with a characteristic energy [116]. In more complex systems, however, the fine structure can extend for hundreds of eV above the edge energy and it is influenced by the atoms surrounding the absorber [116]. In this case, the XAFS can be divided into three different regions, which provide different information about the absorber:

- **Pre-edge and edge.** Limited to the region within a few eV from the edge. The pre-edge is mostly the result of dipole allowed transitions to other bound states and is most evident in oxides. It shows differences in the local structure well, even if the atoms are in the same valence state [116]. The edge energy is related to the oxidation state of the absorber (the higher the oxidation state, the higher the edge energy). The shape of the edge is related to the geometry of the ligand and can be used to identify specific chemical species [116, 124].

- **X-ray Absorption Near Edge Structure (XANES) or Near Edge X-ray Absorption Fine Structure (NEXAFS).** This is the region within ~50 eV of the edge and provides information on the electronic and geometric structures around the absorber [116].

- **Extended X-ray Absorption Fine Structure (EXAFS).** It is the region that extends from the XANES region up to around a thousand eV above it. This region provides information about the geometrical structure around the atomic species being analyzed [116]. In this thesis, however, only the XANES portion of the XAS spectrum was used and, therefore, the discussions that follow will concentrate on this topic.

Although the interpretation of results obtained in the XANES region is more difficult
than the ones from the EXAFS region, the use of this technique has been increasing in the past decades. This is due to several factors (when comparing to EXAFS), such as the stronger spectral features, which makes interpretation less dependent on statistics (and, therefore, beam intensity and quality of the sample). Consequently, XANES is a very interesting choice for natural samples, which are very heterogenous, or diluted samples. Other advantages are that XANES is subject to less temperature effects than EXAFS, and that data acquisition is faster, which makes this technique appropriate for time resolved measurements [116].

3.5.1 The XANES spectra

The analysis of XANES spectra, which typically includes the pre-edge, edge and post-edge regions (fig. 3), provide information on the oxidation state and the environment of the atoms [124], including their coordination chemistry, local structure and ligand symmetry [116]. The different regions within XANES will be examined in more detail in what follows.

Figure 3: Strontium XAFS spectrum with XANES region selected. Also represented the different regions in XANES spectrum: pre-edge, edge and post-edge (multiple scattering region). (Original in colour) .
**Edge**  The position of the edge provides information on the oxidation state of the atom [116, 124]. This is due to the change in the energy necessary to excite an electron when the total number of electrons in the atom changes. If the oxidation state is higher, and, therefore, the number of electrons in the atom is smaller, the overall charge of the atom is more positive and an electron will require more energy to be excited away from its orbital [124]. Therefore, the higher the oxidation, the higher the energy at which the edge appears [116, 124].

**Pre-edge**  The pre-edge features are, mainly, the result of dipole allowed transitions to other bound states in the atom [116]. For the K-edge in particular, the pre-edge is mostly due to transitions from the 1s level to a level with p-symmetry (usually the first available), which is a transition allowed by quantum mechanics ($\Delta l = 1$). Transitions to levels with d-symmetry are not allowed ($\Delta l = 2$) and are rarer [116, 124]. They can be detected in some cases, however, mainly when there is hybridization of p-d levels [116].

Three types of transitions can be used to explain the features of pre-edges:

- local electric quadrupole transitions ($1s \rightarrow 3d$);

- non-local electric dipole transitions ($1s \rightarrow p$), where the empty $p$ states of the absorber are hybridized with the empty $d$ states of the neighbouring atoms;

- local electric dipole transitions ($1s \rightarrow p$), where the empty $p$ states of the absorber are hybridized with its empty $d$ states.

Calculations can be used to determine the contribution of each type of transition to the measured pre-edge [125].

Also, the position of the pre-edge can be used to fingerprint oxidation states for some elements, such as iron, vanadium and chromium [125].
**Post-edge**  The post-edge signal is the result of photoelectrons being excited to the continuum states. The long mean free path for low energy electrons results in the enhancement of multiple scattering terms (full multiple scattering, FMS), in contrast to the EXAFS spectrum, which can be sufficiently explained using a single scattering approximation (see section 3) [116]. By means of fittings and modeling, information on local structure and coordination geometry can be obtained [116].

### 3.5.2 Qualitative and semi-quantitative analysis

The ab-initio calculation for XANES spectra can be very time consuming and requires a very extensive knowledge of its theoretical background. Even though these methods exist, others (qualitative and semi-quantitative) can be used to extract important information without the need for a completely quantitative approach [116]. In the next sections, some of these approaches will be explored.

**Edge shift**  The edge shift method uses the position of the edge (or, in some cases, pre-edge) to rapidly establish the oxidation state of a material. The energy of the edge is compared to the energy of edges of known reference samples and the oxidation state can be easily found [116].

**Linear combination analysis (LCA)**  The linear combination analysis method provides information on the relative amounts of different chemical species in a sample. In order to do that, the normalized spectrum of the unknown sample $\mu_{\text{exp}}$ is fitted with a linear combination of the spectra of reference compounds $\mu_{\text{th}}$:

$$\mu_{\text{exp}} = \sum_j \alpha_j \mu_{\text{th}}$$  (33)

where $a_j$ represents the fraction of absorbers in the jth chemical state [116]. The success of this method depends on having good-quality measurements for the
reference materials. Preferably, these measurements would be gathered as part of the same experimental run as the measurements of the unknown sample, to prevent measurement artifacts from interfering with the results [116].

This method is especially useful for heterogeneous samples, such as objects of cultural relevance, biological specimens, and environmental science samples [116].

**Principal component analysis (PCA)** In the principal component analysis approach, statistical considerations are used to select a set of spectra (principal components) out of an ensemble of reference spectra, in order to perform a linear fit on the data. This method is valuable for routine checks in large data sets, such as for environment monitoring. However, one must be careful while selecting the reference samples and check the final results so as to avoid non-physical answers [116].

**Deconvolution of XANES features** The features seen around the edge region on a XANES spectrum are due to very sharp peaks as a result of transitions to other bound states in the atom over a smooth background of transitions to the continuum. The distribution of peaks is related to the local symmetry and binding geometry in the material. The analysis of these peaks, usually in comparison to references, can be used to obtain information on coordination number and valence state of absorbers [116].

### 3.5.3 Quantitative analysis

**Theory** The discussions in this section will follow reference [116].

X-ray absorption spectroscopy is studied in terms of the transitions between a core state and an occupied level in an atom. The absorption coefficient \( \mu (E) \) is written as
a function of the photon beam energy, and is given by

$$\mu(E) = n_a \sigma(E)$$  \hfill (34)

where \(n_a\) is the density of absorbers in the sample, and \(\sigma(E)\) is the local absorption cross section, which can be calculated using Fermi’s “golden rule” with the dipole approximation:

$$\sigma(\omega) = 4\pi^2 \alpha_0 \hbar \omega \sum_f \left| \left\langle \Psi_f^N | \varepsilon \sum_i r_i | \Psi_g^N \right\rangle \right|^2 \delta (\hbar \omega - E_f^N + E_g^N)$$  \hfill (35)

where \(\varepsilon\) is the polarization vector for the incoming photon, \(r_i\) indicates the position of the \(i^{th}\) electron and the initial and final states are many-body functions. This equation considers all the possible excitations taking place in the atom and the creation of a hole in the level \(\phi_{L_0}^{f}\) with angular momentum \(L_0 = (l_0, m_0)\).

The initial and final states can be approximately written, when spin variables are omitted (i.e., for non-magnetic systems), as:

$$\Psi_f^N (r, r_1, ..., r_{N-1}) = \sqrt{N!} A \sum_{\alpha} \phi_f^\alpha (r) \tilde{\Psi}_{\alpha}^{N-1} (r_1, ..., r_{N-1})$$  \hfill (36)

where \(A\) is the anti-symmetrization operator, \(\phi_f^\alpha\) are functions that describe the excited photoelectron, and \(\tilde{\Psi}_{\alpha}^{N-1}\) are eigenstates of the Hamiltonian \(H^{N-1}\), which describes the remaining \(N - 1\) electrons and has eigenvalues \(E_{\alpha}^{N-1}\):

$$H^{N-1} \tilde{\Psi}_{\alpha}^{N-1} = E_{\alpha}^{N-1} \tilde{\Psi}_{\alpha}^{N-1}.$$  \hfill (37)

The tilde works as a reminder that the relaxed states around the core of the atom are dominant in the expansion presented in equation 36.

Also, the eigenstate \(\Psi_f^N\) of the total Hamiltonian \(H^N\) has eigenvalues \(E_f^N = E_g^N +\)
ℏω and

\[ H^N = -\nabla_r^2 + \sum_i V(r, r') + H^{N-1} \]  \hspace{1cm} (38)

where \( V(r, r') \) is the potential for interaction between the excited photon and the rest of the system.

By combining the results of equations 36 and 37, the amplitude functions \( \phi^f_\alpha \) are given by

\[ (\nabla^2 + k^2_\alpha) \phi^f_\alpha (r) = \sum_{\beta} V_{\alpha\beta} (r, r') \phi^f_\beta (r') \, d^3r' \]  \hspace{1cm} (39)

where

\[ k^2_\alpha = \hbar \omega - (E_{g}^{N-1} - E_{g}^{N}) - (E_{\alpha}^{N-1} - E_{g}^{N-1}) = \hbar \omega - I_c - \Delta E_\alpha \]  \hspace{1cm} (40)

where \( I_c \) is the ionization potential for the core state, and \( \Delta E_\alpha \) is the excitation energy left in the \((N-1)\)-particle system. \( V_{\alpha\beta} \) represents a matrix of potentials between states \( \tilde{\Psi}_{\alpha}^{N-1} \) and \( \tilde{\Psi}_{\beta}^{N-1} \).

The set of equations 39 corresponds to a complete solution for the photoelectron process, with specific boundary conditions determined by how the total energy \( E_{f}^{N} = E_{g}^{N} + \hbar \omega \) is partitioned in the \((N-1)\)-electron system. The solution of the complete system is not achievable. However, the solution of particular cases can be reached, such as the solution for the elastic channel \((\Delta E_\alpha = 0)\), which carries structural information of the material. In this example, the equations belonging to unwanted channels are eliminated, resulting in a single equation for the elastic channel:

\[ (\nabla^2 + k^2_0 - V_c (\vec{r})) \phi_0 (r) = \int \Sigma^{opt} (r, r'; \hbar \omega) \phi_0 (r') \, d^3r' \]  \hspace{1cm} (41)

where \( \Sigma^{opt} \) represents a complex non-local optical potential, with the term \( \hbar \omega \) representing the energy coming from the eliminated channels, and \( V_c \) represents the local Coulomb potential. The calculation of \( \Sigma^{opt} \) is still not possible at the moment and approximations are made based on the system being studied. Using a Green’s
function matrix and considering, again, only the elastic channel (a more detailed
derivation can be seen in [116]), the total absorption cross section for the
unpolarized case can be written as:

$$\sigma(E) = (l - 1) \sigma_{0}^{l+1}(E)\chi^{l+1}(E) + l\sigma_{0}^{l-1}(E)\chi^{l-1}(E)$$  \hspace{1cm} (42)

where $\sigma_{0}^{l}(E)$ is the atomic cross section of the absorber at an edge:

$$\sigma_{0}^{l}(E) = \frac{8\pi^{2}}{3} \alpha k(E + I_{0}) \sin^{2} \delta_{l}^{0} \int_{0}^{\infty} r^{3} R_{L}^{0}(r)\phi_{l_{0}}(r)dr$$  \hspace{1cm} (43)

with $\delta_{l}^{0}$ being the $l$-phase shift for the absorbing atom in a muffin-tin potential, $R_{L}^{0}$
being the regular part of the solution of the Green function at the origin, $\phi_{l_{0}}$ is the
channel function, and $\chi^{l}(E)$ is a structural factor given by

$$\chi^{l}(E) = \frac{1}{(2l + 1) \sin^{2} \delta_{l}^{0}} \sum_{m} \Im \left[ (I - T_{a}G)^{-1} T_{a}^{00} \right]_{lm}$$  \hspace{1cm} (44)

with $T_{a}$ representing a diagonal matrix defined as $T_{a} = \delta_{ij}\delta_{LL'}t_{i}^{l}$ and $G$ representing
the off-diagonal free electron propagators, which are written using spherical
harmonics. This factor contains all the information about the atomic cluster
neighbouring the absorber. The muffin-tin is an approximation for the potential of
an atomic cluster commonly used in multiple scattering calculations. This
approximation assumes that the potential can be divided into three distinct regions
separated by two spheres, one enclosing each atom, and another enclosing the whole
molecule. The potential within the inner sphere and out of the outer sphere are
assumed to be spherical, while the one in between them is assumed to be constant
(position independent).

**Analysis options**  A new fitting method has been proposed in which the XANES
regions are fitted with relation to a series of theoretical calculations using structural
variations on a chosen structure around the absorber. In this method, which is implemented in the software package MXAN [126], the exact calculations of the spectra of hundreds of different geometrical configurations are fitted to the experimental spectrum in order to obtain the smallest square residual function in the parameter space [116]. The fitting uses a set of programs developed by the Frascati theory group for the multiple scattering cross section calculation, and the minimum square residual function is calculated using the MINUIT [127] routines from the CERN library:

\[
S^2 = n \sum_{i=1}^{m} w_i \left[ \frac{\left( y_i^{th} - y_i^{exp} \right) \varepsilon_i^{-1}}{\sum_{i=1}^{m} w_i} \right]^2
\]

(45)

where \( n \) is the number of independent parameters, \( m \) is the number of data points, \( y_i^{th} \) and \( y_i^{exp} \) are the theoretical and experimental values for the absorption, respectively, \( \varepsilon_i \) is the errors for each point in the data and \( w_i \) is the statistical weight [116]. If \( w_i = 1 \) is a constant, then \( S^2 = \chi^2 \) statistical function [116].

This method uses the muffin-tin approximation as was discussed in the previous section. The effects of the non-muffin-tin corrections for XANES are still not well understood, but evidence suggests they are more evident for very low energies and should not interfere with the structural results of the spectrum analysis [116].

The Demeter software package [128] is another option when analyzing XANES data. It is capable of both simple qualitative analysis and quantitative applications (in combination with other software). The package is composed of three softwares:

- **ATHENA.** A program that performs XAS data processing, including the tools for preparing EXAFS data for analysis, and the analysis of XANES spectra. The tools provided include the fitting of linear combinations and the comparison with calculated spectra, which can be imported into the program.

- **ARTEMIS.** A program to analyze EXAFS data, including the fitting of the
spectra.

- HEPHAESTUS. A program that contains tools based on the periodic table and absorption cross sections for various elements. Among other tools, it can display absorption and fluorescence line energies and other relevant chemical information for the elements and show charts containing the transitions associated with absorption lines [128].

The FEFF9 [129] is a code with user interface used to calculate XAS spectra using Green’s functions. Details of how the calculations are performed as well as examples can be seen in [129].

3.6 Conclusions

The use of synchrotron radiation in characterization techniques provides results with high resolution and statistics, and requires short acquisition times when compared to other light sources. This characteristic is essential to the application of such techniques to palaeontology due to the heterogeneity in the composition of specimens. Palaeontological samples have a large quantity of different elemental or molecular species, some of which appear in very low concentrations.

Synchrotron XRF can be used to detect heterogeneities and the distribution of elements at microscopic level. This makes the detailed analysis of the chemical composition and of correlations between elements in fossils possible. XRF can also be used to detect the presence of trace elements. However, the information obtained in most palaeontologic situations is qualitative. Although quantitative measurements can be done with this technique, these become particularly difficult to apply to fossils. Among other factors, fossils usually present irregular surfaces (e.g., due to the presence of canals in bones), resulting in geometric effects on the measurements, which would have to be considered in a quantitative analysis. Even
with these limitations, the technique is very useful to study chemical properties of fossils, such as diagenetic alterations, in the specimens.

While XRF provides information about different chemical elements, XAFS studies their speciation into different compounds and chemical states. This technique can be used to identify characteristics such as the oxidation state, the compound the element belongs to, and mixtures of states. The application of XAFS to palaeontology can be used to suggest the origin of different elements in samples (i.e., biologic or diagenetic), or the path that led to the specimen’s preservation.
4 Chemical diagenesis of *Tyrannosaurus rex*

bones from the Frenchman Formation

4.1 Abstract

*Tyrannosaurus rex* is one of the most widely recognised dinosaur species, probably because of its large size and strength. Among the fossils found for members of this species, there is a relatively complete skeleton (informally known as “Scotty”) that was found in the Frenchman River Valley (Saskatchewan, Canada) in 1991 [140]. “Scotty” is noteworthy because of the large percentage of bones found, which represent approximately 65% of the skeleton. These have preserved microstructures visible, for instance, using optical microscopes, among other techniques; and some of its features can be analyzed through petrographic techniques, such as the one to be discussed in section 4.4.

The goals of this study include the exploration of diagenetic changes and taphonomic history of this specimen. This is accomplished by comparing the chemical analysis of its bones to other bones found in the same stratigraphic interval and geographic region, and a modern (extant) swan is used as a control sample. Another goal is the establishment of a hypothesis to partially explain its preservation.

The results obtained (section 4.4) show that the *T. rex* bones were considerably altered diagenetically, mainly by the addition of yttrium and strontium in the bone tissue, and the presence of a layer of manganese in the transition region between the bone surface and the associated sediment matrix. The manganese layer indicates that the *T. rex*’s tissues were exposed to an aquatic environment where manganese was present, probably as oxides or hydrates. The presence of Fe(III) in the bone tissue offers hints about why this specimen was preserved (see section 2.4.2), and fine details of the diagenetic changes observed.
4.2 Introduction

The first members of the clade Dinosauria probably appeared during Early to Middle Triassic. However, the oldest known specimens date back to the Late Triassic, during the Carnian age (\(\sim 228 \text{ to } \sim 216.5 \text{ Ma}\)) [141]. The members of this clade can be classified into two orders: Saurischia and Ornithischia, named after differences in their hip structures. The dinosaurs belonging to the order Saurischia can be divided into two suborders: Sauropodomorpha, which will not be described here (see [143] and [144]) and Theropoda ("beast-footed ones") [145]. The Theropoda are widely known for containing large predators, such as *Spinosaurus*, *Giganotosaurus* and *Tyrannosaurus*. However, the group also contained species of omnivores and herbivores, as well as dinosaurs among the smallest present in the Mesozoic, such as *Epidendrosaurus*, *Microraptor* and *Mei* [145]. Theropods can be considered the most successful group of dinosaurs, as they were the only dinosaurs to survive the catastrophic extinction event at the end of the Cretaceous, in the form of birds (Aves) [145]. They were strict bipeds and most of them were characterized by ziphodont teeth, long grasping hands with recurved claws (that were usually sharp), chambers and openings in the skull bones associated with air sacs and hollow limb bones and vertebrae [145].

Tyrannosauroidea (tyrant dinosaurs) are a group of theropods best known for their Late Cretaceous members found in Asia and North America, belonging to the family Tyrannosauridae. The early members of Tyrannosauroidea evolved fused nasals, which allowed for a stronger bite, incisiform premaxillary teeth, and a narrow metacarpal III [145]. Tyrannosauridae, in particular, appeared in the Late Cretaceous and were the largest predators in their environments, with most species reaching at least 10 m in length. They were characterized by having large and wide skulls, which allowed for forward-facing eyes (binocular vision); a thick maxillary, and teeth with long roots (which were probably capable of crushing through bone);
large neck muscle attachments; and short forelimbs with didactyl hands [145].
Tyrannosaurids probably depended on their jaws as a primary tool for hunting, and
their long legs allowed for fast running — at least for the lighter members of the
group and juveniles. Among tyrannosaurids, one can find the genus *Tyrannosaurus*
and the species *Tyrannosaurus rex*, which represented the largest predator in
western North America in its time [145].

As organs, bones are responsible for the support and protection of the organs
and tissues in the body, metabolic control of mineral homeostasis, and housing the
bone marrow, which is involved in the production of blood cells. Bones also provide
attachment points for muscles, ligaments and tendons, and store calcium and
phosphate in the body [146].

Bones are composed of organic and mineral components [147]. The organic
components form a framework for the mineralization to occur, and provide tensile
strength and elasticity to the bone. The main organic component of bone is type I
collagen, a triple helix-shaped protein made of two collagen $\alpha_1$ (I) peptide chains
and one collagen $\alpha_2$ (I) peptide chain [147]. Bones also contain other types of
collagen, non-collagenous proteins (e.g., fibronectin, osteocalcin), and other organic
molecules [147]. The mineralization of the bone confers its mechanical rigidity. The
main mineral components are calcium and phosphate in the form of hydroxyapatite
$(\text{Ca}_{10}(\text{PO}_4)_6(\text{OH}_2))$ crystals [147], although other elements chemically similar to
calcium such as strontium and barium can also be found in bone apatite [148].

Bone tissue can be classified into compacta or spongiosa, according to its porosity.
The tissue is considered compacta when its mineral volume is larger than the empty
volume found in its pores, and spongiosa otherwise [146]. In general, compacta tissue
can be found in the outer cortex of bones and spongiosa tissue can be found in its
center (although that is not always the case) [146]. Bones (except their joints) are
usually covered by the periosteum [149, 150]. This membrane is important to the
modelling and remodelling of bone, particularly when repairing fractures [149, 151].

Bone tissues can also be classified according to their structural patterns. These patterns include: lamellar bone, where the tissue consists of thin layers with high density of collagen fibers; woven-fiber bone, where the tissue is composed of disorganized collagen fibers of different sizes with varying orientations; and parallel-fibered bone, where the tissue is composed of a parallel arrangement of tightly packed collagen fibers [146].

The bones are supplied with blood through Harvesian canals, which run the length of the bone (fig. 4), and Volkmann’s canals, which exhibit radial orientation and connect different Havesian canals [146]. When bone is formed or grows, the blood vessels are incorporated in large canals formed by the deposition of matrix around them. These canals can be later filled by concentric deposition of lamellar tissue and these structures (i.e., blood vessels surrounded by lamellar tissue) form what is known as primary osteons (fig. 4). Secondary osteons are the result of changes in the bone during the animal’s life, where bone tissue can be reabsorbed and re-metabolized around a blood vessel, which is, then, surrounded by concentric layers of lamellar tissue [146].

Figure 4: “Bone anatomy”. Modified from [139]. Licenced under the terms and conditions of the Creative Commons Attribution (CC BY). (Original in colour).

The application of synchrotron techniques such as the ones used in this research to
the study of fossils is a relatively recent advancement. It has only become more widely known and used after results were obtained for the primitive bird *Archaeopteryx* [152] in the early 2010s. In that study [152], synchrotron rapid scanning X-ray fluorescence was used to chemically analyze feathers. It showed that these feathers were not just impressions, as it was once believed, but instead were fossilized structures with a chemical composition different from the surrounding material. The use of synchrotron techniques in paleontology (and archeology) studies has been reviewed in [153].

In general, synchrotron X-ray techniques are applied to fossils with the goals of 1) restoring lost information, such as the behaviour and biology of extinct animals; 2) understanding the taphonomic processes that fossils go through, so that it is possible to recreate the original (and lost) information about the material; and 3) predicting the chemical pathways similar objects would follow, making it possible to prevent further damages to the specimens already in collection [153, 154]. These techniques have shown that the materials found in fossil bones are chemically different from the sedimentary matrix where they are found, suggesting that although altered, their chemical information is not completely lost due to diagenesis [155].

Some examples of work developed using synchrotron techniques to study dinosaur bones include the use of synchrotron XRF to map chemical difference between healthy and pathological bone for extant and extinct archosaurs (see section 2.4.1 for bone preservation studies that do not involve synchrotron radiation). This work showed specific features in the regions between different types of bone, like compact and spongy bone, as well as the presence of zinc as a possible biomarker for ossification processes [156]. Another study used a combination of synchrotron XRF for high atomic number (Z) elements, and energy-dispersive X-ray spectroscopy (EDX) for low Z elements, to chemically map the distribution of trace elements in sauropod bones in order to identify the effects of diagenesis. This research showed that even bones that did not show considerable changes at the histological level presented a considerable
degree of diagenetic alteration, including cracks and the presence of mineral filling in pores and canals [157]. Synchrotron XRF, scanning electron microscopy (SEM), and XAFS were also used in [158] to study bones of an artiodactyl and a perissodactyl from Greece. They mapped the distribution of bone apatite using the presence of calcium and phosphorous, and identified oxides with iron filling cavities in the bones. Goethite and pyrite were detected in the samples, and strontium was found to substitute for calcium in the bone apatite. Manganese was also detected in considerable amounts close to cracks, and contaminating the periosteum and medullar cavity.

XRF (not using synchrotron radiation), in combination with other techniques (X-ray diffraction and Fourier transform infrared spectroscopy), have also been used to characterize diagenetic alterations in the bones of Upper Jurassic/Lower Cretaceous to Upper Cretaceous dinosaurs from Spain in [159]. This study detected large amounts of calcium, phosphorus, iron and strontium in the bones, along with the presence of goethite (FeOOH) and celestite (SrSO$_4$) phases. This study was also able to identify elements with higher atomic number (Y, As, Pb, Ti, Mn, Cr, Cu, Zn) in trace amounts.

4.2.1 Scotty, the Saskatchewan *T. rex*

The *Tyrannosaurus rex* specimen (RSKM_P2523.8) analyzed herein is informally known as “Scotty”, and was found in the Frenchman River Valley, close to the town of Eastend in Saskatchewan (Canada), in 1991. However, it was only fully identified in 1994, in the same year that large-scale excavations started [140]. The skeleton was not articulated and was partially encased in a mixture of ironstone and very well-cemented sandstone. Both these matrices made the excavation process more laborious, necessitating two complete summers of fieldwork to extract the specimen [140].

“Scotty” was found in the Frenchman Formation, the youngest Cretaceous
formation in southwestern Saskatchewan [160], dated to the Late Maastrichtian [161]. The formation is a sedimentary succession stratigraphically located between the underlying Battle Formation and the overlying Ravenscrag Formation [162, 163, 164]. Due to the Battle Formation having been eroded in some areas, or not deposited at all in other areas [161], the Frenchman Formation can appear on top of the Bearpaw, Eastend or Whitemud formations in different regions of Saskatchewan [165, 166, 167]. The contact between the Frenchman and Ravenscrag formations is usually conformable, presenting a continuous deposit over the Cretaceous-Paleogene boundary [161]. This transition is rich in iridium and has been interpreted as a result of the meteorite impact event linked to the K-Pg mass extinction [168, 169, 170].

The thickness of the Frenchman Formation varies from 8.5 m to more than 67.7 m, increasing in thickness heading outward from the town of Eastend in both northern and eastern directions [167]. The formation is composed of laterally discontinuous layers of two different facies, one of these is mostly composed of sand, and the other is mostly composed of clay [161, 163, 167]. The sandy facies are predominantly formed by medium-to-fine grained subgreywackes cemented by calcium carbonate [163], forming large sandstone masses in some regions [167]. The clay facies, however, are composed of bentonitic clays, which exhibit a popcorn-like surface when dry [163]. The T. rex skeleton found in the Frenchman Formation and described in the next section was found in sediments of the sandy facies [161].

The RSKM_P2523.8 skeleton discussed in this thesis is one of the most massive specimens found to date, measuring almost 12 metres in length and 4 metres in height, with an estimated weight of 6 tonnes [171]. The specimen is also unique in having been found in association with preserved seeds, leaves, fruits and other plant remains [161]. The presence of plant and animal fossils in the same area is a rare taphonomic event due to differences in conditions required for the preservation of bone and plant
material [161].

Sedimentological and floral evidence from the quarry suggest a mesothermal climate with seasonal droughts and no significant winter frost. The local flora was abundant but deciduous, which raises questions about possible migrations among herbivores during the coldest months, as well as the carnivores that preyed upon them [161, 164, 172].

4.3 Material and methods

Two petrographic slides (50 μm thick) were prepared\(^4\) using two sections of *T. rex* rib bone (RSKM_P2523.8). A third slide was prepared from an ossified hadrosaur tendon (RSKM P2610.1), which was found in the same stratigraphic interval and geographic region of the Frenchman Formation, but not the same quarry. Both samples were still attached to their respective original sedimentary matrix where they were found, and each slide contained both bone and sediment. Epoxy resin was used to prepare the slides; however, measurements of a slide prepared purely with resin did not show any measurable element within the experimental technique used for analyses. A similar sample of a modern swan femur (*Cygnus* sp.; RSKM_A-8637, accession 17602, collected on November 7, 1996 in Regina, Saskatchewan) was also prepared for comparison. After being cleaned of their flesh (the majority of meat and soft tissue was removed from the body with surgical tools, and remaining flesh was consumed by dermestid beetles), the swan bone was cut using a lapidary saw, and wet-polished using a range of abrasive paper grit sizes until any visible major imperfections were removed. Prior to analysis, sample surfaces were cleaned with isopropanol.

The petrographic aspects of the fossils and their sediments were studied by other members of the research group. The same petrographic slides were analyzed,\(^4\)The samples were prepared by Van Petro Inc., 8080 Glover Road, Langley, BC (http://www.vanpetro.com)
alongside the other dinosaur sample and the swan sample, at the VESPERS (Very Sensitive Elemental and Structural Probe Employing Radiation from a Synchrotron) beamline at the Canadian Light Source (CLS) using micro-XRF mapping and XANES techniques [173]. VESPERS is a bending magnet beamline, with an energy ranging between 6 and 30 keV and spot size of 2 to 4 μm, produced by KB mirrors [173]. The XRF measurements were taken using a polychromatic beam, while XANES measurements for strontium were collected in fluorescence mode using a monochromatic beam produced by a crystal and multilayer monochromator.

Data previously taken by other members of the research group from a vertebra of RSKM_P2523.8 were also analyzed. The vertebra sample was prepared by cutting with a water-cooled saw to expose a section of the bone, and cleaned with ethanol. The measurements were also performed at the VESPERS (XRF) and SXRMB (XANES in fluorescence mode for iron, sulfur and calcium) [174] beamlines at the CLS. SXRMB (Soft X-ray Microcharacterization Beamline) is also a bending magnet beamline providing medium energy X-rays (1.7 to 10 keV) that can be used for X-ray photoelectron spectroscopy (XPS), X-ray absorption spectroscopy (XAS) and elemental mapping using XRF. The photon beam energy is selected using a double crystal monochromator with InSb(111) crystals for energies below 3.7 keV and Si(111) crystals for higher energies. The beam collimation is achieved by a sagittal cylindrical mirror with a carbon and platinum bi-layer coating.

The elements calcium, iron, manganese, strontium and yttrium were chosen for the analysis using XRF because they are present in non-negligible amounts in all fossil samples studied. These elements have also been previously researched in the context of fossil bones (see section 4.2). The XRF data were analyzed using PyMCA software [175], developed by scientists from the European Synchrotron Radiation Facility (ESRF). This software allows for peak finding and fitting, and chemical element map reconstruction based on the selection of regions of interest (ROIs) for
each element, once energy calibration is performed. The maps are produced in RGB (red - green - blue) colours and can be plotted together in the same picture for comparison. The average spectra for the bone regions in each of the samples was also computed. For that, 800 pixels of the compact bone region in each sample were selected, their spectra were averaged, and then normalized (for each spectrum, each point was divided by the highest count value in the spectrum, \( y_{\text{norm}} = \frac{y}{y_{\text{max}}} \)).

PyMCA was also used to open and convert XANES data to a format that the software Athena could read for data analysis. This software is present in the Demeter package [176], which contains three programs related to XAS analysis. The data were, then, normalized using the standard method in Athena to eliminate the influence of factors such as sample thickness and detector settings. In the case of iron XANES K-edge measurements, the pre-edge was later fitted using the software Larch [177], with an error function given by:

\[
erf(x) = \frac{1}{\sqrt{\pi}} \int_{-x}^{x} e^{-t^2} dt = \frac{2}{\sqrt{\pi}} \int_{0}^{x} e^{-t^2} dt, \tag{46}
\]

to fit the edge component and a pseudo-voigt function to fit the pre-edge. The pseudo-voigt function is defined as

\[
f(x; A, \mu, \sigma, \alpha) = \frac{(1 - \alpha)A}{\sigma \sqrt{2\pi}} e^{\frac{-(x-\mu)^2}{2\sigma^2}} + \frac{\alpha A}{\pi} \left[ \frac{\sigma}{(x-\mu)^2 + \sigma^2} \right], \tag{47}
\]

which is a weighted sum of a Gaussian and a Lorentzian distribution (weight given by \( \alpha \)) sharing the same amplitude, \( A \), center, \( \mu \), and the same full width at half maximum, thus constraining the parameter \( \sigma \). For the fittings presented here, \( \alpha = 0.5 \).

4.4 Results

The petrographic description of the slides containing \textit{T. rex} (RSKM.P2523.8) and hadrosaur (RSKM P2610.1) shows significant of quartz and plagioclase in the
sediment surrounding all three samples. Both *T. rex* slides present lithic grains and muscovite. The bone in the *T. rex* samples contains osteons with preserved diameters between 0.5 and 1 mm, and with areas that exhibit a silver sheen due to alterations around fractures and between the circular patterns of osteons. The hadrosaur ossified tendon presents osteons with Harvesian canals of 0.1 to 0.5 mm in diameter, with alterations around the perimeter of bone and osteons. All measurements presented in this chapter were taken on regions of compact bone (fig. 5) showing microscope images of the samples studied herein, and the general regions mapped.

Figure 5: Microscope images of a) *T. rex* rib (RSKM_P2523.8), b) *T. rex* rib (RSKM_P2523.8), c) *T. rex* vertebra (RSKM_P2523.8), d) hadrosaur tendon (RSKM_P2610.1), and e) swan femur (RSKM_A-8637). Braces show the general region mapped in each sample, which corresponds in all fossils to the region in the interface between bone and sedimentary matrix. Blue arrows show the region with bone, while red arrows shows regions with sedimentary matrix in the samples. The scale bars correspond to 3 mm. (Original in colour)

XRF maps of two slides of RSKM_P2523.8 *T. rex* rib bones and vertebra are presented in fig. 6, 7 and 8, respectively. In all samples, the bone region is still
attached to the matrix sediment found surrounding the skeletal material.

Figure 6: XRF elemental maps of *T. rex* rib (RSKM_P2523.8), showing transition region from outer cortical bone (left side) to sediment (right side), separated by Mn layer. These maps were measured in steps of 5.0 x 5.0 µm (total area of 880.0 x 219.8 µm) and 2.0 s per point. The colours representing each element are assigned to the right of the respective maps. (Original in colour)

Figure 7: XRF elemental maps of *T. rex* rib (RSKM_P2523.8), showing transition region from sediment (left side) to outer cortical bone (right side), separated by Mn layer. These maps were measured in steps of 10.0 x 10.0 µm (total area of 500 x 400 µm) and 2.0 s per point. The colours representing each element are assigned below the respective maps. (Original in colour)
The elemental maps in figs. 6-8 show a very similar pattern in the distribution of the elements for the three specimens. Calcium appears mostly in the region where bone is present, marking even some of its microstructure (see Harvesian canal in fig. 6). Strontium and yttrium are distributed in the same general region where calcium is present, but also in the areas immediately surrounding these regions, and in cracks and canals (e.g., fig. 8). Iron is present mostly in the regions corresponding to sedimentary matrix and within the osteon canal in fig. 7. Manganese appears in the transition between bone and sedimentary matrix.

The results of the data analysis for the hadrosaur tendon and extant swan bone are presented in figs. 9 and 10, respectively.
Figure 9: XRF elemental maps of hadrosaur tendon (RSKM_P2610.1), showing transition from bone (top) to sediment (bottom). These maps were measured in steps of 5.0 x 5.0 µm (total area of 700 x 750 µm) and 2.0 s per point. The colours representing each element are assigned below their respective maps. (Original in colour)

Figure 10: XRF elemental maps of recent swan femur cortical bone (RSKM_A-8637). These maps were measured in steps of 5.0 µm (total area of 400 x 300 µm) and 2.0 s per point. The colours representing each element are assigned below their respective maps. (Original in colour)

The distributions of calcium, iron, strontium, and yttrium observed for RSKM_P2610.1 in fig. 9 are very similar to what was observed in the Tyrannosaurus bones. The main difference is the manganese distribution, which instead of marking
the transition between bone and sediment, appears to populate osteon canals. This is observed in fig. 10 for the modern swan RSKM_A-8637. For this specimen, all elements seem to appear mostly in the bone region, with some iron and manganese in the osteon canals. Yttrium was not found in the modern swan (RSKM_A-8637).

The average spectra for the bone regions in each of the samples are shown in fig. 11.

![Figure 11: Average normalized spectra of the bone region in T. rex, hadrosaur and swan bones with selected peaks labeled. T. rex rib from fig. 6 is represented in black, rib from fig. 7 in red, hadrosaur in blue, and swan in green. (Original in colour)](image)

The normalized spectra for the different specimens show that the relative amounts of different elements vary from specimen to specimen, even when referring to the different parts of the same animal. The T. rex rib section from fig. 7, for example, shows much more iron present in relation to calcium than its counterpart from fig. 6.

XANES measurements for calcium, sulfur, iron and strontium are shown in fig. 12. In all cases, data were collected from the bone region, from the sediment still attached to the bone, and from the transition between the two regions.
The measurements for calcium (fig. 12-a) show that two very distinct chemical states for this element are present in the bone and sediment, with an intermediate state in the transition region. This difference is best seen in the plot areas marked by the arrows in fig. 12. The spectra for sulfur in fig. 12-b shows the peak position for all measurements at $\sim 2481.7$ eV. However, the chemical state does not seem to be the same for all regions (see feature marked by the arrow in the pre-edge region, for example). The spectra for strontium (fig. 12-d) are slightly different for the different regions in the sample.

The iron K-edge measurements (fig. 12-c) show very different spectra for bone and sediment. The results of the fitting in the pre-edge portions of these spectra can be seen in figs. 13, 14, and 15, for the bone, transition area, and sediment, respectively.
The fitting of the spectra resulted in the pre-edge peak positions of 7115.24 ± 0.15 eV for the bone region, 7114.94 ± 0.15 eV for the sediment region, and 7114.18 ± 0.17 eV for the transition between the two.

Figure 13: Pre-edge fitting for the Fe XANES of *T. rex* bone (RSKM_P2523.8), using an ERF function to fit the edge and a pseudo-voigt function to fit the pre-edge peak. a) shows the data (blue), fitting (red) and residual (x10, in green), b) shows the data (blue), and fitting components (red; the pseudo-voigt is shown with a solid line and ERF function with a dashed line). (Original in colour)

Figure 14: Pre-edge fitting for the Fe XANES of the transition between *T. rex* bone and sediment (RSKM_P2523.8), using an ERF function to fit the edge and a pseudo-voigt function to fit the pre-edge peak. a) shows the data (blue), fitting (red) and residual (x10, in green), b) shows the data (blue), and fitting components (red; the pseudo-voigt is shown with a solid line and ERF function with a dashed line). (Original in colour)
4.5 Discussion

The three-dimensional aspects of the samples measured can generate geometric artifacts in the elemental maps. Even after being polished, the porosity of bone can cause distortions in the surrounding measurements due to differences in the beam penetration depth (if the canals are at an angle with respect to the surface or the incident beam). Other optical effects include differences in the amount of light reflected due to different incident beam angles. In practice, elemental maps will show geometric effects such as the ones seen in fig. 6, in the region below the osteon canal, where all elements seem to present a lower concentration. These are interpreted as purely artifacts of the measurements and do not represent chemical characteristics of the sample.

The first aspect of the analytical data that is worth mentioning is that the swan specimen does not seem to contain any significant amount of yttrium. In the fossil specimens, on the other hand, yttrium was measured in significant amounts. The presence of yttrium in fossil bones can be understood as a result of diagenetic processes.
[178]. As observed in figs. 6 and 7, yttrium seems to be associated with calcium and strontium distributions. However, yttrium is also identified in regions such as inside the Harvesian canals (e.g., fig. 6), which are probably infiltration pathways into bone tissue. Consequently, the presence of yttrium in margins of the osteons canals support previous claims that this element is not original to dinosaur bone tissue [44].

The same general effect can also be observed for strontium. Strontium is also identified inside osteon Haversian canals for dinosaurs. On the other hand, for the swan, strontium is not present in these canals. This supports the hypothesis that strontium in fossils are at least partly the result of diagenetic processes. Strontium in the bone tissue associated with calcium is likely, at least in part, original to the animal, as strontium is known to replace calcium in bone apatite in living vertebrates[179] (strontium is also visible in the swan; fig. 10). In general, these results corroborate previous findings that strontium is added to the bone during diagenesis [41]. A consequence of this diagenetic overprinting is that studies relying on the strontium-to-calcium ratio or strontium isotopes to assess dietary habits of extinct animals will have to take diagenetic strontium into consideration.

The results previously described for yttrium and strontium are possibly related to the alterations (silver sheen) observed around the perimeter of the osteons in the petrographic description of the slides. These perimeter effects can also be seen when comparing the average spectra for the bone regions in each of the samples (fig. 11).

The most interesting feature present in the elemental maps shown in figs. 6, 7 and 8 is the way that manganese appears to form a layer around the transition region between bone and sediment. This layer does not appear in either the hadrosaur or the swan specimens, even though the hadrosaur was found in the same general region and strata as the T. rex and, thus, should have experienced similar taphonomic conditions. For the hadrosaur, manganese seems to be present within the Harvesian canals, and it is probably a diagenetic contaminant [158]. In the case of RSKM_P2523.8, manganese
has likely been deposited on the bones while they were in an aquatic environment, before burial [181]. The current interpretation, based on the type of sediment where RSKM_P2523.8 was found, is that the T. rex was deposited in a low energy section of a broad river, probably on a point bar\(^5\) [161]. Thus, it is likely that the remains were submerged for part of their taphonomic history.

The origin of the manganese in the water could be from the presence of bacteria associated with the decay of the remains, for example. Many types of microbes are known to deposit different minerals, including manganese [183, 181], and manganese concentrations can be associated with rivers [184]. The process that created the manganese coating in the bones could be similar to what occurs in caves, where such coatings are common [181]. In this environment, the wet, alkaline, and oxidizing environment favours the presence of manganese in the form of oxides and hydrates. These insoluble compounds then tend to precipitate, forming crusts and coatings [185]. Thus it is tenable that RSKM_P2523.8 was deposited under a similar set of environmental conditions that included a fluvial environment with oxidizing characteristics, and with presence of manganese.

The spectrum for calcium (fig. 12-a) collected from the T. rex bone (RSKM_P2523.8) is very similar to the spectrum found in the literature for modern bones [186], while that found in the sediment corresponds to calcite [187]. This chemical contrast shows that the dinosaur bone still maintains its original structure even after diagenetic effects, known to increase the overall amount of calcium in the bone tissue due to mineralization (see section 2.2.1).

Although it was not possible to precisely identify the chemical state of the sulfur observed in the bone and sediment regions (fig. 12-b), the peak position for this element is observed in all measurements at \(\sim 2481.7\), and indicates that it is in the

\(^5\)Point bar is the material found as a deposit on the inside of a river bend, composed of transported and eroded material from an outside bend, either the one opposite to the point bar or upstream in the river [182].
form of sulfate [188].

The data collected for iron also show different iron states for all measured regions (fig. 12-c). For iron, I was not able to identify the specific compounds present. However, the most interesting aspect of the iron observations is related to the transition region between bone and sediment. Here, the chemical state of iron cannot be explained as a linear combination of the states observed in the bone and sediment region, which seems to be the case for the other elements analyzed. This different state in the transition zone could potentially be related to the manganese layer observed in the region.

Also, the position of the pre-edge peaks in the iron XANES measurements (see arrow in fig. 12-c) can be related to iron’s oxidation state in the compound. In the case presented, the pre-edge centroid for the transition was measured (using a fitting method, see fig. 14) to be at 7114.18 ± 0.17eV, indicating the presence of Fe(III) [189]. The presence of Fe(III) has been associated with the preservation of dinosaur remains, including soft tissues in previous studies (see [7]). In [7], it was determined the iron was in the form of goethite, which could also be the case for this specimen. For the other measurements in the bone and sediment, the centroid was found at 7115.24 ± 0.15eV (fig. 13) and 7114.94 ± 0.15eV (fig. 15), respectively, which does not provide information on the oxidation state of the iron, but suggests a transition between the 1s level to 3d level6. This transition is also observed in corroded archaeological ferrous objects containing chlorine (see [190] for example).

The chemical state of strontium shown in fig. 12-d could not be measured with the same accuracy achieved for the other elements, resulting in a noisier spectrum. Due to this fact, I was unable to accurately identify the chemical states of strontium in bone and sediment. However, it seems like this chemical state is different between these two regions as it is suggested based on the position of the edge, which is located

---

61s and 3d levels refer to the atomic orbitals, i.e., the possible energy states in an atom.
at slightly different positions in the bone and sediment, and that the sediment edge peak (red arrow in the figure) seems narrower than that measured in bone tissue. The oxidation state is probably the same in both cases, since the strontium measured seems to be \( \text{Sr}^{2+} \), due to the observed edge peak located at the energy \( \sim 16113 \text{ eV} \) [191].

4.6 Conclusions

RSKM_P2523.8 still maintains intact its bone microstructure. This microstructure can be observed both using optical microscopy and chemical mapping of bone samples such as calcium, strontium and yttrium. The findings, presented in this research, show a manganese coating in RSKM_P2523.8 bones. The presence of this coating in the \( T. \ rex \), but not in the hadrosaur specimen studied, may be related to the environment where the dinosaur died, since it is known from sediment analysis that RSKM_P2523.8 died in an aquatic environment, by a river. No other previous mentions of a manganese coating were found for North American dinosaur remains, although the general preservation pattern of RSKM_P2523.8 has been suggested to be similar to that of other \textit{Tyrannosaurus} discoveries [140].

The series of observations developed also points to few modifications of the skeleton as a result of diagenetic effects, including: 1) an increase in the amount of strontium and iron in relation to the amount of calcium; and 2) the appearance of yttrium, which is not found in bones of extant animals. The elemental distributions of strontium (and yttrium, in some cases) show a significant concentration of this element restricted to the walls of canals within the bone. These canals are usually filled with minerals that infiltrate the bone during fossilization and diagenesis, and are also a source for the exchange of materials with the bone tissue.

The XANES measurements indicate that there are clear differences between materials in bones and associated sediments. This includes the fact that the calcium
present in the bone region appears chemically similar to the calcium in extant bones, while it appears as calcite in the surrounding sediment.

Together with the other results discussed above, it is clear that these T. rex bones have more original characteristics preserved than just their general morphology. They also present preserved microstructures and chemical aspects that have been maintained in great details, even after millions of years of burial.
5 Non-destructive chemical analysis of insect inclusions in amber

5.1 Abstract

Amber is polymerized tree resin, and can be responsible for exceptional preservation of trapped biota. It provides protection to insect remains from predation, microbial decay and other environmental factors, favouring their preservation. Previous research has shown detailed morphological preservation in insect inclusions, including soft tissues. However, the chemical analysis of these specimens usually results in their destruction, because the amber must be cracked to remove the insect material for testing. The aim of this study is to apply synchrotron radiation techniques to the non-destructive chemical analysis of insect inclusions in amber; specifically, ants (Hymenoptera: Formicidae) in Baltic amber. The results obtained for iron (section 5.5.1) show that X-ray fluorescence can be used to chemically map the insects while still encased within amber, providing information on the presence of different elements, such as iron and calcium (as long as concentrations are high enough) as well as their distribution in the specimen. The success of the measurements is dependent on the quality of preservation of the insects, and on preparing the samples to guarantee a consistent and thin amber layer on top of the inclusion. The combination of chemical mapping with X-ray micro-CT (as imaging technique), and XANES to define the chemical state of the iron found in the specimens (section 5.5.2) resulted in a more complete study of the preservation of these ants, showing the association between the chemical distribution of elements and preserved tissues within the insects’ bodies. As a result, I was able to determine that all ants studied had soft tissues preserved and that they were associated with the presence of iron or calcium in the corresponding chemical maps. Specifically the iron species was identified as Fe(III) using XANES, which has previously been
associated with the preservation of soft tissues in fossils. A version of this paper has been published in [192].

5.2 Introduction

The main subjects of the research presented in this chapter are three specimens of ant inclusions in Baltic amber. Baltic amber is the name given to the amber found in a series of deposits in Europe, mostly in the coastal region close to the Baltic Sea [193]. It is believed to have originated in a European forest that covered a large continental area. The resin producers have not yet been completely established: although some results suggest the producer to be a pine relative, the chemical analysis of the resin in the amber suggests a composition much closer to that of the resin from extant Araucariaceae or Sciadopityaceae [193, 194]. In chemical terms, Baltic amber can be characterized by a specific feature present in infrared spectra known as the “Baltic Shoulder”. It appears as an absorption band between 1250 and 1175 cm$^{-1}$ that is not seen in any other type of European amber [193].

The amber deposits found today are a result of many transport events. The first transport is believed to have taken place shortly after the resin production occurred near seasonal rivers. This transport took part of the material produced in the forest to depositional environments that offered protection against weathering [193]. The dating of these secondary deposits gives a minimum of 50 million years of age to these amber deposits [193, 195]. Another large transport event occurred in the Pleistocene, around 2 million years ago, when glacial movements transported rocks and debris (including amber pieces) into Northern Europe [193, 195].

Baltic amber, though predominantly yellow, can be found in 200 different colour varieties. The yellow tone ranges from a light yellow to orange and dark yellow and even brown [195]. The animal inclusions found within are mostly from the Arthropoda (98% of inclusions), but other invertebrates, including Protozoa, Nematoda, Annelida
and Mollusca can also be found (0.5%), as well as vertebrate-related material (0.5%) such as bird feathers, mammal hairs and small reptiles. Plants are mostly found as “stellate hairs”, which are predominantly branched epidermal trichomes that are associated with oak tree leaves and flower buds [195]. Trichomes can be found in more than half of all inclusion-bearing specimens, although occasionally other plant types of fragments can also be found. The presence of microorganisms and fungi in Baltic amber is rare [195].

Insects are arthropods characterized by the presence of three major tagmata: head, thorax and abdomen, with the thorax bearing six legs [81]. The head is responsible for sensory input, feeding and neural integration, but it is mostly composed of muscle tissue, leaving little space for the brain, which is usually small. The thorax is used primarily for locomotion and is dominated by muscle tissue, while the abdomen is responsible for digestion, mating, and also some sensory input [81]. As in all arthropods, insects are encased in a chitinized cuticle that provides protection and support for their bodies. The exoskeleton helps in locomotion and communication, prevents water loss, provides a site for waste deposition and offers protection [81]. With this basic body structure, there are no calcified hard parts in insects, and the plates (sclerites) that make up each body region are bound by flexible membranes, so they scatter or decay in many preservational settings (e.g., [197]). Amber is one of the few means of detailed preservation for insects, but the roles of differing amber chemistries, and the sequence of taphonomic events needed to preserve details such as soft tissues are still being investigated [80, 198, 199]. This debate is relevant to the current study, because insects have an open respiratory system, and it is unclear whether soft tissues are more likely to preserve when resin is drawn into the body cavity [199].

The ants belong to the order Hymenoptera together with bees and wasps. This order is characterized by the presence of mandibulate mouthparts and a generalized
ovipositor that can be used to inject eggs in spaces otherwise inaccessible, or in hosts. Within this order, ants belong to the Aculeata, a group with a well-developed ‘waist’, in which the ovipositor was modified to become a stinger. The family Formicidae is thought to have had its origin at approximately 120 million years ago [81]. Based upon the abundance and diversity of inclusions preserved in amber, formicids are only thought to have become a dominant group approximately 70 million years ago. While the focus of the research presented herein is in the Baltic amber specimens, some of the insect inclusions used for comparison specimens in this chapter are from North Carolina amber. This deposit is approximately 83.6 to 72.1 million years old, and it has been recently reviewed [196]. Although Cretaceous ants with the same degree of preservation as the Baltic amber specimens were not available for preparation and analysis as part of this study, comparisons to North Carolina amber specimens provided an opportunity to test whether the same preservational features were present in a much older amber deposit.

Ants are relatively rare as inclusions in Cretaceous amber, and usually belong to the exclusively Cretaceous subfamily Sphecomyrminae [200]. Recent work on Burmese Late Cretaceous amber has demonstrated that members of this extinct subfamily are more abundant and have a more extensive social structure than once thought, but that ants do not occur in the same diversity and abundance that is characteristic of amber deposits in the Eocene and Miocene [201]. The subfamilies that characterize modern ecosystems only appear to have gained ecological dominance by the time that Eocene Baltic amber was produced, and they figure more prominently in Miocene Dominican amber [202]. The genera analyzed in the present study are Ctenobethylus Brues (RSKM P3001.16 and RSKM P3000.17) and Nylanderia Emery (RSKM P3000.15). The former genus belongs to the subfamily Dolichoderinae and is known exclusively from Baltic amber, while the latter genus belongs to Formicinae and has modern representatives [203]. Comparisons were also made to significantly older specimens
with poorer preservation, from Late Cretaceous North Carolina amber (SEMC NC 272-276; Sphecomyrminae: Baikuris Dlussky), and modern formicids.

The focal point for previous soft tissue studies in amber has been larger inclusions of Hymenoptera and Diptera (e.g., [204]; [104]), and these early studies have utilized destructive sampling to analyze tissues preserved within the body cavity. In fact, most chemical analysis of amber inclusions is destructive (i.e., the insect inclusion is deliberately damaged so that measurements can be taken). More recently, non-destructive techniques have been employed more often in amber studies. The most commonly used to characterize amber inclusion compositions has been Confocal Laser Scanning Microscopy (CLSM), which has provided successful results for fungi and plant trichomes (e.g., [205]). Because this technique measures the autofluorescence of the preserved tissues, it is not suitable to be applied to a wide range of inclusions, due to its dependence on their autofluorescence properties [205]. Moreover, the types of molecules studied are limited by the wavelengths (and, therefore, energies) of the laser light available and, in some deposits, the amber also autofluoresces, causing a masking effect on the results (e.g., [206]).

Synchrotron light has been used more commonly as a tool to obtain morphological information on inclusions, using techniques such as synchrotron radiation X-ray microtomography (SR X-ray µCT) as can be seen in [207, 208], for example. This technique is especially useful when studying inclusion within opaque amber, where more conventional techniques (such as optical microscopy) cannot be applied [209]. French Cretaceous amber specimens were the first to be studied using synchrotron µCT [207, 210], but subsequently many others have been imaged [209]. Among the results obtained are larval stages for several groups of insects [209]; and anatomical details of the oldest representative of the beetle family Monotomidae [208]. Other examples of anatomical studies of insect inclusions using synchrotron µCT can be found in [211, 212, 213]. The only taphonomic studies to make use of
synchrotron observations are those of McCoy et al. [198, 199], where the extent of soft tissue preservation visible in CT data is tied to resin type. McCoy also used bubbles within the body cavity are to estimate extent of decay.

As a tool for obtaining chemical information from fossils, synchrotron light has usually been applied to larger specimens in sedimentary rocks, such as the remains of avian and non-avian theropods and reptiles (e.g., [152, 215]). The primary technique for these analyses has been Synchrotron Rapid Scanning X-ray Fluorescence (SRS-XRF), which has been reviewed in [153]. This technique has been used to study the extinct bird Archaeopteryx. The phosphorus distribution, for example, was used to identify teeth that were not apparent in measurements using visible light, and also showed that its feathers were preserved structures instead of impressions [153, 152]. The chemical information from smaller fossils is usually obtained using Scanning Electron Microscopy (SEM) and Energy Dispersive Spectrometry (EDS) (e.g., [216, 217]), but these forms of analysis require exposures that are seldom available in amber without cracking specimens open.

This study investigates the application of synchrotron X-ray fluorescence as a non-destructive technique to obtain chemical information of insect inclusions in amber. The first goal of this research was to understand the potential and limitations of the use of this technique for different amber specimens. In the second portion of this study XRF is used in combination with synchrotron μCT and XANES to study ants in Baltic amber, and how their chemistry is related to their preserved tissues.

5.3 Material and methods

5.3.1 Specimens and preparation

Four ant (Hymenoptera: Formicidae) inclusions in amber and two modern ants were selected for this research. The amber specimens (listed in table 1) were embedded in mineralogical-grade epoxy (Epo-Tek 301) and cut with a water-cooled lapidary saw.
The cuts were made in order to reduce the width of the amber layer on top of the insect inclusion as much as possible, which, in practical terms, was on the order of 1 to 2 mm thick. The cut specimens were polished with lapidary wheels and wet sanding baths to further reduce the thickness of the amber layer. This procedure follows [218] for the preparation of fragile amber pieces and it is a common preparation procedure for obtaining proper anatomical views, and studying amber specimens using light microscopy (e.g., [219, 220]). The final samples (amber blocks) were approximately 2 mm thick in total, including the size of the inclusion. The overlying amber layer in these samples varied between tens to hundreds of micrometers, depending on the specimen and limb orientation. Since polishing to the point of exposing any part of the insect, including appendages, would result in damaging the inclusion, the polished surfaces toward which the appendages were oriented ended up thicker than the others in relation to the insect’s main body. Once this process was finished, the samples were cleaned with isopropanol to remove surface contaminants from handling.

<table>
<thead>
<tr>
<th>Type</th>
<th>Number of specimens</th>
<th>Specimen names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modern ants</td>
<td>2</td>
<td>RSKM P3314.2, RSKM P3314.1</td>
</tr>
<tr>
<td>Baltic amber</td>
<td>3</td>
<td>RSKM P3000.15, RSKM P3000.16, RSKM P3000.17</td>
</tr>
<tr>
<td>North Carolina amber</td>
<td>1</td>
<td>SEMC NC 272-276</td>
</tr>
</tbody>
</table>

Table 1: List of specimens analyzed.

Samples of modern ant specimens were also prepared in order to compare with the results obtained from fossils, and provide a control for some of the materials being examined. In this case, two ants were inserted into the same epoxy resin used in preparing the amber specimens. One of the ants was added to the resin once dead (RSKM P3314.2), while the other was added while still alive (RSKM P3314.1), in order to observe if resin infilling the body cavity due to movement would have an effect on the results, and if observable differences in the fossil specimens could be
associated with the two possible trapping scenarios. These modern samples were prepared (cut and polished) using the same methodology described for the fossils.

The specimens included in this research came from the Royal Saskatchewan Museum (RSKM) Palaeontology Collections, Regina, SK, Canada (samples with P specimen prefixes) and from the Division of Entomology of the University of Kansas Natural History Museum (SEMC), Lawrence, Kansas (sample NC 272-276).

### 5.3.2 Measurements and analyses

The samples were measured at the Soft X-ray Micro-characterization Beamline (SXRMB) at the CLS [174]. This beamline uses a bending magnet to provide medium energy X-rays (1.7 to 10 keV). The beam energy used to collect data is selected using a double crystal monochromator with InSb(111) crystals for energies below 3.7 keV and Si(111) crystals for higher energies. The beam collimation is done by a sagittal cylindrical mirror with a C and Pt bi-layer coating. The resulting beam spot on the sample measures 10 μm x 10 μm (after passing through a K-B mirror pair). The micropobe endstation uses a silicon drift detector for photon detection. Measurements were taken using XRF mapping with a monochromatic beam (see measurement parameters in table 2) for all specimens listed in table 1, and XANES (iron K-edge) for the Baltic amber specimens.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Step size (μm)</th>
<th>Map size (mm)</th>
<th>Time per point (s)</th>
<th>Beam energy (keV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSKM P3314.1</td>
<td>50.0 x 50.0</td>
<td>2.50 x 3.50</td>
<td>3.0</td>
<td>7200</td>
</tr>
<tr>
<td>RSKM P3314.2</td>
<td>60.0 x 60.0</td>
<td>2.60 x 1.90</td>
<td>2.0</td>
<td>7200</td>
</tr>
<tr>
<td>RSKM P3000.15</td>
<td>20.0 x 20.0</td>
<td>1.20 x 1.30</td>
<td>5.0</td>
<td>7200</td>
</tr>
<tr>
<td></td>
<td>15.0 x 15.0</td>
<td>1.65 x 1.05</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>RSKM P3000.16</td>
<td>45.0 x 45.0</td>
<td>2.40 x 3.20</td>
<td>3.0</td>
<td>7200</td>
</tr>
<tr>
<td>RSKM P3000.17</td>
<td>40.0 x 40.0</td>
<td>2.40 x 2.10</td>
<td>3.0</td>
<td>7200</td>
</tr>
<tr>
<td>SEMC NC 272-276</td>
<td>40.0 x 40.0</td>
<td>3.40 x 1.40</td>
<td>3.0</td>
<td>7200</td>
</tr>
</tbody>
</table>

Table 2: Data acquisition parameters for insect inclusion samples used in XRF measurements.
The size of the XRF elemental maps was chosen according to the size of the insect inclusion such that it included the whole body of the insect, plus part of their appendages. Also, for SEMC NC 272-276, the chosen insect was positioned diagonally in relation to the horizontal plane defined by the beamline software. In order to save beam time and avoid mapping a large area devoid of inclusions, the mapping of this specimen was split into two parts. The same strategy of creating two maps was used for the map for RSKM P3000.15 so that the mapping could be done in more detail without generating a single very long scan (increasing the risk of data loss). The acquisition time per point for the measurements was determined according to the statistics observed in a single point measurement prior to the data collection for the map.

The XRF data were analyzed using PyMCA software [175], after correcting the raw data for variations in the beam by dividing the number of counts observed by the beam current measured at the moment the data was collected, as shown in:

\[
data_{corrected} = \frac{data_{raw}}{Beam\ Current}.
\]  

This software integrates the counts in the spectrum for each point within a selected region of interest (ROI), which is established manually by selecting the width of the peak corresponding to the desired element. An exception was made for the map involving the specimen from North Carolina amber, SEMC NC 272-276. In order to attach the two measured maps in one, a program was written using C++ and the Cern ROOT framework [221] to reconstruct the map (see code in appendix ). In this case, the software plotted the spectrum for each point individually and a fit was performed for the background (using the background fitting methods from ROOT) and for the peak of the chosen element (using a Gaussian function). The area of the peak was calculated and normalized using the beam current:
\[ \text{Area}_{\text{plotted}} = \frac{\text{Area}_{\text{peak}} - \text{Area}_{\text{background}}}{\text{Beam Current}} \]  

(49)

The resulting value was used for each pixel in the map.

The elemental maps of the specimens from Baltic amber were combined with their respective tomography images in order to match the features observed using the two techniques. The tomography measurements were taken at the BioMedical and Imaging Therapy (BMIT) Insertion Device (ID) beamline at the CLS [222] by Ryan C. McKellar. The images were reconstructed by Gavin King using the AMIRA software platform (ver 6.3) and the Z-Brush software (ver. 4R7 P3) (more details can be found in [223]). Interpretive drawings of tissues within the tomography data sets were created using AMIRA translucent volume renderings, and then tracing tissue outlines on still images using Adobe Photoshop (ver. CS5) and Autodesk Sketchbook Pro (ver. 6.0.1) software. The final images were combined using the software ImageJ [224] by creating an overlay of the elemental maps (at 80% transparency) on the tomography images. The elemental maps were adjusted in size and angle to match the tomography image.

The XANES data were analyzed using Athena software [128]. The pre-edge region was fitted using an error function (equation 46) to describe the sharp rise of the edge, combined with one or two Gaussian functions to describe the pre-edge structures in the spectrum. The choice of these functions was based on a set that resulted the lowest $\chi^2$/NDF for the fit, with the error function and arctan describing the K-edge and with the Gaussian and pseudo-voigt options for the peaks, which are the curves commonly used for such fittings in previous experiments [189].
5.4 Results

5.4.1 XRF applied to insect inclusions

The elemental maps produced using XRF for the modern ants are presented in fig. 16 for RSKM P3314.1, and in fig. 17 for RSKM P3314.2. Only the elements showing an informative distribution were included in the plots presented. Additional elements in the same energy range were also analyzed, but their maps only resulted in noise with no discernible patterns.

![Elemental maps of Ca, Cl, Fe and K for RSKM P3314.1](image1)

Figure 16: Elemental maps of Ca, Cl, Fe and K for RSKM P3314.1 compared to its microscope image. (Original in colour)

![Elemental maps of Ca, Cl, Fe and K for RSKM P3314.2](image2)

Figure 17: Elemental maps of Ca, Cl, Fe and K for RSKM P3314.2. (Original in colour)
Fig. 16 shows that the amount of calcium, iron and potassium seem to be higher in the region of the insect’s head. The distribution of chlorine is the negative of the distributions of the other elements (i.e., it shows a high amount everywhere but on the region of the insect’s head). Fig. 17 exhibits a similar pattern, but in this case calcium and potassium appear in larger quantities in the abdomen of the insect, while iron appears in the whole body. Again chlorine appears everywhere but in the regions where the other elements are abundant.

Three specimens of ant inclusions in Baltic amber were mapped using XRF: RSKM P3000.15, RSKM P3000.16 and RSKM P3000.17 (figs. 18, 19 and 20, respectively). Iron was the only detectable common element present with a distribution correlated to the inclusion for all specimens studied (including also modern specimens and the Cretaceous North Carolina amber). This element has also been linked to soft tissue preservation in sedimentary settings [7]. Thus, it was the element chosen as the focal point for the study on the applicability of this technique.
Figure 18: Iron elemental distribution (top, split into two scans that correspond to the head and thorax, and the abdomen) for Baltic amber ant RSKM P3000.15 compared to its microscope image (bottom). Arrows show examples of features that can be matched between elemental map and microscope image. (Original in colour)

Figure 19: Iron elemental distribution (left) for Baltic amber ant RSKM P3000.16 compared to its microscope image (right). White arrows show lines with high amount of iron due to the infiltration of minerals in cracks in the amber. (Original in colour)
Figure 20: Iron elemental distribution (left) for Baltic amber ant RSKM P3000.17 compared to its microscope image (right). (Original in colour)

All three specimens from Baltic amber exhibit iron distribution following the region where the insect is located, with the highest iron levels in the region of their heads and thoracic muscles. Some features of the distribution seem to match with features in the microscope images, corresponding to the boundaries of sclerites and following the outlines of the exoskeleton, such as the joints in the abdomen of RSKM P3000.15 in fig. 18. Other characteristics of the distribution do not correspond to any feature visible in the external images. Fig. 19 also shows lines with large amounts of iron (marked by arrows), which are the result of infiltration of minerals in cracks in the amber.

The specimen from North Carolina amber SEMC NC 272-276 contains five partial ant inclusions, of which two were mapped (fig. 21). The area to be mapped was selected based on previous results obtained at the Dr. David Cooper’s Lab (University of Saskatchewan)\(^7\) using preliminary scan data obtained from X-rays imaging in a benchtop µCT scanner.

\(^7\)Cooper Lab: 3D30.3 Health Sciences, 107 Wiggins Rd, Saskatoon, SK S7N 5E5
The iron distribution shown in fig. 21 also follows the shape of the insect inclusion, although not as well as the previous specimens. The contour of the ant in the right-hand side is clearly visible in green in the map, showing it presents more iron than the surrounding amber. The insect on the left side is not as clearly represented in the map as the one on the right, and preservation of the cuticle in this specimen is sporadic. The insect’s body shows elevated levels of iron in comparison to the surrounding material. However, the contours are not as clear, and iron is also present in the regions surrounding the insect.

5.4.2 Preservation of ant inclusions in Baltic amber

It is useful to combine the XRF results with a 3D imaging technique, so that it is possible to match the chemical distribution to physical structures identified in the inclusion at a finer level of detail. In this section, elemental maps for Baltic amber ants, measured using XRF mapping, are combined with results from synchrotron X-ray micro-CT results obtained from the same insects at the Bio-Medical Imaging and Therapy Facility (BMIT) beamline at the CLS. The goal of this data
comparison is to provide a better understanding of what the elemental distribution means for these insects in three dimensions, with clear interpretations of which tissues are being measured. The results for ants RSKM P3000.15, RSKM P3000.16 and RSKM P3000.17 (figs. 22, 23 and 24, respectively). For each inclusion, three images are presented: the first contains a translucent rendering generated from the tomographic data, the second contains the elemental map for that inclusion, and the third contains an overlaid image of both (making it easier to correlate structures with chemical information). The elements being presented in the maps are the ones showing a distribution correlated with the insect’s overall shape (i.e., elements for which the distributions were not random or just noise).

Figure 22: a) CT rendering (ventral view) and b) XRF results for Baltic ant P3000.15. The elemental maps show iron in red and calcium in green. The overlay of the images can be seen in c). In a), the arrows show the two surfaces visible in the image. The inner and outer surfaces are represented by the blue and red arrows, respectively. In c), the white arrow shows the regions where iron and calcium are present in large quantity in the insect’s head. The orange arrows show areas with higher iron concentrations in the insect’s abdomen. The blue arrow shows the region with large quantity of calcium in the insect’s abdomen. (Original in colour)
Figure 23: a) CT rendering (lateral view) and b) XRF results for Baltic ant P3000.16. The elemental map shows iron in red and calcium in green. The overlay of the images can be seen in c). (Original in colour)

Figure 24: a) CT rendering (ventral view) and b) XRF results for Baltic ant P3000.17. The elemental map shows iron in red and calcium in green. The overlay of the images can be seen in c). (Original in colour)

The tomographic renderings in figs. 22–24 show details of the anatomy and preservation of the inclusions. The ants bodies are shown in detail, including preserved inner soft tissues. Because the scope of this study was limited to the detection of soft tissues and the comparison with the chemical information, these
structures were not characterized in detail. However, as an example, fig. 25 shows in more detail the head of RSKM P3000.15 and the corresponding diagram for the preserved tissues made by Ryan McKellar. In this image, it is possible to see the preserved cuticle reinforcements (in blue, the tentorium), and traces of what could be either the brain or digestive glands (in white). The pink area in the right side of 25-b likely represents preserved mandibular muscles. The tomographic renderings also show two surfaces for all insects (marked with arrows in fig. 22-a, as an example), where the external impression of the exoskeleton is recorded in the amber, and the shrivelled remains of the exoskeleton. This means that several parts of the insect are no longer in contact with the amber surface.

Figure 25: a) CT rendering of the head of Baltic amber ant RSKM P3000.15 (dorsal view) and b) the diagram of the preserved tissues observed in the rendering. In b), the cuticle reinforcements of the tentorium are represented in blue, mandibular muscles in pink, and traces of either brain or digestive glands are in white.

The elemental map for RSKM P3000.15 (fig. 22-b) shows iron and calcium distributed along the ant’s body. Iron is more concentrated in the insect’s head, middle of thorax, and parts of the abdomen (mostly in the lines in the cuticle, but
at higher concentrations within the areas that contains shrivelled tissues inside the abdomen, and a bubble of purge fluid that has escaped the posterior end). The same patterns are observed for RSKM P3000.16 and RSKM P3000.17, except for the abdomen of the latter, which appears to have low amounts of all elements. This apparent low concentration is likely influenced by the angle the insect was positioned within the amber (i.e., the abdomen was farther from the amber surface). The calcium distribution in fig. 22-b seems to be more concentrated in the head and abdomen of the ant. As was the case for iron, calcium is present in larger quantities along the margins of the abdominal sclerites, but it is also found in its much higher concentrations within the posterior region. The other specimens do not present such a clear calcium distribution as RSKM P3000.15, but they also show this element is present. RSKM P3000.16 (fig. 23-b) shows high concentrations of calcium in the region of amber outside of the inclusion, and this appears to trace decay products trapped on a flow line in the amber. The structures present in the elemental maps are shown to match structures present in the tomographic renderings in the overlay images (figs. 22-c, 23-c, and 24-c).

Although low, the statistics collected in the SXRMB beamline were sufficient to provide significant iron K-edge XANES measurements for the insects’ heads. Areas of high iron concentration were investigated for all insects in these measurements. The results obtained for the amber insects, as well as for the modern ant depicted in fig. 16, can be seen in fig. 26. The spectra in fig. 26 were normalized following the Athena software package standard parameters. The amounts of calcium found in these samples were not enough to obtain the corresponding spectra for this material.
Figure 26: Iron K-edge XANES results for measurements taken from the head capsules of Baltic amber ants compared to a modern ant (RSKM P3314.1). The spectra were normalized and are presented a) together and b) stacked with 0.2 interval between different spectra. RSKM P3000.15 is shown in blue, RSKM P3000.16 in purple, RSKM P3000.17 in green, and RSKM P3314.1 in red. The arrows mark the position of the edge. (Original in colour)

These measurements (fig. 26) show similar spectra for all samples, with some differences. The position of the K-edge (see arrows) seems to be constant for all samples. However, the pre-edge region seems to match for all the specimens except RSKM P3000.15. These pre-edge peaks were fitted using an arctan function to describe the edge and Gaussian curves for the pre-edge peaks (as many as necessary to describe the data) and the results can be seen in figs. 27, 28, 29 and 30.

Figure 27: Iron pre-edge fit for Baltic ant specimen RSKM P3000.15, showing a) fitting line and residual and b) a magnified image of the pre-edge region and the individual functions used in the fitting. (Original in colour)
Figure 28: Iron pre-edge fit for Baltic ant specimen RSKM P3000.16, showing a) fitting line and residual and b) a magnified image of the pre-edge region and the individual functions used in the fitting. (Original in colour)

Figure 29: Iron pre-edge fit for Baltic ant specimen RSKM P3000.17, showing a) fitting line and residual and b) a magnified image of the pre-edge region and the individual functions used in the fitting. (Original in colour)
Figure 30: Iron pre-edge fit for Baltic ant RSKM P3314.1, showing a) fitting line and residual and b) a magnified image of the pre-edge region and the individual functions used in the fitting. (Original in colour)

The pre-edge Gaussian peaks were found at 7114 eV for all samples (the exact values for RSKM P3000.15, RSKM P3000.16, RSKM P3000.17 and RSKM P3314.1 are 7114.00 eV, 7114.04 eV, 7114.77 eV and 7114.26 eV, respectively). Specimen RSKM P3000.15 needed a second Gaussian to fit the pre-edge, resulting in another peak at 7119.70 eV.

5.5 Discussion

5.5.1 XRF applied to insect inclusions

Modern ants  Single point measurements\(^8\) obtained from the epoxy resin areas of the samples (without insect contents) showed that out of the elements in the energy range measured, only chlorine was present. This can also be observed in the elemental map for this element, where Cl shows a higher concentration in all points measured except for the ones on top of the insect (figs. 16 and 17). Within these regions, the resin layer is thinner due to the presence of the inclusion. Thus, chlorine cannot be studied using this characterization technique if this type of epoxy resin is used as

\(^8\)Single point measurements are taken at a single position in the sample. The data for these measurements could not be saved, because the software used at the SXRMB beamline at the time of the experiment did not offer this option.
a mountant or amber support. The result of this measurement also shows that the composition of the resin should not interfere with the measurements for iron discussed later in this section, but could be used to investigate possible cracks in the amber. If such cracks are present in the sample, the resin will infiltrate and these will appear as regions with high chlorine concentration on the chemical maps.

Although the epoxy resin composition does not affect the results of the measurements, the same cannot be said about its thickness. The thickness of resin layer (or amber, for it has a similar effect) causes attenuation of the signal both when the X-ray beam is traveling through the material before reaching the inclusion, and in the fluorescence photons that are travelling in the direction of the detector. As a result, areas where the resin or amber layer is thicker will show an apparent lower concentration of the element being measured than other parts of the insect, despite the same real concentration in the specimen itself. Thus, geometric aspects of the insect, such as its shape and angle in relation to the measured surface, may introduce differences in the measured signal. Although inevitable, the attenuation effects discussed can be minimized during sample preparation by positioning the insect as close as possible to a parallel direction relative to the measured surface.

Apart from geometric effects caused by differences in the positioning of the two ant specimens, no significant differences were observed between the ant that was embedded in the resin alive compared to the one that was dead. The measured distributions include the same elements for both samples. Also, comparable chemical patterns detected in both modern ants show that there are no detectable differences in measured elemental composition, based on the way the insect died and interacted with the surrounding resin.

**Baltic amber** The presence of iron as the only detectable element may be the result of the attenuation effects imposed by the amber layer between insect and detector.
Since elements with lower atomic numbers predominantly emit fluorescence photons with lower energy and the penetration depth of light in a material depends on its energy, it is logical that measurements involving elements with a low atomic number will be subject to larger attenuation effects. Thus, the techniques employed here are not suitable to ascertain if lighter elements are absent in these specimens, or if the lack of signal is a result of light attenuation in the sample, or a combination of both factors (e.g., if the insect had initially a very low concentration of an element that cannot be measured due to attenuation effects). This question could possibly be answered after careful calculations involving the properties of amber and the thickness of the layer present. However, such values are difficult to obtain due to the irregular surface of the insect inclusion. Elements with higher atomic numbers than iron could not be detected due to the achievable beam energy range at the SXRMB beamline, which sits outside the energy region needed to excite the atoms of these elements.

Light attenuation effects are also present in the iron measurements. This attenuation effect could lead to differences in the measured concentrations that are independent of the actual composition of the inclusion. Consequently, the maps present the locations where iron is found in the insect, but differences in the attenuation caused by variations in the thickness of the amber overlying the insect could lead to apparent variations in the relative concentration of iron within the inclusion. In order to obtain more conclusive results, it would be useful to include an additional three-dimensional imaging measurement. This could present visual information on the insect’s structures and their positioning and better account for such geometric effects (see section 5.5.2).

The qualitative interpretation of these results provides valuable distribution information, which is not attainable through conventional characterization techniques. Apart from the surrounding amber being shaped and prepared, it is a non-destructive and non-invasive measurement that leaves the insect inclusion
intact. Iron is an element of biological relevance for virtually all organisms, including insects [225]. It is found in significant amounts in insects while they are still alive, but iron is also produced as a result of soft tissue decay after their death [226]. The careful study of iron could, therefore, provide more taphonomic information about the preservation of insect inclusions in amber. Although XRF can be used in a quantitative fashion, it is improbable that such an approach could be taken in the context of the specimens studied here, due to effects such as the ones introduced by the thickness of the amber layer on the sample, insect orientation and shape, and the chemical heterogeneity present in these specimens.

**North Carolina amber** It is possible to faintly see the outline of the inclusions in the iron distribution present in the map, in fig. 21. However, its definition is not nearly as clear as those observed in the maps of Baltic amber ants (figs. 18 to 20). This can be explained by differences in the preservation of these insects. Baltic amber is known for the exceptional quality of preservation of its inclusions [81], while the North Carolina amber specimens are also much older [227] and visibly not as well preserved, at least at the exoskeleton level. In these specimens, the exoskeleton has become carbonized and broken up into sheets of cuticle [80], leading to a decrease in the amount of tissue preservation and iron remaining in the specimen. Nevertheless, some iron content seems to have been preserved within the highly altered SEMC NC 272-276 remains as can be observed from the iron distribution shown in the elemental map. This trace of iron suggests that the technique could be successfully applied to a wider range of inclusions, as far back in time as the Cretaceous.

SEMC NC 272-276 could not be mapped with the same quality as the specimens discussed previously, because the amber surface on top of the insects had a round shape that could not be cut or polished without destroying the inclusions. This meant that the thickness of the amber layer was variable during data acquisition,
causing possible beam focusing issues\textsuperscript{9} that can impact the overall quality of the measurements obtained here. The general correspondence between cuticle presence and iron concentrations seems to match the patterns observed in younger specimens, but a wider range of Cretaceous samples prepared for this form of analysis are required to make detailed inferences.

5.5.2 Preservation of ant inclusions in Baltic amber

The tomographic renderings of the three Baltic amber ants show that all of them present preserved internal tissues (see fig. 25, for detailed example), which appear to be correlated with areas that exhibit higher concentrations of iron or calcium in the maps. Preserved soft tissues in amber inclusions have been previously identified in several specimens (e.g., [104, 229, 198]). For Baltic amber specifically, a summary from 2017 showed that out of 27 specimens studied using techniques that would allow for identification of soft tissues, 11 of them had preserved internal structures [198].

Iron is expected to be found in large quantities in insect tissues, since it is present in their vascular system [230] and is important to their metabolism [231]. This can be observed in the images provided such as in fig. 22-c that shows iron preserved in large quantities in the ant’s head (white arrow in fig. 22), which is correlated with the preserved mandible muscles visible in the CT data. The same figure also shows areas with higher iron concentrations in the insect’s thorax, correlated with the thoracic muscles (bright area in middle of thorax in fig. 22). Elevated concentrations in the insect’s abdomen are correlated with regions of the cuticle and shriveled organs (orange arrows in fig. 22). The iron measured in the insects, therefore, is almost certainly original to the insects or, perhaps, a result of a taphonomic process responsible for the preservation of soft tissue structures (as

\textsuperscript{9}If the beam is not properly focused, the beam spot in the sample becomes larger and more dispersed than what it should be. Thus, the measurements would be performed over an area larger than expected. The practical result of this is that the resulting image loses definition and becomes more blurry.
opposed to iron that has infiltrated). If iron had been introduced, it would probably be more evenly distributed within the insects, and exhibit a clear path from an infiltration point, instead of correlating to just the preserved remains of organs and cuticle. In fig. 23, iron also appears in the region of amber outside of the insect in diagonal line close to the insect’s head. This is line is most likely caused by a crack in the amber, resulting on infiltration of minerals, which are not related to the inclusion.

In RSKM P3000.15 (fig. 22), the calcium distribution is mainly correlated with iron in areas where the mandible tissues appear preserved (white arrow in fig. 22) and in the posterior portion of the ant’s abdomen where the concentration of iron is not high (blue arrow in fig. 22). Calcium in modern insects can be found as part of the haemolymph, and is distributed in lower concentrations within their soft tissues [232]. Figs. 16 and 17, for example, show calcium distributed in the insect’s body. In the mandibles in particular, insects are known to accumulate metals, such as the iron and calcium to increase the strength and reduce wear on these structures [233]. This would explain the presence of calcium in the area where muscle tissues were preserved. Calcium is also deposited in insects’ midguts [232], which could suggest that the high concentration observed in the abdomen could be related to a collapsed midgut in the insect. This is corroborated by the presence of preserved internal tissue visible in the tomographic images that exactly match the distribution of calcium in the area.

The calcium distribution seems to be more consistent with insect soft tissue structures in the RSKM P3000.15 (fig. 22) than in the other specimens examined. In the case of RSKM P3000.17 (fig. 24) the distribution could be due to the fact that the insect is at an angle relative to the surface of the amber, with its abdomen farther from the surface than its head. This orientation causes an attenuation effect in the iron distribution compared to other parts of the insect, an effect that is
intensified for calcium due the lower energy of the photons emitted from these atoms. Since the abdomen is where most of the Ca is located in RSKM P3000.15, I cannot conclude from these measurements whether RSKM P3000.17 actually possesses a comparable distribution of this element or, if I am simply unable to measure such a distribution due to sample geometry differences. From what the measurements show, it seems Ca in RSKM P3000.17 is a taphonomic product of the decay and expulsion of the ant’s digestive system. In the case of RSKM P3000.16 (fig. 23), however, the calcium distribution seems to be completely outside of the insect, as vesicles within the surrounding amber. This external distribution could have been caused by haemolymph or decay products leaking out into the resin, or by infiltration from surrounding minerals with carbonate content.

Another common characteristic for all analyzed Baltic amber inclusions (although more visible in RSKM P3000.15 and RSKM P3000.17) is that two surfaces can be seen in the tomographic renderings. The outmost surface corresponds to the original outline of the insect, while the inner surface corresponds to the shriveled remains of the cuticle. These two surfaces are preserved by solidification of the resin into amber before the insect underwent dehydration and loss of volume. This feature suggests that the insect was trapped in the resin and completely encased before it had time to undergo significant decay, or to dehydrate due to exposure. Thus, the taphonomic changes observed were reduced by the presence of a surrounding amber layer, resulting in excellent preservation. In turn, the level of preservation found in these insects is what makes it possible to obtain high quality synchrotron data — which means that observing the inclusion withdrawn from its original outline in the surrounding amber can be used as a way to estimate which samples are more likely to yield better defined elemental map scans using synchrotron light. Since beam time at synchrotron facilities is limited, this taphonomic feature can be used to reduce wasted time with insects that will not provide clear results.
The iron spectra in fig. 26 show that all samples have slightly different curves, although the position of the edge (black arrows in the figure) is maintained for all of them. In particular, RSKM P3314.1 has an edge with a very different shape (red spectra in the figure) compared to the other samples. Although the compounds to which the iron belongs in each specimen could not be identified with confidence, the iron oxidation state can be obtained based on the position of the pre-edge peak. This peak seems to be the same for all samples (considering the noise in the measurements), except for sample RSKM P3000.15.

The results for pre-edge energies (figs. 27-30) at around 7114 eV suggests that the iron is in a Fe(III) state, while the peak at 7119.70 eV does not provide information on the oxidation state of the iron [189]. It is important to notice that the iron in the fossil ants is found in the same oxidation state as the iron in the modern ant, suggesting that the materials measured are originally from the insects, or a slightly modified version of this original material. Moreover, ferric iron in insects is linked to the presence of proteins such as transferrin and ferritin [228], and, thus, the measurements presented could be the result of preserved or partially-preserved structures that were parts of proteins in these animals.

5.6 Conclusions

XRF mapping can be used to study insect inclusions in amber in a non-destructive and non-invasive way. This provides not only compositional information (limited by concentration levels within the sample and possible attenuation factors), but also the elemental distribution in the insect’s body. These results can, then, be combined with imaging techniques to provide insights about preservation without destroying the specimen. Potential applications include determining if the iron found is associated to a particular structure that has been well preserved. However, the quality of the measurements obtained depends on the quality of the preservation of the insects, but
also on preparing the amber pieces so that only a thin and relatively consistent layer of amber remains over the insects.

These measurements showed that the Baltic amber specimens presented much more defined iron elemental distributions than the North Carolina specimen. This is most likely due to the latter being so much older and not as well preserved as the former. The results also showed that there is no detectable difference in the elemental distribution of iron for insects that died before or after being trapped in the resin when using the technique discussed herein. Therefore, the distribution of iron and preserved soft tissues in insect inclusions probably does not depend on how they are trapped, as long as significant drying and decay does not occur before the specimen is completely covered by resin.

The results discussed in section 5.5.2 show that the measured iron and calcium distributions are strongly correlated with preserved insect tissues visible in the tomography images. Iron seems to be correlated to muscles, thicker or reinforced sections of the cuticle, and other soft tissues present in the abdomen and thorax of ants. On the other hand, results from XANES measurements show that iron in the fossil and modern ants are in the same oxidation state (Fe(III)), a state that can possibly be related to proteins. Together, all these measurements suggest that the insects are preserved with tissue that retains traces of its original composition, indicating a very high level of preservation for fossils that are approximately 50 million years old. Although no extra information was acquired for calcium, the location of high concentration in the preserved mandible muscles and what could be a collapsed midgut also suggest that this element is original to the insects (except in specimen RSKM P3000.16, which presents calcium in drying lines and bubbles in the surrounding amber that can be the result of infiltrations from the environment). Although more research is necessary to confirm these findings, the initial results here suggest that synchrotron techniques can be used to non-destructively study...
preserved internal structures and their composition in insects. With detailed comparisons to modern species, it may be possible to determine differences and similarities among extant and extinct species.

This study has also provided some possible strategies to facilitate specimen selection. All measured specimens that exhibited cuticle withdrawn from the surrounding amber also presented exceptional soft tissue preservation. This feature allows us to suggest that insects that underwent dehydration in the amber, instead of decay, have a particular preservational appearance that allows us to select specimens with a higher probability of yielding high quality results in future analyses.
6 Chemical diagenesis of turtle shells

6.1 Abstract

Turtles and their relatives have existed at least since the Late Triassic. These animals are mainly known for their shells (used for protection), and their ability to retract into this shelter. Many studies to understand their fossil exemplars have been developed, but most of this work has focused on morphological aspects of the shells and how they are related to the group’s evolutionary history. However, some of the few studies on the chemistry of turtle fossils have shown interesting outcomes that resulted in more information about extinct species of turtles and the diagenetic alterations their bones experience. The goal of the research presented herein is to understand aspects of turtle taphonomy, by comparing the diagenetic chemical alterations in the different layers of bone tissues in their shells, as well as by comparing specimens separated by time and in differing depositional conditions, including material from: the Ravencrag Formation (Paleocene), Frenchman Formation (Cretaceous) and Dinosaur Park Formation (Cretaceous). The results (section 6.4) show that, within the same sample, the cancellous bone undergoes the highest amount of diagenetic alteration, likely due to porosity, and thus favouring exchanges with the environment. Many differences were detected between different deposits, but, also, among specimens found in the same formation, which suggests that localized environmental differences play a more important role in the diagenetic alterations endured by turtle bones than differences in age and geographic locality. Finally, chemical maps have shown that an effect of diagenetic alterations in turtle bones is the accumulation of manganese within the walls. This accumulation may be a possible criterion to qualitatively compare the levels of alteration experienced by different specimens.
6.2 Introduction

Turtles are reptiles known for their shells, and their ability to retract into this shelter [234]. The oldest known turtle fossil with a complete shell belongs to the genus *Proganochelys* from the Late Triassic of Germany [235]. Although this turtle had a complete shell, it could not retract into it. It was about a metre in length, had protective spines along its neck, and had a spiked tail with a clubbed end. For a long time, understanding of turtle evolution was limited, because transitional fossils were not known. This changed when an even older fossil from the Late Triassic of southwestern China, the *Odontochelys semitestacea* [236], was found. This specimen is approximately ten million years older than *Proganochelys* and presents a completely formed plastron (bottom shell), while the carapace is poorly developed, consisting of, only, neural plates [236]. *Odontochelys semitestacea* (which means toothed turtle with half-shell) had expanded ribs and some bony plates along the spinal cord, but not osteoderms, and, thus, represents an evolutionary stage prior to the development of turtles as we know them today. The species shows that in evolutionary terms the plastron formed first, while the carapace formed later, from the broadening of ribs and ossification of the neural plates [236].

Turtle shells are made of plates of bone that envelop the turtle’s internal organs, and can be divided into two parts: the plastron, which is the bottom (ventral) part of the shell; and the carapace, which is the top (dorsal) part [234]. They are connected to one another by a bridge of bone present along each side of the turtle body. The shell also incorporates the ribs and the central region of the spinal column bones. These are fused to the carapace, and surround the turtle’s pectoral girdle [234]. The bones in the shell are composed of three different layers: the external cortex, cancellous bone, and internal cortex. This structure is similar in both the plastron and carapace [237].

Besides providing a successful defense strategy for these organisms, the shell also
provides muscle attachment sites and functions as a mineral reserve for elements such as calcium, phosphorus, magnesium and sodium [234]. The turtle skeleton constitutes a very high percentage of their body mass when compared to other animals. Bones can reach values close to 40% of the whole animal’s weight for the painted turtle (*Chrysemys picta*), for example, with 32% of the body weight corresponding only to the shell [234]. In terms of composition, the turtle shell is composed of both dermal bones (resulting from ossification within the skin) and endochondrial bones (resulting from the ossification of cartilage). The bones of the shell are made of crystals of calcium phosphate bound in a collagen matrix, and they behave as a living organ that can grow and remodel, exhibiting living cells, nerves and blood vessels [234].

There are 348 [238] known living species of turtles, including aquatic and terrestrial species (sometimes called tortoises), belonging to the order Testudines. Differences in environment and shell type is a determining factor in the shape of the shell for each of the major groups of turtles. Tortoises possess a heavy, dome-shaped shell that is very hard to crush and offers considerable protection against predators, due to its shape and strength. Aquatic turtles, on the other hand, have flatter shells for easier swimming. There are also soft-shelled turtles living in muddy regions with flatter shells and a reduced amount of bone, providing the animals with more flexibility to move in their environment [234].

Most studies of fossil turtles focus upon their most unique characteristic: the shell. The majority of these studies focus on morphological aspects of the shells that relate to the group’s evolutionary history [239, 240] or the taxonomy of the fossils involved (e.g., [241]).

Chemical studies of fossil turtles are uncommon, but some show very promising and informative results. In [242], which investigated the chemical composition of the skin of a 55-million-year-old leatherback turtle (*Eosphargis*) using Time-of-Flight Secondary Ion Mass Spectroscopy (ToF-SIMS), Scanning Electron Microscopy
(SEM) and Energy-Dispersive X-ray Spectroscopy (EDX), researchers found evidence of organic residues in ovoid bodies present in the layer. These bodies were consistent in shape and size with the melanosomes present in extant lizards and bird feathers, and were also associated with measurements of ToF-SIMS that closely match those for eumelanin. Together, these data, suggest that the turtle presented dark dorsal colouring [242]. Another study involving the use of Field Emission Gun Scanning Electron Microscopy (FEG-SEM), Transmission Electron Microscopy (TEM), in site immunohistochemistry, ToF-SIMS and Infrared (IR) Microspectroscopy examined a juvenile sea turtle (Tabacka danica) from the Eocene [243]. It also showed evidence of eumelanin overlying the carapace bones, as well as other molecules of possible organic nature. Despite the interest in pigmentation, none of these studies focused on the composition of the underlying carapace bones.

In terms of synchrotron techniques, [244] used Near-Edge X-ray Absorption Fine Structure (NEXAFS) and X-ray Photoelectron Spectroscopy (XPS) to analyze the calcium content in the external cortex and cancellous bone in the shell of a gigantic tortoise (Titanochelon bacharidisi) from the Pliocene from Greece. They found that although the external cortex, like in extant exemplars, is composed of hydroxyapatite (partially transformed into chlorapatite), the cancellous bone was subjected to more extensive diagenetic changes and show a concentration of ~35% calcite. Not only do these results suggest that cancellous bone is possibly more vulnerable to chemical alterations, but also that synchrotron NEXAFS and XPS techniques can be used to answer taphonomic questions and study bone alteration in turtles [244].

### 6.3 Material and methods

Fossilized turtle shell and bone fragments were chosen from the Paleocene Ravenscrag Formation (RF), the Late Cretaceous Frenchman Formation (FF) and the Late Cretaceous Dinosaur Park Formation (DPF). A shell fragment from a
modern (extant; deaccessioned RSM specimen, *Trionyx* sp.) turtle (MT) was used for reference. From the RF, seven specimens were prepared, including: two sections of costal elements (i.e., shell with rib bone attached; RSKM_P3314.1, RSKM_P3314.2), a section of nuchals (i.e., shell with a vertebra attached; RSKM_P3314.3), two sections of shell (RSKM_P3314.4, RSKM_P3314.5), a section of the pectoral girdle (RSKM_P3314.6) and a section of a humerus (RSKM_P3314.7). One section of plain turtle shell was chosen from FF (RSKM_P3314.8), DPF (uncatalogued shell fragment) and the modern turtle. All specimens were cut using a water-cooled lapidary saw and polished using a series of lapidary wheels, except for the DPF specimen (because it was very brittle and could not be polished). After cutting and polishing and before measurements, all samples were cleaned with isopropanol with no further chemical treatment to the fossil samples. In addition to the steps described above, the modern turtle shell was already bleached before being cut (bleaching with the goal of whitening the bones and removing lipids had already been performed, before I had access to the specimen).

The VESPERS beamline at the Canadian Light Source [173] was used for micro-X-ray fluorescence (µXRF) measurements with a polychromatic beam. The data collection parameters are shown in Table 3. For the chemical maps presented herein, the individual spectra were normalized to the beam current as the measurements were being taken, and were then analyzed using the PyMCA software [175] in order to generate elemental maps for each sample (see eq. 48). Some of the maps produced were chosen to compare the average spectra of different regions of the shell. For these analyses, 125 points from the map in the region of interest were manually selected (to avoid the presence of Haversian canals or spaces between trabeculae), and their spectra were averaged to produce a single spectrum for the specimen or region of the shell. The spectra were normalized to the beam current, the same
amount of points was selected in each area and the data acquisition time per point was always the same (except for RSKM_P3314.4). This approach generated spectra with the same acquisition time, same conditions relative to background rate, and same intensity of the incident beam. Under these conditions, the intensity of the peaks in the spectra can be used to qualitatively compare elemental concentrations between different regions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Step size (μm)</th>
<th>Map size (mm)</th>
<th>Time per point (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ravenscrag - RSKM_P3314.1</td>
<td>15 x 15</td>
<td>0.84 x 3.39</td>
<td>2.0</td>
</tr>
<tr>
<td>Ravenscrag - RSKM_P3314.2</td>
<td>15 x 15</td>
<td>1.32 x 3.52</td>
<td>1.0</td>
</tr>
<tr>
<td>Ravenscrag - RSKM_P3314.3</td>
<td>15 x 15</td>
<td>0.75 x 1.50</td>
<td>1.0</td>
</tr>
<tr>
<td>Ravenscrag - RSKM_P3314.4</td>
<td>15 x 15</td>
<td>0.44 x 2.09</td>
<td>1.0</td>
</tr>
<tr>
<td>Ravenscrag - RSKM_P3314.5</td>
<td>15 x 15</td>
<td>0.77 x 2.42</td>
<td>1.0</td>
</tr>
<tr>
<td>Ravenscrag - RSKM_P3314.6</td>
<td>15 x 15</td>
<td>0.99 x 1.10</td>
<td>1.0</td>
</tr>
<tr>
<td>Ravenscrag - RSKM_P3314.7</td>
<td>15 x 15</td>
<td>1.10 x 1.54</td>
<td>1.0</td>
</tr>
<tr>
<td>Frenchman - RSKM_P3314.8</td>
<td>10 x 10</td>
<td>0.80 x 0.87</td>
<td>1.0</td>
</tr>
<tr>
<td>Dinosaur Park - DPF</td>
<td>10 x 10</td>
<td>0.84 x 0.42</td>
<td>1.0</td>
</tr>
<tr>
<td>Modern - MT</td>
<td>10 x 10</td>
<td>0.33 x 0.44</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 3: Elemental maps data acquisition parameters for turtle samples at the VESPERS beamline.

6.4 Results

The elemental distributions for calcium, iron, manganese, strontium and yttrium are presented in figs. 31–37, for the Ravenscrag Formation turtles; fig. 38 for the Frenchman Formation turtle; fig. 39 for the Dinosaur Park Formation turtle; and fig. 40 for the modern turtle specimen. The choice of these chemical elements was based on the general chemical elements observed in the spectrum from each of the samples, known bone composition (which should include Ca, Mn, Fe and Sr, for example), and known effects of bone diagenesis (e.g., increase of strontium concentration [41] and the appearance of yttrium [44]; see also section 2.4.1).
Figure 31: Elemental maps for Ravenscrag Fm. turtle (RSKM_P3314.1), which includes part of the shell and of a rib bone. Orange arrows show presence of strontium within marrow spaces in the shell. (Original in colour)

Figure 32: Elemental maps for Ravenscrag Fm. turtle (RSKM_P3314.2), which includes part of the shell and of a rib bone. (Original in colour)
Figure 33: Elemental maps for Ravenscrag Fm. turtle (RSKM_P3314.3), which includes part of the shell and of a vertebra. (Original in colour)

Figure 34: Elemental maps for Ravenscrag Fm. turtle (RSKM_P3314.4), which includes part of the shell. (Original in colour)
Figure 35: Elemental maps for Ravenscrag Fm. turtle (RSKM_P3314.5), which includes part of the shell. (Original in colour)

Figure 36: Elemental maps for Ravenscrag Fm. turtle (RSKM_P3314.6), which includes part of a pectoral girdle. (Original in colour)
Figure 37: Elemental maps for Ravenscrag Fm. turtle (RSKM_P3314.7), which includes part of a limb bone. (Original in colour)

Figure 38: Elemental maps for Frenchman Formation turtle (FF, RSKM_P3314.8), which includes part of the shell. Orange arrows indicate examples of regions within the marrow spaces with high amounts of iron. (Original in colour)
The results for the fossil turtles show that calcium, strontium, and yttrium are mostly located in the regions where bone tissue is found. These elements mark the
details in the microstructure of the bones, such as their Haversian canals and marrow spaces. Meanwhile, iron and manganese appear mostly within the canals (except for RSKM_P3314.6). In the case of the modern turtle, manganese and calcium seem to appear in the same regions, with iron particles present in some locations. Although strontium and calcium appear in the same regions for the modern turtle, the areas with the highest amount of each of these elements do not correspond to one another. Yttrium was not detected in the modern turtle specimen.

Using the calcium distribution as a reference, 125 points in each region of the samples (exterior and interior cortex and cancellous bone, depending on what was present in each measurement) were selected and their spectra combined and averaged. The selected regions did not contain osteons canals, marrow spaces, or areas with abnormally high concentrations of calcium. Figs. 41 to 45 show the spectra for turtles RSKM_P3314.1, RSKM_P3314.3, RSKM_P3314.4, RSKM_P3314.5 and RSKM_P3314.8, with representatives of each mappable shell region, in order to compare the element quantities among them.

Figure 41: Average spectra for 125 points in the external cortex, cancellous bone and rib of the Ravenscrag Fm. RSKM_P3314.1 turtle specimen in linear (left) and logarithmic (right) scale. Peaks for the elements discussed in this chapter are marked. The red line corresponds to external cortex (EC), black corresponds to cancellous bone (CB) and green corresponds to the section of rib bone (RB). (Original in colour)
Figure 42: Average spectra for 125 points in the cancellous bone and vertebra (Vert) of the Ravenscrag Fm. RSKM_P3314.3 turtle specimen in linear (left) and logarithmic (right) scale. Peaks for the elements discussed in this chapter are marked. The black line corresponds to cancellous bone (CB) and green corresponds to the section of vertebra bone (Vert). (Original in colour)

Figure 43: Average spectra for 125 points in the external cortex, cancellous bone and internal cortex of the Ravenscrag Fm. RSKM_P3314.4 turtle specimen in linear (left) and logarithmic (right) scale. Peaks for the elements discussed in this chapter are marked. The red line corresponds to external cortex (EC), black corresponds to cancellous bone (CB) and blue corresponds to the internal cortex (IC). (Original in colour)
The comparison of the spectra with respect to a single region of the bone across different specimens is shown in figs. 46 and 47 for the cancellous bone and external cortex, respectively. In these plots, RSKM_P3314.5 was chosen to represent the turtles from RF, because it consists of a similar shell section (without rib bone) to specimens from the other formations, and unlike RSKM_P3314.4, the acquisition time per point was consistent with that used for the other shells. A comparison including the internal cortex was not attainable because this region was only mapped in one of the specimens.
studied (RSKM_P3314.4, fig. 34). Comparison was only possible among the outer cortices of specimens from the Ravenscrag and Frenchman formations as this region of the bone was not sampled from the Dinosaur Park Formation and modern turtle.

Figure 46: Average spectra for 125 points in the cancellous bone for a specimen from each deposit and the modern turtle in linear (left) and logarithmic (right) scale. Peaks for the elements being discussed in this chapter are marked. The green line corresponds to the spectra of the modern turtle (MT; RSKM comparative collection), cyan corresponds to DPF (uncatalogued), orange corresponds to RF (RSKM_P3314.5), and purple corresponds to FF (RSKM_P3314.8). (Original in colour)

Figure 47: Average spectra for 125 points in the external cortex for a specimen from Ravenscrag Formation (RSKM_P3314.5) and the Frenchman Formation (RSKM_P3314.8) in linear (left) and logarithmic (right) scale. Peaks for the elements being discussed in this chapter are marked. The orange line corresponds to the spectra of RSKM_P3314.5 and purple corresponds to RSKM_P3314.8. (Original in colour)
6.5 Discussion

The maps in fig. 31–37 show the elemental distribution for calcium, iron, and manganese in RF turtles. The calcium (or rather its absence) clearly defines the location of the Haversian canals and marrow spaces (shown as regions with no calcium) in the turtle shell. Considering the size and quantity of the spaces present, distinct regions of the shell can be identified (see arrows). These correspond to the known regions of bone in the turtle shells [237]: internal cortex (IC, few and small osteons), cancellous bone (CB, trabeculae producing a very porous bone, with marrow spaces in between), the external cortex (EC, few and small osteons) and bone sections through the rib (figs. 31 and 32) and vertebra (fig. 33). Therefore, calcium can be used to establish the distribution of the bone tissue and its regions, and serve as reference for the distribution of other elements in the following discussions.

The iron distribution shows that this element is primarily located inside the osteons and marrow spaces, most likely result of its infiltration through these empty canals and accumulation on their walls. This is the main mechanism for chemical modifications during diagenesis in the central and less accessible parts of the bone, changing the composition of the bone tissue itself as it will be discussed later on this section. Another unverified possibility is that at least part of this iron is remnant from blood flowing through the blood vessels along the canals. On the other hand, manganese seems to be associated with calcium in some of these samples (RSKM_P3314.3, RSKM_P3314.6, RSKM_P3314.7 and in parts of RSKM_P3314.1 and RSKM_P3314.4) and associated with the positions of marrow spaces in others (RSKM_P3314.1, RSKM_P3314.2, RSKM_P3314.5 and parts of RSKM_P3314.4). In the latter, manganese is mostly located around Haversian canal and marrow space walls, much like iron (but not following the same distribution). Since the modern turtle specimen (fig. 40) also have a distribution similar to that found in
RSKM_P3314.3, RSKM_P3314.6 and RSKM_P3314.7, and, also, the FF turtle RSKM_P3314.8 (fig. 38) to some extent, the results suggest that specimens RSKM_P3314.1, RSKM_P3314.2 and RSKM_P3314.5 had a larger Mn intake during diagenesis. One would expect the infiltrated manganese to accumulate in the osteon and marrow space walls and, if this concentration is large enough, it would overprint the original distribution present in the bone tissue, as seen in the modern specimen. This is confirmed by adjusting the saturation in order to properly view the lower intensity portion of the distribution (fig. 48). Compared to the saturation used before (fig. 48-a), the changes in saturation seen in fig. 48-b show that manganese is, in fact, distributed in the same region where calcium is found, but in a much smaller quantity than that found in the osteons and marrow spaces. The same effect is observed in the DPF turtle specimen (fig. 39).

![Figure 48](image-url)

Figure 48: Elemental map for calcium and manganese for the Ravenscrag Formation turtle RSKM_P3314.5 with a) the same saturation for Mn used in fig. 35 and b) the saturation changed so that the portion of the distribution with lower intensity is shown in more detail. (Original in colour)

The data provided were not enough to explain the reason(s) for such differences in the manganese absorption by different specimens. Considering that specimens found
in the same formation have very different distributions for this element, one could establish that differences in age or general location play a less important role than local variations in the deposit site. For example, animal remains deposited in water would have a higher probability of absorbing minerals from the neighbouring sediments. Variations in the remains themselves may also have had a strong influence (e.g., a shell with cracks and exposed canals early in its diagenetic history would probably experience more of these changes than intact shell). The fossils in this study were all found as fragments, but there is no information regarding when in the fossil’s history such fragmentation took place. The effects of local environment and fossil history are unique to each specimen, and make it difficult to draw general conclusions.

Strontium shows a distribution very similar to that of calcium, since it can substitute for the latter in bone apatite [179]. In living animals, strontium is mainly acquired through diet and, thus, its abundance in bones depends on the animals dietary habits [180]. In fossils, strontium is typically added to the bone during diagenesis [41], and thus it can also be seen within some Haversian canals and marrow spaces, where calcium is not present in measurable quantities (it appears as a blue outline around the canals and spaces; e.g., see orange arrows in fig. 31). However, when considering the modern turtle strontium distribution (fig. 40), one can see that, although it also appears in the same areas where calcium is found, strontium also seems to appear in the osteons, marrow spaces, and other bone areas where the amount of calcium is smaller. This does not invalidate the previous discussion about strontium distribution in the fossils being partly controlled by diagenetic processes, but suggests that there could be a contribution from the original material in the strontium present inside the Haversian canals and marrow spaces. This is expected because the blood going through the canals is the main transport of strontium into the bones. However the relative quantities of strontium when compared to calcium in fossils is much higher than in the extant turtle shell.
This feature is discussed in greater detail later in this chapter, and it suggests that diagenetic processes are most likely the dominant explanation for the distribution observed here.

The last maps for the RF turtles show the distribution of yttrium compared to the distributions of calcium. It is possible to observe in these maps that yttrium has a behaviour very similar to the one seen for strontium, matching the distribution of calcium, but it is also present within the osteons and marrow spaces, usually closer to their walls. While yttrium was detected in all the other samples, no yttrium was observed in the modern turtle (fig. 40). This results from the fact that yttrium is an element with a purely diagenetic origin [44]. The maps for the FF RSKM_P3314.8 (fig. 38) and DPF (fig. 39) turtles show a similar distribution of yttrium as those for RF turtles, except for a smaller amount of this element in the bottom half of the DPF turtle, which is probably due to differences in the deposit and sediment conditions in these regions during diagenesis.

The maps for the DPF specimen (fig. 39) present distributions very similar to the RF turtles (figs. 31-37), with calcium and strontium marking the distribution of bone tissue, while iron is present in larger quantities inside osteons and marrow spaces. The maps for the FF specimen (fig. 38) indicate what seems to be a very different distribution, with iron present in the same regions as calcium and strontium. This is probably due to a smaller amount of diagenetic iron in the fossil, which is not enough to hide the original distribution in the bone of the animal (i.e., the opposite effect of what was discussed previously for manganese). In the FF specimen, most of the osteons and marrow spaces are depleted of iron, with this element distributed in trace amounts within the bone tissue. Regions with higher concentrations of iron are present inside some marrow spaces as indicated by the orange arrows in fig. 38. A similar distribution can be observed for the modern turtle. Iron is present in the same regions where calcium is present, but it is not exactly correlated and appears
in small clusters with higher concentration in some parts of the bones.

Although these measurements were taken with a polychromatic beam, and, therefore, cannot be easily interpreted in a quantitative fashion, a qualitative comparison between spectra is still possible. The plots in figs. 41-45 compare different regions within the same specimen. In all plots, the cancellous bone region presents a higher amount of iron when compared to the other regions of the shell (external and internal cortex). This strongly suggests that a large amount of iron found in these samples is of diagenetic origin and accumulated due to increased porosity of cancellous bone. The spongy bone in this region increases the surface area available for exchanges between bone and environment during diagenesis, increasing the amount of iron absorbed by the bone tissue in this region of the shell. The only measured region that present a higher iron concentration than the cancellous bone is the rib section in RSKM P3314.1 (fig. 41), which is also very porous (as can be seen in its elemental map, fig. 31). The other elements are much more similar between different bone regions than iron — even for other diagenetically controlled materials, such as strontium and yttrium. However, the cancellous bone region still seems to show a slightly larger (or at least equal) amount of diagenetic elements when compared to external and internal cortices. The same is true for the calcium concentration.

One can also compare the spectra with respect to a single region of the bone across different specimens (figs. 46 and 47). The comparison between the cancellous bone for different turtle specimens (DPF, FF, modern, and the RSKM_P3314.5 representative for RF; fig. 46), shows how the relative concentration of calcium in relation to other elements is overwhelmingly higher for the modern turtle shell. For the modern specimen, the amount of calcium present is much higher than any other element, while for the fossils the amount of calcium is comparable to the amounts of strontium and yttrium, for example. If the modern turtle is taken as a reference,
this result suggests that the fossils lost calcium during diagenesis, mostly through yttrium and strontium replacement. On the other hand, yttrium and strontium seem to appear in larger relative amounts in the fossil shells.

Another difference between the modern turtle and fossils is the amount of iron present. It is much higher in the number of counts in the fossils (fig. 46-right, for the peak corresponding to iron). This suggests that most of the iron measured in these fossils is the result of diagenetic processes. The RF turtle in particular presents a much higher iron concentration even with respect to the other fossils, both in its cancellous bone (fig. 46) and in its external cortex (fig. 47). Since the peaks for the other elements (Ca, Sr and Y) are fairly similar, this suggests that sedimentary deposits with certain characteristics favoured iron absorption by this shell when compared to the other fossils.

6.6 Conclusions

The results presented in this chapter show maps comparing different specimens of turtles from the Ravenscrag Formation, Frenchman Formation, and the Dinosaur Park Formation with material from a modern turtle. The elemental maps and XRF spectra show clear differences not only between deposits, but also among specimens from the same formation, and between different bone layers in the same specimen. Here, it is shown that the cancellous bone in turtle shells undergoes more chemical taphonomic alterations than the more compact bone layers (external and internal cortices), due to its higher porosity. It is also shown that local differences in the deposit’s environment can sometimes have similar or greater effects than those found between specimens from different formations and ages. The results seen here for the turtles suggest that the same is possibly true for fossils from other species. The characteristics of fossils are difficult to generalize, because they depend strongly on the local conditions of the deposit. As a result, fossils found very close to one another
with the same approximately the same age can have completely different taphonomic alterations.

Furthermore, the manganese distribution results show that all fossil bones start with a distribution of manganese consistent with the bone tissue distribution in modern turtle shell (i.e., there is strong potential that original manganese can still be found in the fossils). This was shown for the Ravenscrag Formation turtle RSKM_P3314.5 (fig. 48), for example. During diagenesis, infiltration of minerals through the canals in the shells causes the accumulation of manganese in the osteon and marrow space walls, as well as the increase of its amount in the bone tissue due to absorption effects (see fig. 46 for a comparison between the amount of manganese in the modern turtle and the fossils). Thus, the presence of large amounts of manganese in the osteon and marrow space walls can be used as a criterion to qualitatively compare the levels of alteration or replacement between different bones. If there is a large quantity of manganese, then the bone has potentially endured significant chemical changes during diagenesis.
7 Chemical diagenesis of individual structures in hadrosaurid skin layer

7.1 Abstract

Most fossils from deep time are preserved remains of animals’ hard parts (i.e., tissues already mineralized while the animals are alive, such as bones, and teeth). Rarely, the conditions are such that the preservation of soft tissues (such as muscle and integument) is possible. Here, I study the chemistry of a fossilized hadrosaurid (Edmontosaurus sp.) skin with detailed anatomical preservation, as part of a larger project involving other researchers. The research previously performed in this specimen had shown skin-like layers formed by substructures [245]. My goal, in particular, was to tentatively study the chemistry of a single example of the substructures in order to hypothesize on the mechanisms behind their preservation. The results (section 7.4) show the presence of carbon, calcium and iron within the substructure. Calcium, in the form of carbonate, was found in large quantity in the region surrounding the substructure. This occurrence was probably a result of mineral infiltration, and calcium is also present in smaller amounts within the structure. This could be the result of an early mineralization process that outpaced microbial decay. The mineralization process contributed to the highly-detailed preservation of the skin specimen and for the potential entrapment of molecules with biological origin. The extent of this preservation is observed through the analysis of the carbon form identified in the skin. Iron (in Fe(III) oxidation state) was found, oddly, only in the middle of the substructure, and may also be part of the mechanism that resulted in the preservation of this specimen.
7.2 Introduction

Hadrosaurids are members of the clade Ornithopoda. This clade includes all ornithischian dinosaurs that are more closely related to the hadrosaurid *Edmontosaurus* than to the ceratopsian *Triceratops* [246]. Ornithopoda was once used to group all dinosaurs that did not belong to one of the known groups, but this is not the case anymore. Most ornithopods possess kinetic skulls, ventrally offset premaxillary and jaw joints relative to the maxillary tooth row, a mandibular fenestra that is closed or reduced, and a rectangular obturator process on the ischium [246]. The most primitive members of this clade are the hypsilophodontids known to have lived in the Late Jurassic, but believed to exist since the Middle Jurassic at least. This group included the genera *Hypsilophodon* and *Thescelosaurus* and existed until the end of the Cretaceous [246].

Within Ornithopoda, hadrosaurids are part of the clade Iguanodontia, which included ornithopods more closely related to *Edmontosaurus* than to *Thescelosaurus*. Iguanodontia is characterized by a transversely expanded premaxilla lacking teeth, a deep dentary with parallel dorsal and ventral margins, a missing phalanx from digit III in their hands and a deep and transversely compressed anterior process in the pubis [246]. The earliest evidence of a member of the Iguanodontia is a single femur from the Middle Jurassic in Europe [246].

More specifically, hadrosaurids are members of the clade Hadrosauroidia, which is part of Iguanodontia and includes the family Hadrosauridae. This clade includes the subfamilies Hadrosaurinae and Lambeosaurinae as well as other basal taxa [246]. hadrosaurids are members of the Hadrosaurinae subclade and are a very well-known group, with fossils having been found in many regions of the world, including nearly complete skeletons, skin fossils, remains of eggs, embryos, hatchlings and coprolites [54, 247, 248, 249, 250]. They were large animals, estimated to be between 7 and 12 metres long and having an average mass of 3 tons, with some species that were much
heavier. They were characterized by using both bipedal and quadrupedal postures and by highly modified tooth rows of dental batteries with as many as sixty teeth organized in files of 3 to 5 closely packed teeth each [246].

The most commonly preserved fossils are structures that were mineralized when the animal was alive, such as the teeth and bones. However, soft-tissues are also rarely preserved. The most common soft-tissue preservation is of structures made of more durable materials such as the protein keratin, which is the main component of external skin layers (scales), claws and feathers [251]. Animal skin has roles that include protection (it must be able to resist abrasion), defense against microbial invasion, and maintaining water balance. This is achieved through the presence of keratin (present as alpha and beta forms in dinosaurs [251]) as the main structure of the skin external layers. Keratin possesses great durability and is insoluble in water, making it resistant to decay through hydrolytic damage [251].

Even though dinosaur skin has properties that can lead to preservation in the fossil record, most specimens found have been classified as only skin impressions in sediments (e.g. [252] and references therein). This can still lead to an understanding of how dinosaur skin dealt with dermal pathology [253], and the establishment of a set of criteria to identify skin impressions from other phenomena that can mimic these impressions [254]. However, rare occurrences of actual fossilized skins have also been documented. These specimens have been used to provide insights on dinosaur behaviour and physiology.

A deep cross section through the dermis of a *Psittacosaurus* found in the Jehol Group, China, showed visible collagenous fiber layers [255]. The study of this specimen indicates that 25 well-preserved and 15 poorly-preserved fiber layers were estimated to be present, suggesting a very thick skin. Also, the fibers in the layers were organized into left- and right-handed geodesic helices, which would have improved the skin’s resistance to stresses and strains. This suggests that this
dinosaur's skin was highly adapted for protection [255].

Another example of fossilized skin was found in an exceptionally preserved three-dimensional specimen of nodosaurid ankylosaur (*Borealopelta markmitchelli*) from the Early Cretaceous in Alberta, Canada [56]. Mass spectroscopy studies of this specimen showed the preservation of melanin, revealing a lighter pigmentation on the animal’s parascapular spines and a pattern of countershading on the animal’s body. This pigmentation suggested that the spines were probably used for display while the larger-scale color patterns were an adaptation against predation [56].

The most relevant previous fossil discovery to the specimen described herein is a hadrosaurid (*Edmontosaurus* sp.) skin fossil from the Hell Creek Formation (North Dakota, U. S. A.) [54]. This specimen was described as containing partially preserved epidermal microstructures, including the mineralized skin cell boundaries. FTIR measurements suggested the presence of compounds with amide groups [54]. The exceptional preservation was explained by the rapid burial and mineralization, which outpaced tissue decay and preserved structures with high fidelity. The mineralization was assisted by the presence of bicarbonate in the system due to an anoxic environment with high methanogenesis, and by the dissolved reduced iron and rock fragments (caused by reducing porewaters resulting from the decay of plant material). The reduced iron-rich solution rapidly replaced soft tissues with carbonate minerals [54].

Previous measurements collected from the hadrosaurid under study by other members of the research team indicated the presence of carbon-rich layers that appear to be composed of substructures with layering patterns similar to those found in epithelial tissue of chicken leg skin. Assuming this to be true, I tentatively selected one of these supposed substructures for further studies. The aim was to chemically characterize a single substructure in order to possibly obtain information relevant to its preservation mechanism. This is the focus of the work discussed in
this chapter.

7.3 Material and methods

The measurements described in this chapter are part of a series of measurements taken on samples of the same hadrosaurid skin, and performed by different members of the research group, including myself, using several different techniques. The results presented here are based on the measurements performed as part of my thesis research and developed by me, but will also include some of the other results, where they are relevant to the discussion. Section 7.3.1 provides a description of the specimen used in this study along with some information concerning the location where it was discovered. The methodology used for the measurements discussed in this chapter is described in section 7.3.2.

7.3.1 Specimen and geological settings

The specimen used in this study is a sheet of fossilized integument (skin) found associated with UALVP 53290 [245]. UALVP 53290 is an incomplete articulated-to-associated hadrosaurid skeleton, tentatively classified as *Edmontosaurus cf. regalis* (the only hadrosaurid species known from this stratigraphic interval) [245]. The skeleton was found in the Red Willows Fall locality of the Upper Cretaceous Wapiti Formation near Grande Prairie (Alberta, Canada), and consists of most of the thoracic region, forelimb and pelvic elements, among other bones. The integument was associated with the forelimbs of UALVP 53290, presumably from the dorsal and anterior surface of the forearm [245]. The skin presented scales identical to the ones found in the upper arm regions of other *Edmontosaurus* specimens (e.g., [54]) [245].

The Wapiti Formation is a nonmarine deposit of interbedded fluvial sandstone, siltstone and mudstone deposited during the early Campanian to the Late
Maastrichtian [256]. The formation can be divided into five stratigraphic units, as described in [256], unit 4 being the one where the hadrosaurid specimen was found [245]. Unit 4 deposits consist of repeating sequences of crevasse-splay, muddy and organic-rich overbank deposits, and minor sandy channel fills [256]. The stacking pattern suggests a fluvial environment, and together with common coal seams, it indicates the presence of high-water table conditions and highly vegetated, poorly drained grounds [257]. Many fossils have been found in the carbonaceous mudstone layers of this unit, including duck-billed and horned dinosaurs (remains and tracks), and insects in amber (see [256] and references therein).

7.3.2 Methodology

A piece of a pristine fossilized hadrosaurid skin (from skeleton UALVP 53290) with the attached sediment matrix was sectioned using a microtome (Leica EM UC7) in a water bath with the goal of obtaining a slice of 100 nm thickness. However, due to the brittleness of the specimen, this process resulted in flakes and debris of the sample. These fragments were collected and deposited on a silicon nitride membrane and air-dried at room temperature before the measurements.

Scanning transmission X-ray microscopy (STXM) measurements were taken at the Soft X-ray Spectromicroscopy Beamline (SM) [258, 259] at the Canadian Light Source. This technique, as the name suggests, is performed in transmission mode and results in a series of XAS spectra: one for each pixel measured in the selected region of the sample. This beamline uses a beam produced by a 75 mm generalized Apple II Elliptically Polarizing Undulator, with energy range between 130 and 2700 eV and spot size on the sample for STXM measurements of 30 nm, with wavelength selected by a slit-less plane grating monochromator with single grating substrate with three stripes.

A carbonyl map of the region with the skin layer (fig. 49-left) was used to
tentatively select a supposed single substructure for measurements. A 5 x 5 μm area with one of the substructures is believed to be located in its centre was selected for the measurements. This region is depicted in fig. 49, where the dark regions represent the presence of material that stops the passage of X-rays. Meanwhile, the white regions represent empty regions in the sample (regions transparent to the type of X-ray beam used), since measurements were obtained in transmission mode. The area was measured using 200 x 200 pixels of data (pixels here refer to each point that composes the image and for which STXM spectra was collected). The same region was used for all measurements shown herein.

Figure 49: Region (20 x 20 μm) of the skin sample associated with UALVP 53290 containing a layer of approximately with substructures (left) and the measured region (5 x 5 μm) containing one of these substructures (inset and to right). The images were obtained in transmission mode and, thus, dark areas correspond to detected matter. The scale bar corresponds to 0.5 μm in the images. (Original in colour)

The data collected resulted in image sequences [260] for varying energies around the edges of carbon (K-edge), potassium (L-edge), calcium (L-edge), iron (L-edge) and manganese (L-edge), as observed in Table 4. These images present transmission signatures (i.e., the black areas show the presence of signal, because it indicates that the incident light was absorbed by the sample, while the white areas denote no signal and the incident light passed through the sample without being absorbed; see, for example, fig. 50-a). In order to change these images to the most common absorption
mode, they were converted using optical density (OD; also known as absorbance), according to the equation:

\[
OD = -\ln \left( \frac{I}{I_0} \right)
\]  

(50)

where \(I_0\) is the total flux received by the material (estimated by selecting an area where no sample was present in the image), and \(I\) is the flux transmitted by the material (i.e., signal measured at each point) [261]. The resulting images show the signal as white and the no-signal areas as black (fig. 50-b). The images were aligned and regions showing similar behaviour with energy changes were selected using the software aXis2000 [262]. The average spectra for these regions were obtained and then used to fit the whole image, identifying the measured regions where such spectra were present. The result is a map showing the regions where each chemical state (represented by a spectrum) could be found, and its amount (indicated by the intensity of the colours representing the material). The quantity of material (proportional to the material’s concentration) in each pixel is measured by the software using the intensity of its spectrum in each point of the sample. Thus, since I did not measure any standards to establish the relationship between these values and actual concentration for the samples, the obtained values are to be interpreted qualitatively, as showing regions with more or less concentration of each chemical state.

<table>
<thead>
<tr>
<th>Element</th>
<th>Energy range (eV)</th>
<th>Dwell time (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>342 to 360</td>
<td>0.725</td>
</tr>
<tr>
<td>Carbon + Potassium</td>
<td>280 to 320</td>
<td>1.040</td>
</tr>
<tr>
<td>Iron</td>
<td>699 to 730</td>
<td>1.024</td>
</tr>
<tr>
<td>Manganese</td>
<td>635 to 665</td>
<td>1.016</td>
</tr>
</tbody>
</table>

Table 4: Energy range and dwell time for the STXM measurements of different elements.
7.4 Results

In the region of interest, STXM measurements were taken in the energy regions corresponding to the edges for calcium, carbon and potassium, iron and manganese (see table 4). The stacks of images were then analyzed for each element and the different XAS spectra were obtained for different regions in the measured area (figs. 51, 52, 53 and 54, for calcium, carbon, iron and manganese, respectively). Manganese and potassium could not be detected in the measured region.

Figure 51: Calcium XAS spectra for different regions of the substructures found in the UALVP 53290 skin layer. The colour of each spectrum (right) corresponds to the colour of the region in the image (left), where white areas represent a strong signal and black represents no signal. The vertical line represents the energy in the plot for which the image in the left was taken. (Original in colour)
Figure 52: Carbon and potassium XAS spectra for different regions of the substructures found in the UALVP 53290 skin layer. The colour of each spectrum (right) corresponds to the colour of the region in the image (left), where white areas represent a strong signal and black represents no signal. The vertical line represents the energy in the plot for which the image in the left was taken. None of the spectra found in this image show the presence of potassium. (Original in colour)

Figure 53: Iron XAS spectra for different regions of the substructures found in the UALVP 53290 skin layer. The colour of each spectrum (right) corresponds to the colour of the region in the image (left), where white areas represent a strong signal and black represents no signal. The vertical line represents the energy in the plot for which the image in the left was taken. Only the dark green spectrum had any identifiable iron. (Original in colour)
Figure 54: Manganese XAS spectra for different regions of the substructures found in the UALVP 53290 skin layer. The colour of each spectrum (right) corresponds to the colour of the region in the image (left), where white areas represent a strong signal and black represents no signal. The vertical line represents the energy in the plot for which the image in the left was taken. Manganese was not found in any region of the measured area. (Original in colour)

In fig. 51 the presence of two different chemical states for calcium are apparent within the sample: the one in red (referred to as calcium-1 hereafter), and the other corresponding to the other colours (calcium-2 hereafter). These spectra can be used to fit the spectra for each point in the image, assuming each point’s spectrum is a linear combination of the individual spectra for each chemical state. The fitting is done in order to identify the specific areas in the sample where each state can be found (fig. 55). These regions were used to create a mask and select the areas in the sample where each of the spectra are stronger, thus calculating the average spectrum for each of these areas. Details can be seen in fig. 56, and the peaks from each spectrum are listed in table 5.
Figure 55: Map showing the distribution of the two different states of calcium in fossil skin associated with UALVP 53290, where a) shows the distribution of the spectrum found in red in fig. 51 (calcium 1), b) shows the distribution of the other spectrum (calcium 2) and c) shows a combination of both distributions. The circle in a) shows the approximated region where the substructure is believed to be. The arrow shows the region of strong calcium 1. (Original in colour)

Figure 56: Average spectra of calcium 1 (blue) and calcium 2 (red) in fossil skin associated with UALVP 53290, calculated based on the regions where the spectra are found. Blue arrows point at the main peaks of the spectra, while black arrows point at the secondary peaks. The red arrow points at detail at the second main peak, where two “bumps” can be observed. (Original in colour)
<table>
<thead>
<tr>
<th>Spectrum</th>
<th>Peaks (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium 1</td>
<td>347.7, 348.2, 348.8, 350.1, 352.2, 353.4</td>
</tr>
<tr>
<td>Calcium 2</td>
<td>347.8, 349.0, 349.9, 350.1, 352.3, 353.1, 353.4</td>
</tr>
</tbody>
</table>

Table 5: Calcium peaks for each spectrum in fig. 56.

Fig. 55 shows that the distribution of the two states of calcium are different from one another. Even though both seem to be present in small quantities in the whole measured region, the areas where they are in larger quantities are different depending on the state. For calcium 1, the highest amounts can be found in a “lens” in the bottom, left corner of the mapped region (see white arrow), followed by some presence in the area where the substructure is found. Meanwhile, the calcium 2 distribution shows its highest amounts approximately in the middle and in the surrounding areas of the structure. The fact that these two states of calcium are different is also visible when comparing their spectra in fig. 56. In this image, the position and relative intensity of the peaks (also seen in table 5) for the two spectra are clearly different.

Fig. 52 shows the presence of three types of carbon spectra present in the mapped region, exemplified by the spectrum in dark green (referred to as carbon-1 hereafter), the one in pink (carbon-2 hereafter) and the one in light green (carbon-3 hereafter). The distribution of these three states can be seen in fig. 57 and the average spectrum for each distribution is shown in fig. 58. Table 6 shows the position of the peaks identified in the spectra shown in fig. 58.
Figure 57: Map showing the distribution of the three different states of carbon in fossil skin associated with UALVP 53290, where a) shows the distribution of carbon-1, b) shows the distribution of carbon-2, c) shows the distribution of carbon-3 and d) shows a combination of all distributions. (Original in colour)

Figure 58: Average spectra of carbon 1, 2 and 3 in fossil skin associated with UALVP 53290, calculated based on the regions where the spectra are found. (Original in colour)
Table 6: Carbon peaks for each spectrum in fig. 58.

<table>
<thead>
<tr>
<th>Spectrum</th>
<th>Peaks (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon 1</td>
<td>288.5</td>
</tr>
<tr>
<td>Carbon 2</td>
<td>288.6</td>
</tr>
<tr>
<td>Carbon 3</td>
<td>285.6, 288.6, 290.7</td>
</tr>
</tbody>
</table>

The three states of carbon detected show very different distributions in the measured region, as seen in fig. 57. Carbon 1 appears mostly in the region close to the border of the substructure and in small zones between them. Carbon 2 appears with higher intensity in the middle of the substructure, while carbon 3 seems to have a more homogeneous distribution, with some higher amounts in the areas surrounding the substructure. These carbon states are clearly different from each other, as can be seen in fig. 58.

Fig. 53 shows that only one spectrum of iron seems to be present in the mapped regions. The corresponding map showing its distribution can be seen in fig. 59 and the average iron spectrum based on this distribution is presented in fig 60. Iron is only present in one state in the mapped region, and it is only present in a small area approximately in the middle of the substructure (fig. 59).

Figure 59: Map showing the distribution of the only measured state of iron in fossil skin associated with UALVP 53290 as seen in the green spectrum of fig. 53. (Original in colour)
Maps comparing the distribution of different identified elements in the regions under study can be observed in figs. 61 and 62, using carbon-1 and carbon-2, respectively. A similar map for carbon-3 was not produced because its distribution covers most of the map and the only visible feature is an increase in intensity in the same region where the calcium is abundant.
7.5 Discussion

The main peak of L₃-edge calcite (at 350.1 eV) was arbitrarily set at the energy of 349.3 eV, as was done in [263], in order to compare the peak positions with the standards described in this reference. Using the information displayed in table 5, this results in peaks at positions (in eV): 346.9, 347.4, 348.0, 349.3, 351.4 and 352.6 for calcium-1, being a perfect match for the described calcite (CaCO₃) spectrum. Therefore, the material so far called calcium-1 can be identified as calcite, and is likely associated with sedimentary materials surrounding the skin. Fig. 55 shows that calcite distribution sits in larger quantities outside the substructure, forming an egg-shaped structure encapsulating it (see white outline in the figure). It is probably a calcite-rich crystal from the sedimentary rock. The distribution also shows that calcite is present within the substructure in smaller quantities. The presence of calcite within the substructure could be the result of an early-mineralization process, which may have contributed to the preservation of these structures [7]. However, calcium carbonates are not often known for detailed preservation of soft-tissues in taphonomic studies: this preservation is usually a result of calcium phosphate deposition (phosphatization) [39]. The two processes
can occur in the same settings, due to a switch in the environmental conditions brought about by a change in environmental pH due to decay processes [264]. The presence of calcium carbonate suggests that, at least in part of its taphonomic history, this fossil has been under “open” conditions, within a more alkaline environment that favoured carbonate precipitation [39].

The spectrum for calcium-2 is composed of two different states of calcium, as can be seen around 353 eV in the $L_2$ peak in fig. 56, where two bumps at the top of the peak can be observed (red arrow in fig. 56), suggesting this spectrum is the result of the combination of two spectra with $L_2$ peaks at slightly different energies. One of these states is believed to be calcite, since it has already been determined that this material is present in the sample. Also, considering that the energies corresponding to the components forming each of the main peaks (blue arrow in fig. 56) in the spectrum (at 349.9 and 350.1 eV for the first peak, and 353.1 and 353.4 eV for the second), and the higher energy components are compatible with the measured peaks for calcite (350.1 and 353.4 eV), the peaks associated with the unknown state should be located at 349.9 and 353.1 eV. Although these two peak positions are not compatible with any of the materials examined in [263] or any other reference found, they seem compatible with aragonite ($\text{CaCO}_3$) due to their relatively weak secondary peaks (black arrows in fig. 56), and the main peaks’ lower energies with respect to the corresponding peaks for calcite [265]. The differences in energy between main and secondary peaks for $L_3$ and $L_2$ edges ($349.9 - 349.0 = 0.9$ eV and $353.1 - 352.3 = 0.8$ eV, respectively) are also compatible with the aragonite difference (0.8 eV) given in [265]. As a comparison, the values found here for calcite in the spectrum for calcium 1 are 1.3 eV and 1.2 eV, which are compatible with the 1.2 eV and 1.3 eV found in [265]. Thus, the spectrum for calcium 2 is a combination of two calcium carbonate materials.

The lack of evidence for the presence of calcium phosphate suggests that the
preservation of skin layers in the hadrosaurid specimen is not a result of phosphatization, and that the presence of calcium carbonate cannot be explained by the pH switch described previously and discussed in [264]. The precipitation of phosphates would be favoured over that of carbonates if the conditions were “closed”, resulting in an anoxic environment with an acidic pH. The absence of phosphatization in spite of the presence of a nearby source of phosphorous (the animal’s carcass), and the presence of calcium carbonate instead, indicates different taphonomic conditions during its mineralization stage. The skin specimen was found in an area believed to have exhibited freshwater conditions, with a high water table, high amount of vegetation and poorly drained soils [257]. This was an “open” environment, with presence of water and decaying vegetation, creating conditions similar to the ones described in [54] (see also section 7.1.2). The setting would favour the rapid replacement of soft tissue with carbonate minerals. The presence of substructures believed to be remnants of the original tissues suggest the replacement was faster than microbial decay. The rapid mineralization resulted in the high-fidelity preservation of skin substructures. As discussed in the following paragraphs, rapid mineralization ensured that products from organic molecules were preserved within the fossil.

The position of the peaks measured in the spectra for carbon-1 and 2 are compatible to the ones attributed to the carboxyl group, which should appear between 288.0 and 288.7 eV [266]. These are organic forms of carbon. The presence of organic carbon in the hadrosaurid skin layer suggests that I could be measuring the remains of original organic material from the dinosaur, or material from the decay process, or maybe subsequent biological activity. However, with the use of the techniques attempted here alone, it is not possible to determine exactly which compound is being measured and, therefore, it is not possible to exactly determine its source.
For carbon-3, the spectrum shows the presence of the same peak discussed in the previous paragraph, corresponding to the presence of the carboxyl group. The peak at lower energies (285.6 eV) is compatible with an aromatic (C = C) functional group that could potentially have originated from an biological process. The peak at 290.7 eV is most likely indicative of carbonates [267], as suggested by the previous observation of calcite in the sample. In fact, the region in the map shown in fig. 57-c with the highest concentration of this carbon state matches the area where calcite was predominant in the sample (fig. 55-a).

Although not presented in this thesis, other measurements have been performed on this specimen by other members of the research team using other techniques such as Fourier-transform infrared spectroscopy (FTIR). The measurements through this technique also strongly support the evidence for the presence of organic material in the regions of the specimen where the skin layer is located [245]. The unpublished data further corroborates the discussion presented here. Ultimately, it supports the idea that the observed carbon is the result of some biological process experienced by the animal after its death.

The main peak in the spectrum shown in fig. 60 is found at 709.8 eV, indicating iron in Fe(III) oxidation state [260]. Although the mechanism that led to this form of iron in the sample is not understood, the presence of Fe(III) has been associated with exceptional preservation of fossils in the past, especially with preservation of soft-tissues in dinosaurs [7]. The fact that its distribution indicates a concentration of this compound in the skin layer substructures, and nowhere else in the mapped region, seems to strongly corroborate this hypothesis.

The maps in figs. 61 and 62 show that the presence of Fe(III) is concentrated within the region in the center of the substructure, which also corresponds to the highest concentrations of organic carbon in both images. In all the maps it is possible to see that calcium carbonate is also present in small quantities within the
substructure, but is present in higher amount in the regions surrounding the structure. This calcium carbonate distribution is probably the result of infiltration of cementing minerals through voids in the layer.

### 7.6 Conclusions

The results shown here suggest that the exceptional preservation of the skin layers from a hadrosaurid is the result of an unknown mechanism involving the presence of Fe(III) in combination with the rapid mineralization caused by the replacement of soft tissues with carbonate minerals. The effect of this iron state has already been described in the literature as being related to the preservation of dinosaur soft tissues. Mineralization outpacing microbial decay was responsible for a highly detailed preservation of the morphological structure of the skin layer. It was also responsible for the preservation of molecules of biological origin identified by the presence of organic carbon groups in the region where one of the substructures that make up the skin layer is found. Although these organic compounds cannot be precisely identified, they strongly suggest the presence of preserved tissues when combined with the structured distributions observed.

Regardless of the processes involved, the findings described here present evidence that the layers of material identified in the hadrosaurid skin retain structure that reflect the preservation of fossilized skin layers. This is backed by both the physical structure of these layers, similar to those found in skins of living avian species, and its chemical composition.
8 Synchrotron applied to taphonomic studies

8.1 Abstract

The previous chapters discuss the results of the research involving the application of synchrotron-based techniques to taphonomic studies concerning different animal groups, types of fossils and different types of fossilized tissues. The goal of this chapter is to combine these results and analyses to obtain information about fossils in general, their differences and similarities, and the possible associations they may have that can be explored using these techniques.

8.2 Introduction

Several different specimens were studied in the previous sections. Here I present comparative analyses involving the bones and tendon previously discussed with respect to the relative concentration of the elements of interest and their correlations. Please, refer to these sections for specifics of individual projects, specimens, and techniques covered in this synthesis.

8.3 Results

The peak area of iron, manganese, strontium and yttrium relative to calcium was calculated for the bone specimens mentioned in the previous chapters, namely T. rex, hadrosaur, swan, and turtle (extant and fossil) bones, using the equation

\[ \text{Rel amount} = \frac{\text{Area}_{\text{element}}}{\text{Area}_{\text{calcium}}} \] (51)

where \( \text{Area}_{\text{element}} \) refers to integrated counts under the peak of the element being calculated (iron, manganese, strontium, or yttrium), and \( \text{Area}_{\text{calcium}} \) is the integrated counts under the peak corresponding to calcium. These areas for turtle shells and
dinosaur bones are listed on table 7. They were calculated based on a selected area of the map containing only bone tissue. It is important to notice, however, that what is being computed here is not the relative concentration, but the relative area of the peaks. This distinction is necessary because the concentration for each element was not obtained and the integrations under the peaks identified in each spectrum are only proportional to the actual element concentration and do not give the real concentrations. The values used for the calculations include the Ca K-alpha and K-beta peaks (3,691.68 eV and 4,012.7 eV) for the area of calcium, Fe K-beta (7,057.98 eV) for iron and K-alpha peaks for the other elements (5,898.75 eV for manganese, 14.165 eV for strontium and 14,958.4 eV for yttrium). The Fe K-alpha peak was not used because it is located at the same energy as the Mn K-beta peak. Therefore, since K-alpha and K-beta peak intensities are proportional, there is no loss of information by selecting one or the other. In this way, the results obtained for the area of iron are independent of the area of manganese present in the sample.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Iron</th>
<th>Manganese</th>
<th>Strontium</th>
<th>Yttrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swan femur</td>
<td>0.112043</td>
<td>0.0392676</td>
<td>1.62247</td>
<td>-</td>
</tr>
<tr>
<td>Extant turtle</td>
<td>0.00350424</td>
<td>0.00228511</td>
<td>0.105049</td>
<td>-</td>
</tr>
<tr>
<td>Hadrosaur tendon</td>
<td>0.339417</td>
<td>0.151169</td>
<td>0.667739</td>
<td>1.56149</td>
</tr>
<tr>
<td><em>T. rex</em> rib (fig. 6)</td>
<td>0.0779487</td>
<td>0.83652</td>
<td>0.530047</td>
<td>0.221752</td>
</tr>
<tr>
<td><em>T. rex</em> rib (fig. 7)</td>
<td>0.412866</td>
<td>2.61926</td>
<td>0.381296</td>
<td>0.224441</td>
</tr>
<tr>
<td><em>T. rex</em> vertebra</td>
<td>0.109736</td>
<td>0.26276</td>
<td>0.457367</td>
<td>0.37145</td>
</tr>
<tr>
<td>Turtle DPP</td>
<td>0.344965</td>
<td>0.579493</td>
<td>1.03543</td>
<td>0.365736</td>
</tr>
<tr>
<td>Turtle FF</td>
<td>0.093124</td>
<td>0.0407651</td>
<td>1.18513</td>
<td>1.99849</td>
</tr>
<tr>
<td>Turtle T1</td>
<td>0.210233</td>
<td>0.0718282</td>
<td>1.22575</td>
<td>1.57938</td>
</tr>
<tr>
<td>Turtle RF RSKM_P3314.5</td>
<td>1.36944</td>
<td>0.256526</td>
<td>0.798125</td>
<td>1.24094</td>
</tr>
</tbody>
</table>

Table 7: Relative areas of Fe, Mn, Sr and Y relative to Ca for dinosaur bones and turtle shells used in the research presented in previous chapters.

The distribution of the ratio between iron, manganese, strontium, and yttrium in relation to calcium are shown in figs. 63-66. For these plots, only four specimens (*T. rex* rib (fig. 6), *T. rex* rib (fig. 7), DPP turtle and RF RSKM_P3314.5 turtle) were chosen as representative of the other samples.
Figure 63: Distribution of the ratio iron/calcium for a) *T. rex* rib RSKM_P2523.8 (fig. 6), b) *T. rex* rib RSKM_P2523.8 (fig. 7), c) DPP turtle (uncatalogued) and d) RF turtle RSKM_P3314.5. The numbers on the axes correspond to the number of steps in each direction. (Original in colour)
Figure 64: Distribution of the ratio manganese/calcium for a) *T. rex* rib RSKM_P2523.8 (fig. 6), b) *T. rex* rib RSKM_P2523.8 (fig. 7), c) DPP turtle (uncatalogued) and d) RF turtle RSKM_P3314.5. The numbers on the axes correspond to the number of steps in each direction. (Original in colour)
Figure 65: Distribution of the ratio strontium/calcium for a) *T. rex* rib RSKM.P2523.8 (fig. 6), b) *T. rex* rib RSKM.P2523.8 (fig. 7), c) DPP turtle (uncatalogued) and d) RF turtle RSKM.P3314.5. The numbers on the axes correspond to the number of steps in each direction. (Original in colour)
Figure 66: Distribution of the ratio yttrium/calcium for a) *T. rex* rib RSKM_P2523.8 (fig. 6), b) *T. rex* rib RSKM_P2523.8 (fig. 7), c) DPP turtle (uncatalogued) and d) RF turtle RSKM_P3314.5. The numbers on the axes correspond to the number of steps in each direction. (Original in colour)

Fig. 67 and 68 show the correlation plots for calcium and iron for the dinosaur (*T. rex* and hadrosaur) and turtle bones studied in chapters 4 and 6, respectively. The correlation plots for calcium and manganese for the *T. rex* and hadrosaur, and the turtle bones can be seen in fig. 69 and 70, respectively.
Figure 67: Scatter plots relating calcium and iron for dinosaur bones. The quantities are represented in arbitrary units, corresponding to the number of counts under the peak associated to a given element, corrected for variations in the electron beam current which affects the X-ray beam intensity at the synchrotron facility.

Figure 68: Scatter plots relating calcium and iron for turtle bones. The quantities are represented in arbitrary units, corresponding to the number of counts under the peak associated to a given element, corrected for variations in the electron beam current which affects the X-ray beam intensity at the synchrotron facility.
Figure 69: Scatter plots relating calcium and manganese for dinosaur bones. The quantities are represented in arbitrary units, corresponding to the number of counts under the peak associated to a given element, corrected for variations in the electron beam current which affects the X-ray beam intensity at the synchrotron facility.

Figure 70: Scatter plots relating calcium and iron for dinosaur bones. The quantities are represented in arbitrary units, corresponding to the number of counts under the peak associated to a given element, corrected for variations in the electron beam current which affects the X-ray beam intensity at the synchrotron facility.
8.4 Discussion

8.4.1 Relative concentrations

The technique used to map the elemental distribution in the different types of fossils, XRF, as used here, cannot easily generate quantitative results for the concentrations of different elements. This is due to the use of a polychromatic beam with an unknown spectrum and, also, due to geometric aspects of the samples used. Geometric aspects can include, for example, the fact that bones have cavities and other irregularities on their surface, so that the angle between the incident synchrotron radiation beam and sample is not the same across the mapped regions. If the angle changes, so does the beam spot size in the sample, which changes the area being probed as well as the beam intensity per unit area. This variation makes the final image blurry and causes a false variation in the measured concentration, respectively. The cavities in the bone can also change the beam focus, by changing the distance between sample and beam source. This effect is similar to what is observed when using an optical microscope to examine a sample with an irregular surface: the focus setting that works well for some parts of the sample causes blurry images for others, and needs to be adjusted according to what is being visualized. Also to be considered is the fact that differences in composition of the bones, after fossilization, may change the depth of penetration of the incident radiation. However, some of these factors cancel out when computing relative areas. This is because the same effects are present for all the different chemical elements identified in each measured point on the sample.

The results in table 7 show a very large variation in the relative areas of the elements even when the same animal is considered, as, for example, in the case when comparing the T. rex vertebra and rib results. This is probably due to the high influence of very local environmental conditions and the specific characteristics of each bone. The local environmental conditions could include factors such as the
animal being only partially under water or buried, which could alter completely the
early taphonomic changes the fossil undergoes, even within the same piece of bone.
The changes can also be very different if a bone is broken or cracked, or between a
more dense or porous tissue. With that in mind, it becomes very difficult to interpret
such results without the knowledge of the full history of what has occurred to the
bones since the animal’s death. The large differences seen in the table above could
be explained by many different phenomena that cannot be accurately or completely
tested for (such as the ones just mentioned). Thus, the differences seen in the numbers
cannot be explained with certainty.

Some characteristics seem to be consistent between the specimens such as the
higher areas of yttrium and strontium in the turtle shell fossils when compared to
the *T. rex* bones. This is true even for the Frenchman Formation turtle, a specimen
coming from the same formation as the *T. rex* skeleton. This is indicative that the
turtle shell specimens were more readily altered than the *T. rex* bones, which could
be an effect of the presence of a large microbial population protecting the tissues of
the *T. rex* from alterations, as proposed in chapter 3. Also, the areas of iron and
manganese in the bones of most specimens are lower than those of strontium and
yttrium, probably due to association of the latter with the structure of bone apatite.
Both strontium and yttrium seem to be concentrated in the lamellae and trabeculae
(solid regions of bone) within the samples, selected for these calculations, distributed
differently from manganese and iron (usually present in higher quantities within the
osteon canals).

Although the quantitative differences between specimens is obvious (table 7), the
ratios between the areas of these elements to calcium follow a very similar qualitative
distribution in all specimens (see fig. 63 for iron). These figures show a very similar
pattern in the ratio distribution, yielding smaller ratios in the bone regions and higher
ratios inside osteons and in the sediment. The same type of pattern across different
samples can also be observed for the other elements: manganese (fig. 64), strontium (fig. 65) and yttrium (fig. 66).

While the quantitative XRF analysis of fossil samples may prove not to be accurate in the general studies of differences between species, formations and eras, the qualitative distributions provide patterns that are easier to understand and can be used to explain general taphonomic alterations. They also make it easier to identify important effects that can cause changes in such patterns. However, the quantitative analysis can be an useful tool to study the history of a particular fossil such as analyzing the differences in preservation between different parts of the same animal or, even, the same bone. These results could lead to better taphonomic models and a better understanding of specific diagenetic changes and their relationship with the environment, consisting in an interesting future research project.

8.4.2 Correlation plots

The values obtained for the area of each element can also be used to study the correlation between different materials. In this case, the values used are also not concentration values, but quantities related to them. Therefore, only distribution patterns and not their absolute values are to be considered. In the correlation plots presented in this section, the quantities are displayed in arbitrary units (a. u.), representing counts under a given chemical element peak corrected for beam current intensity used for the experiments at the synchrotron facility.

The plots for the turtle bones (fig. 68) show a very similar distribution between the modern turtle and the fossils for all ages and locations: they all have a positive correlation\textsuperscript{10} between calcium and iron, although this correlation is more

\textsuperscript{10}A positive correlation means that when the amount of one of the quantities increase, the other also increases. A negative correlation means that when the amount of one of the quantities increase, the other decreases.
pronounced in the modern specimen. The fossil turtles show more scattered points with higher areas of iron, which are probably due to the presence of iron within the osteons and, to a lesser extent, to diagenetic alterations in the bone. The dinosaur case is very different, though, as the studied maps include regions corresponding to bone tissue and surrounding sediment. Thus, two correlation lines are visible in this samples: one corresponding to the sediment composition (lower areas of calcium); and the other corresponding to the bone region (higher areas of calcium). In these specimens, calcium and iron are also correlated in the bones, but present a negative correlation unlike that observed for turtles. This could be due to differences in the initial composition between the bones of the different species, but this is unlikely. A more likely explanation is that the larger size bones from the *T. rex* and hadrosaur can lead to a different taphonomic history relative to turtle bones. Some of the dinosaur bones can have larger osteon canals than those found in turtle bones and can be more prone to infiltrations from the surrounding environment, favouring alterations in the bone composition. Being larger, the *T. rex* and hadrosaur specimens would also contain more organic matter, and the effects of its decay on bone preservation can lead to different results from those encountered in turtle bones. The extant swan femur does not show any significant correlation between calcium and iron.

In fig. 70, again, the turtle specimens present a positive correlation between calcium and manganese. This is also observed for the extant turtle specimen. Some of the turtles, DPP and RF RSKM_P3314.5 turtles in particular, show a more scattered distribution towards higher values of manganese when compared to the other turtle specimens. This results from taphonomic alterations and manganese infiltrations in the bone osteons. The plots of the dinosaur bones, on the other hand, display considerably different patterns. The manganese content in the hadrosaur sample is approximately constant in the sediment region and gives higher values in the bone
area in a way that is not directly correlated to the area of calcium. In the T. rex samples, again, two correlated distributions can be seen, one for the sediment region and another for the bone region. However, the higher areas of manganese appear to be associated with moderate values of calcium, which is a result of the manganese layer found in the transition zone between bone and sediment as described in chapter 4 (section 4.5). Again, in this case, the swan femur does not show any correlation between calcium and manganese.

Although the images are not depicted here, calcium and strontium and calcium and yttrium appear positively correlated for all specimens of turtle and dinosaur bones, except for the swan femur. Since these two elements seem to substitute calcium in the bone apatite, it makes sense that where apatite is present in larger quantities (i.e., more calcium is present), the areas of strontium and yttrium would also increase.

8.5 Conclusions

Although these plots confirm some of the observations made in the previous chapters for specific specimens (see, for example, sections 4.4 and 6.3), a general pattern for different animals, different formations, or different ages of fossils has not been observed. This lack of an over-arching pattern seems to suggest that local effects and characteristics of environment and specimens such as bone size exert a stronger control over the final results of taphonomic alterations than the general aspects, such as the type of animal and the stratigraphic formation of burial and preservation.
9 Conclusions

9.1 Summary of the findings

The work presented in this thesis represents one more step in a broader use of synchrotron radiation and other multidisciplinary techniques in palaeontology. Although these techniques have been used in the past, they have only recently become more common, and there are still limited results obtained from synchrotron radiation applications for many taxa or fossil types, such as turtles or amber inclusions. However, the work developed here has shown that these techniques can be very powerful to obtain chemical data that can help identify taphonomic changes and preservation mechanisms. More than the specific results from each group of specimens, this research has shown the potential of multidisciplinary studies of fossils to resolve previous issues in palaeontology, and to extract new information from specimens.

Some of these analyses showed a manganese layer covering *T. rex* bones, with the conclusion that this layer is likely related to deposition within a fluvial environment. They have also showed that these bones have undergone extensive chemical changes during diagenesis, although significant preservation of bone microstructure can still be observed. Overall, some of the results have indicated potential preservation mechanisms for the dinosaur fossils, such as the presence of calcium carbonates in the hadrosaur skin. This feature could be the result of early mineralization processes that would have been key to the preservation of the specimen involved.

I was also able to non-destructively study elemental distributions in insect inclusions, combining these with data from synchrotron microtomography. This method can provide a better understanding of the different types of preserved tissue without exposing or modifying the insects, and, therefore, damaging the inclusions.
This will provide researchers with one more technique that can prove invaluable when dealing with rare or important specimens.

Finally, I examined the preservation of turtle shells and bones, comparing the results across different types of bone, ages and deposits. This work showed that local environmental conditions may be more influential with respect to the taphonomic alterations experienced by these fossils than large-scale differences in age and geographic location. These results are probably applicable to fossils in general, showing that generalizations are not always possible when discussing taphonomic alterations. Fossils with the same age and from the same geographic location and formation could undergo distinct changes due to more localized conditions, such as humidity or pH, for example.

Furthermore, iron in the Fe(III) oxidation state was found in the insect inclusions and hadrosaur skin. This form of iron has been associated with the exceptional preservation of fossils in the past, including soft tissue [7]. Although the mechanism of production of iron and how it contributes to fossil preservation are still to be fully comprehended, previous research and the results presented in this thesis show it to often be present when fossils are preserved in great detail. However, other factors may have contributed to the preservation of tissues in this study, such as the presence of resin layers protecting insect inclusions, and early mineralization of the hadrosaur skin. Thus, it is not clear if the presence of Fe(III) is one of the prerequisite conditions for the exceptional preservation of fossils, or if it is simply a consequence of when this preservation occurs.

In this work, I showed that synchrotron radiation can be a useful technique to examine the chemical components that could have been involved in fossil preservation. This could help the development of theories explaining the preservation mechanism for different fossils. Also, this research has laid the groundwork for non-destructive analyses on the pigmentation of feathers and insects and structural reinforcement of
insect cuticles in amber inclusions.

The knowledge of fossil chemistry and a better understanding of taphonomic processes and preservation mechanisms, including those caused by localized factors, could possibly lead to methods to obtain information on the original chemistry of remains. Thus, synchrotron radiation can be used to investigate the original content of remains, their taphonomic alterations, and the mechanisms that caused their preservation, helping with the development of a comprehensive picture of fossils and the animals that originated them.

One of the main messages transmitted by the work in this thesis addresses the power of synchrotron radiation techniques, specially XRF and XAS, in the study of fossil chemistry and its implications in preservation and taphonomy. The results obtained from the application of these techniques in this thesis, as well as in the literature, strongly indicate that synchrotron radiation techniques will play an important role in the understanding of taphonomic alterations and fossil preservation in future studies. In this context, my investigation has the intention of guiding future similar studies by providing information on the strengths and pitfalls of using synchrotron radiation in taphonomy research. This thesis explores a new aspect of synchrotron radiation as applied to fossils, and helps researchers to frame new questions while preparing samples to get the data that they seek.

9.2 Future work

The potential shown for the application of synchrotron techniques to study fossils provides remarkable possibilities for future studies. Many fossils in museum collections, and many others to be found, could be studied using these techniques. While some fossils may not provide significant new information, others might provide details on their taphonomic history and preservation. The study of a significant number of fossils from the same formation and geographic location could
also be used to better understand environmental factors associated with their taphonomy, and how these factors affect different specimens variably. Ultimately, detailed chemical data from fossils from several different formations could be used to “fingerprint” a specific location in the world, as has been done in Mongolia [268]. This could be done with the construction of a large database identifying the characteristics of each location (or formation), which then could be compared to the chemical results from an unknown fossil. Not only could this be a tool for identifying specimens in museums of unknown origin, it could also be used to prevent poachers from selling fossils from location where this practice is illegal. An algorithm, such as likelihood function, could be developed to match the chemical signature of a fossil to that of a certain location listed in the database.

For the work presented in this thesis, the next logical steps on the research into *T. rex* bones would likely involve measurements of chemical standards and other sediment pieces, to identify the state of the elements measured using XAFS. It would also be interesting to map bones from other parts of the body of the animal to investigate if manganese coating is also present elsewhere (or in the whole specimen). Assuming that manganese was deposited due to the fluvial environment surrounding the bones, measuring this layer in different parts of the dinosaur body could tell if the whole animal was submerged or only some parts of it were.

For the insect inclusions, the next steps would include measurements of other insect types in Baltic amber to explore if the observed preservation of soft tissues is peculiar to certain taxon. The chemical mapping could also be used to identify elements that characterize specific structures in insects. Together with a three-dimensional imaging technique, this could be used to study the preservation of determined structures in the insects. These techniques could also be used for dedicated studies of amber types could be performed to verify how important specific resin properties are in specimen preservation. Another possible application
could be examining vertebrate amber inclusions that do not present an exposed surface, applying the same strategies used here for insects to bones and soft tissues.

In the case of the turtle shells, the measurement of the composition of the sedimentary matrix could be used to possibly explain the origin of the differences in the chemistry of fossils from the same formation. Variations in the surrounding environment could have led to different availability of minerals during their mineralization and diagenesis. The study of the sediments could also provide information on environment at the time the remains were deposited (i.e., fluvial, marine, or terrestrial, among others). This could also lead to explanations of those differences related to depositional environments and a better understanding of the fossils’ taphonomy.

For the hadrosaur skin, one of the next steps would be to accurately identify all the chemical states of the elements detected in the sample that could not be identified in this research. This would require the measurement of standards and comparative samples. It would also be very useful to develop micro-tomography images of the skin area and associate it with the chemical map, as was done for the insect inclusions. With these results, I would be able to better determine what was preserved, and maybe identify the structures from which the preserved carbon originated. To help with this, other techniques could also be used, such as mass spectroscopy.

Although this work has shown that the generalization of taphonomic alterations is not always easy (or even possible) even for fossil from the same formation and geographic area, a deeper understanding on how local characteristics of the deposits alter fossil chemistry could be obtained. This could be used to better understand the history of the remains and perhaps allow for more information on their original chemistry. One of the possible applications of this knowledge would be the investigation of animals’ diet habits using strontium/calcium ratios, for example.
References


[137] Duller, G. et al. A High Precision, High Stability, Four Bounce Monochromator for Diamond Beamline I20. Available at:


[245] Barbi, M., Bell, P. R., Fanti, F., Dynes, J. J., Buttigieg, J., Kolaceke, A., Currie, P. J. Epidermal cell layers in exceptionally preserved hadrosaur (Dinosauria: Ornithischia) skin. Unpublished manuscript.


A Electron focusing magnetic devices used in synchrotron light sources

The following derivations, definitions and discussions can be found on [119].

The deflection angle, $\alpha$, in optics, is given by:

$$\alpha = -\frac{r}{f}$$

(52)

where $r$ is the distance between the light ray incidence point and the centre of the lens and $f$ is the focal length, as shown in figure 1. Similarly, one can write the equation for an azimuthal magnetic field acting on charged particles:

$$\alpha = -\frac{\ell}{\rho} = -\frac{e}{\beta E} B_\phi \ell = \frac{e}{\beta E} g r \ell$$

(53)

where $\rho$ is the bending radius of the trajectory, $\ell$ is the path length of the particle’s trajectory in the azimuthal magnetic field $B_\phi$, $g = dB_\phi/dr$ or $B_\phi = gr$ is the magnetic field gradient, $e$ is the charge of the electron, $\beta = v/c$ is the electron velocity and $E$ is the electric field. This calculation assumes the length $\ell$ to be short when compared to the focal length, so that changes in $r$ within the magnetic field are negligible.

For this magnetic lens to behave as desired, a linear dependence in $r$ is necessary, which can come either from $B_\phi$ or $\ell$, while the other remains constant. In general, the choice is the magnetic field, which, then, has to vary linearly with the distance $r$. The most common choice of device which respects this property, while, at the same time, providing an aperture in which the particles can pass through, is the quadrupole magnet. The scalar potential for it, in cartesian coordinates, is given by

$$V = -g xy$$

(54)

which can be used to determine the magnetic fields.
\[ B_x = -\frac{\partial V}{\partial x} = gy \quad (55) \]

\[ B_y = -\frac{\partial V}{\partial y} = gx \quad (56) \]

These fields exhibit the desired property of varying linearly with the distance between the particle and the optical axis. Figure 71 shows a schematic representation of the field pattern present in a quadrupole magnet.

\[ k = \frac{ec}{\beta E} g \quad (57) \]

and the focal length of the quadrupole magnet is given by

\[ f^{-1} = k\ell \quad (58) \]

Maxwell’s equations imply that quadrupole magnets can only focus the beam in one plane, while defocus it on the other. In order to use these devices in synchrotron sources, a combination of quadrupoles is necessary. In this case, the total focal length
$f$ resulting from the combination of two lenses with two different focal lengths $f_1$ and $f_2$ is

\[ \frac{1}{f} = \frac{1}{f_1} + \frac{1}{f_2} + \frac{d}{f_1 f_2} \]  

(59)

where $d$ is the distance between the two lenses.
B Devices used to generate synchrotron light

B.1 Bending Magnets

The known circular trajectory electrons describe in synchrotron accelerators is achieved with the use of magnets. If not for their action, the electrons would, according to inertia laws, move in a straight line. The Lorentz force resulting of the interaction between magnets and charged particles in the accelerator acts as a centripetal force causing a circular trajectory (which connects stretches of straight trajectories to generate the trajectory observed in accelerators) according to the equation

\[ m\gamma v^2 \kappa + e [v \times B] = 0 \]  \hspace{1cm} (60)

where \( \kappa = (\kappa_x, \kappa_y, 0) \) is the local curvature vector in the trajectory:

\[ \kappa_{x,y} = \frac{1}{\rho_{x,y}} \]  \hspace{1cm} (61)

with \( \rho_{x,y} \) is the local radius of the trajectory [119].

In order to simplify the calculations for the particle beam dynamics, it is considered that only the transverse component of the magnetic field (the component normal to the velocity of the particles) acts on the particles. Considering that the velocity components normal to the trajectory of the beam are negligible compared to the total particle velocity, using equation 60, the bending radius for the electron trajectory is given by

\[ \frac{1}{\rho} = \left| \frac{e}{p} B \right| = \left| \frac{e c}{\beta E} B \right| \]  \hspace{1cm} (62)

where \( p = \gamma mv \) and the so-called cyclotron or Larmor frequency, which represents the frequency of revolution of a particle in its orbit, is [119]
\[ \omega_L = \left| \frac{e c^2}{E B} \right|. \tag{63} \]

When going through the bending magnets the two effects discussed previously happen: the electron beam is bent into a curved trajectory and radiation is emitted as the charged particles travel in a curved path [116].

Since radiation is emitted during the whole curved trajectory the beam goes through, the collimation around narrow angle is lost in the horizontal direction, but maintained in the direction perpendicular to the particle beam plane. A slit is used in experiments to select the size of the horizontal radiation beam desired. The radiation is, then, collected and summed incoherently in the collection angle given by

\[ \Delta \theta = \frac{w}{D} \gg \psi \tag{64} \]

where \( w \) is the width of the slit used, \( D \) is the distance from the electron orbit and the slit and \( \psi \) is the vertical angle in which the radiation is distributed [116].

The power radiated in the synchrotron when relativistic electrons travel at a circular orbit is given by the Schwinger’s formula:

\[ P_e = \iint P(\lambda, \psi) d\lambda d\psi = \frac{2}{3} \frac{e^2 c}{R^2} \left[ \frac{E}{mc^2} \right]^4 \tag{65} \]

where \( \lambda \) is the wavelength of the radiation emitted and \( R \) is the curvature radius in the particles’ trajectory. This equation shows that \( P_e \propto E^4/R^2 \), which means that, if one wants to increase the energy in the storage rings, while maintaining the necessary radiated power, one must also increase the radius of the ring. This equation also shows that \( P_e \propto m^{-4} \) and, therefore, electrons, due to their lower mass, will produce more power radiated than more massive particles, such as protons, and that is the reason most synchrotron facilities choose electrons to compose their particle beams.
The radiation emitted by using a bending magnet has a broad spectrum, going from x-rays to infrared, for which the describing function is called universal synchrotron radiation function ($G_1$). The synchrotron radiation spectral distribution can be characterized by the critical wavelength $\lambda_c$, which is given by

$$\lambda_c = \frac{4}{3} \pi R \gamma^{-3}$$

(66)

where $\gamma = \psi^{-1}$. The critical wavelength determines the point in the radiation spectrum that divides the radiation power emitted in half, i.e. 50% of the power is emitted in wavelengths below the critical value, while the other 50% is emitted in wavelengths above it [116].

This wide and continuous distribution spectrum can be explained by statistical fluctuations on the energy of the electrons in the beam and around their orbit. They are also influenced by the statistical nature of the light emission itself [116].

The number of photons with wavelength band of width $\Delta\lambda/\lambda$, centered at $\lambda$, and emitted by each electron moving in an arc with length $R\Delta \theta$ is given by

$$N(\lambda) = \frac{3^{1/2}}{2\pi} \frac{e^2}{hR} \gamma G_1 \frac{\Delta\lambda}{\lambda} \Delta \theta$$

(67)

where $h$ is Planck’s constant and $G_1$ is the universal synchrotron radiation function. Thus, the total number of photons emitted by a storage ring is given by equation 67 multiplied by the number of electrons traveling in the ring, which is:

$$N_e = \frac{I (2\pi R)}{e c}$$

(68)

where $I$ is the beam current. Then,

$$N_{total}(\lambda) = \frac{3^{1/2}}{hc} e \gamma I G_1 \frac{\Delta\lambda}{\lambda} \Delta \theta$$

(69)
This equation describes the spectral distribution of the universal synchrotron radiation function, where it is noticeable that the distribution falls exponentially when \( \lambda \ll \lambda_c \) and falls slowly for \( \lambda \gg \lambda_c \), being, in this last case, mostly determined by the electron beam current [116].

Another important parameter used to characterize a synchrotron source is the critical energy, which is given by

\[
\epsilon_c = \frac{3hc\gamma^3}{4\pi R} \tag{70}
\]

The radiation energy achievable by a synchrotron source is typically a few times the critical energy [116].

The radiation emitted using bending magnets is mostly linearly polarized. The polarization direction varies with respect to the angle in relation to the beam plane. On the horizontal plane, the radiation emitted is completely polarized on the horizontal direction (parallel to the beam plane). As one moves above or below such plane, a perpendicular component appears [116].

### B.2 Wigglers

Bending magnets must always be present in synchrotron rings, since they are responsible for generating its circular trajectory. However, after the first generation sources, insertion devices started being used to generate radiation. These devices do not interfere with the overall circular trajectory of the beam and, therefore, can be inserted in the ring along its straight pieces. They generate radiation by creating small oscillations in the electron beam, using alternating magnets, maintaining, however, the beam’s direction of movement along the ring. These devices not only increase the amount of beamlines allowed in the facility, since they can be inserted anywhere along the trajectory, but also allow for higher radiation energies (due to
the possibility of smaller angles of curvature); for higher radiation intensities, by increasing the amount of oscillations (and, thus, magnetic poles present in the device) the beam goes through; and for the increase in spectral brightness [116].

Wigglers were the first insertion devices developed to be used in synchrotron sources, since their second generation. K. W. Robinson was the first to theorize the use of such a device to generate radiation in synchrotron facilities in an unpublished report in 1956 [130]. The first wiggler was built a decade later, in 1966, at the Cambridge Electron Accelerator (CEA-14) [131] as a damping system for their beam storage system. In 1979, the first wiggler was used in a synchrotron source at the Stanford Synchrotron Radiation Lightsource (SSRL) and its success prompted other synchrotron across the world to develop similar devices [130].

Wigglers are built as a set of magnets with alternating poles, which cause the electrons to wiggle around a straight line. The magnetic field applied is usually perpendicular to the beam plane, which results in the horizontal oscillation of the electrons. Since these magnets are not being used to maintain the electron beam in their orbit, they can be tuned to obtain a smaller radius of curvature when compared to bending magnets, which results in the emission of radiation with higher energy [116].

In commonly used units, the instantaneous radius of curvature of a charged particle under the effect of magnetic field is

\[ R(m) = \frac{3.34E[GeV]}{B[T]} \]  

(71)

where \( E \) is the electron energy given in GeV and \( B \) is the intensity of the magnetic field given in teslas. Using equation 70, also substituting the constants by their values, and the previous result, the critical energy for a wiggler is given by

\[ \epsilon_c(keV) = 0.665E^2[GeV]B[T]. \]  

(72)
This result shows that the critical energy, and therefore the maximum photon energy that can be reached at the storage ring, can be tuned according to the intensity of the magnetic field chosen, independently of the energy of the electrons in the beam [116].

The amount of magnetic poles, and, consequently, electron oscillations, present in the wiggler will determine the gain in intensity compared to a bending magnet. However, the construction of a wiggler must be such that the beam trajectory is not changed once it goes through the device. For this to be possible, the first and last magnets must have their effects mirrored and the magnetic field of each must be half of that present in the intermediary magnets so the electrons will only complete half of an oscillation [130].

One can see a wiggler device as a series of bending magnets, and, therefore, they both produce radiation with the same spectral characteristics of a broad wavelength bandwidth. However, since the radiation in the wiggler is produced by multiple beam oscillations, it appears as a series of short pulses, instead of the single pulse produced by a bending magnet. That is the cause for the device’s increase in radiation intensity when compared to bending magnets [116].

In order to mathematically differentiate wigglers and undulators, one can use the dimensionless parameter $K$, which is defined as “the ratio between the wiggling angle of the trajectory, $\alpha$ and the natural aperture of synchrotron radiation $1/\gamma$” [116]. Both variables are defined in figure 72, which shows the electron oscillations in a wiggler as well as the path described by the emitted radiation. The parameter $K$ is, then, written as

$$K = \alpha \gamma$$  \hspace{1cm} (73)

which means that an electron moving in a sinusoidal trajectory will have $K$ given by
\[ K = \frac{e}{2\pi mc} \lambda_u B = 0.934\lambda_u [cm]B[T] \]  

(74)

where \( \lambda_u \) is the period of the device.

In wigglers, the angle of trajectory is much larger than the opening angle \( \psi = 1/\gamma \), which means that \( K \gg 1 \). In this case, the effects caused by interference between radiation emitted in different poles can be neglected and the intensity of the radiation produced can be obtained by integrating over the contributions of each oscillation [116].

**B.3 Undulators**

The theoretical idea of undulators was first proposed by V. L. Ginzburg, in 1947 [133] and they were first built by Hans Motz and coworkers in 1953 [134]. Soviet scientists installed undulators in two synchrotrons in the 1970s for tests and gathered most of the initial information on the properties of synchrotron light produced by these devices [130].

Undulators have similar technology to that of wigglers, with the difference that the magnetic field generated by each pole is designed such that the angle of the trajectory \( \alpha \) is smaller than, or close to, the opening angle \( \psi \), in a way that the emission cone remains within the device. In consequence, instead of the series of short pulses of
radiation seen with the use of wigglers, undulators produce a single long pulse. Also, for undulators, $K < 1$ [116].

Since the emission angle is small in undulators, the interference between radiation produced in different oscillations must be taken into account and the wavelength for which the interference is constructive is given by the following equation when the radiation is observed at an angle $\theta$ in relation to the undulator axis:

$$\lambda = \frac{\lambda_u}{2\gamma^2} \left(1 + \frac{K^2}{2} + \gamma^2\theta^2 \right).$$

(75)

As consequence of the interference, the spectrum of the radiation produced has a very narrow wavelength bandwidth, centered on the peak photon energy $h\nu_c$, which is given by (derivation can be found on [116]):

$$h\nu_c \approx \frac{2\gamma^2hnc}{L_u}$$

(76)

where $L_u$ is the period of the undulator, $\gamma$ is obtained with the use of Lorentz contraction and Doppler effect arguments and can be written as

$$\left(\frac{c + v}{c - v}\right)^{1/2} = \left(\frac{(c + v)^2}{c^2 - v^2}\right)^{1/2} = \left(1 + \frac{v}{c}\right) \left[\frac{1}{1 - \frac{v^2}{c^2}}\right]^{1/2} \approx 2\gamma$$

(77)

and $n$ is an integer that corresponds to the n-th harmonic of the wavelength that suffer constructive interference $\lambda_n = \frac{\lambda}{n}$ [116]. The amount and intensity of the harmonics increase with $K$, while when $\theta = 0$, i.e. on the undulator axis, only odd harmonics can be produced [116].

Since $K \propto B$ (equation 74), the wavelength $\lambda \propto B^2$. Therefore, one can tune undulators to produce the desired wavelength by changing their magnetic field. And while in wigglers the radiation emitted in each period adds up, increasing with $2N$, where $N$ is the number of periods, in undulators the radiation adds coherently, increasing with $N^2$ [116].
The small emission angle together with the increase in intensity with $N^2$ mean that the brightness reached with undulators is much higher than what can be achieved with any other devices [116]. The choice of the method to produce radiation, however, depends on the experiment intended: if brightness is the most important characteristic, undulators are the best choice, but if a broad spectrum is necessary, wiggles should be used.


C  X-ray interactions with matter and optics

Interactions between X-rays and matter are particularly interesting in the study of materials due to the characteristics of this type of electromagnetic radiation, since it has wavelengths in the same order of magnitude as the distance between atoms in solids and liquids and it has a relatively large penetration depth in samples. Different kinds of interactions include absorption, scattering or diffraction, refraction and emission and they will be explained in more detail in the next sections [123].

C.1 Scattering

Classically, the scattering of an X-ray by a single electron is described as the interaction between the incident wave and the electron, which suffers the action of electromagnetic force causing its acceleration and consequent emission of a scattered wave with the same wavelength as the incident one. Therefore, classically, this process is always elastic. Considering quantum aspects, the scattering may be inelastic, since the photon can transfer energy to the electron, resulting on a scattered photon with energy smaller than the incident one. This phenomenon is known as Compton effect. Considering the elastic case and the fact that momentum can be transferred to the electron, in this case, as well, one can define

\[
\hbar Q = \hbar k - \hbar k'
\]

(78)

where \(\hbar k\) and \(\hbar k'\) represent the initial and final momenta for the photon, respectively, and the vector \(Q\) is the wavevector transfer or scattering vector, which is commonly used to describe scattering processes [135].

According to classical principles, in the simplest scattering case, the interaction of an X-ray with a single electron would make it vibrate, making it act like a dipole
antenna, emitting radiation. The radiated field at a point \(X\) at a distance \(R\) from the electron and at angle \(\psi\) with respect to the direction of the incident light can be derived using Maxwell’s equation (see [135] for derivation):

\[
E_{\text{rad}}(R,t) = -\frac{-e}{4\pi \epsilon_0 c^2 R} a_X(t')
\]

(79)

where \(-e\) is the charge of an electron, \(c\) is the speed of light, \(\epsilon_0\) is the vacuum permittivity and \(a_X(t')\) is the acceleration seen by the observer at \(X\), measured at a time \(t'\) before the observation time \(t\), given by \(t' = t - R/c\) [135].

The acceleration \(a_X(t')\) is measured from the observer, which means that if \(\psi = \pi/2\), \(a_X(t') = 0\), while it reaches its maximum value when \(\psi = 0\). This behaviour is explicit in the equation for the acceleration:

\[
a_X(t') = -\frac{e}{m} E_{\text{in}} e^{-i\omega t'} \cos \psi = -\frac{e}{m} E_{\text{in}} e^{i\omega(t')/c} \cos \psi
\]

(80)

where \(E_{\text{in}} = E_x e^{-i\omega t'}\) is the electric field of the incident X-ray. Using this result, equation 79 can be rewritten as

\[
E_{\text{rad}}(R,t) = -\frac{-e}{4\pi \epsilon_0 c^2 R} \frac{-e}{m} E_{\text{in}} e^{i\omega(t')/c} \cos \psi
\]

(81)

and the ratio between radiation emitted and incident is given by:

\[
\frac{E_{\text{rad}}(R,t)}{E_{\text{in}}} = -\left(\frac{e^2}{4\pi \epsilon_0 mc^2}\right) \frac{e^{ikR}/R}{} \cos \psi
\]

(82)

where \(k = \omega/c\), \(e^{ikR}/R\) represents a spherical wave and the term in brackets that precedes it is called Thomson scattering length (or classical electron radius):

\[
r_0 = \left(\frac{e^2}{4\pi \epsilon_0 mc^2}\right) = 2.82 \times 10^{-4}\text{nm}
\]

(83)

This scattering length determines the ability an electron has to scatter an incident
A wave [135].

An important result of equation 82 is related to the negative symbol, which means that the two waves, incident and radiated, have a phase difference of 180° or, in radians, $\pi$ [135].

This classical description of the interaction between the X-ray and the electrons is called Rayleigh-scattering. For incident radiation with electric field randomly distributed in direction:

$$I_{\text{scat}} = I_0 \frac{1}{r^2} \left( \frac{e^2}{m_0 c^2} \right)^2 \left( 1 + \cos^2 \theta \right)$$

(84)

where $I_{\text{scat}}$ is the intensity of the scattered radiation, $I_0$ is the intensity of the incident light, $r$ is the distance to the observation position, $e$ is the charge of the electron, $m_0$ is the mass of the electron and $\theta$ is the scatter angle. According to this equation, then, the energy of the scattered light is not different from the energy of the incident light, however its intensity depends on the scatter angle [123].

If the X-ray is interacting with an atom instead, the binding energy between the electron and the atom must be considered. If the incident energy is approximately the same as the binding energy of the electron to the atom, then one would expect that the scattering length to decrease considerably. The length increases as the incident beam energy increases or as the binding energy decreases (i.e., if the electron is farther from the nucleus). For beam energies much higher than the binding energy, the electrons can be treated as free [135].

In the case of a single molecule, the scattering length is much smaller than for an atom and measurements cannot be taken using any X-ray source available today. In order to measure molecules, they must be organized into crystals to amplify the signal. In this case, the scattering only happens in determined angles, according to Bragg’s law:

219
\[ m\lambda = 2d\sin \theta \]  \hspace{1cm} (85)

where \( m \) is an integer, \( d \) is the distance between planes in the crystal, and \( \theta \) is the incidence angle. When on the Bragg angle, the light interferes constructively and the signal gets stronger than for a single molecule. For all other angles the interference is destructive and a signal cannot be measured [135].

The previous discussion is based on elastic scattering of light by electrons, which, as already mentioned, is the classical description of the interaction. However, quantum mechanics allow for inelastic collisions. For these conditions one must assume the incoming X-ray is composed of a beam of photons. In this case, energy can be transferred from the incoming photon to the electron and, therefore, the scattered photon will have an energy smaller than the initial one [135, 136]. This is the Compton effect and it is mathematically described by the following equation [136]:

\[ \lambda_2 - \lambda_1 = \frac{h}{mc} \left( 1 - \cos \theta \right). \]  \hspace{1cm} (86)

This equation shows that the change in the energy of the scattered radiation depends on the scatter angle, such that it is minimum when \( \theta = 0 \), i.e. there is no collision, and maximum at \( \theta = 180 \), i.e. when the collision is head-on [123].

While both types of interaction happen in a sample, the relative intensity between inelastic and elastic scatterings depends on the composition of the sample. While elastic scattering is mostly independent on the sample matrix, the inelastic portion depends on the atomic number of the elements present in it. The intensity of the inelastic scattering is large for low atomic numbers and decreases as the atomic number increases. This relationship can also be used to estimate the average atomic number in the sample matrix [123].
C.2 Absorption

When a photon interacts with an atom, it can also be absorbed. The photon’s energy is, then, transferred to an electron, which will either move to a higher energy level within the atom or, if the energy is enough, will leave the atom completely, becoming a free electron and leaving the atom ionized. This mechanism is mathematically described by the linear absorption coefficient $\mu$. The intensity of radiation going through a sample is given by

$$-dI = I(z) \rho_a dz \sigma_a = I(z) \mu dz$$  \hspace{1cm} (87)$$

where $\mu dz$ corresponds to the attenuation of the beam through a sample of thickness $dz$ and the intensity is measured at a distance $z$ from the surface of the material [135].

The attenuation coefficient can be written as:

$$\mu = \rho_a \sigma_a = \left( \frac{\rho_m N_A}{A} \right) \sigma_a$$  \hspace{1cm} (88)$$

where $N_A$ is Avogadro’s number, $\rho_m$ is the mass density and $A$ is the atomic number of the element in the sample [135].

The solution of this equation is

$$I(z) = I_0 e^{-\mu z}$$  \hspace{1cm} (89)$$

where $I_0$ is the intensity of the beam at $z = 0$. That means $\mu$ can be obtained experimentally by measuring the intensity of the beam with and without going through a sample [135].

The mass attenuation coefficient for a compound material is given by the average

$$\mu_{\text{compound}} = \sum w_i \mu_i$$  \hspace{1cm} (90)$$
where \( w_i \) is the mass fraction of element \( i \) and \( \mu_i \) is the mass attenuation coefficient for element \( i \) [123].

Analyses using X-ray absorption were the first to be developed, virtually at the same time that X-rays themselves were discovered by Roentgen. The most famous X-ray image, of his hand with a ring, is an example of the use of X-ray absorption to visualize internal structures in people, which is still, probably, the most common application of X-rays. Since people’s bodies are made up of different tissues, with different attenuation coefficients, the amount of radiation able to go through the body depends on the kind of tissue it finds in its way, which generate patterns related to the disposition of tissues in the body [123].

Using the same absorption principle, but taking images from different directions of the sample, one can reconstruct the whole sample in three dimensions, including internal structures. This technique is called X-ray tomography and it is another application of X-ray absorption to characterize materials [123].

A common requirement for all absorption techniques, however, is the choice of radiation energy. The energy should be such that X-rays can pass through the sample chosen, while, at the same time, providing enough contrast between different structures, i.e. it must provide attenuation coefficients different enough for each studied structure [123].

C.3 Refraction

There are two ways that a scattered wave can interact with the incident wave, depending on their phase difference: when there is no phase shift between them, the refractive index \( n > 1 \); and when there is a phase shift of \( \pi \) between them, the refractive index \( n < 1 \). In the case of incident x-rays, the phase difference is \( \pi \) [135], as previously discussed.

Assuming there is no absorption by the material and the interface between a
medium with a homogeneous distribution of scatterers, and vacuum is flat and well
defined, with the scatterers producing a spherical scattered wave with amplitude \( b \),
one can derive the equation that relates the index of refraction \( n \) to the scattering
properties of the sample as

\[
n = 1 - \delta
\]  

(91)

where

\[
\delta = \frac{2\pi \rho r_0}{k^2}
\]  

(92)

with \( \rho \) being the density of electrons, \( r_0 \) being the scattering length per electron and
\( k \) being the wavevector, which is related to the wavelength \( \lambda \) by \( k = \frac{2\pi}{\lambda} \). Typically,
for X-rays, \( \delta \sim 10^{-6} \), which produces a refraction index very close to 1 [135].

Snell’s law relates the angles of incident and refraction of a light ray, according to
the refraction index:

\[
\sin \theta_1 = n \sin \theta_2
\]  

(93)

or

\[
\cos \alpha = n \cos \alpha'
\]  

(94)

where the first form is the most common and uses the incident and refracted angles
in relation to the normal to the surface [136], and the second form uses the angle
in relation to the surface itself [135]. The critical angle \( \alpha_c \) for which the light ray is
completely reflected (total reflection) and does not refract is found by making \( \alpha' = 0 \),
which means, using equations 91 and 92,
and incident light at any angle smaller than $a_c$ will be completely reflected. For X-rays, the typical values for $a_c$ are in the order of milliradians [135].

If absorption processes are also considered as the light goes through the medium, the refraction index becomes a complex value and is written as

$$n = 1 - \delta + i\beta$$  \hspace{1cm} (96)

with

$$\delta = \frac{2\pi\rho f(0)r_0}{k^2}$$  \hspace{1cm} (97)

and

$$\beta = \frac{\mu}{2k}$$  \hspace{1cm} (98)

where $\rho$ is the electron density in the medium, $f(0)$ is the atomic scattering factor evaluated when $Q = 0$ ($Q$ is the scattering vector), $r_0$ is the scattering length per electron, $k$ is the wavevector and $\mu$ is the absorption coefficient [135].

In the case of X-rays, both $\delta$ and $\beta$ are much smaller than one, which limits the pertinent study to small angles. Assuming the incident light wavevector $k_I$ and the transmitted and reflected wavevectors $k_T$ and $k_R$, respectively and the wave amplitudes $a_I$, $a_T$ and $a_R$ following the same notation, one can derive Snell’s law and Fresnel equations by imposition the continuity of the wave and its derivative at the boundary $z = 0$. This means that

$$a_I + a_R = a_T$$  \hspace{1cm} (99)
and

\[ a_I k_I + a_R k_R = a_T k_T \]  \hspace{1cm} (100)

where in vacuum, \(|k_I| = |k_R| = k\) and in the material \(|k_T| = nk\) \cite{135}. Separating the previous equation in components perpendicular and parallel to the surface of the medium:

\[ - (a_I - a_R) k \sin \alpha = -a_T (nk) \sin \alpha' \]  \hspace{1cm} (101)

\[ a_I k \cos \alpha + a_R k \cos \alpha = a_T (nk) \cos \alpha'. \]  \hspace{1cm} (102)

where \(\alpha\) is the incident and reflected angle and \(\alpha'\) is the transmitted angle. Then, combining the results from equations 99 and 102:

\[ \cos \alpha = n \cos \alpha' \]  \hspace{1cm} (103)

which is Snell’s law \cite{135}.

One can also combine the results of equations 99 and 101, resulting in

\[ \frac{a_I - a_R}{a_I + a_R} = \frac{n \sin \alpha'}{\sin \alpha} \approx \frac{\alpha'}{\alpha} \]  \hspace{1cm} (104)

for small angles. From this relation, the Fresnel equations can be obtained:

\[ r \equiv \frac{a_R}{a_I} = \frac{\alpha - \alpha'}{\alpha + \alpha'} \]  \hspace{1cm} (105)

\[ t \equiv \frac{a_T}{a_I} = \frac{2\alpha}{\alpha + \alpha'} \]  \hspace{1cm} (106)

where \(r\) and \(t\) are the amplitude reflectivity and transmittivity, respectively \cite{135}.
C.4 Reflection

The reflected amplitude discussed in the previous section using Fresnel equations is valid when X-rays are shone onto an infinite slab of material, i.e., there is only one interface to be crossed (usually, between vacuum and medium). This result changes when the material is finite, since, in this case, multiple interfaces exist, resulting in infinite possible reflections (figure 73) [135].

![Figure 73: Schematic representation of incident light on a finite slab of material and some of its possible reflections [113].](image)

As can be seen in figure 73, a reflection can happen directly at the first interface between vacuum and material, which is the same being considered on a infinite slab. However, others are possible: the light can be transmitted through the material, reflected on the interface between material and vacuum, transmitted again through the material and pass through the vacuum-material interface again. In this case, the final reflected amplitude must be made of a series of transmitted and reflected amplitudes to describe the light’s path and interactions. It must also contain a phase factor $p^2 = e^{iQ\Delta}$ [135]. Since infinite series of transmissions and reflections are allowed, the total reflectivity amplitude must be given by

$$r_{slab} = r_{01} + t_{01}t_{10}r_{12}p^2 + t_{01}t_{10}r_{10}r_{12}^2p^4 + t_{01}t_{10}r_{10}^2r_{12}^3p^6 \ldots$$

(107)
\[ r_{slab} = r_{01} + t_{01} t_{10} r_{12} p^2 \sum_{m=0}^{\infty} (r_{10} r_{12} p^2)^m \]  

(108)

where the index 01 means interface 0 to 1 and so on \[135\].

Equation 108 is a geometric series, which can be summed to result on

\[ r_{slab} = r_{01} + t_{01} t_{10} r_{12} p^2 \frac{1}{1 - r_{10} r_{12} p^2} \]  

(109)

which can be simplified using equations 105 and 106 resulting on

\[ r_{slab} = \frac{r_{01} + r_{12} p^2}{1 + r_{01} r_{12} p^2} \]  

(110)

where \( p^2 = e^{iQ_1 \Delta} \) and \( Q_1 = 2k_1 \sin \alpha_1 \).

C.5 Emission

The absorption phenomenon discussed in the previous section can also generate the emission of photons. When the photon is absorbed by an electron (considering the energy of X-rays, this electron can even belong to the atom inner shells), this electron will move to a higher energy level or leave the atom. This will leave a hole that must be filled by an electron in a higher energy level, which will emit a photon with the corresponding energy difference. The emitted photon is also, typically, in the X-ray energy range. This process is called X-ray fluorescence and the energy of the emitted photon \( E \) is related to the atomic number \( N \) of the atom according to Moseley’s law

\[ E = C_1 (Z - C_2)^2 \]  

(111)

where \( C_1 \) and \( C_2 \) are constants, which depend on the energy shells involved in the process \[123\].

The intensity of the radiation emitted depends on the intensity of the incident
radiation, on the mass attenuation coefficient for the material and on the probability of the specific transition of the electron in a higher energy level to the lower level. Also, it depends on the probability that an Auger electron is emitted. That happens when instead of the excess energy being released as a photon, it is transferred to another electron of the atom, giving it energy to leave [123]. Fluorescence yield $\omega$ is what the probability of the X-ray emission is also called, and it depends on the atomic number of the atom and on the electron transition involved, and it is mathematically given by

$$\omega = \frac{X_s}{X_s + A_s}$$

(112)

where $X_s$ is the emission probability of a fluorescence photon and $A_s$ is the emission probability of a Auger electron [116].

The total de-exitation probability (i.e., emission of a fluorescence photon or Auger electron added) is inversely proportional to the core-hole lifetime $\tau_h$, which is smaller for atoms with higher atomic number and, consequently, larger number of upper energy levels and options of electrons, which can occupy the hole. This value is related to the energy width of the excited state $\Gamma_h$ due to the uncertainty principle by $\Gamma_h \tau_h \approx h$, which is related to the resolution of absorption peaks. For a fixed edge, the larger the atomic number, the smaller the core-hole lifetime and the larger the energy width [116].

X-ray fluorescence can be used in materials characterization, as will be discussed in the next sections of this paper (see section 2.6), to determine the composition of samples. It can be used in qualitative way, in order to determine which elements are present, and also in a quantitative way, since the number of photons detected for a certain energy depends on the concentration of the element in sample [123].
C.6 Diffraction

When the X-ray scattering happens in a periodic crystal, the scattered light from different planes in the crystal interfere such that the resulting light can only be measured in some angles, where the interference is constructive. This angle depends on the energy of the radiation and it is given by Bragg’s law:

\[ n\lambda = 2d \sin \theta \]  

(113)

where \( n \) is an integer, \( \lambda \) is the wavelength of the scattered light, \( d \) is the interplanar distance for the crystal and \( \theta \) is the scatter angle [123].

Bragg’s law for X-ray diffraction offers the possibility of using different techniques for materials characterization. The law presents only three parameters, making it possible to design experiments such that one parameter is fixed, one is measured and, then, the other can be calculated. One of these techniques is X-ray diffraction (XRD), where, typically, the wavelength is fixed, the angle is measured and the interplanar distance is calculated providing information on the lattice structure of the material being measured [123].
D Detectors and detector artifacts

Energy dispersive detectors (i.e., detectors capable of obtaining information on a photon’s energy) are necessary for X-ray fluorescence measurements. The different types of these detectors can be classified into gas filled detectors and semiconductor detectors.

Gas filled detectors use the ionization of the gas resulting of the interaction with the incident X-rays to detect the photons. They are filled with an inert gas, which will not react with the radiation, such as argon, kryptonium or xenon and a quench gas, responsible for avoiding avalanche processes in the gas. Since quench gases can change chemically during the detection, they are consumed with the use of the detector and, therefore, in a sealed detector, they determine the detector’s lifetime. A way to prevent that is to use a flow counter, which introduces gas in the detector as necessary to maintain a constant level. This last kind of detector is specially used when detecting elements with low atomic number, since the detector in this case must have a very thin entrance window, which does not avoid completely atmospheric contamination and, therefore, the continuous flow of quench gas is necessary for maintaining the detecting properties of the detector [123].

The proportional scintillation counter is a combination of gas and scintillation detectors. In this case, the detector uses the recombination energy (i.e., when the free electrons recombine with the ions from the gas after being ionized by the radiation), typically in the order of 20 to 40 eV. These photons are captured and amplified by the scintillator, which improves the energy resolution of the detector. However, due to the difficulty in producing these detectors, they are not available commercially [123].

The most common detectors for X-ray fluorescence measurements, however, are semiconductor detectors. These detectors can be built using a series of materials, but the most common choice is silicon, because it provides a good combination of parameters important for an efficient detection, such as a small amount of energy
necessary for the creation of a charge carrier pair, which has a relatively long lifetime, resistance against radiation damage and facility in production [123].

Silicon-based detectors have a better energy resolution than gas filled detectors, typically in the range of 120 to 200 eV, but they lose in terms of count rate capabilities and detection areas, which are usually in the order of a few 10 mm² [123].

In energy dispersive detectors, the number of charge carriers produced is proportional to the energy of the photon detected:

\[ N = \frac{E}{\varepsilon} \]  

where \( N \) is the number of charges, \( E \) is the energy of the absorbed radiation and \( \varepsilon \) is the amount of energy necessary to produce each charge. Therefore, for each detection, the charge carrier pulse heights are measured and assigned to an energy channel. Since photons with the exact same energy can be affected by different noises, the height of peaks from photons with the same energy will differ slightly and, as result, the heights corresponding to each energy are given by a Gaussian distribution [123].

The detected energy spectrum from a sample can show structures, which are not the result of fluorescence emissions, but are caused by other factors [123]. They will be discussed in the following sections, which are based on reference [123], unless otherwise marked.

D.1 Detector response function

The detector response function influences the spectrum intensity distribution, according to various factors, such as the broadening of the measured peaks discussed above, due to the statistical fluctuations in the energy detected caused by noise in the detector.

Another factor that may contribute to the response function is incomplete charge
collection, which can be caused by the recombination of charges along the path from where they are generated until they reach the cathodes. This effect would decrease the amount of measured charges, causing a continuous contribution to the lower energies of a peak called shelf; and by the impossibility of measuring all the charges created by a photon when its absorption happens very close to the detector entrance window (some of the charges end up in the window), which causes a tail also on the low energy side of the peak that depends on the energy of the incident photon due to the fact that lower energy photons have an increased probability to be absorbed close to the entrance window when compared to higher energy ones. The first effect can be reduced by using a detector material with better quality and the second can be reduced by using special window materials.

D.2 Escape peak

Escape peaks are peaks produced when the incident photon produces a fluorescence photon in the detector material, which, then, leaves the detector. When this happens, the peak measure by the detector appears to have an energy smaller than it should, considering the energy of the absorbed photon:

\[ E_{esc} = E_{inc} - E_{fluor} \] (115)

where \( E_{esc} \) is the energy where the escape peak appears, \( E_{inc} \) is the energy of the incident photon and \( E_{fluor} \) is the energy of the fluorescence photon produced in the detector.

The probability of an escape peak being formed depends on the energy of the incident photon. If the photon has energy below the edge of the detector material, the probability is zero, since this photon will not be able to cause the excitation of the atom’s electrons. If the energy is above the edge energy for the material, then lower
energy photons will have a larger probability to generate escape peaks, since they are usually absorbed closer to the surface of the detector when comparing to higher energy photons. When the photon is absorbed farther from the detector surface, it has to travel a larger distance to escape the detector, increasing the probability that the produced fluorescence photon will still be absorbed by the detector and will not escape.

D.3 Pile up peaks

When two photons are detected at the same time (i.e., when the time interval between them is smaller than the time resolution of the detector) the signal from the two pulses may be added. The result is a peak at the energy corresponding to the sum of the energy of the two detected photons.

The probability of such an overlap increases for more intense energy peaks, since they are result of more photons being detected at such energy. The pile up peak intensity, therefore, is given by

\[ I_{\text{Sum}} = I_{\text{Mother}_1} I_{\text{Mother}_2} P \]

(116)

where \( I_{\text{Mother}_1} \) and \( I_{\text{Mother}_2} \) refer to the intensity of the mother peaks (i.e., peaks with the same energy as the photon being overlapped) and \( P \) is the probability for sum peak as a function of shaping time and energy.

The pile up peaks need to be identified as such so that they are not assigned to an element in the spectrum analysis. One way of doing so is by changing the excitation intensity and plotting the results normalized in relation to a peak, which is surely identified as an element’s spectral line. In this case, as the excitation intensity increases or decreases, the intensity of the elemental peaks will also increase or decrease proportionally, maintaining a constant ratio. However, since
pile up peak intensities are the result of a multiplication of the intensities of two other peaks, their change with excitation intensity will be squared. Thus, when comparing the normalized spectra, all the peaks will look the same, except the pile up peaks, which will have increased or decreased in intensity much more than the elemental peaks.

Pile up peaks can also be handled mathematically, using equation 116 to calculate their intensity and applying these values to the spectrum fitting. This approach is necessary when a pile up peak coincides with the spectral line of an element measured and it is a way to isolate their intensities.

D.4 Scattering peaks

Scattering peaks are usually a small effect, but can still cause misidentifications in the spectrum analysis. They are the result of X-rays being scattered in the spectrometer and are not a result of sample measurement. Some common effects include the excitation of the argon in the atmosphere, resulting on a peak in its corresponding energy in the final spectrum (this can happen when the measurement is not taken under vacuum conditions); the scattering of fluorescence radiation at the detector’s collimator or envelope, which usually generates small peaks for uncommon elements, such as zirconium; and scattered radiation form the sample, which excites parts of the spectrometer, resulting on small peaks, which can be misconstrued as trace elements.

D.5 Diffraction peaks

If the sample is crystalline or has crystalline areas, the appearance of diffraction peaks is possible. The energy of the radiation diffracted is given by

\[ E = 1,242 \frac{n}{2d} \sin \theta \]  

(117)
where $n$ is an integer, $d$ is the interplanar distance in the crystal and $\theta$ is the angle between beam and sample.

For polycrystalline samples (i.e., samples in which the crystals are oriented in many different directions), a large energy range can be diffracted in small quantities. These diffractions, however, involve a small portion of the beam and do not have large intensity. In this case, the result is an overall increase of the background for the energy range in question. In micro-XRF, however, since the beam shines in a very small region, not all directions of crystals are present, resulting in higher intensity peaks with very specific energies, which do not correspond to elemental spectral lines.

It is difficult to correct the measured spectra for diffraction peaks, so the best procedure is to try to avoid them at the time of the measurement or to find experimental ways to identify them. These techniques can include measuring the same sample in different scatter angles, which would also change the energy of the diffraction peaks, but not the energy of the fluorescence peaks; identifying the peaks, using the fact that diffraction peaks may not correspond to any element or, when it does, they will not present the other lines in the element series, or that diffraction peaks are usually not Gaussian in shape; and using filters to reduce the radiation in the continuous part of the spectrum.
E  Software used to reconstruct ant map

This is the code used to reconstruct the map for the North Carolina amber inclusion (NC 272-276) from chapter 5. The software was written in C++ using the Cern ROOT framework.

/** Program developed to fit spectra from SXRMB beamline from CLS – map reconstruction, gluing two maps. Using fitting background methods from TSpectrum and fitting peaks with Gaussians. */

#include <stdio.h>
#include <fstream>
#include <iostream>
#include <string.h>
#include <TApplication.h>
#include <TCanvas.h>
#include <TSpectrum.h>
#include <TMath.h>
#include <TH1.h>
#include <TLegend.h>
#include <TF1.h>
#include <TH2.h>
#include <TH2F.h>
#include <TFile.h>
#include <TPolyMarker.h>

TApplication myapp("myapp", 0,0,0);  
using namespace std;

int size =2048;
int nElement;
int pointsx1;
int pointsy1;
float lowx1;
float highx1;
float lowy1;
float highy1;
int add1;
int pointsx2;
int pointsy2;
float lowx2;
float highx2;
float lowy2;
float highy2;
int add2;
char kind[4];
float current;
char nameElement[20];
float posElement;
float areaError;
float area;
float_t *pSpec=new float[size];

TH1 *rawSpec; //raw spectrum
TH1 *backSpec; // spectrum without the background
TH1 *background; // background
TH1 *concentration=new TH1F("Concentration", "Concentration", 1000, 0, 2);
TH2 *map;

canvas *c=new TCanvas();
canvas *cBack=new TCanvas();
canvas *cMap=new TCanvas();
canvas *cConc=new TCanvas();
TF1 *gaus1;
TList *functions;
TPolyMarker *tpm;
TSpectrum *sp;

ifstream input1;
ifstream input2;
ifstream elementData;
ifstream corrFile1;
ifstream corrFile2;
TFile *file;

// openFile opens the input File
bool openFile(char inDir[], char inName1[], char inName2[], char outDir[] , char name[]){
    input1.open(Form("%s%Bruker_1.mca", inDir, inName1));
    if(!input1){
        std::cout << "ERROR − Input 1 file does not exist" << std::endl;
        return false;
    }
    input1.clear();
    input1.seekg(0, ios::beg);
    input2.open(Form("%s%Bruker_1.mca", inDir, inName2));
    if(!input2){
        std::cout << "ERROR − Input 2 file does not exist" << std::endl;
        return false;
    }
    input2.clear();
    input2.seekg(0, ios::beg);
    file=new TFile(Form("%s%root", outDir, inName1, name), "RECREATE");
    if(!file){
        std::cout << "ERROR − Could not create output file" << std::endl;
    }
    return true;
}
return false;
}
elementData.open("ListElements.dat");
if (!elementData){
    std::cout << "ERROR - Element data file does not exist" << std::endl;
    return false;
}
elementData.clear();
elementData.seekg(0, ios::beg);
corrFile1.open(Form("%s%s_1.dat", inDir, inName1));
if (!corrFile1){
    std::cout << "ERROR - Correction file" << inDir << inName1 << " does not exist" << std::endl;
    return false;
}
corrFile1.clear();
corrFile1.seekg(0, ios::beg);
corrFile2.open(Form("%s%s_1.dat", inDir, inName2));
if (!corrFile2){
    std::cout << "ERROR - Correction file" << inDir << inName2 << " does not exist" << std::endl;
    return false;
}
corrFile2.clear();
corrFile2.seekg(0, ios::beg);
return true;
}

bool initiateVar(int nx1, float lx1, float hx1, int ny1, float ly1, float hy1, int ad1, int nx2, float lx2, float hx2, int ny2, float ly2, float hy2, int ad2, char name[], char type[]){
    pointsx1=nx1;
    pointsy1=ny1;
lowx1=lx1;
highx1=lx1;
lowy1=ly1;
highy1=ly1;
add1=ad1;
pointsx2=nx2;
pointsy2=ny2;
lowx2=lx2;
highx2=lx2;
lowy2=ly2;
highy2=ly2;
add2=ad2;

strcpy(nameElement, name);
strcpy(kind, type);
if((strcmp(kind,"lin")!=0)&&(strcmp(kind,"log")!=0)){
    std::cout << "Parameter for kind of map invalid. Options: log and lin"
    << std::endl;
    return false;
}
for(int i=0; i<36; i++){
    string line;
    getline(corrFile1, line);
}
for(int i=0; i<36; i++){
    string line;
    getline(corrFile2, line);
}
std::cout << "Initiating variables" << std::endl;
std::cout << "Map size: " << (pointsx1+add1) << " X " << (pointsy1+
    pointsy2-1) << std::endl;
map=new TH2F("Map", Form("Map - %s", nameElement), (pointsx1+add1),
    lowx1, highx2, (pointsy1+pointsy2), lowy1, highy2);

return true;
}

bool readFile(int mapN){
    char temp[20];
    int vbin;
    int bin=0;
    if(mapN==1){
        input1 >> temp;

        do{
            input1 >> temp;
            vbin=atoi(temp);
            rawSpec->Fill(bin+1,vbin);
            pSpec[bin]=vbin;
            bin++;
        }while(bin<size);
    }
    else{
        input2 >> temp;

        do{
            input2 >> temp;
            vbin=atoi(temp);
            rawSpec->Fill(bin+1,vbin);
            pSpec[bin]=vbin;
            bin++;
        }while(bin<size);
    }
}

```cpp
char pars[21][21];
}

char pars[21][21];
std::cout << "Beam current (correction): " << current << std::endl;
std::cout << "================================================================================File

================================================================================" << std::endl;
return true;
}

bool closeFile()
{
elementData.close();
input1.close();
input2.close();
corrFile1.close();
corrFile2.close();
return true;
}

// fitRawData draws and fits the histograms and save fitting parameters.
// It also calculates the area under the fitted peaks bool dra=false;

bool fitRawData()
{
  // draw histogram
  elementData.clear();
elementData.seekg(0, ios::beg);
c->ed();
c->SetLogy(1);
}
rawSpec->Draw();
sp->Background(pSpec, 2048, 20, TSpectrum::kBackDecreasingWindow,
     TSpectrum::kBackOrder2, kFALSE, TSpectrum::kBackSmoothing3, kTRUE);
for (int i=0; i<size; i++)
    background->Fill(i+1,pSpec[i]);
background->SetLineColor(2);
background->Draw("SAME L");
cBack->cd();
for (int i=0; i<size; i++)
    backSpec->Fill(i+1, rawSpec->GetBinContent(i+1)-background->
                   GetBinContent(i+1));
backSpec->Draw();
std::cout << "Background removal... DONE" << std::endl;
sp->Search(backSpec, 2.5, "nobackground", 0.008);
functions=backSpec->GetListOfFunctions();

****** If automatic algorithm could find peaks, it will identify
the desired peak by comparing it to the expected energy and use
the energy from the peak finder to fit and calculate the area.
In case the algorithm cannot find the desired peak, the
theoretical value for the position will be used instead ******

if (functions->FindObject("TPolyMarker")){
    tpm=(TPolyMarker*) functions->FindObject("TPolyMarker");
    tpm->SetMarkerSize(1.5);
    tpm->SetMarkerColor(kGreen);
    Double_t* posPeak=tpm->GetX();
    int nPeaks=tpm->GetN();
    std::cout << nPeaks << " peaks found" << std::endl;
    nElement=nPeaks;
    char name[20];
    char tEnergy[10];
    float fEnergy;

    243
```cpp
int foundPeaks[30];
int pCount=0;

do{
    elementData >> name >> tEnergy;
    fEnergy=atof(tEnergy);
    if(strcmp(nameElement, name)==0)
        posElement=fEnergy/5+95;
    if(strcmp(name, "***")==0)
        continue;
    for(int cnt=0; cnt<nPeaks; cnt++)
        if(((posPeak[cnt]-95)*5 < fEnergy+50) && ((posPeak[cnt]-95)*5 > fEnergy-50))
            std::cout << "Element: " " name << " - Energy: " " (posPeak[cnt]-95)*5 " eV" << std::endl;
            if(strcmp(nameElement, name)==0)
                posElement=posPeak[cnt];
        foundPeaks[pCount]=cnt;
        pCount++;
} while(strcmp(name,"***")!=0);

for(int i=0; i<nPeaks; i++)
    bool unk=true;
    for(int t=0; t<pCount; t++)
        if(i==foundPeaks[t])
            unk=false;
        if(unk)
            std::cout << "Unknown peak - Energy: " " posPeak[i]*10/2 " eV" << std::endl;
} else{
    std::cout << "Peaks could not be found" << std::endl;
```
std::cout << "Using theoretical values for calculating area" << std::endl;
char name[20];
char tEnergy[10];
float fEnergy;
do{
    elementData >> name >> tEnergy;
    fEnergy = atof(tEnergy);
    if(strcmp(nameElement, name)==0){
        posElement = fEnergy / 5 + 95;
        std::cout << "Element: " << name << " - Energy: " << fEnergy << " eV
             " << std::endl;
    }
    if(strcmp(name, "***") == 0)
        continue;
}while(strcmp(name, "***") != 0);

// fit peaks
if(strcmp(nameElement, "Nikalpha") == 0){
    std::cout << "Fitting ... " << nameElement << std::endl;
    backSpec->Fit(gaus1, "Q", ",", posElement - 20, posElement + 30);
    area = gaus1->Integral(posElement - 20, posElement + 30);
    areaError = gaus1->IntegralError(posElement - 20, posElement + 30);
} else if(strcmp(nameElement, "Kkalpha") == 0){
    std::cout << "Fitting ... " << nameElement << std::endl;
    backSpec->Fit(gaus1, "Q", ",", posElement - 20, posElement + 20);
    area = gaus1->Integral(posElement - 20, posElement + 20);
    areaError = gaus1->IntegralError(posElement - 20, posElement + 20);
} else if(strcmp(nameElement, "Cakalpha") == 0){
    std::cout << "Fitting ... " << nameElement << std::endl;
    backSpec->Fit(gaus1, "Q", ",", posElement - 40, posElement + 20);
    area = gaus1->Integral(posElement - 30, posElement + 20);
areaError=gaus1->IntegralError(posElement-30, posElement+20);
}
else{
std::cout << "Fitting ... " << nameElement << std::endl;
backSpec->Fit(gaus1, "Q", ",", posElement-30, posElement+30);
area=gaus1->Integral(posElement-30, posElement+30);
areaError=gaus1->IntegralError(posElement-30, posElement+30);
}
return true;
}

bool plotMap(int xpos, int ypos){
    if(strcmp(kind, "lin")==0)
        if(area/current*100<1)
            map->SetBinContent(xpos, ypos, area/current*100);
else
    map->SetBinContent(xpos, ypos, 0);
    if(strcmp(kind, "log")==0)
        map->SetBinContent(xpos, ypos, TMath::Log10(area/current));
    std::cout << "Current: " << current << std::endl;
    std::cout << nameElement << " area: " << xpos << ", " << ypos << " = "
            << area << std::endl;
    std::cout << nameElement << " normalized area: " << map->GetBinContent(
            xpos, ypos) << std::endl;
    if(area!=0)
        concentration->Fill(area/current*100);
return true;
}

bool MakeMap(char outDir[], char inName1[], char opt[]){
    for(int j1=0; j1<pointsy1; j1++)
    for(int i1=0; i1<pointsx1; i1++)
        rawSpec=new TH1F("Raw Spectrum", "Raw Spectrum", 2048, 0, 2047); //
        raw spectrum
backSpec = new TH1F("Spectrum (no background)", Form("Spectrum (no background) − %i,%i", i1+1, j1+1), 2048, 0, 2047); // spectrum without the background

background = new TH1F("Background", "Background", 2048, 0, 2047); // background
gaus1 = new TF1("gaus1", "gaus", 0, 2048);
sp = new TSpectrum(100);
if (!readFile(1)) {
    std::cout << "Error reading file 1" << std::endl;
    return false;
}
if (fitRawData())
    std::cout << "Area calculated successfully!" << std::endl;
else {
    std::cout << "Fitting could not be performed" << std::endl;
    return false;
}
file >> cd();
rawSpec->Write();
backSpec->Write();
if (plotMap(i1+1,j1+1))
    std::cout << "Map plotted" << std::endl;
else {
    std::cout << "Error plotting map" << std::endl;
}
}

for (int b=0; b<add1; b++){
    area = 0;
    if (plotMap(pointsx1+b+1,j1+1))
        std::cout << "Map plotted" << std::endl;
    else {
        std::cout << "Error plotting map" << std::endl;
    }
}
rawSpec->Delete();
backSpec->Delete();
background->Delete();
gaus1->Delete();
sp->Delete();
}

for(int j2=0; j2<pointsy2; j2++){
    for(int b=0; b<add2;b++){
        area=0;
        if(plotMap(b+1,pointsy1+j2+1))
            std::cout << "Map plotted" << std::endl;
        else{
            std::cout << "Error plotting map" << std::endl;
        }
    }
}

for(int i2=0; i2<pointsx2; i2++){
    rawSpec=new TH1F("Raw Spectrum", "Raw Spectrum", 2048, 0, 2047); // raw spectrum
    backSpec=new TH1F("Spectrum (no background)", Form("Spectrum (no background) \(-%i, %i\)\), i2+1, j2+1), 2048, 0, 2047); // spectrum without the background
    background =new TH1F("Background", "Background", 2048, 0, 2047); // background
    gaus1=new TF1("gaus1", "gaus", 0, 2048);
    sp=new TSpectrum(100);
    if(!readFile(2)){
        std::cout << "Error reading file 1" << std::endl;
        return false;
    }
    if(fitRawData())
        std::cout << "Area calculated successfully!"<< std::endl;
    else{

        248
std::cout << "Fitting could not be performed" << std::endl;
return false;
}
file ->cd();
rawSpec->Write();
backSpec->Write();
if(plotMap(add2+i2+1,pointsy1+j2+1))
std::cout << "Map plotted" << std::endl;
else{
    std::cout << "Error plotting map" << std::endl;
}
rawSpec->Delete();
backSpec->Delete();
background->Delete();
gaus1->Delete();
sp->Delete();

file ->cd();
map->Write();
cMap->cd();
map->SetStats(0);
map->Draw("COLZ");
cConc->cd();
concentration->SetTitle("Concentration");
concentration->Draw();
if(strcmp(opt, "no")==0){
cMap->SaveAs(Form("%s_%s_map-complete.png", outDir, inName1, nameElement));
cConc->SaveAs(Form("%s_%s_conc-complete.png", outDir, inName1, nameElement));
} else {
cMap->SaveAs( Form("%s%s_%s_map-complete.png", outDir, inName1, 
    nameElement, opt));
cConc->SaveAs( Form("%s%s_%s_conc-complete.png", outDir, inName1, 
    nameElement, opt));
}
closeFile();
return true;

// Main function calling all the action. Stops program if anything goes 
    wrong
int main(int argc, char* argv[]){
    std::cout << "Parameters: " << argc << std::endl;
    std::cout << "Element: " << argv[16] << std::endl;
    if(argc!=22){
        std::cout << "Invalid number of parameters\nProgram will be closed" << 
            std::endl;
        return 0;
    }
    if(openFile(argv[1], argv[2], argv[9], argv[18], argv[16]))
        std::cout << "Files opened successfully" << std::endl;
    else{
        std::cout << "Invalid input file" << std::endl;
        return 0;
    }
    initiateVar(atoi(argv[3]), atof(argv[4]), atof(argv[5]), atoi(argv[6]), 
        atof(argv[7]), atof(argv[8]), atoi(argv[9]), atoi(argv[10]), atof( 
            argv[11]), atof(argv[12]), atoi(argv[13]), atof(argv[14]), atof( 
                argv[15]), atoi(argv[16]), argv[17]);
    std::cout << "Making map" << std::endl;
    if(MakeMap(argv[18], argv[2], argv[19]))
        std::cout << "Map made successfully" << std::endl;
    else{

250
```cpp
std::cout << "Error in map" << std::endl;
return 0;
}

std::cout << "***************END PROGRAM***************" << std::endl;
myapp.Run(1);
```